Volume 5, Issue 2, 2021 ISSN: 2617-3530
DOI: https://doi.org/10.31058/j.as.2021.52003

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

Oshibanjo Olusegun Debola[1*], Adelowo Victoria Olayinka[2], Adetunji Joshua[1], Philips Goholshak[3], Ajimohun Felicia Folashade[3], Joel Elizabeth[7], Ugwu Kingsley[4] Ajibola Ololade[5], Yusuf Uten[6], Sallah Wanvangbapi Danka[2]

[1] Department of Animal Production, University of Jos, Jos, Nigeria
[2] Department of Animal Production, Federal College of Animal Health and Production Technology, National Veterinary Research Institute (NVRI), Vom, Nigeria
[3] National Veterinary Research Institute (NVRI), Rabbitary Division, Vom, Nigeria
[4] Department of Medical Laboratory Science, Bayero University Kano, Kano, Nigeria
[5] Department of Basic Science, The Federal school of Medical Laboratory Technology Jos, Jos, Nigeria
[6] National Veterinary Research Institute (NVRI), Poultry Division, Vom, Nigeria
[7] Department of Animal Science, University of Ibadan, Ibadan, Nigeria

Email Address

oshibanjoo@unijos.edu.ng (Oshibanjo Olusegun Debola)
*Correspondence: oshibanjoo@unijos.edu.ng

Received: 4 September 2021**; Accepted:** 30 September 2021**; Published:** 21 October 2021

Abstract:

A total of 24 cross breed weaner rabbits of 6 weeks of age of both sexes was divided into three dietary treatments (Treatment 1; control, Treatment 2; 25 % inclusion level of moringa leaf meal (MLM) and Treatment 3; 50% MLM) of 8 rabbits in 4 replicate of two rabbit each in a completely randomized design. 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 α0.05. There are no significant differences in all the haematological indices analysed. No differs observed in white blood differentials count but rabbits on 50% Moringa-based diet are higher in neutrophils and Lymphocytes with least neutrophils obtained in rabbit fed control diet. Similar trend was observed for the monocytes and eosinophils count. Meanwhile, basophils count was higher in rabbits fed 25% Moringa -based diet and least count was obtained in rabbits fed 50% Moringa -based diet. Both the total protein and cholesterol was significantly higher in rabbits fed control diet with Moringa leaf meal while least values were obtained in rabbits fed 25% and 50% Moringa -based diet respectively. Triglyceride (mmol/L) levels in rabbits fed Moringa-based diet decreases as the Moringa leaf meal inclusion levels increased. The same trend was observed for high density lipoprotein and low density lipoprotein Moringa leaf meal can be fed to rabbits up to 50% level of inclusion without any detrimental effect on haematology and serum biochemistry of rabbits while total cholesterol triglyceride and low density lipoprotein were reduced and the physiological and oxidative status of the rabbits was improved.

Keywords:
Moringa-Based Diet, Heamatology, Serum Biochemistry, Blood Morphology and Weaned Rabbit

1. Introduction

Protein supplementation is often important to improve livestock performance, and this needs to be done with respect to the requirements of the animal in addition to the balance of other nutrients available. Soyabean meal and fish meal have been widely and successfully used as conventional protein sources for livestock. However, the prices of these protein sources have been escalating continuously in recent times, whilst availability is often erratic. The problem has been worsened due to the increasing competition between humans and livestock for these protein ingredients as food. According to [1] the rapid growth of human and livestock population, which is creating increased needs for food and feed in the less developed countries, demand that alternative feed resources must be identified and evaluated. There is the need, therefore, to explore the use of non-conventional feed sources that have the capacity to yield the same output as conventional feeds, and perhaps at cheaper cost. Hence, any similar high protein ingredient which could partially or completely be used as a substitute for soyabean meal or fishmeal is desirable. This strategy could help reduce the cost of production, and ensure cheaper meat production thereby making available the major crops for human consumption. The economization of feed cost using cheaper and unconventional feed resources [2,3] is an important aspect of commercial rabbit production. One possible source of cheap protein is the leaf meals of some tropical legume browse plants. Recently, there has been interest in the utilization of moringa (Moringa oleifera) commonly called horseradish tree or drumstick tree, as a protein source for livestock [4,5]. Moringa leaves have quality attributes that make it a potential replacement for soyabean meal or fish meal in non-ruminant diets. Moringa can easily be established in the field, has good coppicing ability, as well as good potential for forage production. Furthermore, there is the possibility of obtaining large amounts of high quality forage from moringa without expensive inputs due to favourable soil and climatic conditions for its growth. [5] reported that moringa foliages are a potential inexpensive protein source for livestock feeding. The advantages of using moringa for a protein resource are numerous, and include the fact that it is a perennial plant that can be harvested several times in one growing season and also has the potential to reduce feed cost. Moringa oleifera is in the group of high-yielding nutritious browse plants with every part having food value [6]. Despite the high crude protein content of moringa leaf meal, there is little information available on the use of this unconventional feed resource, especially as an alternative protein supplement for rabbit production. The present study aimed at assessing the possibility of replacing soyabean meal either partially or completely with moringa leaf meal for weaner rabbits as it affects its heamatology, serum biochemistry and blood morphology.

2. Materials and Methods

2.1. Experimental Site

The research was carrying out at Dagwom farm division of the National Veterinary Research Institute (NVRI) Vom, Jos south Local Government Area of Plateau State Nigeria. The site of the farm is located at Kaduna Vom a village on the out skirts of Jos, the capital of Plateau State. 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, mean annual rainfall of 131.75cm to 146cm with the highest rainfall is usually recorded during the wet months of July and August (NVRI).

2.2. Experimental Animal

A total of 24 cross breed weaner rabbits of 6 weeks of age of both sexes was divided into three dietary treatments of 8 rabbits in 4 replicates of two rabbit each in a completely randomized design.

The initial weight of all the treatments was taken before the beginning of the experiment. Feed (Moringa-based diet) and clean drinking water was given ad-libitum. Dewormer was administered to all the rabbits at the beginning of the study. The experimental animal was housed two per hutch.

2.3. Test ingredients

Moringa 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.

2.4. Experimental diet

The feed composition fed to weaner rabbits is shown on Table 1.

Table 1. *Feed composition.*

Ingredients	Control	25% Moringa-based diet	50% Moringa-based diet
Maize	38.50	33.00	30.75
Full fat soya	17.00	5.00	0.00
Wheat offals	15.00	14.75	10.00
Rice offals	16.50	10.00	5.00
Palm kenle cake	8.75	8.00	0.00
Moringa Leaf Meal	0.00	25.00	50.00
Moringa 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
Metheonine	0.10	0.10	0.10
Lysine	0.10	0.10	0.10
Total	100.00	100.00	100.00
Crude Protein	15.89	16.25	17.99
Metabolizable energy	2591.68	2601.75	2644.04
Crude fibre	8.96	9.40	9.05

2.5. Experimental Design

Complete randomize Design was used in carrying out the research

2.6. Data Collection Procedure

2.6.1. 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 [7].

2.6.2. Hemoglobin Determination

N/10 HC1 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 it 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 [8].

2.6.3. Red Blood Cell Count

For 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 [8].

RBCs was calculated by using the following formula:

RBC (million/mm) = Cells counted x 10x200

2.6.4. Packed Cell Volume (PCV)

Packed cell volume will be measured using the heparinized blood in the plain capillary tubes (75mm x I mm). Tubes will be filled approximately 1 cm from the end. Holding it in the flame sealed. Care most be 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 [8].

2.6.5. Red Blood Cell Indices

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

2.6.6. Mean Corpuscular Volume (MCV)

MCV expresses the average volume of the individual RBC and is calculated from the formula as given by [8] and [9].

MCV = Hematocrit X 10/R.B.C.

MCV is expressed in femtoliter.

2.6.7. 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 [8] and [9].

MCH = Hemoglobin X 10/R.B.C.

It is expressed in picogram.

2.6.8. 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 [8] and [9].

MCHC = Hemoglobin X 100/ Hematocrit

2.6.9. White blood cell differential count

The white blood cell differential count was carried out measured such as White cell count ($x10^9$/L), Neutrophils ($x10^9$/L), Lymphocytes ($x10^9$/L), Monocytes ($x10^9$/L) and Eosinophils ($x10^9$/L L).

2.6.10. Serum biochemistry indices

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

2.6.11. Data 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

Effect of moringa-based diet on heamatology of weaner rabbit is represented on Table 2. No significant differences were observed in all the haematological indices analysed. Figure 1 shows the white blood cell differentials. Although, no differs was observed in the white blood differentials count but rabbits on 50% moringa-based diet are higher in neutrophils and lympocytes with least neutrophils value in rabbit fed control diet and lymphocytes value in rabbits fed 25% moringa-based diet. Similar trend was observed for the monocytes and eosinophils count. Meanwhile, basophils count was higher in rabbits fed 25% moringa-based diet and least count was obtained in rabbits fed 50% moringa based diet.

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

Parameters	Control	25% Moringa-based diet	50% Moringa-based diet	SEM
Packed Cell Volume (%)	40.00	42.00	43.33	0.83
Red blood cell ($x10^{12}$/l)	6.66	7.00	7.22	0.14
Haemoglobin (g/dl)	13.33	14.00	14.44	0.28
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 ($x10^9$/l)	6.73	6.40	7.23	0.53

[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.

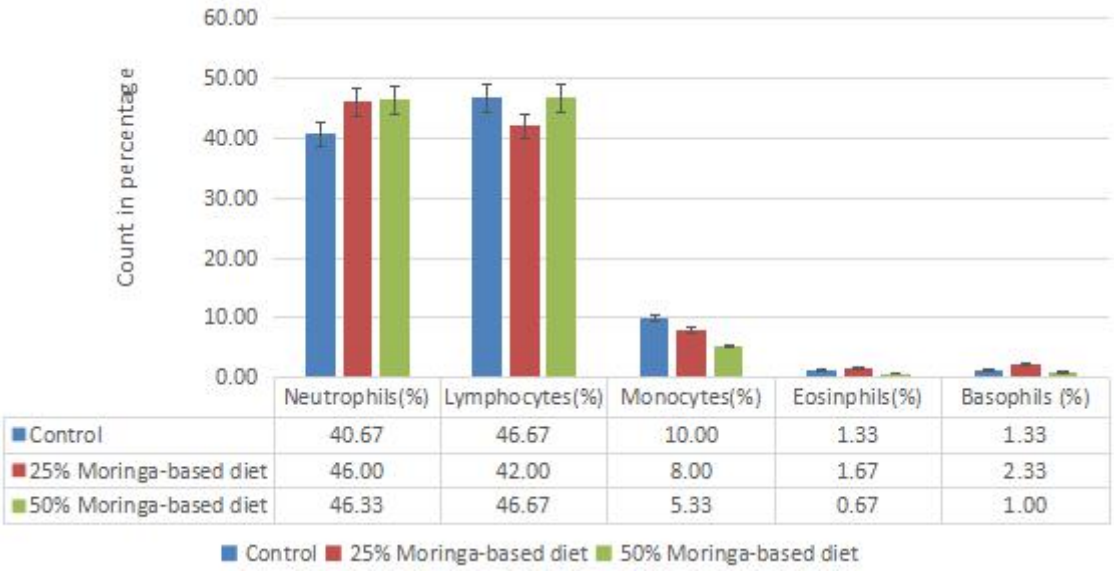

Figure 1. *White blood cell differential count.*

Table 3 shows the effect of moringa-based diet on selected serum biochemistry of weaned rabbit. Both the total protein and cholesterol was significantly higher in rabbits fed control diet while least values were obtained in rabbits fed 25% and 50% moringa-based diet respectively. Triglyceride (mmol/L) levels in rabbits fed moringa-based diet decreases as the moringa leaf meal inclusion levels increased as showed in Figure 2. The same trend was observed for high density lipoprotein and low density lipoprotein as shown in Figure 2.

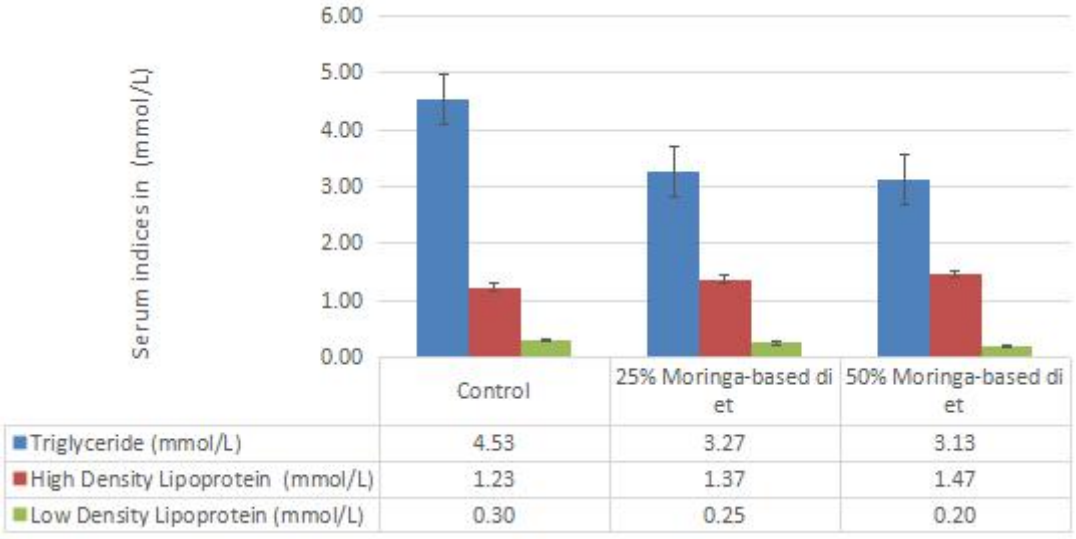

Figure 2. *Effect of moringa-based diet on serum biochemistry of weaner rabbit.*

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

Parameters	Control	25% MML	50% MML	SEM
Total protein (g/L)	108.67[a]	112.00[a]	56.00[b]	13.67
Albumin (g/L)	38.00	39.00	36.33	6.17
Total cholesterol (mmol/L)	6.30[a]	5.17[ab]	4.97[b]	0.27

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

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

Parameters	Control	25% Moringa-based diet	50% Moringa-based diet
Red blood cell	MA +,Hypo+, MI ++, HYPER +	MI ++, HYPER +, MA +	MA ++,SC +, MA ++, HYPO +
White blood cell	N, NTG+	RS ++	NTG +, RL+, LL +
Platelet	N	N	L +

Keys: HYPER= HYPERCHROMASIA, HYPO= HYPOCHROMASIA, L= LARGE, LS = LEFT SHIFT, MA= MACROCYTES, MI= MICROCYTES, N= NORMAL, NTG = NOT-TOXIC NUETROPHILIC GRANULATIOS, SC = SCHISTOCYTES, LL = LARGE LYMPHOCYTE and RS = RIGHT SHIFT

3.2. Discussions

In this study, Packed cell volume (PCV) which is a measure of the relative mass of blood according to [10] study shows that PCV of rabbits were not affected by dietary treatment and were within the normal range reported by [11]. The normal PCV value is suggestive of adequate nutritional status of the rabbits [12]. This study result is in line with the findings of [13] and [14], who observed no significant (P >0.05) effect of feeding Moringa oleifera leaf meal in the diet on PCV of Rabbit.

The main function of Red blood cells (RBC) is to transport oxygen. The values of RBC obtained in this study shows no difference and were within the normal range [11]. Similarly [14] and [13], who found no significant influence of diet on RBC of rabbit fed Moringa oleifera leaf meal. Although there were no differs reported in this result but an increase in RBC was observed as the inclusion level of moringa increased. It has been reported by Hack Barth that increased RBC values were associated with high quality dietary protein and disease-free animal.

No difference was observed in haemoglobin (Hb), the mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCH), and mean corpuscular haemoglobin concentration (MCHC).value of rabbits fed control and test ingredient. Values however, were within the standard range (10.0- 17.0g/dl) recommended for clinical healthy rabbits [11].

The neutrophils, eosinophils, monocytes, basopils and lymphocytes were not significantly (P>0.05) affected by dietary treatments, although, an increase in neutrophils and lymphocytes was observed as the inclusion level of moringa increased thus indicating probably normal physiology of the animals fed Moringa oleifera leaf meal. It also implies that the immune system of rabbits was adequate and no fear of imminent hazard that may be associated with the use of Moringa oleifera leaf meal.

Total protein shows significant different (p>0.050). Values obtained fall within the normal range recommended [11] and the values obtained in the study indicate nutritional adequacy of the dietary protein. The mean values of albumin obtained in the study were also not significantly (p>0.05) influenced by dietary treatments. Increase in triglyceride level may be a risk factor for atherosclerosis, pancreatitis and liver disease. In this present study, there is decrease in level of triglycerides as the level of moringa leaf meal increased. This result is in line with the data obtained by [15] who got a reduction in triglyceride level in rabbits fed 15% gliricidia-based diet compared to the rest test diets. This result obtained could be due to presence of some

bioactive compounds in moringa leaf meal which impaired fat absorption and consequent fat depletion. This findings also supported the reduction in cholesterol and Low-Density Lipoprotein level (LDL) in rabbits fed 50% gliricidia-based diet compared to the control diet in this study as this is also of health benefit to the consumers, especially those predisposed to heart disease. The inclusion of moringa - based diet in the diets lower the uptake of cholesterol or increased loss or catabolism of cholesterol as stated by [16] which further confirms the health benefits of including moringa leaf meal in rabbit's diet. However, the present findings agreed with earlier reports of [17], [16] and [18], who all reported a reduced cholesterol level in rabbits fed Alchornea cordifolia leaf meal based diets, processed cocoa pod husk meal based diet and pawpaw leaf meal inclusive diet, respectively. The reduction of cholesterol level in this study may be linked to the presence of saponins, one of the components of gliricidia which exerts inhibitory effects on cholesterol uptake in the gut through intra-lumenal physiochemical interaction as stated by [19].

Blood picture of rabbits used for this study showed varying morphology upon observation. Both control and test group were observed to show macrocytosis. The increasing unusual morphologies is in tandem with the report of [20] certain adverse effect could be happened with dosage of moringa 50% due to the high content of some phytochemical compounds (phenols, cumares, alkaloids and tanins) which are naturally occurring at high levels of MOLM [21].

Although, the leukocytes counts were not significantly ($P>0.05$) affected by dietary treatments, some cells showed non-toxic nuetrophilic granulations (NTGs), which could result from physiologic response to stress vis-a-vis existence of a recent infection, usually with bacteria [22].

Thrombocytes appeared normal except for rabbits on 50% Moringa-based diet which agreed with the findings of [21].

4. Conclusions

The data obtained in this study showed that moringa leaf meal can be fed to rabbits up to 50% level of inclusion without any detrimental effect on haematology and serum biochemistry of rabbits while total cholesterol triglyceride, and low density lipoprotein were reduced and the physiological and oxidative status of the rabbits was improved.

Conflicts of Interest

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

Author Contributions

Conceptualization: O.O.D.; P.G.; Methodology: P.G.; A.V.O.; Validation: O.O.D.; Formal analysis: A.J.; Investigation: A.F.F.; A.O.; Y.U.; S.W.D.; Resources: S.W.D.; O.O.D.; Data Curation: O.O.D.; Writing – all authors; Supervision: O.O.D.; A.V.O.; P.G.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

References

[1] Odunsi, A.A. Assessment of Lablab (Lablab purpureus) leaf meal as a feed ingredient and yolk colouring agent in the diet of layers. *International Journal of Poultry Science,* 2013, 2(1),71-74.

[2] Vasanthakumar, P.; Sharma, K.; Sastry, V.R.B.; Kumar, R. Effect of graded levels of neem (Azadirachta inidica) seed kernel cake on carcass characteristics of broiler rabbits. *Asian-Australasian Journal of Animal Science,* 1999, 12, 1246-1250.

[3] Muriu, J.I.; Njoka-Njiri, E.N.; Tuitoek, J.N.; Nanua, J.N. Evaluation of sorghum (Sorghum bicolor) as replacement of maize in the diet of growing rabbit (Oryctolagus cuniculus). *Asian-Australisian Journal of animal science,* 2002, 15, 565-569.

[4] Makker, H.P.S.; Becker, K. Nutrients and anti quality factors in different morphological parts of the Moringa oleiferatree. *Journal of Agric. Sci.* 1997, 128, 311-322.

[5] Sarwatt, S.V.; Kapange, S.S.; Kakengi, A.M. Substituting sunflower seed cake with Moringa oleifera leaves as supplemental goat feed in Tanzania. *Agro-forestry systems,* 2002, 56, 241-247.

[6] Duke, A.J. Moringaceae. Handbook of energy crops 1998. Available online: Http://www/hort.purdue/edu./newcrop/duke_energy/moringa.html (accessed on 4 May 2008).

[7] 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.

[8] Wintrobe, M.M. Clinical Hematology, 1967, 6th ed. Lea and Febiger, Philadelphia, USA.

[9] Diem, K.; Clenter L. Scientific Tables 1970., 7th ed. Geigy Pharmaceuticals, Basel, Switzerland.

[10] Bakers F.J.; Kilshaw D. Introduction of medical laboratory technology 6th ed, 1985.

[11] Brunett, N.; Mathura, K.; Metivier, K.S.; Holder, R.B.; Brown, G.; Campbell, M. An investigation into haematological and serum chemistry parameters of rabbits in Trinidad. *World Rabbit Sci.* 2003, 14, 175-87.

[12] Church, J.P.; Jude, J.J.; Young, C.W.; Kebay, J.I.; Kim, W.W. *Amer. J. of clinical Nutr.* 1984, 40, 1338-1344.

[13] Okeke, G.C.; Obiocha, F.G.; Odeogu, A.E. *Nutr. Rep. Int,* 2009, 1(32), 130-148.

[14] Terzungwe, A.; Adakole, H.; Abu, Lois K. Iorgilim. Physiological responses of rabbits fed graded levels of Moringa oleifera leaf meal (MOLM): Some aspects of haematology and serum biochemistry. *Archives of Applied Science Research,* 2013, 5(2), 172-176

[15] Oloruntola, O.D.; Johnson, O.A.; Simeon, O.A.; Eyanlola, S.A.; Olajumoke, T.D.; Deborah, A.O. Gliricidia leaf meal and multi-enzyme in rabbits diet: effect on

performance, blood indices, serum metabolites and antioxidant status. *Journal of Animal Science and Technology,* 2018, 24, 60.

[16] Oloruntola, O.D.; Ayodele, S.O. Pawpaw leaf meal and exo-enzyme in rabbit diet: effect on haematological and serum biochemical indices. *Asian J Adv Agric Res*. 2017, 2(4), 1-8.

[17] Oloruntola, O.D.; Ayodele, S.O.; Agbede, J.O.; Oloruntola, D.A.; Ogunsipe, M.H.; Omoniyi, I.S. Effect of Alchornea cordifolia leaf meal and enzyme supplementation on growth, haematological, immunostimulatory and serum biochemical response of rabbits. *Asian J Bio Life Sci.* 2016, 5(2), 190-5.

[18] Adeyeye, S.A.; Agbede, J.O.; Aletor, V.A.; Oloruntola, O.D. Processed cocoa (Theobroma cacao) pod husks in rabbit diet: effect on haematological and serum biochemical indices. *Asian J Adv Agric Res.* 2017, 2(4), 1-9.

[19] Yilkal, T. Important anti-nutritional substances and inherent toxicants of feeds. *Food Sci Quality Manag.* 2015, 36, 40-7.

[20] Etchu, A.K.; Tientcheu, B.L.; Ghomsi, M.O.S.; Enow, J.T.; Tuedom, N.M.; Enamou, G. Effect of Moringa Oleifera Leaf Meal (Molm) on the Growth, Carcass,Heamatology and Biochemical Parameters of Rabbits. *SOJ Vet Sci,* 2017, 3(3), 1-5.

[21] El-Badawi, A.Y.; Omer, H.A.A.; Abedo, A.A.; Yacout, M.H.M. Response of Growing New Zealand White Rabbits to Rations Supplemented with Different Levels of Moringa oleifera Dry Leaves. *Global Veterinaria.* 2014, 12, 573-582.

[22] Jiwuba, P.C.; Ikwunze, K.; Dauda, E.; Ugwu, D.O. Haematological and Serum Biochemical Indices of Growing Rabbits Fed Diets Containing Varying Levels of Moringa oleifera Leaf Meal. *British Biotechnology Journal,* 2016, 15(2), 1-7.