FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 8 No. 3, June, 2024, pp 396 - 400
DOI: https://doi.org/10.33003/fjs-2024-0803-3922

EFFECT OF VITAMIN C, E AND SELENIUM ON HAEMATOLOGICAL AND SERUM BIOCHEMISTRY OF ADULT MALE GUINEA PIGS

***Ibrahim Bature and Gaddafi Sani**

Department of Animal Science, Federal University Dutsin-Ma, Katsina State, Nigeria

*Corresponding authors' email ibature@fudutsinma.edu.ng Phone: +2347067212353

ABSTRACT

The study was conducted to assess the biopotency of antioxidant supplements on physiological, testicular, and oxidative biomarkers in adult male guinea pigs (*Cavia porcellus*). A total of 60 adult male guinea pigs of three months of age with an average body weight of 500±20g were randomly allocated into three dietary treatment groups of vitamin C, E and Selenium and each comprised four varying levels with five animals per group in a completely randomized design (CRD). Feed and water was provided *ad-libitum.* Haematological concentration of guinea pigs in this study indicated a significant ($P<0.05$) variations in Packed cell volume, Haemoglobin, White blood cell and differential counts of guinea pigs supplemented with varying levels of vitamin C, E and Selenium. The result also indicate a non-significant ($P>0.05$) difference on Plasma Albumin, Globulin, Total protein, Alanine aminotranferase and Alkaline phosphate of animal supplemented with varying dosage of vitamin E and Selenium. However, a significant ($P<0.05$) difference occur in Plasma ascorbic acid, Plasma vitamin E, Plasma selenium and Testesterone in guinea pig supplemented with vitamin C, E and Selenium with only exception on Plasma ascorbic acid and Plasma selenium in guinea pigs supplemented with vitamin E and C for Plasma ascorbic acid and Plasma selenium respectively. It could be concluded that vitamin C, E and Selenium can be incorporated into guinea pigs without deleterious effect in both heamatological and serum biochemical profile. Farmers are therefore recommended to use upto 300mg/kg vitamin C, 45mg/Kg vitamin E and 0.3mg/kg selenium in guinea pigs diet for outstanding performance and healthy blood profile.

Keywords: Guinea pigs, Haematology, Serum, Vitamins, Selenium

INTRODUCTION

Information on guinea pig production is scarce in Nigeria, which hampers rapid development of micro-livestock sector, perhaps due to lack of knowledge and technical know-how, less acceptability of guinea pig by people and guinea pig market and product acceptability is generally scarce in Nigeria. Even though they are suitable for breeding, they require less management and capital for investment (Gaddafi *et al.,* 2020).

The guinea pig is best known from a nutritional standpoint, by its requirement for dietary vitamin C, this feature has made the guinea pig particularly useful in studies of collagen biosynthesis, wound healing, and bone growth. Scientific researchers showed that vitamin C has hepatoprotective property. This is linked to its antioxidative property. Vitamin C was reported to attenuate hepatic damage induced by some chemical agents especially in animals. This is supported by the work of Bashandy and Alwasel (2011). These authors reported that vitamin C normalized levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, blood hydroperoxide and malondialdehyde in liver of carbon tetrachloride induced hepatotoxicity in rats.

Vitamin C is a water soluble antioxidant. In contrast to many mammals, humans and guinea pigs are unable to synthesize vitamin C due to the lack of the enzyme L-gulono-gamma-lactone oxidase (Jesse *et al.,* 2007)..Vitamin C is found in citrus, soft fruits and leafy green vegetables. Kidney and liver are good animal derived sources of vitamin C (Stangeland *et al.,* 2008). Vitamin C can be administration orally or intravenously (Padayatty *et al.,* 2008). It is well absorbed efficiently in the small intestine via a saturable active transport mechanism. Vitamin C is widely distributed in all tissues of the body, with higher levels found in the adrenal glands, pituitary and retina. Kidney and muscle tissues have lower level of vitamin C (Wandzilak *et al.,* 2013). Selenium is an essential micronutrient required for normal growth and maintenance in livestock. Recently, selenium (Se) has been recognized as an essential dietary nutrient. Dietary selenium is an essential trace element for animals and humans with a variety of biological function (Surai and Sparks, 2006). These compounds are necessary for growth, fertility, immune system, hormone metabolism, cell growth and antioxidant defense systems in animals and humans (Pappas and Zoidis, 2012).

Since vitamin E acts as a tissue antioxidant and aids in quenching free-radicals produced in the body, any infection or other stress factors may exacerbate depletion of the limited vitamin E stores from various tissues. The protective effects of vitamin E on animal health may be involved with its role in reduction of glucocorticoids, which are known to be immunosuppressive. Vitamin E also most likely has an immune enhancing effect by virtue of altering arachidonic acid metabolism and subsequent synthesis of prostaglandin, thromboxanes and leukotrienes. Under stress conditions, increased levels of these compounds by endogenous synthesis or exogenous entry may adversely affect immune cell function (Gaddafi *et al.,* 2020). The main objectives of this study is to determine the effect of vitamin C, E and selenium supplementation on haematological and serum biochemical profile of guinea pigs.

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at the teaching and research farm of the Department of Animal Science Ahmadu Bello University, Samaru, Zaria. The farm is located between latitude 11° 9' 45''N and 7° 38'8'' E longitude at an altitude of 610m above sea level (Ovimaps, 2015). Zaria town is part of Northern Guinea Savannah between latitudes 11° and 12°N and between longitude 7° and8' E at an altitude of 640m above sea level. The climate of the area is sub-humid; rainfall is well distributed during the rainy season, with a mean annual rainfall of 1100mm. The rainy season starts from late April or early mid-October and about 70% of the rain occurs during the months of July and August, followed by the hamattan period of cool and dry weather which usually lasts from October to February. This is then followed by hot-dry weather from March to April, known as pre-rain period. The average ambient temperature and relative humidity during the wet season are 24.7°C and 72% respectively. This is followed by the hot weather when temperatures fluctuate during day from 13 to 36°C with a mean relative humidity of 20-37% (IAR 2016)

Experimental Animals and Design

A total of 60 adult male guinea pigs of three months of age with an average body weight of 500±20g were randomly allocated into three dietary treatment groups of vitamin C, E and Selenium and each comprised four varying levels with five animals per group in a completely randomized design (CRD). Feed and water was provided *ad-libitum.*

Experimental Diet and Management

The diet was formulated to meet the dietary recommendation for guinea pig under tropical condition (NRC, 1995). Twelve different diets were formulated comprising four levels of vitamin C (0 mg, 100 mg, 200 mg and 300 mg of vitamin C/kg of diet), vitamin E (0 mg, 15 mg, 30 mg and 45 mg of vitamin E/kg) and selenium (0 mg, 0.1 mg, 0.2 mg and 0.3 mg of selenium/kg).

Data Collection

Haematological Parameters

Blood was collected at the end of experiment (12 weeks post feeding) by severing the *jugular veins* of two (2) fasted guinea pigs per treatment into labeled bottles, which contained ethylene diamine tetraacetic acid (EDTA). The blood samples were placed on an ice pack and transported to the Clinical Pathology unit of the Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for determination of haematological parameters. Such as: haemoglobin content, white blood cells, red blood cells, neutrophils, lymphocytes, eosinophils, monocytes, basophils and packed cell volume (PCV) was determined by the procedure outlined by Dacie and Lewis (2001).

Biochemical Parameters Analysis

Blood and serum samples were collected at the end of experiment (week 12) from Jugular veins of two (2) guinea pigs from each treatment. A quantity of 5 ml of blood was collected into labeled sterile sample bottles without anticoagulant and used for the serum biochemical analysis. The sample was centrifuged at 3000 rpm for 15 minutes. Separated sera were stored frozen at -20°C in sample bottles without anticoagulant until the time of analysis for Vitamin C, E and Selenium content. Vitamin C and Vitamin E were determined using Spectrophotometer (UV-2550™) from a previously modified technique described by Rutkowski *et al.* (2005), while plasma selenium was determined using the procedure described by Ursine *et al.,* (1985) at the Basic Research Laboratory of National Research Institute for Chemical Technology Basawa, Zaria. The serum biochemical indices to be determined were serum Albumin, globulin, total protein, alkaline phosphate (ALP), Alanine aminotranferase (ALT), Aspartate aminotransferase (AST), were analyzed using spectrophotometric linked reaction method (Cheesbrough, 2004).

Statistical Analysis

The data collected from all the experiment was subjected to analysis of variance (ANOVA), using the General Linear Model Procedure of SAS (2002). The significant differences among the treatment means were separated using the Duncan`s Multiple Range Test in the SAS Package.

RESULT AND DISCUSSION

Effect of vitamin C, E and Selenium on Haematological Parameters of Adult Male Guinea Pigs

Effect of vitamin C, E and selenium on haematological parameters of adult male Guinea pigs is presented in table 1. Packed cell volume (PCV), Red blood cell (RBC), Haemoglobin (Hb), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) and eosinophils were statistically similar (P>0.05) in guinea pigs supplemented varying levels of vitamin C and selenium. The significantly higher concentration of white blood cells, lymphocyte, monocytes, nuetrophils in the blood of adult guinea pigs supplemented with varying dosage of vitamin C. All the values measured are within the normal haematological range of healthy guinea pigs reported by Williamson and Festing (1971) who's reported healthy guinea pigs had 37-51%, 11-15 g/dl, 6-10 x 10^9/mm^3, 39-72%, 3-12% and 28-44% for WBC, Lymphocyte, Monocytes and Neutrophils respectively.

While Red blood cell, Packed cell volume, heamglobin, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and eosinophils concentration in the blood of adult male guinea pigs supplemented with varying levels of vitamin C were similar (P>0.05). These values obtained also fall within the normal range of 4.5-8.0g/dl, 37-51%, 11-15g/dl, 20-25µ/dl, 30-35% and 1-5% for red blood cell, PCV, Hb, mean corpuscular haemoglobin, mean corpuscular concentration and Eosinophils respectively as reported by Deniels (1996).

White blood cell (77.00%), neutrophils (8.93x10^9/I), monocyte (42.33%), lymphocytes (1.67%) and basophils (4.7%) are higher (P<0.05) in guinea pigs supplemented 0mg, 200 mg, 100 mg, 0 mg and 300 mg vitamin C/kg diets. Higher (P<0.05) PCV (49.00%), RBC (8.20g/dl), Hb (16.33 g/dl), WBC (7.70x10^9/i), Neutrophils (53.33%) and Lymphocyte (67.67%) values in Guinea pigs supplements 45 mg, 45 mg, 45 mg, 15 mg, 30 mg and 0 mg vitamin E/kg fed diet. The result revealed a significantly differences in Packed cell volume, heamaglobin, Red blood cell, White blood cell, Neutrophils, eosinophils, Lymphocyte and Basophils in vitamin E supplemented adult male guinea pigs in table 1 While mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and monocytes concentrations in blood of adult male guinea pigs supplemented with vitamin E indicated similar values. The irregular and inconsistent trend of white blood cell and some differential counts in vitamin E group may be as a result of hind-limb paralysis observed in some Guinea pigs during the experimental period or as a result of detrimental effect of environmental temperature fluctuation during the study period.

Neutrophils (48.67%), basophils (15.67%) was higher (P<0.05) in Guinea pigs fed diets supplemented 0.3 and 0 mg/kg selenium diet. The effect of selenium supplementation on the haematological parameters of adult male guinea pigs is presented in Table 1. There were similar values in packed cell volume, red blood cell, heamoglobin, eosinophils, monocyte, MCH, MCHC and White blood cell in Guinea pigs fed diets supplemented varying levels of selenium. Basophils concentration of guinea pigs was significance in this group, this can be attributed to the effect of selenium on immune system. (Wintergerst *et al.,* 2007) reported that selenium is indispensable in the production of lymphocyte and basophil migration inhibition factor and interleukin 2 which accelerates the proliferation, maturation and activity of T lymphocytes.

Table 1: Effect of Vitamin C, E and Selenium on Haematological Parameters of Adult Male Guinea Pigs

Treatments	PCV (%)	RBC (g/dl)	Hb (g/dl)	MCH (µ/dl)	MCHC (%)	WBC (x10⁹/l)	Neut (%)	Eosi (%)	Mono (%)	Lympho (%)	Baso (%)
Vit. C											
0mg/kg	48.67	8.03	15.90	20.04	33.29	8.93^{a}	20.33^{c}	0.00	0.33^{bc}	77.00^{a}	1.0^{b}
100mg/kg	48.67	8.17	14.23	19.77	33.29	7.40^{a}	31.67^{b}	1.33	1.67^{a}	60.33^{b}	3.7^{ab}
200mg/kg	45.00	7.27	15.03	19.64	33.26	4.47^{c}	42.33^{a}	0.67	0.00^{c}	54.33^{c}	3.7^{ab}
300mg/kg	50.00	8.20	16.57	20.03	33.25	6.20^{b}	38.00^{a}	0.00	1.00^{ab}	54.33^{c}	4.7^{a}
SEM	0.35	0.14	0.36	0.01	0.01	0.21	0.95	0.21	0.10	0.28	0.0
LOS	NS	NS	NS	NS	NS	*	*	NS	*	**	*
Vit. E											
0mg/kg	42.67^{c}	7.27^{b}	14.47^{b}	20.15	34.55	7.67^{a}	27.00^{b}	0.00	3.67	67.67^{a}	2.67
15mg/kg	47.33^{ab}	7.73^{b}	16.07^{a}	20.37	33.27	7.70^{a}	27.33^{b}	0.67	4.33	63.67^{a}	2.00
30mg/kg	45.00^{bc}	7.63^{b}	14.97^{b}	34.27	34.27	4.93^{b}	53.33^{a}	0.00	3.67	40.67^{c}	2.00
45mg/kg	49.00^{a}	8.20^{a}	16.33^{a}	33.26	33.26	5.13^{b}	47.33^{a}	0.00	0.67	50.00^{bc}	1.00
SEM	0.35	0.07	12.26	0.65	0.38	0.23	2.80	0.08	0.55	2.80	0.0
LOS	**	**	*	NS	NS	**	**	NS	NS	*	NS
Selenium											
0mg/kg	48.00	7.80	15.87	20.25	35.32	12.93	33.33^{a}	4.00	2.33	38.67	15.67^{a}
0.1mg/kg	48.67	7.90	16.37	20.08	34.22	12.40	28.93^{a}	0.00	4.00	44.67	8.67^{ab}
0.2mg/kg	44.67	7.80	15.23	21.07	33.93	15.37	4.37^{b}	0.00	1.33	47.33	2.33^{b}
0.3mg/kg	46.67	8.17	15.70	19.00	33.93	8.47	48.67^{a}	0.00	1.00	48.67	1.33^{b}
SEM	0.30	0.10	0.17	0.27	0.51	0.953	3.67	0.0	0.50	2.49	0.0
LOS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	*

^{abc}Mean with different superscripts within columns differed significantly, * significant at P<0.05, NS= Not significant, SEM= Standard error of mean, PCV= Packed cell volume, RBC= Red blood cell, Hb= Heamoglobin, MCH= Mean corpuscular heamoglobin, MCHC=Mean corpuscular heamoglobin concentration, WBC=White blood cell.

Effect of Vitamin C, E and Selenium on Some of the Serum Biochemical Parameters of Adult Male Guinea Pigs

Table 2 shows effect of vitamin C, E and selenium on some of the serum biochemical parameters of adult male Guinea pigs. In vitamin C group, no significant (P>0.05) difference was observed for plasma Albumin and plasma globulin. These values obtained fall within the normal range of plasma albumin and globulin for healthy guinea pigs reported by Wiliamson and Festing (1971) (2.1 to 3.9 g/dl and 1.7 to 2.6g/dl plasma albumin and globulin respectively).

Significant (P<0.05) differences was observed in total protein, ALT, AST while ALP indicated a non-significant (P>0.05) increased for adult guinea pigs supplemented with varying dosage of vitamin C. The plasma total protein values falls within the normal range reported by Williamson and Festing (1971) and Mohanta *et al.,* (2014) 4.6-6.2g/dl and 5.66-5.94g/dl respectively. The high level of ALT and AST observed in this study supported the findings of Bashandy and Alwasel (2011). They reported that vitamin C normalized levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, blood hydroperoxide and malondialdehyde in blood and liver of carbon tetracholide induced hepatotoxicity in rats.

Plasma Ascorbic acid, vitamin E, and Testosterone are highly significant (P<0.05) different in an adult guinea pigs supplemented with vitamin C. There are numerically linear increases of plasma ascorbic acid this is similar with the findings of Hill *et al*., (2017), who reported 0.0µmol/L to 59±14µmol/L of ascorbate concentrations in plasma of guinea pigs. The highest value of 1.900µmol/L for plasma vitamin E obtained in guinea pigs supplemented with vitamin C also falls within the range of 0.5-2.5µmol/L alpha tocopherol concentration in plasma of guinea pigs. Plasma testesterone concentration in Table 2 For a guinea pigs supplemented with vitamin C shows a highly significant (P<0.05) increase with increase in dosage level of vitamin C (5.300ng/ml to 9.533ng/ml for 0mg/kg to 300mg/kg vitamin C respectively) this values obtained for plasma testosterone was inconformity with Koss-slob *et al.* (1978) who reported 5.0ng/ml to 10.2ng/ml plasma testosterone level of guinea pigs at 55 to 65 days of age respectively. This findings is in contrast with the report of Rigaudiere *et al.* (1976) who reported a relatively low concentration of plasma testosterone in adult guinea pigs. This may be as a result of vitamin C beneficial effect, absence of induced stress that will interfere with the endocrine secretion of the testis or plane of nutrition Koss-slob *et al.* (1979) reported that undernourished guinea pigs had very low plasma testosterone levels (1.7 ng/ml).

In vitamin E group, Albumin, glubolin, total protein ALT and ALP values were in conformity with the normal range for healthy guinea pigs as reported by Williamson and Festing (1971).

A significant difference was observed in this study for plasma AST of adult male guinea pigs supplemented with varying dosage of vitamin E. This depletion of AST values as shown on Table 4.6. This may be as a result of environmental factors that were not identified and which might possibly affects the vitamin E groups, and this was probably a reflection of vitamin E response against stress amelioration. However, this may also support the findings of Sattar *et al.* (2007) that "vitamin E supplementation has been reported to improve humoral immune responses to bacterial, viral antigen and effect on thermal stress amelioration. Plasma ascorbic acid shows non-significant (P>0.05) with increased dosage of vitamin E while plasma vitamin E, selenium and testosterone in this study showed significantly higher value. Guinea pigs showed a linear increase of plasma vitamin E concentration with increased dosage level of vitamin E (1.300 µmol/L, 1.933 µmol/L, 2.300 µmol/L and 2.700 µmol/L for 0, 15, 30 and 45 mg/kg respectively).

This clearly indicates that vitamin E metabolism, absorption and retention tends to increase with increased dosage of vitamin E, hence there is a need for further studies on guinea pigs. Plasma vitamin E concentration obtained in this study agreed with 0.5-2.5 µmol/L and 0.7-2.8 µmol/L α-tocopherol concentration of adult male guinea pigs observed during this study was in agreement with the adult guinea pig range of 5.0 ng/mg to 10.2 ng/ml reported by Koss-slob *et al.,* (1978). This is clearly reflected that guinea pigs supplemented with varying dosage of vitamin E are properly nourished since Koss-slob *et al.,* (1979) reported very low plasma testosterone levels of 1.7ng/ml for undernourished guinea pigs. Also this concurred with Azzi and Stocker (2000) reports on vitamin E physiological role who said "vitamin E is a dietary factor for animal nutrition, which is important for normal reproduction. Selenium group, indicated that guinea pigs supplemented with varying dosage of dietary selenium had similar values of plasma albumin, glubolin, total protein, ALT and ALP. This normalization of some serum biochemical parameters indicate the physiological activities played by dietary selenium in this experiment. This is also in agreement with statement made by Surai, (2006) who said "dietary selenium is essential trace element for animal with a variety of biological functions. Significant difference for AST, plasma vitamin C, E, selenium and Testosterone were significantly different with dosage level of selenium of adult male guinea pigs.

Table 2: Effect of Vitamin C, E and Selenium on Some of the Serum Biochemical Parameters of Adult Male Guinea Pigs

Treatment	Alu (g/dl)	Glu (g/dl)	TP (g/dl)	ALT (ul^{-1})	AST (ul^{-1})	ALP (ul^{-1})	PAA (µmol/l)	PE (µmol/l)	Pse (µ/g)	Tes (ng/ml)
Vit. C										
0mg/kg	3.367	2.400	5.767	26.00	22.00^{b}	72.67	29.80^{c}	1.00^{b}	5.50	5.30^{b}
100mg/kg	2.433	2.433	4.867	27.00	19.00^{b}	72.67	36.93^{bc}	1.53^{bc}	6.30	5.40^{b}
200mg/kg	2.833	3.433	3.933	19.33	15.67^{b}	70.33	44.80^{b}	1.90^{a}	7.33	5.57^{b}
300mg/kg	3.200	2.300	5.500	36.00	32.33^{a}	73.33	60.07^{a}	1.77^{a}	6.50	9.53^{a}
SEM	0.105	0.306	0.055	2.187	1.098	3.586	1.498	0.062	0.305	0.237
LOS	NS	NS	NS	NS	**	NS	**	*	NS	**
Vit. E										
0mg/kg	2.600	2.367	4.967	19.67	33.00^{ab}	55.00	31.27	1.30^{c}	6.10	7.47^{ab}
15mg/kg	2.400	2.333	4.733	17.00	25.67^{b}	64.00	28.87	1.93^{b}	7.40	5.80^{b}
30mg/kg	2.667	1.967	4.633	17.67	12.00^{c}	75.67	36.07	2.30^{a}	7.00	8.63^{a}
45mg/kg	2.933	2.200	5.133	18.67	41.67^{a}	65.67	34.40	2.70^{a}	7.27	8.53^{a}
SEM	0.480	0.312	0.438	1.255	1.590	3.775	1.567	0.047	0.136	0.355
LOS	NS	NS	NS	NS	**	NS	NS	**	NS	*
Selenium										
0mg/kg	2.533	2.533	5.033	14.00	19.67^{a}	48.67	28.47^{c}	0.87^{b}	7.63^{bc}	5.00^{c}
0.1mg/kg	3.467	1.867	5.333	15.67	10.07^{b}	123.00	31.17^{b}	1.70^{a}	7.23^{c}	7.83^{b}
0.2mg/kg	3.000	2.000	5.000	8.33	0.027^{c}	96.00	37.53^{a}	0.77^{b}	8.03^{b}	10.43^{a}
0.3mg/kg	3.067	2.100	5.167	15.33	0.047^{c}	92.33	39.20^{a}	1.60^{a}	8.83^{a}	9.70^{a}
SEM	0.142	0.169	0.121	3.428	1.330	15.230	0.168	0.071	0.010	0.054
LOS	NS	NS	NS	NS	**	NS	**	**	**	**

[abc]Mean with different superscripts within columns differed significantly, * significant at P<0.05, NS- Not significant, SEM-Standard error of mean. Alu -Albumin, Glu - Glubomin, TP - Total protein, ALT- Alanine aminotransferase, AST- Aspartate aminotranseferase, ALP- Alkaline Phosphatase, PAA -Plasma ascorbic acid, PE-plasma vit. E and PSE- Plasma selenium

CONCLUSION

It could be concluded that vitamin C, E and Selenium can be incorporated into guinea pigs without deleterious effect in both heamatological and serum biochemical profile. Farmers are therefore recommended to use upto 300mg/kg vitamin C, 45mg/Kg vitamin E and 0.3mg/kg selenium in guinea pigs diet for outstanding performance and healthy blood profile.

REFERENCES

Azzi, A., and Stocker, A. (2000). Vitamin E: non-antioxidant role. *Lipid Research,* 39:231-255. https://doi.org/10.1016/S0163-7827(00)00006-0

Bashandy, S.A. and Alwasel, S.H. (2011). Carbon tetrachloride-induced Hepatoxocity and Nephrotoxicity in rats: protective role of vitamin C. *Journal of Pharmacology and Toxicology,* 6(3):283-292.doi10.3923/jpt.2011.283.292.

Cheesbrough, M. (2004). District laboratory practice in tropical countries. Part 2 university press Cambridge United Kingdom, 266-342. https://www.cabidigitallibrary.org/doi/full/10.5555/20063178567

Dacie, J.V. and Lewis, S.M. (2001). Practical heamatology 9th edition Churchill livingstone, London, p.633. https://www.scirp.org/reference/referencespapers?referenceid=52040

Daniels, LA. (1996). Selenium metabolism and bioavailability. *Biology of Trace Element Research,* 54:185-199. https://www.scirp.org/reference/referencespapers?referenceid=1108177

Gaddafi, S., Garba, M.G., Abdulrshid, M., Zahraddeen, D., Daudou, O.M. and Iyeghe-Erakpotobor, G.T. (2020). Effect of antioxidant supplements on testicular histo-morphology in adult male guinea pigs (*cavia porcellus*), *Nigerian Journal of Animal Production*, 47(5): 29-40. https://njap.org.ng/index.php/njap/article/view/1272

Hill, K.E., Montine, T.J., Motley, A.K., Li, X., May, J.M and Burk, R.F. (2017). Combined deficiency of vitamin E and C causes paralysis and death in guinea pigs. *The American Journal of Clinic Nutrition,* 77:1484-1488. Downloaded from ajcn.nutrition org at Nigeria: ASNA sponsored on August 19, 2017. https://doi.org/10.1093/ajcn/77.6.1484

IAR (2016). Meteorological unit, weather report, Institute for Agricultural Research. Ahmadu Bello University Zaria. https://journals.uniosun.edu.ng/ogr/article/view/98

Jesse, B., Nick, H., Robert, P., Kevin, A.C., Keith, D.T., Penny, J., Alex, G., Nicholas, G., and Mary, R.L. (2007). Sparing effects of selenium and ascorbic acids on vitamin C and E in guinea pig tissues. *Nutrition journal,* 6:7. https://doi:10.1186-2811-6-7

Koss-Slob, A., Vreeburg, J.T.M and Vanderwerfftenboseh, J.J (1978). Body growth, puberty and undernutrition in the male guinea pig. *British Journal of Nutrition.* 41:231-237. Downloaded from https://www. Carbridge.0rg/core. Ip address:197.210.227.229, on 21 aug 2017 at 11:46:39. https://doi.org/10.10779/BJN19790032.

Ovimaps (2015). Map version 01.28.107. Nokia® Corporation.

Mohanta, R.K., Garg, A.K., Dass, R.S. and Behera, S.K. (2014). Blood biochemistry thyroid hormones, and oxidant/antioxidant status of guinea pigs challenged with sodium arsenite or arsenic trioxide. *Biological Trace Element Research,* 14:41-45. https://doi:10.1007/s12011-014-0041-5

NRC (1995). National Research Council. Nutrient Requirement for laboratory Animals, fourth Revised Edition, 1995. Washington DC: *The National Academics press.* https://doi:10.17226/4758

Padayatty, S.J., Katz, A. Wrang, Y., Eck, P. and Kwon, O. (2008). Vitamin C as an antioxidant Evaluation of its role in disease prevention. *Journal of Animal Nutrition.* 22:18-35. https://doi.org/10.1080/07315724.2003.10719272

Pappas, A.C. and Zoidis, E. (2012). The role of selenium in chicken physiology: New insight in: Chicken physiology, Diseases and farming practices, Kapur, I. and Mehra, A. (Eds.). Nova science publishers inc; New York USA; ISBN-13:9781620810279, PP.51-69. https://scholar.google.com/scholar?cites=14349258689788496464&as_sdt=2005&sciodt=0,5&hl=en

Rigaudiere, N., Pelardy, G., Robert, A. and Delost, P. (1976). Changes in the concentrations of p testosterone and androstenedione in the plasma and testis of the guinea pig from birth to death. *Journal of Reproduction and Fertility,* 48, 291-300. https://rep.bioscientifica.com/view/journals/rep/48/2/jrf_48_2_012.xml

Rutkowski, M., Grzegorczyk, K. and Paradowski, M.T. (2005). Kolorymetryczna metoda Oznaczania calkowitej vitaminy E w osoczu krwi-modykikacjawlasna metody Tsena (Colorimetric method of blood plasma total vitamin E determination- the own modification of Tsen method). *Diagnostic Laboratory,* 41, 375 (in polish). https://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-72e614e4-f8be-401e-b693-3a991d0b86ac

Sattar, A., Mirza, R.H. and Hussain, S.M.I. (2007). Effect of prepartum treatment of vitamin E- Selenium on postpartum reproductive and productive performance of exotic cows and their calves under subtropical condition. *Parkistan Veterinary Journal,* 27(3):105-108. https://www.pvj.com.pk/pdf-files/27_3/page%20105-108.pdf

SAS Institute, Inc. (2002). SAS User`s guide: Statistics. Version 9. SAS Institute, carry, NC 27513, USA.

Stangland, B., Henson, D.E., Block, G., and Levine, M. (2008). Ascorbic acid: biologic functions.Stangland, B., Henson, D.E., Block, G., and Levine, M. (2008). "Ascorbic acid: biologic functions and relation to cancer". *Journal of National Cancer Instution,* 83(8):547-50. PMID 1672383. https://agris.fao.org/search/en/providers/122621/records/6473968d3ed73003714cce51

Surai, P.F. and Sparks, N.H.C. (2006). Tissue-specific fatty acid and α-tocopheral profile in male chickens depending on dietary tuna oil and vitamin E provision. *Poultry Science,* 79:1132-1143. https://doi.org/10.1093/ps/79.8.1132

Wandzilak, T.R., D`Andre, S.D., Davis, P.A., Williams, E.A., and Oputri, D. (2013). Heparisprotective effect of vitamin C (Ascorbic acid) pharmacology 4(1) Article ID:275089. http://dx.doi.org/10.4236/pp.2013.41012

Wiliamson, J. and Festing, M. (1971). A note on some haematological parameters in three strains of guinea pig. *Newsletter,* 2:7-11. https://www.cabidigitallibrary.org/doi/full/10.5555/19710105073