

Physiological Responses of Rabbits Fed Graded Levels of Leucaena -Based Diet

Philips M. Goholshak¹, Oshibanjo Debola Olusegun^{2*}, Adelowo Victoria Olayinka³, Adetunji Joshua², Yusuf Uten⁴, Tyem Binshal¹

¹ National Veterinary Research Institute (NVRI), Rabbitry Unit, Vom Jos Plateau state, Nigeria

² Department of Animal Production, University of Jos, Jos, Nigeria

³ Department of Animal Production, Federal College of Animal Health and Production Technology, N.V.R.I., Vom, Plateau State, Nigeria

⁴ National Veterinary Research Institute (NVRI), Poultry Division, Vom Jos Plateau state, Nigeria

Email Address

oshibanjoo@unijos.edu.ng (Oshibanjo Debola Olusegun)

*Correspondence: oshibanjoo@unijos.edu.ng

Received: 4 August 2021; **Accepted:** 25 August 2021; **Published:** 9 September 2021

Abstract:

Sourcing for unconventional feed resources at a reduced cost to feed farm animals is imperative to solving competition between man and animals with greater consideration for the health status of these animals. A total of 24 unsexed cross breed weaner rabbits of 6 weeks old was divided into three dietary treatments (Treatment 1; control, Treatment 2; 25 % inclusion level of Leuceana leaf meal (LLM) and Treatment 3; 50% LLM) in a completely randomized design. Each treatment had 8 rabbits and 4 replicate of two rabbits each. Blood samples was collected into labeled Ethylene-deamine-tetra-acetic acid (EDTA) treated tubes for haematological analysis and into tubes without anticoagulant for serum biochemical evaluation. Data were analysed using descriptive statistic and ANOVA at $\alpha 0.05$. There were no significant differences in all the haematological indices analysed. Rabbits on 25% Leuceana-based diet had higher significant variation in neutrophils while least neutrophils count was observed in rabbit fed control diet. Lymphocytes was higher in rabbits fed the control diet while rabbits on 25% Leuceana-based diet had the least lymphocytes counts. Similar trend was observed for the monocytes and basophils count. Meanwhile, eosinophils count was higher in rabbits fed with 25% Leuceana-based diet and least count was obtained in rabbits fed with 50% Leuceana-based diet. The total protein was significantly higher in rabbits fed control diet without leuceana leaf meal while least values was obtained in rabbits fed 50% leuceana -based diet. Triglyceride (mmol/L) levels in rabbits fed leuceana -based diet decreases as the leuceana leaf meal inclusion levels increases. The same trend was observed for low density lipoprotein as shown in Figure 4. The high density lipoprotein was higher in the rabbits fed with 25% leuceana -based diet but least in rabbits fed 50% leuceana-based diet. Total cholesterol triglyceride and low density lipoprotein were reduced with improved physiological and oxidative status of the rabbits were observed. Therefore, Leucaena leaf meal can be fed to rabbits at 50% level of inclusion without detrimental effect on their haematology and serum biochemistry.

Keywords:

Leucaena-Based Diet, Hematology, Serum Biochemistry, Blood Morphology and Weaned Rabbit

1. Introduction

In rabbit production, feed accounts for about 70 % of total costs and if commercial feeds are used solely in raising rabbits on concentrate diets it cannot be economically viable. The search for alternative feed resources as a way of reducing production costs is therefore imperative. Rabbits are pseudo-ruminants and their potential to utilise forages can be exploited in production systems [1]. Although rabbits can survive on all forage diet, optimum performance can only be ensured in a mixed feeding regime involving forage and formulated feeds [2] of such forage is *Leucaena leucocephala*. *Leucaena leucocephala* leaves and seeds contain lipids, crude protein and carbohydrates. The seeds contain tannin and oxalic acid [3,4]. The kernel contains oil of about 17-20 %. The leaves and seeds also contain a toxic and non-protein substance known as mimosine. The seeds of *leucocephala* have great medicinal properties and are used to control stomach ache, as contraception and abortifacient. The seed gum is used as a binder in tablet formulation [5,6], Sulfated glycosylated form of polysaccharides from the seeds was reported to possess significant cancer chemopreventive and anti-proliferative activities [7]. *L. leucocephala* is one of the highest quality and most palatable fodder trees in the tropics, often being described as the 'alfalfa of the tropics'. It serves as a good potential, cheaper plant protein source with high nutritive value. Hence, need to study its effects on the physiological status of rabbits fed with *L. leucocephala*. This study evaluates the effect of leucaena-based diet on hematology, serum biochemistry and blood morphology of weaner rabbit.

2. Material and Method

2.1. Study Location

The research was carried out at Dagwom farm division of the National Veterinary Research Institute (NVRI) Vom, Jos south Local Government Area of Plateau State Nigeria. Vom lies on longitude 8°45 East and latitude 9°48 North and has an altitude of about 1280m above sea level. The average temperature is between 19°C to 22°C (NVRI).

2.2. Experimental Animal

A total of 24 cross breed unsexed weaner rabbits of 6 weeks old were divided into three dietary treatments. Each of the treatments had 8 rabbits with four replicates of two rabbits each in a completely randomized design. The initial weight of all the animals in each treatment were taken before the beginning of the experiment. The test ingredient *Leucaena* was harvested fresh, air dried and mill into meal. Feed (*Leucaena*-based diet) and clean drinking water was given *ad-libitum*. The experimental diets consist of the control, 25% *Leucaena*-based diet and 50% *Leucaena*-based diet, they were all compounded to meet the nutritional status of the animal. The composition is shown in Table 1. Dewormer was administered to all the rabbits at the beginning of the study. The experimental animal was housed two per hutch.

Test ingredients

Leuceana leaves was harvested from Dagwom farm division of the National Veterinary Research Institute (NVRI), by hand picking, washed with distill water and air dried under shade to prevent the leaves from being denatured until they are crispy to touch. The leaves were thereafter crushed with hammer mill before incorporation in the test diets at 25% and 50% inclusion levels.

Experimental diet

Table 1. *Experimental diets of weaner rabbits.*

Ingredients	Control	25% Leuceana-based diet	50% Leuceana-based diet
Maize	38.50	33.00	27.00
Full fat soya	17.00	5.00	0.00
Wheat offals	15.00	14.75	10.00
Rice offals	16.50	10.50	8.75
Palm kernel cake	8.75	7.50	0.00
Leuceana leaf meal	0.00	25.00	50.00
Leuceana Tigs meal	0.00	0.00	0.00
Bone meal	2.50	2.50	2.50
Lime stone	1.00	1.00	1.00
Salt	0.30	0.30	0.30
Premix	0.25	0.25	0.25
Methionine	0.10	0.10	0.10
Lysine	0.10	0.10	0.10
Total	100.00	100.00	100.00
Crude Protein	15.89	15.99	17.44
Metabolizable energy	2591.68	2639.12	2639.12
Crude fibre	8.96	8.90	9.35

Blood sample collection

Blood samples was collected into labeled Ethylene-deamine-tetra-acetic acid (EDTA) treated tubes for haematological analysis and into tubes without anticoagulant for serum biochemical evaluation. Evaluations was conducted according to the method described by [8].

Hemoglobin Determination

N/10 HCl was taken into an ordinary pipette and will be poured in the graduated dilution tube up to 20% mark. The heparinized blood was filled into the hemoglobin pipette up to 0.02 ml and transferred into the dilution tube. The blood and HCl was stirred in the dilution tube with the stirrer. Distilled water was added until the colour of the dilution and standard tubes matched with each other. The reading was noted which gave hemoglobin as g/dl of blood according to [9].

Red Blood Cell Count

RBC counting blood with an anticoagulant was used. Blood was drawn into the RBC diluting pipette exactly to the 0.5 mark, using gentle suction on the mouth piece. The lip of the pipette was wiped free of blood before inserting it in to the diluting fluid (Toission Solution). The diluting fluid was drawn up to the mark 101 above the bulb. The tube was rotated in a horizontal position to ensure uniform dispersion of the blood cells in the pipette [9].

RBCs was calculated by using the following formula:

$$\text{RBC (million/mm)} = \text{Cells counted} \times 10 \times 200$$

Packed Cell Volume (PCV)

Packed cell volume was measured using the heparinized blood in the plain capillary tubes (75mm x 1 mm). Tubes was filled approximately 1 cm from the end. Holding it in the flame sealed. Care was taken not to heat the blood. Capillary tubes were fixed in the hematocrit centrifuge machine. Then centrifugation was done at 13000 rpm for 5 minutes [9].

Red Blood Cell Indices

From the values of PCV; Hb and RBC count (following useful erythrocyte indices) was empirically calculated.

Mean Corpuscular Volume (MCV)

MCV expresses the average volume of the individual RBC and is calculated from the formula as given by Wintrobe, M. M. (1967), Diem and Clenter (1970).

$$\text{MCV} = \text{Hematocrit} \times 10 / \text{R.B.C.}$$

MCV is expressed in femtoliter.

Mean Corpuscular Hemoglobin (MCH)

MCH is the amount of hemoglobin by weight in average Red blood cell count and is calculated by the formula as given by [9]; [10].

$$\text{MCH} = \text{Hemoglobin} \times 10 / \text{R.B.C.}$$

It is expressed in picogram.

Mean Corpuscular Hemoglobin Concentration (MCHC)

MCHC is the concentration of hemoglobin in the average red blood cells or the ratio of weight of hemoglobin to the volume in which it is contained and is calculated from the formula as given by [9,10].

$$\text{MCHC} = \text{Hemoglobin} \times 100 / \text{Hematocrit}$$

White blood cell differential count

The white blood cell differential count was carried out measured such as White cell count ($\times 10^9/\text{L}$), Neutrophils ($\times 10^9/\text{L}$), Lymphocytes ($\times 10^9/\text{L}$), Monocytes ($\times 10^9/\text{L}$) and Eosinophils ($\times 10^9/\text{L}$).

Serum biochemistry indices

Serum biochemistry indices such as Total protein (g/ 100ml), Albumin (g/ 100ml), Total cholesterol (mg/100ml) was measured.

Statistical analysis

Data obtained was subjected to analyses of variance using SPSS statistical package version 25. Significant differences between treatment means were separated using Duncan's Multiple Range Test.

3. Results and Discussions

3.1. Results

Table 2 shows the effect of Leuceana-based diet on heamatology of weaner rabbit. There were no significant differences in all the haematological indices analysed. The white blood cell differentials are shown on Figure 1 where rabbits on 25% Leuceana-based diet are significantly higher in neutrophils with least neutrophils count in rabbit fed control diet. Lymphocytes was higher in rabbits fed the control diet while rabbits on 25% Leuceana-based diet had the least lymphocytes counts. Similar trend was observed for the monocytes and basophils count. Meanwhile, eosinophils count was higher in rabbits fed 25% Leuceana-based diet and least count was obtained in rabbits fed 50% Leuceana-based diet.

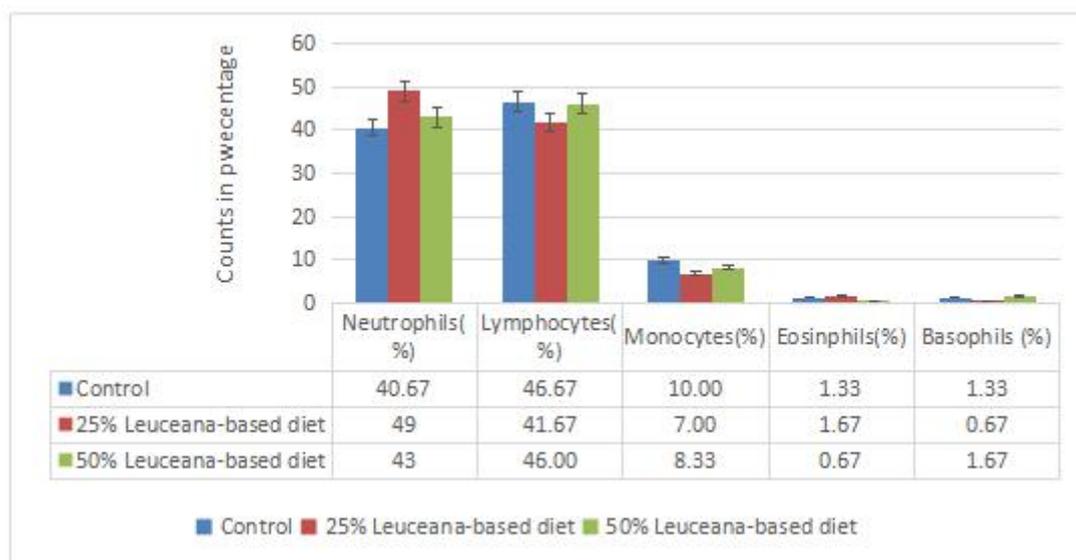


Figure 1. Effect of leuceana-based diet on White blood differentials count of weaner rabbit.

Table 2. Effect of leuceana-based diet on heamatology of weaner rabbit.

Parameters (%)	Control	25% Leuceana-based diet	50% Leuceana-based diet	SEM
Packed Cell Volume (%)	40.00	32.67	37.67	1.58
Red blood cell (x1012/l)	6.66	5.44	6.28	0.26
Haemoglobin (g/dl)	13.33	10.89	12.56	0.53
MCHC (g/dl)	33.33	33.33	33.33	0.15
MCH (pg)	60.02	60.02	60.02	0.34
MCV (fl)	20.01	20.01	20.01	0.11
White blood cell (x109/l)	6.73	7.87	6.60	0.55

^{a, b} Means in the same row not sharing superscript are significantly different at $P < 0.05$.

MCV=Mean corpuscular volume MCH=Mean Corpuscular Haemoglobin MCHC=Mean Corpuscular Haemoglobin Concentration, SEM: Standard error of the mean.

The effect of leuceana-based diet on selected serum biochemistry of weaned rabbit could be seen on Table 3. The total protein was significantly higher in rabbits fed control diet without leuceana leaf meal while least values were obtained in rabbits fed 50% leuceana -based diet. Triglyceride (mmol/L) levels in rabbits fed leuceana -based diet decreases as the leuceana leaf meal inclusion levels increased as showed in Figure 2. The same trend was observed for low density lipoprotein as shown in Figure 4. The high density lipoprotein in Figure 3 was higher in the rabbits fed 25% leuceana -based diet with least in rabbits fed 50% leuceana-based diet.

Table 3. Effect of leuceana-based diet on serum biochemistry of weaned rabbit.

Parameters	Control	25% Leuceana-based diet	50% Leuceana-based diet	SEM
Total proteing/L)	108.67 ^a	113.67 ^a	65.00 ^b	10.40
Albumin (g/L)	38.00	44.00	36.50	2.50
Total cholesterol (mmol/L)	6.30	5.87	4.45	0.47

^{a, b} Means in the same row not sharing superscript are significantly different at $P < 0.05$. SEM: Standard error of the mean.

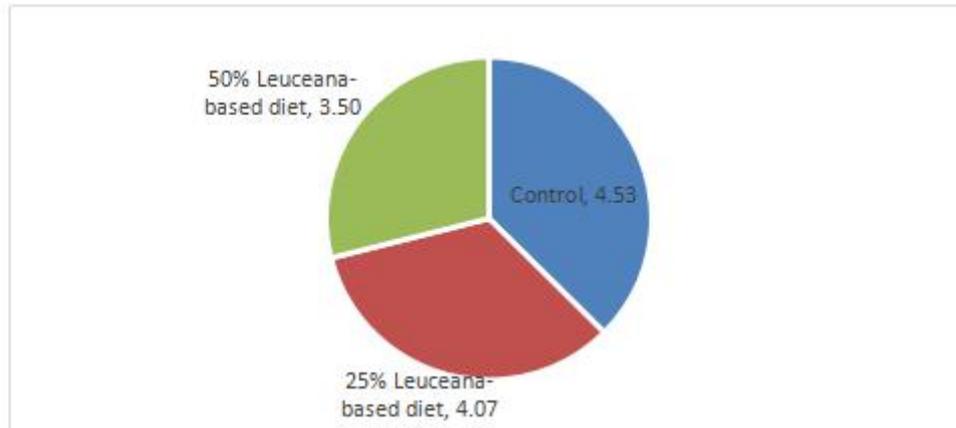


Figure 2. Triglyceride (mmol/L) levels in rabbits fed leuceana-based diet.

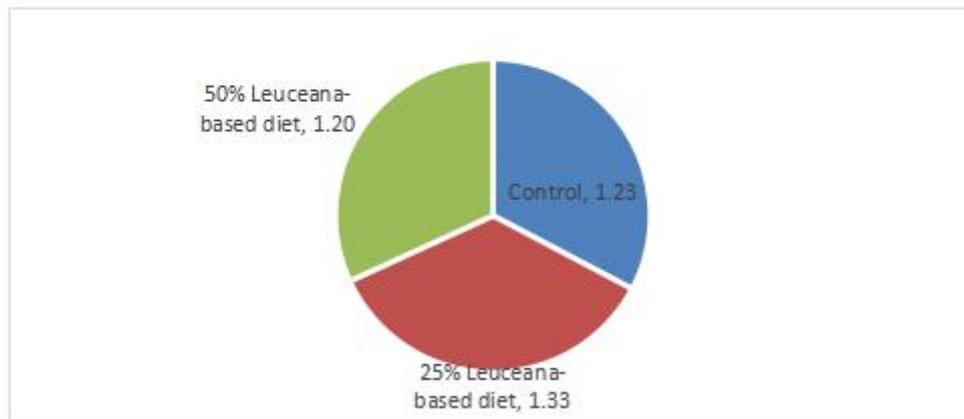


Figure 3. High Density Lipoprotein (mmol/L) levels in rabbits fed leuceana-based diet.

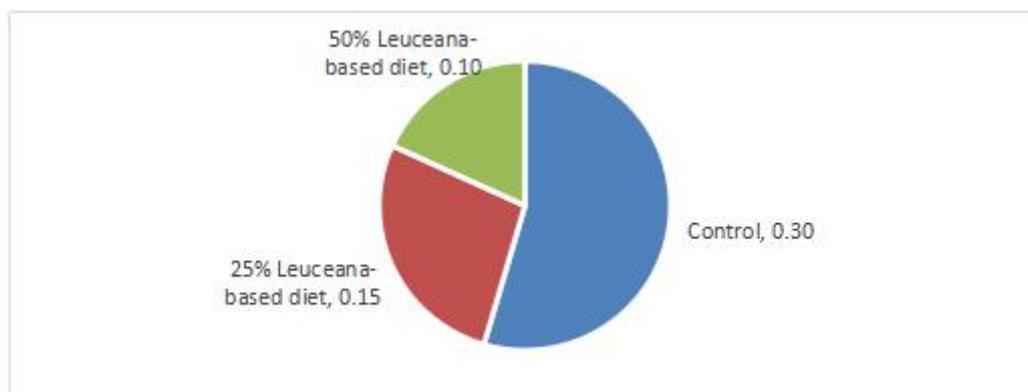


Figure 4. Low Density Lipoprotein (mmol/L) levels in rabbits fed leuceana-based diet diet.

Table 4. Effect of Leuceana-based diet on blood morphology of weaner rabbit.

Parameters	Control	25% Leuceana-based diet	50% Leuceana-based diet
Red blood cell	N, MA +, HYPO +, MI ++, HYPER +,	TC ++, STO +, BURR +, SPURR +, TC +, SPH +	TC +, N, BITE +, OVA ++
White blood cell	N, NTG +	NTG, RL, N	N, RS
Platelet	N	N, L	N, L

Keys: BURR = BURR CELLS, ELIP= ELIPTOCYTES, HYPER= HYPERCHROMASIA, HYPO= HYPOCHROMASIA, L= LARGE, MA= MACROCYTES, MI= MICROCYTES, N= NORMAL, NTG = NOT-TOXIC NUETROPHILIC GRANULATIOS, SPURR = SPURR CELLS (ACANTOCYTES). OVA = OVALOCYTES, RL = REACTIVE LYMPHOCYTE,

RS = RIGHT SHIFT, SC = SCHISTOCYTES and SPH = SPHEROCYTES

3.2. Discussions

The blood profiles have been used widely to establish the health status of animals particularly when they are subjected to dietary treatment that could affect their well-being. Thus, [11,12] identified haemoglobin (Hb), PCV, WBC, RBC, MCH and MCHC among others as blood parameters that are useful in feed toxicity and feed quality, monitoring their effect on health status of the animals. In this study, the heamatology result obtained after feeding the Leuceana-based diet revealed that the control was not different from the experimental diets thus no negative effects on the blood parameters, so Leuceana can be fed to rabbits at 50% without detrimental effect on the heamatology of the animal. The non-significant difference observed among most of the haematological parameters measured implies that the haematopoietic activity was enhanced identically by the dietary treatments and by extension the health status of the rabbit was not compromised by feeding with leuceana. Rabbits are said to be pseudo-ruminants according to [1] which stated that they have potentials to utilize forages, hence from the result obtained they utilize the control diet with 0% Leuceana equally as the Leuceana based diet and it corroborate the study of [2] that rabbits can survive on all forage diet and that optimum performance can only be ensured in a mixed feeding regime involving forage and formulated feeds. For the serum biochemistry; total protein value was higher in the control and 25% Leuceana-based diets compared with the 50% Leuceana-based diet. The results obtained for total protein was in line with the results obtained by [13]. Furthermore, the results obtained for total protein was in range with the report of [14] (68.00 – 74.00 g/L) in the serum of the rabbits in all the treatments which was agreement with the reports of [15] in their study of the growth performance, haematology and serum biochemistry of females rabbits (*Oryctolagus cuniculus*) fed dietary fumonisin. The result obtained was an indication of the nutritional adequacy of dietary proteins since serum protein synthesis is related to the amount of available protein in the diet [16]. The range of values obtained for albumin was in line with the reports of [14,17,18]. Triglyceride (mmol/L) levels in rabbits fed leuceana-based diet was observed to be lower as the leuceana increased in the diet. Similar trend was obtained for high density lipoprotein whereas the low density lipoprotein decreased with increase in the inclusion level of leuceana in the diet. The result obtained could be due to the antioxidant activity presence in the leuceana leave meal.

4. Conclusions

Leucaena leaf meal can be fed to rabbits at 50% level of inclusion without detrimental effect on their haematology and serum biochemistry as the total cholesterol triglyceride and low density lipoprotein were reduced with improved physiological and oxidative status of the rabbits were observed.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgments

The authors would like to acknowledge the Staff of the National Veterinary Research Institute (NVRI), Rabbitry Unit, Vom Jos Plateau state, Nigeria for the success of this research.

References

- [1] Aduku, A.O.; Olukosi, J.O. Rabbit Management in the Tropics: Production Processing, Utilization, Marketing, Future Prospects. Abuja, Nigeria Living Books services. 1990.
- [2] Oshibanjo, D.O.; Goholshak, P.M.; Akinfolarin, O.; Akwashik, M. A.; Adediran, O.; Adesope, A.I.; Abegunde, L. Evaluation of Reproductive Performance of Rabbits Fed Graded Levels of Moringa oleifera Leaves and Twigs Meals. *International Journal of Research and Innovation in Applied Science*, 2018, 3(12), 01-04
- [3] Azeemoddin, G.; Jagan, M.; Rao, S.; Thirumala, R.SD. Amino acid composition of subabul (*Leucaena leucocephala*) seed kernel proteins. *J Food Sci Technol*, 1988, 25, 158.
- [4] Padmavathy, P.; Shobha, S. Effect of processing on protein quality and mimosine content of subabul (*Leucaena leucocephala*). *J Food Sci Technol*, 1987, 24, 180-2.
- [5] Deodhar, U.P.; Paradkar, A.R.; Purohit, A.P. Preliminary evaluation of *Leucaena leucocephala* seed gum as a tablet binder. *Drug Dev. Ind. Pharm*, 1998, 24(6), 577-582.
- [6] Verma, P.R.P.; Balkishen, R. Studies on disintegrant action of *Leucaena leucocephala* seed gum in ibuprofen tablet and its mechanism. *Journal of Scientific and Industrial Research*, 2007, 66, 550-557.
- [7] Gamal-Eldeen, A.M.; Amer, H.; Helmy, W.A.; Ragab, H.M.; Talaat, R.M. Antiproliferative and cancer-chemopreventive properties of sulfated glycosylated extract derived from *leucaena leucocephala*. *Indian J Pharm Sci*. 2007, 69, 805-11.
- [8] Bitto, I.I; Gemade, M. Preliminary investigations on the effect of Pawpaw peel meal on growth, visceral organ and endocrine gland weights, testicular morphometry and the haematology of male rabbits. *Global J.P. & Appl. Sci*. 2001, 7(4), 611-625.
- [9] Wintrobe, M.M. Clinical Hematology 1967., 6th ed. Lea and Febiger, Philadelphia, USA .

- [10] Diem, K.; Clenter L. Scientific Tables, 1970.7th ed. Geigy Pharmaceuticals, Basel, Switzerland.
- [11] Aro, S.O.; Akinmoegun, M.B. Haematology and red blood cell osmotic stability of pigs fed graded levels of fermented cassava peel based diets. *Proceedings 17th Annual Conference of Animal Science Association of Nigeria*, 2012; pp. 152-153.
- [12] Isaac, L.J.; Abah, G.; Akpan, B.; Ekaette, I.U. Haematological properties of different breeds and sexes of rabbits. *Proc. of the 18th Annual Conference of Animal Science Association of Nigeria*, 2013; pp. 24-27.
- [13] Adejumo, D.O. Performance and serum chemistry of rabbits fed graded levels of cassava peels, *Leucaena leucocephala* and *Gliricidia sepium* leaves based diets. *Global Journal of Pure and Applied Sciences*, 2006, 12(2), 171-175.
- [14] Audu, R.; Tijjani, A.; Ibrahim, A.A.; Amin, A.B.; Gumel, I.A.; Suleiman, A.T. Evaluation of haematology and serum biochemistry of weaner rabbits fed diets containing *Ficus sycomorus* and *Parkia biglobosa* leaf meals. *Nigerian Journal of Animal Production*, 2018, 45(5), 30-38.
- [15] Gbore, F.A.; Akele, O. Growth performance, haematology and serum biochemistry of female rabbits (*Oryctolagus cuniculus*) fed dietary fumonisin. *Veterinary Archive*, 2010, 80, 431-443.
- [16] Iyayi, E.A.; Tewe, O.O. Serum total protein, urea, creatinine levels as indices in cassava diets for pigs. *Tropical Veterinary*, 1998, 8, 11-15.
- [17] Annon. Guide to Care and use of experimental animal. Canadian Council of Animal Care, Ottawa, Ontario, Canada, 1980, 1, 185-190.
- [18] Medirabbit. Complete blood count and biochemical reference values in rabbits. Available online: www.medirabbit.com (accessed on 7 September 2018).



© 2021 by the author(s); licensee International Technology and Science Publications (ITS), this work for open access publication is under the Creative Commons Attribution International License (CC BY 4.0). (<http://creativecommons.org/licenses/by/4.0/>)