



HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF GROWING RABBITS FED ON GRADED LEVELS OF DRIED OKRA (*ABELMOSCHUS ESCULENTUS*) LEAF MEAL

Imade, A. A.<sup>1</sup>, Familola, O. E.<sup>2</sup>, Adetoboye, O. O.<sup>3</sup>, Sarumi, B. B.<sup>1</sup>, Osokolo, E.<sup>1</sup>, Abiodun, O. A.<sup>1</sup>, Familola, O. T.<sup>1</sup>, Ganiyu, A. A.<sup>1</sup> and Adekiya, P. O.<sup>1</sup>

<sup>1</sup>Federal Institute of Industrial Research, Oshodi, Lagos

<sup>2</sup>Nigerian Stored Products Research Institute, Yaba, Lagos

<sup>3</sup>Department of Pharmacology, University of Lagos

\*Corresponding Author: erica.noriega@dps.texas.gov; imadeafiange@yahoo.com

Abstract

A ten-week trial was conducted to evaluate the effects of dried okra leaves on haematological and serum biochemical indices of growing rabbits. The blood parameters were packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC) counts and white blood cell (WBC) counts. Others are mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Forty-five unsexed rabbits (Chinchilla x New Zealand White) of 5- 6 weeks old with average weight of 720g were used for the study. The rabbits were divided on weight equalization basis into 5 dietary groups containing dried okra leaves (DOL) at (0.00%, 2.50%, 5.00%, 7.50%, and 10.00%) inclusion levels. Each treatment consisted of 9 rabbits and replicated thrice with each replicate consisting of 3 rabbits. The rabbits were supplied with the diets in mash form and clean drinking water *ad libitum*. The blood analysis was carried out at the 9<sup>th</sup> week of the experiment. Red blood cell count was significantly ( $P < 0.05$ ) largest for rabbits fed diets containing 5.00% DOL ( $7.50 \times 10^{12}/L$ ) and least for 0.00% and 7.50 % ( $6.30 \times 10^{12}/L$ ) each group.

**Keywords:** Blood parameters, rabbits and dried okra leaves

INTRODUCTION

In most developing countries, particularly Nigeria, average consumption of animal protein is very low, and is estimated at 4.5g per day as against the minimum requirement of 35g per day (FAO, 2010). Yusuf *et al.* (2009) reported that high cost of conventional animal feed might be responsible for the low protein consumption. This reduced animal protein intake has far-reaching implications on the health status and well-being of the populace (Alu *et al.*, 2009). Therefore, efforts are being directed towards exploring all reasonable options to meet the recommended level of animal protein consumption at a reduced cost. The role of rabbits to provide a regular supply of high quality protein and income under sustainable systems that utilize renewable resources at a minimal cost is presently recognized in many parts of the world (Amaravadhi *et al.*, 2012). Rabbits can thrive on high fibre diets and hence have a comparative advantage over other monogastric animals. Rabbit is increasingly becoming an important meat source and it is recommended for production in countries that are experiencing meat shortage (Ewuola *et al.*, 2012). Rabbit is a mini livestock that is easy to manage, highly prolific and has a short gestation interval of thirty days (Adeyemi *et al.*, 2008). It is also fast

becoming a substitute for red meat which is believed to have slower rate of digestion and hence under investigation for cause of colon cancer (Corpet, 2011). Rabbit meat is reputed to be low in fat, (unsaturated fatty acid is about 63% of the total fatty acid) low in sodium and cholesterol and has high protein/energy ratio and is relatively rich in essential fatty acid (DalleZotte and Spendro, 2011). Rabbit has a greater ability to efficiently convert leaf meal and agro-industrial by-products into meat compared to other ruminant livestock Omoikhoje *et al.*, (2006). This has necessitated the need to seek for alternative feed sources in forages. The use of okra leaves as forage is among the possible alternatives, Doreddula *et al.* (2014). This is especially so because of the greater availability of okra leaves and ability of rabbits to convert okra leaves into meat for human consumption (Iyeghe-Erakpotobor *et al.*, 2006). Okra (*Abelmoschus esculentus*) is one of the most well-known and utilized species of the family (*Malvaceae*). It is also a vegetable crop grown for its immature pods that can be consumed as a fried or boiled vegetable or may be added to salads, soups and stews (Roy *et al.*, 2014). World production of okra as fresh vegetables is estimated at six million tonnes per year (Sergius and Esther, 2014). Okra leaves are commonly used both as food and for

curative purposes showing low calories, a good source of edible fibre, contains important bioactive compounds such as carotene, folic acid, thiamine, riboflavin, niacin, vitamin C, oxalic acid and amino acids (Roy *et al.*, 2014). Okra leaves have essential B vitamins for creating and maintaining new cells and foliate, a vital substance for optimum pregnancy. Doreddula *et al.* (2014). Vitamin C aids in preventing birth defects and enables the foetus to develop completely (Habtamu *et al.*, 2014). The high quantity of foliate present in okra leaves is helpful for the foetus while developing. Foliate is a vital nutrient that increases the growth and development of the foetus's brain. The high quantity of folic acid within okra performs a huge role in the formation of the foetus through the fourth to the twelfth weeks of pregnancy in humans (Zaharuddin *et al.*, 2014). Fresh okra leaves are high in beta carotene and ascorbic acid (Habtamu *et al.*, 2014). Beta carotene as important as pro-vitamin A for the maintenance of normal tissue structure and other important physiology functions such as vision and reproduction (Habtamu *et al.*, 2014). The antioxidant activity of the okra leaves is due to its content of phenolic compounds which are effective antioxidants and can be used in the prevention of degenerative processes such as cancer, cardiovascular diseases and diabetes (Doreddula *et al.*, 2014) Okra leaves play a significant role in human nutrition by providing carbohydrates, protein, fat, minerals and vitamins that are generally deficient in basic food (Oyelade *et al.*, 2003). Okra leaves are used in making soup, salad and for flavouring when dried and powdered. The tender leaves contain minerals especially calcium, magnesium, iron and phosphorus, protein, vitamins A and C including riboflavin as well as high mucilage (Ndaeyo *et al.*, 2005).

## MATERIALS AND METHODS

### Experimental Site and Location

The experiment was carried out at the rabbitary unit of the Directorate of University Farms (DUFARMS), Federal University of Agriculture (FUNAAB), Alabata road, Abeokuta, Ogun State, Nigeria. The University is located on latitude 7° 10'N, longitude 3°2'E (Google Earth 2016) and in altitude of 76 m above sea level. It lies between South-Western part of Nigeria with a prevailing tropical climate with mean annual rainfall of 1,037 mm, an average temperature of 34.7°C. The vegetation in the University represents the interphase between the tropical rainforest and the derived savannah with relative humidity of 82%.

### Experimental Design and Management of Experimental Animals

Forty-five (45) unsexed crossbred rabbits (Chinchilla X New Zealand White) of 5 - 6 weeks of age and weight range of 720g - 750g were assigned to 5 treatment groups using Completely Randomized Design and on weight equalization basis. Each treatment group consisted of 9 rabbits with 3 rabbits per replicate. The 3 rabbits representing an experimental unit were housed together serving as replicate in each wooden cage measuring 76cm x 62cm x 42cm.

Before the start of the experiment, the rabbits were de-wormed using Ivermectin injection 0.1ml/kg BW and administered coccidiostat (Ampro-victracycline) via drinking water. The rabbits were acclimatized for 14 days during which they were fed concentrates and forages *ad libitum*. Drinkers and feeding trough were provided in each cage. The drinkers were washed daily. The rabbits were fed daily in two feeding periods (08.00 hours and 16.00 hours). Feed leftover was weighed before the morning feeding. Fresh water was supplied to the rabbits *ad libitum*. The hutches were thoroughly cleaned weekly throughout the experimental period to maintain a good hygienic environment. The experiment lasted for 70 days. The experimental diets are shown in Table 1.

### Preparation of dry okra leaves meal and feed formulation

The forage okra (*Abelmoschus esculentus*) leaves were purchased from local farmers in Igboora, Oyo State. The fresh okra leaves were air-dried by spreading on concrete floor until crispy to touch. The dried okra leaves were milled before being incorporated into the concentrate diet. In this study, 5 diets were formulated to meet the nutrient requirement for growing rabbits (NRC, 1994). Diet 1 was a basal diet 17% crude protein and 2700 ME/kg (control). Diets 2, 3, 4, and 5 consisted of basal diet plus 2.50% 5.00% 7.50% and 10.00 % of dried okra leaves (DOL) respectively. 100g of wilted *Tridax procumbens* were supplied to each replicate daily as supplement.

### Collection of blood samples

At the 10<sup>th</sup> week of the experiment, one rabbit was selected per replicate and blood samples were collected. A volume of 2.5ml of blood was withdrawn from the ear vein of each rabbit by means of sterile hypodermic needle and 2.5 ml was used for haematological parameters and stored in Bijou bottles with ethylene diamine tetra acetate (EDTA) as anticoagulant while the other 2.5ml was stored without coagulant (allowed to clot) for serum Biochemistry analysis. Sample bottles containing blood samples were placed on ice packs to maintain a cool and stable temperature and immediately sent for laboratory analysis. Plasma was harvested subsequently by centrifuging the blood samples at

3000rpm for 15 minutes in a centrifuge. The heparinised plasma samples were stored at 20 °C in Eppendorf tubes and analysed. The serum biochemical measurements determined are serum, total protein, globulin, albumin, glucose, urea, creatinine, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides

### Haematological indices

Haematological indices such as packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC) and differential leukocyte counts were determined according to the procedure described by Jain *et al.*, (1986). Erythrocyte indices (MCV, MCHC, and MCH) was calculated using appropriate formulae:

$$\text{MCV} = \text{PCV} / \text{RBC} \text{ (litre/cell)}$$

$$\text{MCH} = \text{Hb} / \text{RBC} \text{ (gram /cell)}$$

$$\text{MCHC} = \text{Hb} / \text{Hct} \text{ or } = \text{MCH}/\text{MCV} \text{ (gram /litre)}$$

**Packed cell volume:** The percentage of the packed red cells in the blood was determined using the haematocrit centrifuge method as described by Dacie and Lewis, (1995). A capillary tube was dipped into preposition to fill it about three-quarter length. Excess blood on the side of the capillary tube was wiped off in other to keep accurate reading. One end of the tube was sealed over a Bunsen burner. The capillary tube was put into a micro-haematocrit reader and the levels of packed cells were regarded as the packed cell volume.

**Red blood cell (RBC):** Blood was diluted with 0.9 % NaCl. The diluted blood was mounted on a haemocytometer. Calculated erythrocytes were expressed in million per cubic meter.

**White blood cell (WBC):** The estimate of the total number of white blood cells was carried out immediately after collection of blood sample from experimental animals using Neubauer haemocytometer counting chamber (Jain, 1986). From blood sample of test animal 0.2 ml of blood sample was pipette and mixed with 4 ml of WBC diluting fluid made up of 3 % aqueous solution of acetic acid and 1 % gentian violent). The sample was put into the haemocytometer and cell counted and expressed as  $10^6$  WBC per litre of blood.

**Haemoglobin (Hb):** The Hb concentration of each blood sample was determined by cyanmethaemoglobin method as described by (Jain, 1986). From each blood sample of experimental animal, 20  $\mu$ l of blood was mixed with 4ml of modified Drabkin's solution prepared by mixing 200 mg potassium ferricyanide, 50 mg potassium cyanide and 140 mg potassium dihydrogen phosphate; volume was made up of 1 litre with distilled water and pH adjusted to 7.0). The mixture

of blood sample of experimental animal and Drabkin's solution was allowed to stand for 3 minutes before reading the haemoglobin concentration using a spectrophotometer at wavelength of 540nm. The acute value haemoglobin was extrapolated from a standard curve.

**Mean corpuscular haemoglobin concentration (MCHC):** The mean corpuscular haemoglobin concentration is a measure of the concentration of haemoglobin in a given volume of packed red blood cells. It was reported as part of a standard complete blood count. It was calculated by dividing the haemoglobin by the packed cell volume (PCV) (Jain, 1986).

$$\text{MCHC} = \text{Hb} / \text{PCV} \text{ (grams/litre)}$$

**Mean corpuscular haemoglobin (MCH):** The mean corpuscular haemoglobin or mean cell haemoglobin (MCH) is the average mass of haemoglobin per red blood cell in a sample of blood. It was calculated by dividing the total mass of haemoglobin by the number of red blood cells in a volume of blood. It was calculated using the formulae below:

$$\text{MCH} = \text{Hb} / \text{RBC} \text{ (g/cell)} \text{ (Jain, 1986)}$$

**Mean corpuscular volume (MCV):** The mean corpuscular volume or mean cell volume (MCV) is a measure of the average red blood cell volume that was reported as part of a standard complete blood count. It was calculated using the formulae below:

$$\text{MCV} = \text{PCV}/\text{RBC} \text{ (litre/cell)} \text{ (Jain, 1986)}$$

**Total serum protein:** The following was prepared according to Kaneko (1989):

Solution 1: It contained 45g of sodium potassium titrates (Rochelle salt) dissolved in 400ml of 0.2N NaOH in a beaker. 1.5g of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and 5g of potassium iodide were added and dissolved completely by stirring. The mixture was rinsed with 0.2N NaOH and poured into a flask. The solution was made up to a litre with 0.2N NaOH.

Solution 2: This consists of 0.5% potassium iodide (KI) in 0.2N NaOH working biuret reagent. An aliquot of solution 1 was diluted to 250 ml with solution 2. An aliquot of 0.1 ml was pipetted into test tube and 2.9 ml of water was added. The blank consisting of 3.0 ml distilled water was pipetted into the test tube. To each test tube, 3.0 ml of working biuret reagent was added and the tubes were incubated in 37°C water bath for 10 minutes.

Protein of each sample was calculated from the formula below:

$$\text{TSP (g/100ml)} =$$

$$\frac{\text{Optical density of test} \times \text{Concentration of standard}}{\text{Optical density of standard}}$$

### Serum albumin

The bromocresol green (BCG) method was used to determine the serum albumin adopted by (Peters *et. al* 1982). The bromocresol green is a stable complex with absorbance maximum at 600 nm. The intensity of the colour produced is directly proportional to the albumin concentration of the sample. 4 ml of bromocresol green was added to 0.2 ml of each of the serum samples. Bromocresol green solution (4 ml) was used as a blank. The content of each tube was mixed and left at room temperature for 10 minutes at pH 4.2±0.05. After 10 minutes, the test solution was read at a wavelength of 640 nm in a spectrophotometer set to zero with the blank solution. Values of the samples were calculated using the formula

Serum albumin (g/100 ml) =

$$\frac{\text{Optical density of test X Concentration of standard}}{\text{Optical density of standard}}$$

#### Serum globulin

Serum globulin was calculated as adopted by (Peters *et.al.*, 1982):

Serum globulin (g) =

Total Serum Protein (g) – Serum albumin (g/100 ml).

#### Serum urea

This was determined using a kit (Quinica spam) having a linear measurement of about 566.6 ml per litre of urea concentration. (Bush, 1991) The serum was determined calorimetrically. The spectrophotometer (Model SP6-400 Ur Pyeunicam) was set at 600 nm wavelength and equivalent wavelength of sample read. Serum urea was calculated by the method adopted by (Bush, 1991).

$$\text{Urea (mg)} = \frac{\text{Sample Optical Density X 40}}{\text{Optical density of standard}}$$

#### Serum creatinine

This was analysed using calorimeter method. 1.0 ml of trichloroacetic acid and 1.0 ml of serum was mixed and centrifuged at 250 rpm for 10 minutes and the supernatant poured off. The mixture was allowed to cool for 20 minutes at a temperature of 25°C. The absorbance of the sample and standard was measured against the blank. (Peters *et. al.*, 1982).

Concentration of Creatinine (mg/dl) =

$$\frac{\text{Absorbance of sample x 2}}{\text{Absorbance of standard}}$$

#### Serum cholesterol

The cholesterol of the serum was determined using enzymatic endpoint method as described by Roeschlau *et al.* (1974). The absorbance of the sample was measured against the blood reagent within 60 minutes with the reading taken at wavelength 520 nm (Peters, *et al.*, 1982).

#### High density lipoproteins (HDL)

About 10 µl of test sample was precipitated and left for 10 minutes. The supernatant was centrifuged at 4000 rpm for 10 minutes. Sample supernatant of 100 µl was added to 1000 µl cholesterol reagent, mixed well and incubated for 10 minutes at 37 °C. The absorbance was read against blank on a spectrophotometer at 505 nm (Randox, 2012).

Concentration of HDL (mg/dL) =

$$\frac{\text{Absorbance of test x Concentration of std}}{\text{Absorbance of standard}}$$

#### Low density lipoprotein (LDL)

About 10 µl of sample test was mixed with 1000 µl of LDL cholesterol reagent and incubate for 10 minutes at 37°C with the standard. Absorbance was read against blank on a spectrophotometer at 505 nm (Randox, 2012).

Concentration of LDL (mg/dL) =

$$\frac{\text{Absorbance of test x Concentration of standard}}{\text{Absorbance of standard}}$$

#### Serum triglycerides

This was analysed using a spectrophotometer by measuring the absorbance of alkali hydrolysis forming glycerol which is oxidized to form periodate of test sample after incubation for 10 minutes at 37°C and read against the blank at 505 nm and standard reagents (Randox, 2012).

$$\text{Concentration of triglyceride (mg/dL)} = \frac{\text{Absorbance of test x Concentration of standard}}{\text{Absorbance of standard}}$$

#### Statistical analysis

Data collected were subjected to one-way analysis of variance. Data were analysed using SAS Package (2014) Significant means were separated and compared using Duncan Multiple Range Test of the SAS package at 5% level of probability.

## RESULTS

Effect of diets containing okra dried leaves on haematological indices of growing rabbits is presented in Table 1. There were no significant affected ( $P>0.05$ ) variations among the treatment groups in packed cell volume, haemoglobin, white blood cell, however, significant difference occurred in the red blood. 5.00% dried okra leaves had the largest value of  $7.50 \times 10^{12}$  while 0.00 % and 7.50 % of dried okra leaves have the least values of ( $6.30-7.13 \times 10^{12}$ ) each. Effect of diets containing varying levels of dried okra leaves on biochemical indices is presented in Table 2. There was no significant difference ( $P>0.05$ ) between the diets on all the serum parameters measured.

Table 1: Haematological parameters of rabbits fed diets on graded levels of dried, okra leaves (*Abelmoschus esculentus*)

Parameters	Normal values	Levels of Inclusion of dried okra leaves (%)					SEM	P Values
		0.00	2.50	5.00	7.50	10.00		
PCV (%)	30.00-50.00	38.00	40.00	43.00	42.00	40.67	0.76	0.388
RBC(X10 <sup>12</sup> /l)	5.00-8.00	6.30 <sup>b</sup>	6.67 <sup>ab</sup>	7.50 <sup>a</sup>	6.30 <sup>b</sup>	7.13 <sup>ab</sup>	0.17	0.256
Hb(g/dl)	10.00-17.00	12.70	13.33	13.83	15.00	13.03	0.34	0.442
WBC (X10 <sup>9</sup> /L)	5.00-12.00	6.10	5.67	6.33	7.17	8.20	0.42	0.043
Neutrophil (%)	25.00-55.00	29.00	27.00	27.00	28.00	30.67	0.92	0.398
Lymphocyte (%)	30.00-80.00	67.33	69.00	70.33	70.33	66.00	0.89	0.321
Eosinophil (%)	1.00-4.00	1.00	1.33	1.33	0.67	1.33	0.26	0.306
Basophil (%)	1.00-7.00	2.33	0.67	0.33	0.67	1.00	0.28	0.500
Monocyte (%)	1.00-4.00	0.33	2.00	1.00	0.33	1.00	0.23	0.571
MCHC (g/dl)	31.00-35.00	32.00	33.33	32.13	35.70	32.20	0.66	0.386
MCH (g/dl)	17.00-24.00	20.17	20.00	18.50	24.00	18.23	0.69	0.310
MCV (fl)	60.00-68.00	63.30	60.00	57.43	67.00	57.20	1.40	0.217

Ab = Means in the same row with different superscripts differ significantly (P<0.05); SEM = Standard error of means; PCV = Packed Cell Volume; RBC = Red Blood Cell; Hb = Haemoglobin; WBC = White Blood Cell; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; MCHC = Mean corpuscular haemoglobin concentration; Source of Reference for normal values: Fraser and Mays (1986)

Table 2: Serum biochemical indices of growing rabbits fed diets on grade levels of dried okra leaves (*Abelmoschus esculentus*)

Parameters	Normal values	Levels of Inclusion of dried okra leaves (%)					SEM	P Values
		0.00	2.50	5.00	7.50	10.00		
Total Protein (g/l)	5.00-8.00	7.90	5.47	6.87	5.97	6.10	0.35	0.211
Albumin (g/l)	2.50-4.00	4.30	3.07	4.07	3.20	3.57	0.19	0.467
Globulin (g/l)	2.40-4.70	3.16	2.40	2.80	2.77	2.53	0.19	0.467
Cholesterol (mg/dl)	20.00-83.00	78.00	84.33	74.33	82.33	75.00	2.22	0.737
Triglycerides (mg/dl)	30.00-90.00	83.67	81.00	82.33	81.33	86.00	3.93	0.835
Creatinine (mg/dl)	0.50-5.00	0.87	1.43	0.93	0.80	1.17	0.12	0.304
HDL (mg/dl)	30.00-50.00	39.87	44.73	38.50	40.67	38.17	1.82	0.637
LDL (mg/dl)	15.00-40.00	21.40	21.40	19.37	21.80	19.63	0.79	0.580
VLDL (mg/dl)	10.00-30.00	16.73	18.20	16.47	20.47	17.20	0.79	0.580

SEM = Standard error of means; HDL = High Density Lipoproteins; LDL = Low Density Lipoproteins; VLDL = Very Low Density Lipoprotein; Source of Reference for normal values: Fraser and Mays (1986)

## DISCUSSION

Packed cell volume (PCV) is a measure of the relative mass of blood (Baker and Silverston, 1985). The PCV values obtained in this study falls in line with the report by Ogbuewu *et al.*, (2010) on the effect of dietary inclusion of *africana indica* (goose grass) leaf meal on haematological characteristics of New Zealand White and Chinchilla buck rabbits. The PCV values obtained in this study were however; higher than the values reported by Etim and Oguike, (2011) who worked on rabbit does fed *Aspilia africana* leaf meal and Ojebiyi *et al.*, (2013). Aikhuomobhogbe and Orheruata (2006) asserted that low PCV results in anaemia that causes reduced oxygen carrying capacity of blood, increase pulse

rate and consequently heart failure. Reduction in the concentration of PCV in the blood usually suggests the presence of a toxic factor, for example. Haemagglutinin which has adverse effect on blood formation (Oyawoye and Ogunkunle, 1998).

Haemoglobin concentration obtained in this study is in agreement with the report by Ogbuewu *et al.*, (2010) on Newzealand white and Chinchilla buck rabbit. Similarly, the values obtained in this study are similar to the findings of Esonu *et al.*, (2006) on female rabbits fed *Aspilia africana* leaf meal based diet and that of the report by (Mufwa *et al.*, 2012). This implies that okra leaves protein is of higher quality. Pellet and Young (1980) demonstrated the

existence of positive correlation between haemoglobin concentration and quality protein in the diet. According to the report of (Etim and Oguike 2011 and Ojebiyi *et al.*, 2013) on haematology profile of non-pregnant and pregnant doe rabbits (9.51-10.41 g/dl and 9.84 -11.33 g/dl) were slightly lower than the haemoglobin concentration of dried okra leaves obtained in this study. Adejumo (2004) reported that haematological traits especially packed cell volume (PCV) and haemoglobin (Hb) were correlated with the nutritional status of the animal. Archetti *et al.*, (2008) reported that decrease in number of white blood cells below the normal range is an indication that the rabbits were stressed at the time of collection or an indication of allergic conditions anaphylactic shock and certain parasitism, while elevated values (leucocytosis) indicate the existence of a recent infection, usually bacteria (Ahamefule *et al.*, 2008). White blood cell values obtained for dried okra leaves in this study is in agreement with the findings of Esonu *et al.* (2006). However, the value obtained in this study is higher than the value reported by Ojebiyi *et al.*, (2013) The values obtained in this study showed that these animals were well nourished and were able to provide essential amino acids and minerals necessary for the normal functioning of the haematopoietic tissues (Ezeagu *et al.*, 2002).

The red blood cell counts (RBC) values recorded in this study falls within the recommended value of (3.8-7.9 X10<sup>6</sup> ) reported by (Hewitt *et al.*, 1989 : Medi rabbits, 2014). Increase in red blood cell counts were associated with high quality protein and disease free animal. Adeyemi *et al.*, (2008) observed that the red blood cells and haemoglobin are positively correlated with protein quality and protein level in the diet.

The Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values fall within the recommended value by Hewitt *et al.*, (1989). The Mean cell volume (MCV) value obtained in this study falls within the recommended value by (Hewitt *et al.*, 1989). This showed that the rabbits responded positively to the test ingredients. Thompson (2006) suggested that all the rabbits irrespective of the diets had normochromic and normocytic anaemia, meaning that the leaf meal 2.5 % to 10 % did not affect iron utilization by the rabbits. MCHC has shown to be the most accurate value that indicates anaemic condition (Thompson, 2006). The neutrophils counts obtained in this study fall within the normal recommended value of reported by Medi rabbits (2014). The lymphocyte count falls within the range value lymphocytes for buck and does rabbits reported by Ogbuewu *et al.*, (2010). The results recorded in this study was in agreement with the statement of (Ogbuewu *et al.*, (2010) who reported

that non elevated value of neutrophils and lymphocyte ratio suggest that the animals were clinically sound. The higher the value of leucocyte count, the better the ability of the animal to fight diseases (Robert *et al.*, 2003). One of the major functions of lymphocyte is their response to antigen (foreign bodies) by forming antibodies that circulate in the blood or in the development of cellular immunity. From the result of the differential counts, the higher value obtained for lymphocyte in the test group suggests a more effective antibody production. Reduction in the value of leucocytes may be due to low protein intake or liver damage or anaemia.

Monocytes, Eosinophils and Basophils counts were not significantly (P>0.05) influenced by dietary treatments. Eosinophils counts are in agreement with the report by Hewitt *et al.* (1989) for clinically healthy rabbits. The normal eosinophils levels in this study indicate that the animals did not suffer from parasitic infections during the experiment. Basophils component plays a significant role in some types of immunologic hypersensitive reactions and increase in lymphocytes and monocytes indicate viral or bacterial infection (Robert *et al.*, 1993). The presence of basophils counts in this study agree with the statement that basophil is normally presents in small or moderate number in the peripheral blood system of rabbits (Odeys, 1996: Ogbuewu, 2010). The monocyte counts obtained in this study when compared with the reference value (0-4%) reported by Medi rabbits, (2014) indicate that the animals did not react to any infections during the experimental period. The presence of monocyte in the rabbit fed dietary treatments contradicts the observations of (Ogbuewu, 2010) who recorded total absence of monocytes in male rabbits fed pawpaw peel meal and absence of monocytes in buck and doe rabbits fed graded levels of *Africana indica* (goose grass) leaf meal.

According to Otesile *et al.*, (1991), serum biochemistry is a generalized medium of assessing the health status of animals. Dietary components have been shown to have measureable effects on blood components (Awosanya *et al.*, 2000) hence serum biochemical metabolites are used to detect the existence of heart attack, liver damage and to evaluate protein quality and amino acid requirements in animals (Haper *et al.*, 1999). Chineke *et al.*, (2002) also reported that differences in serum biochemistry parameters might as well be caused by nutrition, environmental and hormonal factors.

The mean cholesterol value obtained in this study is similar to the findings reported by (Ogbuewu *et al.*, 2008). The creatinine value obtained in this study was found to be within the recommended value

reported by (Medi, 2014). Creatinine content has been shown to depend on the quality and quantity of dietary protein (Esonu *et al.*, 2001). The non-significant differences observed in creatinine were in line with the report of protein retained in animal by Akintola and Abiola (1999) and Awosanya *et al.* (2000). Abdel Hameed *et al.* (2013) reported that serum protein concentration at any given time in turn is a function of hormonal balance, nutritional status, water balance and other factors affecting health. Serum total protein has been reported as an indication of the protein retained in the animal body. Akintola and Abiola (1999) and Esonu *et al.* (2001). The values recorded for serum total protein of all treatments in this study is in agreement with the report by Hillyer (1994). The values in this study were higher than the values reported by Ogbuewu (2008) and Nuhu (2010). Generally, higher biochemical values observed could be attributed to the high nutritional value of the diets. The biochemical values of albumin and globulin obtained in this study were higher than the values reported by Ogbuewu *et al.* (2010). Total protein, albumin and globulin are influenced by the quality and quantity of protein supplied in the diet. Onifade and Tewe (1993) and Esonu *et al.* (2001).

## CONCLUSION

The haematology and serum biochemistry analysis of the experimental rabbits fed dried okra leaves manifested the superiority over the control diet. Okra leaves did not have adverse effects on the rabbits and it is assumed that the decrease or increase in the values of blood parameters investigated may be attributed to the stimulation of erythropoiesis and leucopoiesis. Dietary inclusion of dried okra leaves up to 10.00 % tended to improve various haematological parameters such as haemoglobin, white blood cell, MCH, MCHC, MCV and neutrophils. This shows that the leaves have the potential to increase immunity and some serum chemistry such as serum total protein, and albumin.

## REFERENCES

- Abdel-Hameed, A. A., Salih, A. M., FadelElseed, A. M. and Amasab, E. O. 2013. Effect of feeding Untreated or Urea Treated Groundnut Hull Supplemented with different protein sources on blood parameters of Sudan Desert Lambs. *Online Journal of Animal and Feed Research* 3(1): 40 -46.
- Adejumo, D. O. 2004. Performance organ development and haematological of rats fed sole diets of graded levels of cassava flour and soyabean flour (Soy gari) as substitutes for energy and protein concentrates. *Tropical Journal of Animal Science*.7 :57-63.
- Adeyemi, O. A., Sobayo, R. A., Aluko, F. A. and Oke, D. B. 2008. Utilization of rumen filtrate fermented corn-cobs by weaner rabbits. *Nig. J. Anim. Prod.* 35 (1): 69-75.
- Ahamefule, F. O., Obua, B. E., Ukweni, I. A., Oguike, M. A., and Amaka, R. A. 2008. Haematological and biochemical profile of weaner rabbits fed raw of processed pigeon pea seed meal based diets. *African Journal of Agricultural Research*, 3 (4): 315-319.
- Aikhuomobhogbe, P. U. and Orheruata, A. M. 2006. Haematology and blood biochemical indices of West African Dwarf goats vaccinated against pests des petit ruminants (PPR). *African Journal of Biotechnology*, 5 (9); 743-748.
- Akintola, S. O. and Abiola, S. S. 1999. Blood Chemistry and carcass yield of cockerels fed melon husk diets. *Tropical Journal of Animal Science* 2 ; 39-39.
- Alu, S. E., Ruma, R. S., Ubugadu, A. A. U., Aua, M. M and Makinde, O. J. 2009. The effect of different dietary fibre sources on haematological parameters and serum biochemical variable of growing rabbits. *Proceedings of Annual Conference of Animal Science Association of Nigeria (ASAN)*, Pp 274 – 276.
- Amaravadhi, S. C., Maalam, M., Manthani, G. P. and Komireddy, K. R. 2012. Effect of dietary supplementation of probiotics and enzymes on the haematology of rabbits reared under two housing systems. *Veterinary World*, 5 (12); 748-753.
- Archetti, L., Tittarelli, C., Cerioli, M., Brivio, R., Grilli, G. and Lavavzza, A. 2008. Serum chemistry and haematology values in commercial rabbits: Preliminary data from industrial farms in Northern Italy. Ethology and welfare 9<sup>th</sup> World Rabbit Congress. June 10-13, 2008. Verona, Italy. Pp. 237-243.
- Awosanya, B., Joseph, J. K., Apata, D. F. and Ayoola, M. A. 2009. Performance blood chemistry and carcass quality attributes of rabbits fed raw and processed Puereria seed meal. *Tropical Journal of Animal Science*.2 ;89-96.
- Baker, F. and Silvertown, J. 1985. Introduction to Medical Laboratory Technology (6<sup>th</sup> Edition). Butterworth Scientific, London, UK.
- Chineke, C. A., Adeniran, F. A., Oluogun, A. G., Ikeobi, C. O. N. and Oseni, O. A. (2002). Analysis of some serum biochemical parameters in New Zealand white rabbits and their crosses. In: Aletor, V. A. Onibi, G. E. (Eds). (2002) *Proc. 27<sup>th</sup> Anim. Conf. Nig. Soc. Anim. Prod.* March 17-21, FUTA, Akure, Nigeria. Pp.5 -7.
- Corpet, D. E. 2011. Red meat and colon cancer: Should we become vegetarians or can we make meat safer? *Meat Science*, 89:310-316.

- DalleZotte, A. and Spendro, Z. 2011. The role of rabbit meat as functional food. *Meat Science*, 88:319-331.: 443-455.
- Doreddula, S.K., Bonam, S.R. Gaddam, D.P., Desu, B. S.R., Ramarao, N., Pandey, V. 2014. Phytochemical Analysis, Antioxidant Antistress and Nootropic. Activities of Aqueous and Methanolic seed Extracts of ladies finger (*Abelmoschus esculentus*) in Mice *Sci. World J.* 2014: 519848
- Esonu, B. O., Emenelom, O. O., Udedibie, A. B. I., Herbert, U., Ekpo, C. F., Okoli, I. C. and Iheukwumere, F. C. 2001. Performance and blood chemistry of weaner pigs fed raw Mucuna (Velvet bean) meal. *Tropical Animal Production Invest*, 4: 49 -54.
- Esonu, B. O., Opara, M. N., Okoli, I. C., Obikaonu, H. O., Udedibie, C. and Iheshiulor, O. M. 2006. Physiological responses of laying birds to Neem (*Azadirachta indica*) leaf meal based diets, body weight, organ characteristics and haematology. *Online Journal Health and Applied Science* 2; 4 <http://www.ojhas.org/issue18/2006-2-4.htm>.
- Etim, N. N. and Oguike, M. A. 2011. Haematology and serum biochemistry of rabbits does fed *Aspila africana*. *Nigerian Journal of Agriculture, Food and Environment*, 7 (4) : 121-127.
- Ezeagu, I. E., Ologhobo, A. D., Akinsoyinu, A. O., and Tona, G. O. 2002. Haematobiochemistry of Albino rats fed African Kudzu (*Puerariaphaseolides Roxbenth*) seed diets. *Tropical Journal of Animal Science*. 5 (2); 109 -114.
- Ewuola, E. O., Jimoh, O. A., Atuma, O. V. and Solpe, O. D. 2012. Haematological and serum biochemical response of growing rabbits fed graded levels of *Moringa oleifera* leaf meal. *Proceedings of 10<sup>th</sup> world rabbit congress*. September 3-6, 2012 Sharm El-Sheikh, Egypt, Pp 679-683.
- Food and Agriculture Organization 2010. Improving Nutrition through Home gardening. A training package for preparing field workers in Africa, Nutrition programme service Rome available online at <http://www.fao.org/docrep/007/X3996/X399p18.htm>
- Frandsen, R. D. 2003. Anatomy and Physiology of Farm Animals. Chapter 31, Pp.462.
- Fraser, C. M. and Mays, A. 1986. The Merck veterinary manual. A handbook of diagnosis, therapy, disease prevention and control for the Veterinarian. 6<sup>th</sup> Edition Marck and Co., Inc, Rahway, New Jersey, USA. Google earth 2016: <http://www.googleEarth>.
- Habtamu, F. G., Negussie, R., Gulelat, D. H. and Ashagrie, Z. W. 2014. Nutritional quality and health benefits of okra (*Abelmoschus esculentus*); A Review. *Global Journal of Medical Research*. 14 (5):28-37.
- Harper, A. E., Rodwell, B. and Mayers, P. A. 1999. Review of physiological chemistry. 17<sup>th</sup> ed. Lang medical Los Altos, California 9442, Pp 60 -81, 188 -216
- Hillyer, E. V. 1994. Rabbits. *Small Anim. Pract.*, 24; 25-65.
- Hewitt, C. D., Innes, D. J., Savory, and Wills, M. R. 1989. Normal biochemical and haematological values of New Zealand white rabbits. *Clinical Chemistry*. 35/8.
- Ivegbe-Erakpotobor, G. T, Aliyu, R., Uguru, J. and Van Soest, P.J. 2006. Nutritional ecology of the ruminant (2nd ed) Cornell University Press. Pp. 476.
- Mufwa, B. J., Zaklag, D. U., Alade, F. T., Nyameh, J. and Antyevev, M. 2012. Varying levels of Neem (*Azadirachta indica*) leaf meal on haematology and biochemical components of weaner rabbits. *Journal of Agricultural Sciences and Policy Research*: 2, (1).
- Ndaeyo N. U., Edu S. U and John N. M. 2005. Performance of Okra as Affected by Organic and Inorganic fertilizers on A Ultisol In: Orheruata A. M. Nwokoro, S. O., Ajayi, M. T. Adekunle, A. T. and Asumugha G. N. (Eds). *Proceedings of the 39<sup>th</sup> Annual Conference of the Agricultural Society of Nigeria*. Pp. 206-209.
- Nithanantham, S., Selvakumaar, S., Sidohuraju, P. 2012. Total phenolic content and antioxidant activity of 2 different extracts from raw and processed legumes. *J. Food Composition*. 27 (1): 52-60.
- Nuhu, F. 2010. Effects of Moringa leaf meal on Nutrient digestibility, Growth, Carcass and Blood Indices of Weaner Rabbits. Masters thesis, Faculty of Agriculture and Natural Resources, Department of Animal Science, Kwame, Nkrumah University of Science and Technology, Kumasi, Ghana.
- Ogbuewu, I. P. 2008. Physiological resources of rabbits fed graded levels of Neem (*Azadirachta indica*) leaf meal. Msc. Dissertation, Federal University of Technology, Owerri, Imo State. Nigeria.
- Ojebiyi, O. O., Shittu, M. D., Oladunjoye, I. O., Omotola, O. B. and Olaniyi, S. A. 2013. Haematology, carcass and relative organ weights of growing rabbits on skip- a- day concentrate feeding regime. *International Journal of Applied Agricultural and Apiculture Research IJAAAR* 9 (1 & 2); 167-174, 2103.
- Omikhoje, S. O., Bamgbose, A. M. Aruna, M. B. and Ammashahun, R. A. 2006. Response of weaner rabbits to concentrate supplemented with varying levels of *Syndrellanodiflora*

- forage. *Pakistan Journal of Nutrition* 5 6: 577-579.
- Onifade, A. A. and Tewe, O. O. 1993. Alternative tropical energy feed resources in rabbit diets: Growth performance, diet digestibility and blood composition. *World Rabbit Science*, 1:17-24.
- Oyawoye, E. O. and Ogunkunle, M. 1998. Physiological and Biochemical effects of raw jack beans on broiler. *Proceedings of Nigerian Society of Animal Production*, 23: 141-142.
- Roberts, K. M., Murray, D., Daryl, K., Grammer, K and Rodwell, W. 2013. Harper Biochemistry 29<sup>th</sup> edition.
- Roy, A., Shrivastava, S.L., Mandal, S. M. 2014. Functional properties of okra. Traditional claims and scientific evidences. *Plant Sci.* 1 (3) 121-130.
- Sagar, V. R. and Suresh, K. P. 2010. Recent advances in drying and dehydration of fruits and vegetables: A review. *Journal of Food Science and Technology*, 47: (10, 15-26).
- Sergius, U. O. and Esther, D.U. 2014. Screening of *Abelmoschus esculentus* and *Abelmoschus callei* cultivars for resistance against okra leaf curl and okra mosaic viral diseases under field conditions in South Eastern Nigeria. *Afr. J. Biotechnol.* 13 (48): 4419-4429.
- Thompson, R. B. (2006). A short textbook of haematology (7<sup>th</sup> edition) in Garden City Press Ltd Letchworth, Hertfordshire. Pp217.
- Yusuf, A. M., Garba, M. H., Olafadehan, O. A., Inuwa, M., Orokanulo, U. O., Meduna, A. J. and Adekojo, N. 2009. Performance of growing rabbits feed diets containing graded levels of mango seed kernel meal. *Proceedings of 14th Annual conference of Animal Science Association of Nigeria*. Pp. 446 – 447.
- Zaharuddin, N. D., Noordin, M. I. and Kadivar, A. 2014. The Use of *Hibiscus esculentus* Okra). Gum in Sustaining the Release of Propranolol Hydrochloride in a Solid Oral Dosage. Form. *BioMed Research International* Article ID 735891.