Archives of Current Research International

15(1): 1-10, 2018; Article no.ACRI.44105
ISSN: 2454-7077

Serum Biochemistry and Histological Studies in Growing Rabbits Fed Diets Supplemented with *Mucuna pruriens* Leaf Meal

O. D. Oloruntola[1*]

[1]Department of Animal Science, Adekunle Ajasin University, Akungba Akoko, Nigeria.

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/ACRI/2018/44105
Editor(s):
(1) Dr. Sung-Kun Kim, Department of Natural Sciences, Northeastern State University, USA.
Reviewers:
(1) Anand Kumar Pathak, Sher-e-Kashmir University of Agricultural Sciences and Technology, India.
(2) Pravin Rathod, Post Graduate Institute of Veterinary & Animal Sciences, Maharashtra Animal and Fishery Sciences University, India.
(3) Othmani-Mecif Khira, University of Sciences and Technology Houari Boumediene, Algeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/26847

Original Research Article

Received 24 August 2018
Accepted 19 October 2018
Published 25 October 2018

ABSTRACT

Aims: To study the serum biochemistry, hepatic and renal histology of rabbits fed diets supplemented with Mucuna leaf meal.
Study Design: Completely Randomised Design.
Place and Duration of Study: The experiment was carried for 12 weeks at Agricultural Technology Department Teaching and Research Farm, The Federal Polytechnic, Ado Ekiti, Nigeria. One hundred and twenty, 35-day old crossbreed (Chinchilla x New-Zealand) weaner rabbits (equal sexes; average weight 694±5 g) were randomly allotted to 4 experimental diets (30 rabbits/treatment; 3 rabbits/replicate).
Methodology: A basal diet was formulated to meet the nutritional requirement of the rabbit. The basal diet was divided into 4 equal portions and designated diets 1, 2, 3 and 4. Diets 1 to 4 were supplemented with 0, 0.4, 0.8 and 1.2% Mucuna leaf meal (MLM), respectively. On day 119 of age, blood samples were collected from 10 rabbits/experimental group for serum biochemical indices determination. On the same day, the rabbit was selected from each replicate and sacrificed. The liver and kidney were surgically removed for histological studies.
Results: The total protein, creatinine, and bilirubin level of the rabbits were not affected (P>0.05) by

**Corresponding author: E-mail: oloruntoladavid@gmail.com, olugbenga.oloruntola@aaua.edu.ng;*

MLM supplementation. The serum cholesterol levels of the rabbits reduced (P<0.05) at 0.8% and 1.2% MLM supplementation (Diets 3 and 4). The aspartate aminotransferase concentration in rabbits fed 1.2% MLM supplemented diets significantly (P<0.05) increased compared to those fed 0% MLM supplemented the diet. The liver of rabbits fed MLM supplemented diets shows some remarkable histological alterations which vary from hepatitis, interstitial oedema (0.4% MLM supplemented diet), portal triad with congested blood vessels, mild periportal inflammation (0.8 MLM supplemented diet), and congested blood vessels of hepatic parenchyma and portal traid (1.2% MLM supplemented diets).

Conclusion: Dietary MLM supplementation at 0.8% and 1.2% levels reduced the serum cholesterol concentration of the rabbits. However, dietary MLM supplementation at 1.2% level for the duration of 12 weeks caused some observable histological alteration in the liver of the rabbits.

Keywords: Rabbits; serum biochemical; histology; mucuna; phytogenic feed supplements.

1. INTRODUCTION

There is a ban in several countries on the use of synthetic growth promoters such as antibiotics in meat production [1]. Alternatives such as dietary supplementation with phytogenic/phytochemical feed additives, botanical compounds and phytobiotics are presently being considered [2,3]. Therefore, the use of phytogenic feed supplements and ingredients in meat production to improve the growth performance, antioxidant status and general health status of the animals are becoming more popular [3,4].

The phytochemical feed additives or ingredients have numerous biological properties such as antioxidant, anti-stress, anti-microbial and nutrigenomic effects on the immunity development; that made them useful as growth promoters in animal production [5,6].

The growth and productive performance being promoted by phytochemical additives have been related to several biological activities such as improved antioxidant status, increased appetite stimulation, decreased antimicrobial colonisation and possible excitation of the olfactory nerve and taste buds [3,7].

Phytogenic feed additives and ingredients such as leaf meals from *Alchornea cordifolia* [6], pawpaw [8], gliricidia [9], neem [10] among others have been used in monogastric animal nutrition.

Mucuna parts have potentials for usage as feed supplement due to their amino acids, minerals, vitamins and phytochemicals profiles [11]. All parts of Mucuna plant are reported to possess medicinal value, and it is a constituent of over two hundred Indian indigenous drug formulations [12,13].

Although the use of phytochemicals as feed additives are acclaimed to be safe, proper toxicological studies are required to ensure their safety in animal production [14]. At present, there is dirt of information concerning the histological effect of the use of *Mucuna pruriens* leaf meal as the feed supplement in rabbits. Therefore, this study evaluated the histological effect of MLM supplementation in rabbits' diet.

2. MATERIALS AND METHODS

2.1 Experimental Site

The feeding trial was carried out within October, and December 2017 at the Rabbitary, Agricultural Technology Department Teaching and Research Farm, The Federal Polytechnic, Ado Ekiti, Nigeria after the right to conduct the research was granted by Agricultural Technology Department Research Committee of the same Institution, within October and December 2017. The average temperature and average relative humidity of the rabbit house were 29.6°C and 77.5%, respectively.

2.2 Preparation of Mucuna Leaf Meal

Fresh *Mucuna pruriens* leaves were harvested within the premises of The Federal Polytechnic, Ado Ekiti, Nigeria. The leaves were washed, drained, chopped and spread lightly on tarpaulin under the shed for 7 days to air-dry. The dried Mucuna leaves were thereafter milled with 2 mm hammer mill to *Mucuna pruriens* leaf meal (MLM) and analysed for terpenoid [15], cardiac glycoside [15], steroid [16], tannin [17], saponin [18], alkaloid [19], and flavonoid [18].

2.3 Experimental Diets, Design and Rabbit Housing

A basal diet was formulated to meet the nutritional requirements of the growing rabbit [20] (Table 1). The basal diet was divided into 4 equal portions and designated diets 1, 2, 3 and 4. Diets 1 to 4 were supplemented with 0, 0.4, 0.8 and 1.2% MLM, respectively. All diets were pelleted to 4 mm diameter and 8 mm long and analysed for proximate composition [21]. The gross energy was determined against thermo-chemical grade benzoic acid using combustion calorimeter (Model:e2k combustion calorimeter, www.cal2k.com).

Table 1. The composition of the basal diet (%)

Ingredients	Inclusion level
Maize	8.00
Wheat offals	8.00
Soybean meal	16.10
Maize husk	22.00
Cassava peels	22.00
Brewers dried grain	21.70
Bone meal	1.10
Premix	0.25
Methionine	0.20
Lysine	0.10
Salt	0.25
Vegetable oil	0.30
Chemical composition (%)	
Crude protein	16.88
Crude fibre	17.59
Gross energy (Kcal/Kg)	4108.49

The recommendations and guidelines for applied nutrition and experiments in rabbits were followed in the management of the rabbits [22]. One hundred and twenty, 35-day old crossbreed (Chinchilla x New-Zealand) weaner rabbits (equal sexes; average weight 694±5 g) were randomly allotted to 4 experimental diets (30 rabbits/treatment; 3 rabbits/replicate). The rabbits were housed in wire meshed cage, inside a well-ventilated pen. Experimental diets and water were provided *ad libitum* throughout the period of the experiment.

2.4 Blood Analysis

Blood samples were collected from 10 rabbits/experimental group on day 119 of age from overnight fasted selected rabbits as described by Burnett et al. [23] via their prominent ear vein. The serum biochemical indices (total protein, creatinine, cholesterol, aspartate aminotransferase (AST) and bilirubin) were determined with a Reflectron ® Plus 8C79 (Roche Diagnostic, GombH Mannheim, Germany), using Reflectron kits.

2.5 Histological Study

On day 119 of age, one rabbit was randomly selected from each replicate (10 rabbit/experimental group) and sacrificed. The liver and kidney were surgically removed for histological studies. Specimens (liver and kidney) were fixed in 10% neutral buffered formalin, dehydrated in a graded alcohol series (70%, 90%, absolute ethanol), cleared with methyl benzoate and embedded in paraffin wax. Sections of 5 µm were cut and stained with hematoxylin and eosin stain for light microscopic examination [24,25]. Stained sections were examined by light microscope and photographed using a digital camera.

2.6 Statistical Analysis

Completely randomized design with this model: $T_{ij} = \mu + \alpha_i + E_{ij}$ was adopted. Where T_{ij} = any of the response variables; μ = the overall mean; α_i = effect of the *i*th treatment (*i* = diets 1, 2, 3 and 4) and E_{ij} = random error due to experimentation. All data were subjected to analysis of variance using SPSS statistical software package, version 20. Duncan's multiple ranges was used to determine the differences between treatment groups. Statistical significance was assessed at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1 Phyto-constituents of *Mucuna pruriens* Leaf Meal (MLM)

The phytochemical constituent of MLM is shown in Table 2. Phytochemicals: tannin, terpenoid, cardiac glycoside, saponin, steroid, alkaloid, and flavonoid were detected in MLM in this study. Phytochemicals are secondary metabolites in plants and are biologically active [26]. These plant-derived secondary metabolites are reported to provide health benefit, support disease prevention, and support growth performance [3,27]. However, the high concentration of these phytochemicals in diets can produce deleterious effects in animals [26].

3.2 Serum Biochemical Parameters of Rabbits Fed Diet Supplemented with MLM

The effects of MLM supplementation on serum metabolites of growing rabbits on day 119 of age

is shown in Table 3. Enzymes, due to cell damage or destruction leaked out into the surrounding fluids and finally into the blood and thereby increasing their concentration in the blood [28]. In this study, the total protein, creatinine, and bilirubin were not affected (P>0.05) by MLM supplementation. This suggests that supplementing the rabbits' diet with MLM as from 35 days of age to 119 days of age did not affect the production of protein, nor produced renal/cardiovascular disorders, neither does it caused liver damage [29]. The reduction in serum cholesterol levels of the rabbits with increased MLM supplementation level and significant (P<0.05) reduction of cholesterol level (P<0.05) at 0.8% and 1.2% dietary MLM supplementation (Diets 3 and 4) may be a result of decreased uptake of cholesterol due to the dietary treatment applied in this study. This could be the reflection of activities of some phytochemicals present in MLM that are interfering with the normal cholesterol absorption in the rabbits. For instance, a high level of saponin has been reported to reduce cholesterol absorption [30].

The increased concentration of aspartate aminotransferase (liver leakage enzyme) in rabbits fed MLM supplemented diets and significant (P<0.05) increase elevation of this enzyme at 1.2% MLM dietary supplementation indicates hepatocellular damage in the rabbit [29,30]. The use of phytochemicals in the treatment of liver problems had been reported [31]; however, some of these phytochemicals could be hazardous to health and vital internal organs when consumed level beyond the body of the animals could handle [26,32].

Table 2. Phytochemical constituent of *Mucuna pruriens* leaf meal

Phytochemicals	Quantity (mg/g)
Tannin	3.08
Terpenoid	12.40
Cardiac glycoside	9.91
Saponin	30.72
Steroid	9.51
Alkaloid	12.12
Flavonoid	91.15

3.3 Histopathology

3.3.1 Liver

Histopathological sections of the liver of the experimental rabbits are shown in Plates 1 to 4. Photomicrographs showed varying degrees of changes in the appearance of the hepatic cells with graded levels of dietary MLM supplementation.

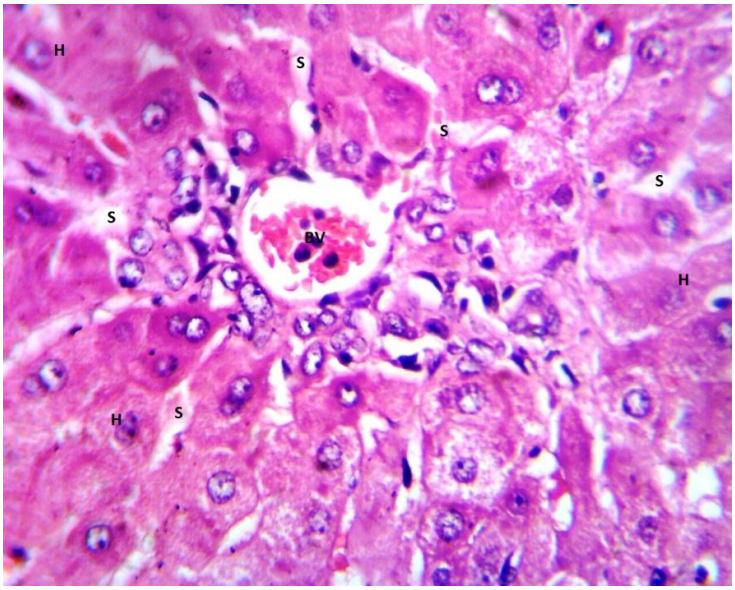

Plate 1. Representative micrograph of liver section (x400) of rabbits fed 0% MLM supplemented the diet

Photomicrograph showed the hepatic tissue composed of the hepatic parenchymal disposed of in sheet (H) separated by the sinusoids (S) that were free from congestion and inflammatory cells. The portal region consists of vessels, bile ducts that are unremarkable

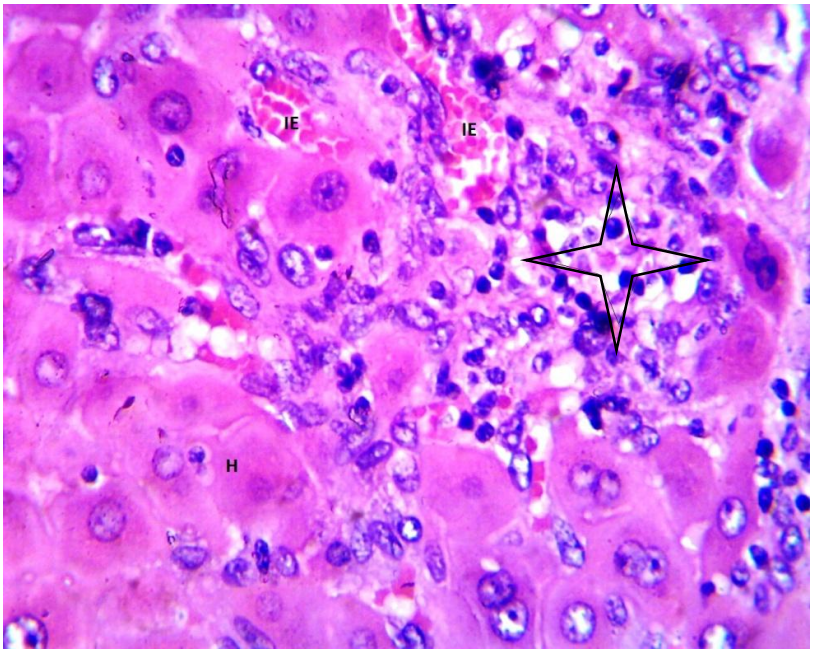

Plate 2. Representative micrograph of liver section (x400) of rabbits fed 0.4 % MLM supplemented the diet

Photomicrograph shows marked hepatitis (star), interstitial edema (IE), the hepatocytes (H) are disposed of in the sheet

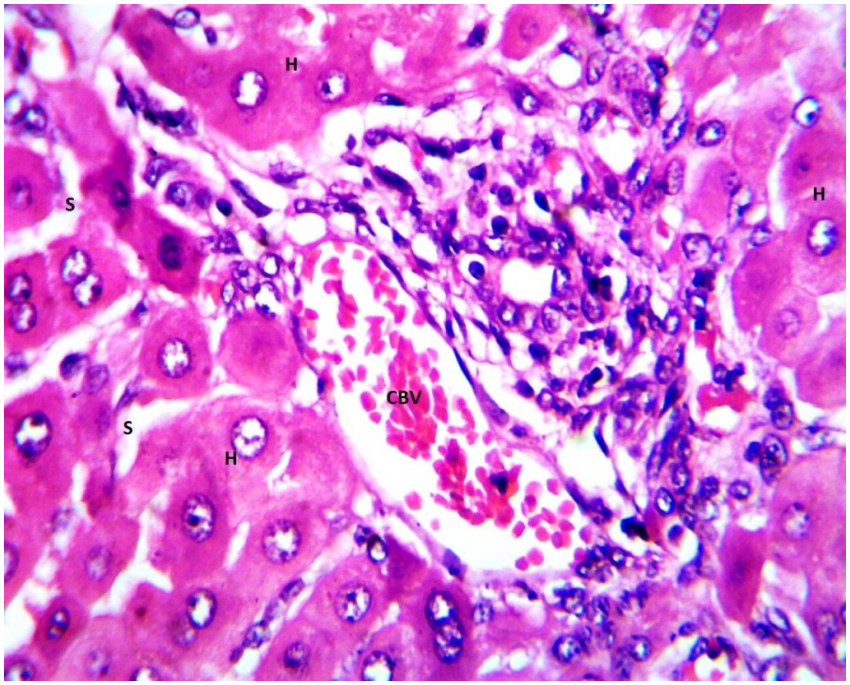

Plate 3. Representative micrograph of liver section (x400) of rabbits fed 0.8 % MLM supplemented diet

The micrograph shows the portal traid with congested blood vessels (CBV) and mild periportal inflammation, the hepatocytes (H) are separated by the sinusoidal space (S) that is free from congestion

Table 3. Effects of MLM supplementation on serum metabolites of growing rabbits on 119d of age

	Diet 1 0.0% MLM	Diet 2 4% MLM	Diet 3 0.8% MLM	Diet 4 1.2% MLM	SEM	P value
No. of rabbits	10	10	10	10		
Total protein (g/dl)	6.70	7.67	7.91	7.90	0.56	0.89
Creatinine (µmol/l)	86.67	84.61	121.27	115.26	8.86	0.36
Cholesterol (mmol/l)	1.70^{a}	0.93^{ab}	0.54^{b}	0.49^{b}	0.18	0.03
Aspertate aminotransferase (IU/L)	87.64^{b}	102.49^{ab}	106.59^{ab}	121.45^{a}	4.54	0.04
Bilirubin total (µmol/l)	9.93	9.69	10.79	13.01	0.76	0.45

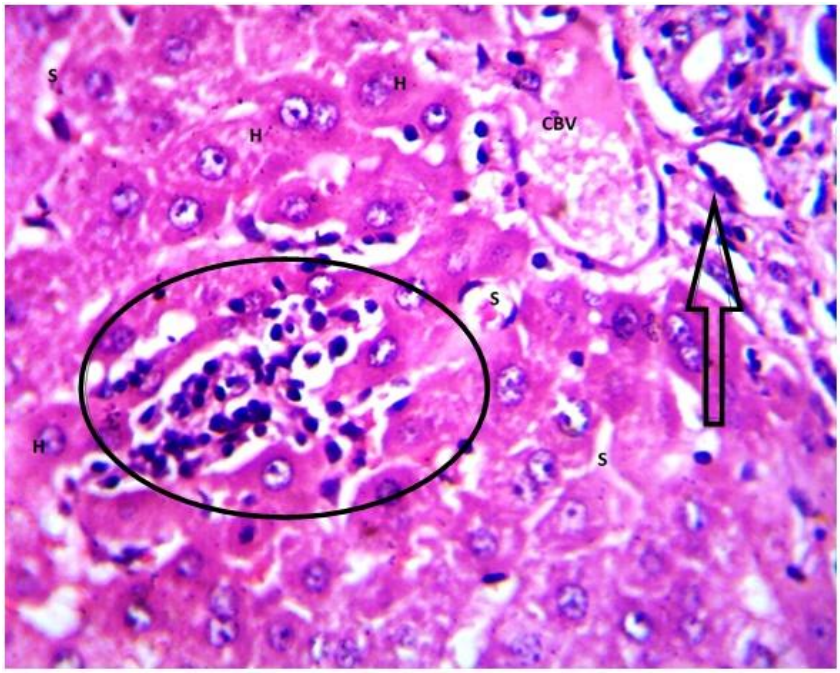

Plate 4. Representative micrograph of liver section (x400) of rabbits fed 1.2 % MLM supplemented diet

The section showed clusters of inflammatory cells within the hepatic parenchyma (Circle), while, the portal traid (arrow) showed congested blood vessels (CBV), the hepatocytes (H) were separated by the sinusoidal space (S)

Functions of the liver in the body are numerous and include the processing of the nutrient absorbed from the small intestine, secretion of bile, which also plays roles in fat digestion and removal of toxic substances from the living organism. The various histopathological lesions in the liver of the experimental rabbits fed MLM supplemented diets could be due to the increased dietary concentration of the phytochemicals by incorporating MLM in the rabbit diets. Soetan and Oyewole [33] had reported that some plants contain phytochemicals/anti-nutritional factors (anti-vitamin factors) whose mode of action is poorly understood but could cause histopathological lesions such as liver necrosis, muscular dystrophy and low concentration of plasma tocopherol [33,34]. The histological alterations observed in the liver of rabbits fed MLM supplemented diets is in harmony with increasing concentration of serum aspartate aminotransferase in rabbits fed 1.2% MLM supplemented the diet. The appearance of histological alterations in these rabbits could be as a result of the long period (12 weeks) of tasking the liver with the processes of detoxification; possibly due to the presence of anti-nutritional factors being introduced into the diet by dietary MLM supplementation.

3.3.2 Kidney

Plates 5 to 8 shows the histopathological sections of the kidney of the experimental rabbits. Photomicrographs showed the varying degrees of morphological changes of the kidney cells at graded levels of dietary MLM supplementation.

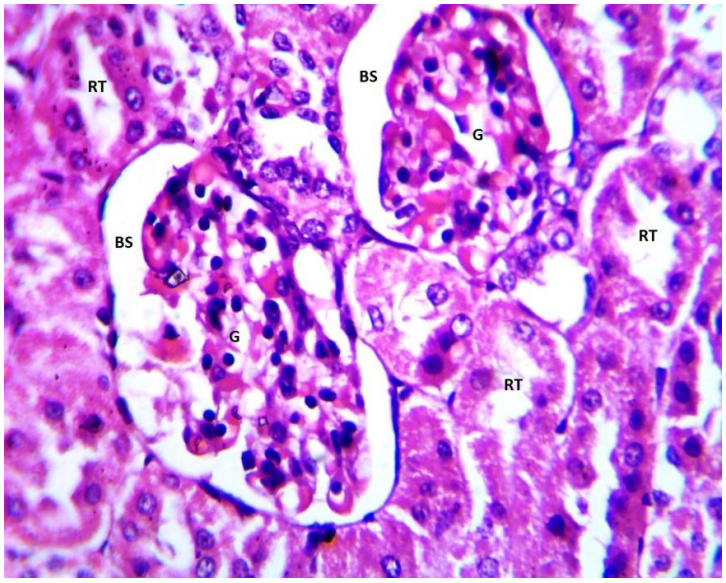

Plate 5. Representative micrograph of kidney section (x400) of rabbits fed 0 % MLM supplemented diet

The section shows the renal tissue composed of a well-defined renal corpuscle and renal tubules (RT). The corpuscle is made of the Bowman's capsule with well-outlined bowman's space, glomerulus, the renal tubules are lined by regular epithelium, columnar-cuboidal epithelium and are separated by the interstitium that is free from congestion and inflammatory cells

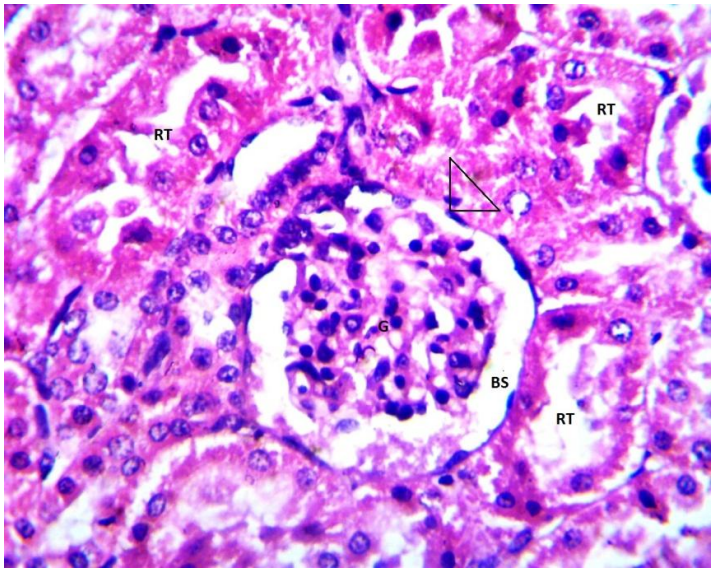

Plate 6. Representative micrograph of kidney section (x400) of rabbits fed 0.4 % MLM supplemented diet

The section shows the renal tissue composed of a well-defined renal corpuscle and renal tubules (RT). The corpuscle is made of the Bowman's capsule with well-outlined bowman's space, glomerulus, the renal tubules are lined by regular epithelium, columnar-cuboidal epithelium and are separated by the interstitium that is free from congestion and inflammatory cells

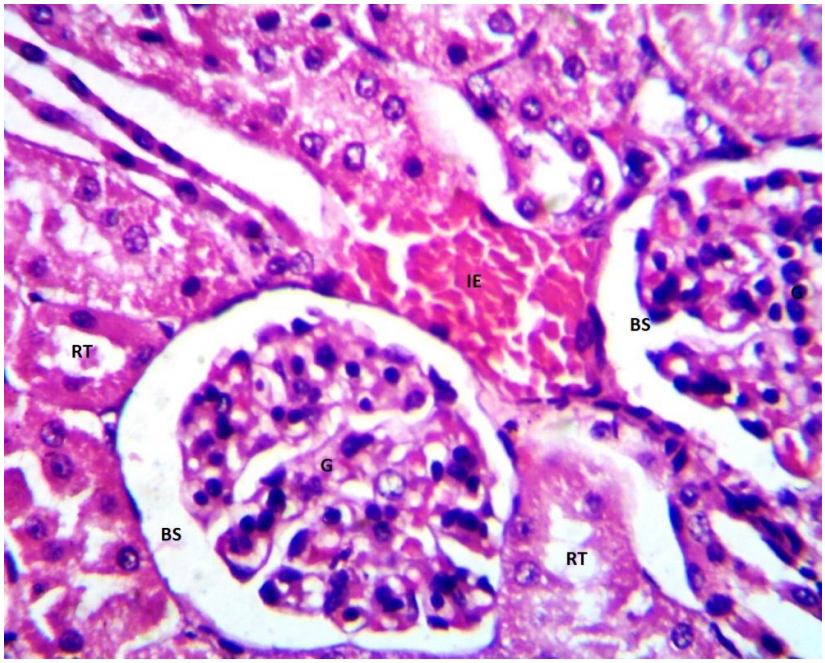

Plate 7. Representative micrograph of kidney section (x400) of rabbits fed 0.8% MLM supplemented diet; stained with HE (X400)

Section shows marked interstitial oedema (IE). The renal tubules (RT), renal corpuscle appear normal

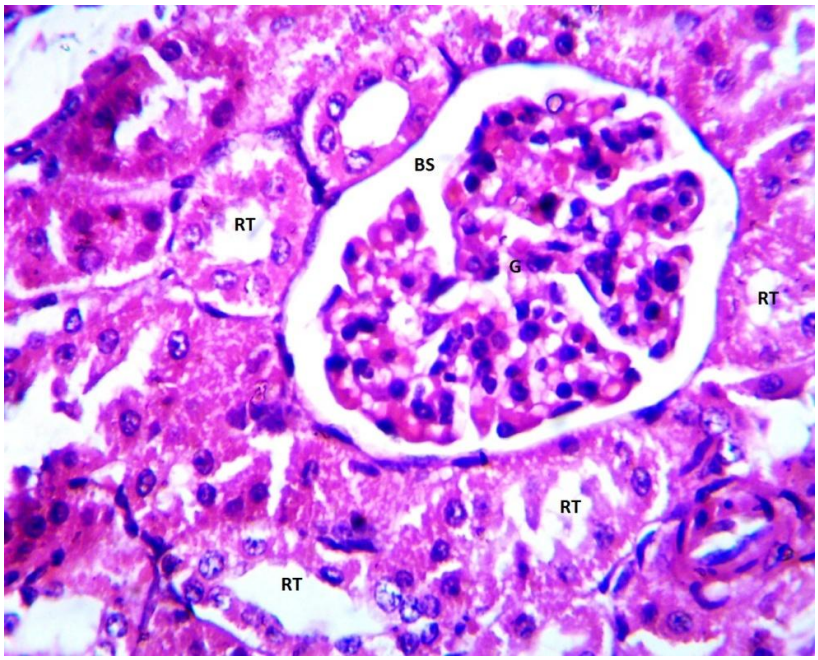

Plate 8. Representative micrograph of kidney section (x400) of rabbits fed 1.2 % MLM supplemented the diet

Photomicrograph shows the renal tissue composed of the renal corpuscle made of the Bowmans capsule, glomerulus (G), the renal tubules (RT) lined by column-cuboidal epithelium. The renal tubules were separated by the interstitium that was free from congestion and inflammatory cells

Kidney functions essentially in removing waste products from the blood and regulating water fluid levels. In this study, dietary MLM supplementation did not cause any major or remarkable histological alteration in the kidney of the rabbits. This implies that at 119 days of age, MLM supplementation did not pose threats to normal functioning of the rabbits' kidney. This is supported by the stability of the serum creatinine (kidney marker) of the rabbits across the various dietary treatments.

4. CONCLUSION

In conclusion, dietary MLM supplementation at 0.8% and 1.2% levels reduced the serum cholesterol concentration of the rabbits. However, 12 weeks long dietary MLM supplementation at 1.2% level caused some observable histological alteration in the liver of the rabbits.

ETHICAL APPROVAL

The author hereby declares that "guide for the care and use of laboratory animals" (National Research Council, Copyright 2011, 8th edition) were followed, as well as specific National Laws where applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Falcao-e-Cunha L, Castro-Solla L, Maertens L, Marounek M, Pinheiro V, Freire J, Mourao JL. Alternatives to antibiotic growth promoters in rabbit feeding: A review. World Rabbit Sci. 2003;15:127-140.
2. Nahak G, Suar M, Sahu RK. Antioxidant potential and nutritional values of vegetables: A review. Res. J. Med. Plant. 2014;8(2):50-81.
3. Valenzuela-Grijalva NV, Pinelli-Saavedra A, Muhlia-Almazan A, Dominguez-Diaz D. Dietary inclusion effects of phytochemicals as growth promoters in animal production. J. Ani. Sci. Techn. 2017;59:8.
4. Oloruntola OD, Ayodele SO, Adeyeye AA, Ogunsipe MH, Daramola OT, Eyanlola SA. Effect of pawpaw leaf meal and multi-enzyme supplementation in the diet on performance, digestibility, and oxidative enzyme status of rabbits. The J. Basic. Appl. Zool. 2018;79:26-33.
5. Hashemi SR, Davoodi H. Phytogenic as new class of feed additive in poultry industry. J. Anim. Vet. Adv. 2010;90:2295-2304.
6. Oloruntola OD, Ayodele SO, Agbede JO, Oloruntola DA, Ogunsipe MH, Omoniyi IS. Effect of *Alchornea cordifolia* leaf meal and enzyme supplementation on growth, haematological, immunostimulatory and serum biochemical response of rabbits. Asian J. Biol. Life Sci. 2016;5(2):190-195.
7. Alipour F, Hassanabadei A, Golian A, Nassiri-Moghaddam H. Effect of plant extracts derived from thyme on male broiler performance. Poultry Sci. 2015;94: 26-30.
8. Oloruntola OD, Ayodele SO. Pawpaw leaf meal and exo-enzyme in rabbit diet: Effect on haematological and serum biochemical indices. Asian J. Agric. Res. 2017;2(4):1-8.
9. Oloruntola OD, Ayodele SO, Agbede JO, Asaniyan EK. Performance and apparent digestibility of broiler starter fed diets containing *Gliricidia sepium* leaf meal. Asian J. Biol. Life Sci. 2016;5(1):97-102.
10. Ansari J, Khan SH, Haq A, Yousaf M. Effect of the levels of *Azadirachta indica* dried leaf meal as phytogenic feed additive on growth performance and haemato-biochemical parameters in broiler chicks. J. Appl. Anim. Res. 2012;40(4):336-345.
11. Sese BT, Okpeku M, Patani I. Tropical velvet bean (*Mucuna pruriens*) leaf meal as unconventional protein supplement in the diet of broiler chicks. J. Anim. Sci. Adv. 2013;3(11):575-583.
12. Warrier PK, Nambiar VPK, Ramankutty C. Indian medicinal plants, a compendium of 500 species. Orient Longman Limited, Madras, India. 1996;4.
13. Ujowundu CO, Kalu FN, Emejulu AA, Okafor OE, Nkwonta CG, Nwosunjoku EC. Evaluation of the chemical composition of *Mucuna pruriens* leaves used in herbal medicine in Southeastern Nigeria. African J. Pharm. Pharmacol. 2010;4(11):811-816.
14. Orhue ES, Idu M, Ataman JE, Ebite LE. Haematological and histological studies of *Jatropha tanjorensis* (J.L Ellis and Soroja) leaves in rabbits. Asian J. Bio. Sci. 2008;1(2):84-89.
15. Ferguson NM. A textbook of pharma-cology. McMillan Company. New Delhi. 1956;191.

16. Edeoga GO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African J. Biotehn. 2005;4:685-688.
17. Van-Burden TP, Robinson WC. Formation of complexes between protein and tannin acid. J. Agric. Food Chem. 1981;1:77.
18. Shad MA, Nawaz H, Rehma T, Ikram M. Determination of biochemicals, phyto-chemicals and antioxidative properties of different part of *Cichorium intybus* L.: A comparative study. The J. Animal and Plant Sci. 2013;23(4):1060–1066.
19. Harborne JB. Phytochemical methods. Chapman and Hall Ltd, London. 1973;49-188.
20. de Blas C, Mateos GG. Feed formulation. In: Nutrition of the Rabbit. 2nd Edition (eds C.de Blas and J. Wiseman). CAB International. The United Kingdom. 2010; 229.
Available:http://dx.doi.org/10.1079/9781845936693.0222
21. AOAC. Official methods of analysis of the Association of Official Analytical Chemists, 15th Ed. AOAC, Washington, D.C. USA; 1990.
22. Fernández-Carmona J, Blas E., Pascual JJ, Maertens L, Gidenne T, Xiccato G, Garcia J. Recommendations and guidelines for applied nutrition experiments in rabbits. World Rabbit Sci. 2005;13:209-228.
Available:http://doi.org/10.4995/wrs.2005.516
23. Burnett N, Mathura K, Metivier KS, Holder RB, Brown G, Campbell M. An investigation into haematological and serum chemistry parameters of rabbits in Trinidad. World Rabbit Sci. 2003;14:175-187.
24. Bancroft TD, Stevens A, Turner DR. Theory and practice of histological technique, 4th Ed. Churchill, Livingstone, New York; 1996.
25. Oloruntola DA, Dada EO, Osho IB, Ogundolie OO. Effects of hydro-ethanolic leaf extract of *Tithonia diversifolia* on parasitaemia level, serum metabolites and histopathology of organs in Swiss Albino mice infected with *Plasmodium berghei* NK65. Asian J. Med. Health. 2017;6(2):1-7.
26. Gemede HF, Ratta N. Antinutritional factors in plant foods: Potential health benefits and adverse effects. Int. J. Nutr. Food Sci. 2014;3(4):284-289.
27. Chukwuebuka E, Chinenye IJ. Biological function and anti-nutritional effects of photochemicals in living systems. J. Pharm. Bio. Sci. 2015;10(2):10-19.
28. Oloruntola OD, Ayodele SO, Agbede JO, Oloruntola DA, Ogunsipe MH, Omoniyi IS. Effect of *Alchornea cordifolia* leaf meal and enzyme supplementation on growth, haematological, immunostimulatory and serum biochemical response of rabbits. Asian J. Biol. Life Sci. 2016;5(2):190-195.
29. Lording PM, Friend SCE. Interpretation of laboratory results. Aust. Vet. Pract. 1991;21(4):188-192.
30. Girling SJ, Campbell-Palmer R, Pizzi R, Fraser MA, Cracknell J, Arnemo J, Rosell F. Haematology and serum biochemistry parameters and variations in the Eurasian Beaver (*Castor fiber*). PLoS ONE. 2015; 10(6):2-15.
DOI: 10.137/journal.pone.0128775
31. Igwe CU, Nwaogu LA, Ujuwondu CO. Assessment of the hepatic effects, phytochemicals and proximate composition of *Phyllanthys amarus*. African J. Biotech. 2007;6(6):728-731.
32. Ayodele SO, Oloruntola OD, Agbede JO. Effect of *Alchornea cordifolia* leaf meal inclusion and enzyme supplementation on performance and digestibility of rabbits. World Rabbit Sci. 2016;24:201-206.
33. Soetan KO, Oyewole OE. The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: A review. African J. Food Sci. 2009;3(9):223-232.
34. Liener IE. In: Advances in legume science. RJ Summerfield and AH. Bunting (eds.), Academic Press, New York, London; 1980.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/26847