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Haematological and Serum Biochemical Indices of Growing Rabbits Fed Diets Containing Varying Levels of *Moringa oleifera* Leaf Meal

P. C. Jiwuba[1*], K. Ikwunze[2], E. Dauda[1] and D. O. Ugwu[1]

[1]Department of Animal Health and Production Technology, Federal College of Agriculture, P.M.B.7008, Ishiagu, Ebonyi State, Nigeria.
[2]Department of Animal Production and Management, Michael Okpara University of Agriculture, P.M.B.7267 Umudike, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author PCJ designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors KI and ED managed the analyses of the study. Author DOU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the influence of *Moringa oleifera* leaf meal (MOLM) on haematological and serum biochemical indices of growing rabbits fed diets containing varying levels of *Moringa oleifera* leaf meal.
Study Design: Completely randomized design.
Place and Duration of Study: At the Rabbit unit of the Teaching and Research farm of Federal College of Agriculture, Ishiagu, Ivo L.G.A., State, Nigeria, between May, 2015 and July 2015.
Methodology: 48 growing rabbits were used for this study. Four diets were formulated such that diets T1, T2, T3 and T4 contain MOLM at 0%, 10%, 20% and 30%, respectively. The diets were offered to the growing rabbits, which were randomly divided into 4 groups of 12 rabbits each, with 4 animals constituting a replicate in a completely randomized design pattern. Each animal received the experimental diet for 49 days. Blood samples were drawn from each animal on the last day of

**Corresponding author: E-mail: jiwubapc@yahoo.com;*

the trial and evaluated for haematological and serum biochemical indices, data obtained were analysed statistically.

Results: Results showed that packed cell volume (PCV), haemoglobin Mean cell haemoglobin concentration and white blood cell differed ($P<0.05$) significantly, while red blood cell, mean cell haemoglobin and mean cell volume were similar ($P>0.05$) among the groups PCV ranged between 35.01–36.59%. Haemoglobin was improved ($p<0.05$) by *Moringa oleifera* leaf meal at 30% inclusion level. White blood cell (WBC) count of weaner rabbits in treatment groups was significantly ($p<0.05$) higher and better than the control. All the serum biochemical indices were significantly ($P<0.05$) except creatinine. Cholesterol was lowest at 30% inclusion indicating the anti-diabetic properties of the test ingredient.

Conclusions: All the parameters studied fell within the normal range reported for clinically healthy rabbits; an indication that MOLM had a beneficial effect on health status of rabbits.

Keywords: Moringa oleifera; leaf meal; blood profile; medicinal plant; unconventional feed resource.

ABBREVIATIONS

MOLM = Moringa oleifera leaf meal; PCV = Packed cell volume; MCHC = Mean corpuscular haemoglobin concentration; MCH = Mean corpuscular haemoglobin; MCV = Mean corpuscular volume; RBC = Red blood cell; WBC = White blood cell; ANOVA = Analysis of variance; DM = Dry matter; NFE = Nitrogen free extract; ME = Metabolisable energy; CP = Crude protein; CF= Crude fibre.

1. INTRODUCTION

Blood indices are considered to be critical indicators of the physiological stages of farm animal, thus reflecting the relationship between their nutrition and health. They are useful for clinical evaluation of various animal diseases and feed quality. According to [1] serum creatinine helps in evaluation of liver function and diseases while serum urea evaluates renal function. Packed cell volume and red blood cells help to determine the feed toxicity and anemia in farm animals. Blood parameters change in relation to the physiological status of an animal. These changes could be as a result of several factors such feeding level, feed quality, age, sex, breed, temperature and physiological status of animals. These differences have further underlined the need to establish appropriate physiological and nutritional baseline values for rabbits, which could help in realistic evaluation of the management practice, nutrition and diagnosis of health of the host animal.

The incorporation of protein from leaf sources in diets for rabbits is fast gaining grounds because of its availability, abundance and relatively reduced cost [2]. Leaf meals do not only serve as protein sources but also provide some vitamins, minerals and also oxycarotenoids. Studies have shown that multipurpose trees can be used as cheap protein source which can improve general performance of animals.

Moringa oleifera belongs to the single genus *monogeneric* family *Moringaceae* and is well distributed in Africa and Asia. Apart from being a good source of protein, vitamins, amino acids, and minerals for rabbits, they also have medicinal uses [3]. *Moringa oleifera,* has reputation for many medicinal properties, possesses hypocholesterolemic properties [4] and could serve as substitute for conventional feedstuffs [5]. The protein content of the leaves are high ranging between 20–35% on a dry weight basis and most importantly, the protein is of high quality having significant quantities of most essential amino acids [6]. The leaves are highly nutritious containing significant amount of Vitamins A, B, C, Ca, Fe, P and protein [7]. The tree has in recently been noted as an outstanding source of highly digestible protein, calcium, iron, vitamin C, and carotenoids suitable for utilization. However, data on haematological and serum biochemical parameters of rabbits fed concentrate from multipurpose tree such as *M. oleifera* are scanty. Therefore, this experiment was carried out to determine the influence of *Moringa oleifera* leaf meal (MOLM) on haematological and serum biochemical indices of growing rabbits.

2. MATERIALS AND METHODS

2.1 Location of Experiment

The research was carried out at the Rabbit Unit, Federal College of Agriculture, Ishiagu, Ivo Local

Government Area of Ebonyi State, Nigeria. The College is located at about three kilometers (3km) away from Ishiagu main town. The College is situated at latitude 5.56°N and longitude 7.31°E, with an average rainfall of 1653 mm and a prevailing temperature condition of 28.50°C and relative humidity of about 80%.

2.2 Sources and Processing of Experimental Material

Fresh leaves of *Moringa oleifera* were harvested within the College environment and air dried for some days to a moisture content of about 10%. The dried leaves were processed and milled.

2.3 Experimental Materials and Management

Forty eight weaner rabbits were randomly divided into four (4) experimental groups of twelve (12) animals each, with four (4) rabbits constituting a replicate. The four treatment groups were assigned the four experimental diets in a Completely Randomized Design. Each rabbit received an assigned diet for 49 days. The animals were provided with feeders and drinkers. Each animal was vaccinated against prevalence under current diseases and were quarantined for 21 days before the commencement of the experiment. They were also dewormed and given accaricides bath prior to the experiment.

2.4 Experimental Diets

Four diets, T1, T2, T3 and T4 were formulated from maize, wheat offal, soybean, fish meal, palm kernel cake, *Moringa oleifera* leaf meal, bone meal, limestone, vitamin premix, methionine, lysine and common salt. Treatment one (T1) did not contain the test ingredients, thereby serving as the positive control. The experimental diets were formulated such that *Moringa oleifera* leaf meal was included at the levels of 0%, 10%, 20% and 30% for T1, T2, T3 and T4 respectively as presented in Table 1.

2.5 Blood Sample

Blood samples (5 ml) were drawn from each animal on the last day of the study. The rabbits were bled through the ear marginal vein. The samples were separated into two lots and used for biochemical and haematological studies. An initial 2.5 ml was collected from each sample in labelled sterile universal bottle containing 1.0 mg/ml ethyldiamine tetracetic acid and used for haematological analysis. Another 2.5 ml was collected over anti-coagulant free bottle. The blood was allowed to clot at room temperature and serum separated by centrifuging within three hours of collection. Serum biochemistry and haematological parameters were measured using Beckman Coulter Ac-T10 Laboratory Haematology Blood Analyzer and Bayer DCA 2000+ HbA1c analyzer, respectively. Mean cells haemoglobin (MCH), MCV and mean cell haemoglobin concentrations (MCHC) were calculated.

2.6 Analytical Procedure

All feeds and experimental materials were analyzed for proximate compositions using the method of [8].

Table 1. Composition of the experimental diets

Ingredients	Dietary levels (%)			
	T1 (0%)	T2 (10%)	T3 (20%)	T4 (30%)
Maize	37.00	37.00	37.00	37.00
Wheat offal	9.00	9.00	9.00	9.00
Palm kernel cake	10.00	10.00	10.00	10.00
Fish meal	3.00	3.00	3.00	3.00
Soybean	35.00	25.00	15.00	5.00
MOLM	0.00	10.00	20.00	30.00
Bone meal	2.00	2.00	2.00	2.00
Limestone	1.50	1.50	1.50	1.50
Vitamin premix	1.50	1.50	1.50	1.50
Salt	0.50	0.50	0.50	0.50
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**

Moringa oleifera leaf meal

2.7 Data Analysis

The results were analyzed using the Special Package for Social Sciences Window 17.0. One - way analysis of variance (ANOVA) was employed to determine the means and standard error. Treatment means were compared using Duncan's new multiple range test.

3. RESULTS AND DISCUSSION

The proximate compositions of *Moringa oleifera* leaf meal and experimental diets are presented in Table 2. Proximate analysis of *Moringa oleifera* leaf meal and experimental diets revealed the presence of dry matter, crude protein, crude fibre, ether extract, ash, nitrogen free extract and metabolizable energy. The dry matter values of the test diets (T2, T3 and T4) compared favourably with the control diet, but however did not show a specific trend. The crude protein of the experimental diets in this study ranged between 18.03 - 19.92% and compared favourably well with the range of 19.11 – 19.25 % reported by [9] for growing New Zealand white rabbits fed supplemented levels of *Moringa oleifera* dry leaves. The crude protein (CP) values tended to decrease with increasing levels of the test diets. The crude fibre contents of the experimental diets increased with increasing levels of *M. oleifera*. The crude fibre content of the diets however met the crude fibre requirement of 14 – 18% on dry matter basis as stated by [10] for rabbits. The Metabolizable energy (ME) content of the diets is in agreement with the reported Metabolizable energy values recorded by [11] for growing rabbits fed grasshopper meal as a substitute for fish meal.

The dry matter content (92.19%) reported for MOLM in this study compared well with 93.63% reported by [12]. The crude protein (CP) content (29.06%) of MOLM reported in this study is in agreement with the normal range of protein content (20-35%) reported by [6] for the same forage. The crude fibre (CF) (17.54%) is comparable with 16.98% reported by [13]. The difference and similarities could be due to the processing method, soil type, age at which the leaves were harvested and analysis.

The heamatological indices of growing rabbit fed diet containing *Moringa oleifera* leaf meal is presented in Table 3. The packed cell volume (PCV), haemoglobin (Hb), mean cell haemoglobin concentration (MCHC) and white blood cell (WBC) were significantly ($p<0.05$) affected by the treatment diets while red blood cell (RBC), mean cell haemoglobin (MCH) and mean cell volume (MCV) were similar ($P>0.05$) among the treatment. PCV is a measure of the relating mass of blood and involved in the transport of oxygen and absorbed nutrients. However, the PCV (%) values for all the treatment groups fell within the normal physiological range of 30.0-50.0% reported by [14] for healthy rabbits, suggesting that MOLM were tolerated across the treatment groups. This agrees with the findings of [15] who reported that normal PCV values are indicators of adequate nutritional status of rabbits.

Haemoglobin functions in transporting oxygen to tissues of animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body of animal [16]. The present study showed significant ($P<0.05$) difference among the treatments with T2 having the highest value and the lowest in T4 suggesting that *Moringa oleifera* leaf meal protein are of higher quality; a view corroborated by [17] that low level haemoglobin (Hb) of treatment could imply that dietary proteins were not of high quality. The MCHC values were significantly ($P<0.05$) affected with the rabbits fed treatment (T2) having the highest value (31.76 g/dl). The values recorded for all the treatment group however fell within the normal range (27-37) reported by [18]. The reported range of the MCHC in this study gave a clear indication of the absence of aneamia among the experimental animals.

White blood cells (WBC) function to fight infections, defend the body against invasion by foreign organisms and to produce or distribute antibodies in immune response. The range for WBC count obtained in this study ranged between 5.55 – 10.66 ($x10^6$/dl) and fell within the normal range for white blood cell (4.5 – 11 (10g/l) reported by [18]. The WBC count was significantly ($P<0.05$) different among the treatments, with the highest in T4 and the lowest in the control. The increase in the level of WBC with the level of MOLM in the diet agreed with the reports of [19]. These results indicated that the animals were healthy because decrease in number of WBC below the normal range is an indication of allergic conditions, anaphylactic shock and certain parasitism, while elevated values (leucocytosis) indicates the existence of a recent infection, usually with bacteria.

Table 2. Proximate compositions of the experimental diets and *Moringa oleifera* leaf meal

Constituents (%)	T1	T2	T3	T4	MOLM
Dry matter	94.05	93.98	95.06	94.79	92.19
Crude protein	19.92	19.12	18.12	18.03	29.06
Crude fibre	14.99	15.64	16.02	16.87	17.54
Ash	10.75	10.89	11.05	10.96	12.27
Ether extract	7.85	8.34	8.60	8.99	8.59
NFE	40.54	39.99	41.27	39.94	24.73
Metabolizable energy (kcal/kg)	2783.35	2777.75	2809.65	2793.10	2612.80

Moringa oleifera leaf meal

Table 3. Haematological indices of growing rabbit fed diet containing *Moringa oleifera* leaf meal

Parameters	T1	T2	T3	T4	SEM
PCV (%)	35.01^{a}	32.57^{b}	36.59^{a}	31.62^{b}	0.77
HB (g/dl)	9.45^{b}	10.49^{ab}	10.01^{ab}	11.60^{a}	0.34
RBC ($x10^{6}$/ul)	4.78	6.05	5.18	4.94	0.28
MCHC (%)	29.56^{b}	31.76^{a}	30.90^{ab}	29.65^{b}	0.4
MCH (pg)	22.81	21.69	20.61	20.69	0.27
MCV (fl)	74.91	68.78	68.02	70.02	1.03
WBC ($x10^{6}$/dl)	5.55^{d}	7.16^{c}	8.66^{a}	10.66^{a}	0.72

$^{a, b, c, d}$ *means in the row with different superscripts are significantly different (P<0.05)*

Table 4. Serum biochemistry of growing rabbits fed diet containing *Moringa oleifera* leaf meal

Parameters	Dietary levels				SEM
	T1	T2	T3	T4	
Total protein (g/l)	72.36^{a}	66.75^{b}	60.76^{c}	60.06^{c}	1.90
Albumin (g/dl)	39.35^{a}	34.66^{b}	32.45^{c}	31.16^{c}	1.19
Globulin (g/l)	31.77^{a}	31.06^{b}	29.67^{c}	26.74^{d}	0.73
Urea (mg/dl)	40.58^{a}	38.32^{b}	36.90^{c}	35.08^{d}	0.77
Creatinine (mg/dl)	0.72	0.59	0.50	0.59	0.03
Cholesterol mg/dl	65.40^{a}	50.50^{b}	46.36^{c}	45.89^{c}	3.01

$^{a, b, c, d}$ *means in the row with different superscripts are significantly different (P<0.05)*

Table 4 above shows the serum biochemistry of growing rabbits fed diet containing *Moringa oleifera* leaf meal. The treatment diets had a positive ($p<0.05$) influence among the treatments for the serum biochemical parameters except for creatinine that showed no significant ($p<0.05$) difference among the treatment groups. The result of total protein revealed that there were significant differences ($P<0.05$) among the treatment groups. T1 (Control) had the highest value (72.36 g/l) while T4 had the lowest value of (60.06 g/l). The values obtained in this study however fell within the normal range for healthy rabbits as reported by [20]; an indication of nutritional adequacy of the dietary proteins in this study.

The albumin and globulin also showed significant ($P<0.05$) difference among the experimental groups and fell within the normal physiological range for healthy rabbits. The range of values (31.16- 39.35/l) for albumin obtained in this present study fell within the normal range reported of (2.5 - 4.5 gl dl^{-1}) reported by [21] which is an indication of proper functioning of the liver in the rabbits. Abnormal serum albumin usually indicates an alteration of normal systemic protein utilization [22]. The globulin also fell within the normal range (1.5-3.3 g/dl) reported by [20] which is indicative of high immunity and good resistance to disease in the experimental animals. This perhaps highlights the ethno-veterinary properties of *Moringa oleifera* as reported by [4].

The cholesterol value in this study was significantly ($P<0.05$) affected by the treatment diets. There was a trend towards a reduction in the cholesterol value as the inclusion level of MOLM in the diet was increased. This

observation agrees with the result of [23] who reported that juice extracted from *Moringa* leaves was found to be a hypocholestrolemic agent. This reduction in serum cholesterol level of rabbit fed the MOLM diet may suggest a general decline in lipid mobilization and maybe suggest that *Moringa* leaf meal diet were capable of reducing serum cholesterol, hence assisting in the reduction and deposition of cholesterol in the muscle; thus production of lean meat. This result also suggested that MOLM diets could be used to produce animal products with reduced cholesterol level. However, the values of cholesterol obtained in this study were found to be within the normal physiological range for rabbits (35.0-66.0mg/dl) reported by [24].

The urea values differed significantly ($p<0.05$) among the treatment groups. The higher level of urea in T1 animal could be as a result of increase in the activities of uric enzymes ornithine carbonoyle transferase and arginase. The low blood urea observed in the test animals are indication that the amino acids of *M. oleifera* are balanced, since high blood urea levels are associated with poor protein quality [25] or excess tissue catabolism associated with protein deficiency [26]. The result of this study is in agreement with the findings of [25] who observed that an amino acid imbalance would result in an increase in blood urea concentration. This perhaps highlights the high quality protein of *M. oleifera* as reported by [6].

4. CONCLUSION

From physiological point, blood profiles of rabbits in all treatment groups are within normal range for rabbit; an indication that the test ingredient enhanced feed quality and inadvertently the nutritional and health status of rabbits.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This paper followed all the guidelines for the care and use of laboratory animal model of the Federal College of Agriculture, Ishiagu, Ebonyi State, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Belewu MA. Ogunsola Fo Haematological And Serum Indices of Goat Fed Fungi Treated *Jatropha curcas* Kernel Cake in A Mixed Ration. Journal of Agricultural Biotechnology and Sustainable Development. 2010; 2(3): 35 – 38.
2. Onu PN, Aniebo AO. Influence of *Moringa oleifera* leaf meal on the performance and blood chemistry of starter broilers. International Journal of Food, Agriculture and Veterinary Sciences. 2011;1(1):38-44.
3. Makkar HPS, Becker K. Nutritional value and anti-nutritional components of whole and ethanol extracted *Moringa oleifera* leaves. Animal Feed Science and Technology. 1996;63:211-228.
4. Fahey JW. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Trees for Life Journal a forum on beneficial trees and plants; 2005. Available:www.TFLJournal.org/article.php/20051201124931586
5. Jiwuba PC, Okechukwu SO, Onyekwere MU, Olabode AD, Ugwu DO, Ogbuewu PI, Ahamefule FO. Performance of West African Dwarf (WAD) goats fed *Moringa oleifera* leaf meal; in proceeding of 41st annual conference for Nigeria Society for Animal Production (NSAP). 2016;711-714.
6. Foidl N, Paull R. *Moringa oleifera*. In: The encyclopedia of fruit and nuts. CABI, Oxfordshire, UK. 2008;509–512.
7. Murro JK, Muhikambele VRM, Sarwatt SV. *Moringa oleifera* leaf meal can replace cottonseed cake in the concentrate mix fed with Rhodes grass (*Chloris gayana*) hay for growing sheep. Livestock Research for Rural Development. 2002;15:11.
8. AOAC. Association of official analytical chemists: Official methods of analysis. 6th Edition. Washington DC, USA; 2000.
9. El-Badawi AY, Omer HAA, Abedo AA, Yacout MHM. Response of growing New Zealand white rabbits to rations supplemented with different levels of *Moringa oleifera* dry leaves. Global Veterinaria. 2014;12(4):573-582.
10. Lebas F. Role of dietary fibre in rabbit nutrition and in digestive troubles prevention. 2d Rabbit Congress of the Americas, Habana City, Cuba, June 19-22; 2002.
11. Njidda AA, Isidahomen CE. Haematology, blood chemistry and carcass

characteristics of growing rabbits fed grasshopper meal as a substitute for fish meal. Pakistan Vet. J. 2010;30(1):7-12.
12. Aye PA, Adegun MK. Chemical composition and some functional properties of Moringa, Leucaena and Gliricidia leaf meals. Agriculture and biology journal of North America. 2013; 2151-7517.
13. Fadiyimu AA, Alokan JA, Fajemisin AN. Digestibility, nitrogen balance and haematological profile of West African dwarf sheep fed dietary levels of *Moringa oleifera* as supplement to *Panicum maximum*. Journal of American Sci. 2010; 6(10):634 -643.
14. Onifade AA, Tewe OO. Alternative tropical energy feed performance in rabbit diets: Growth performance, diets digestibility and blood composition. World Rabbits Sci. 1993;1:17–24.
15. Ahamefule FO, Ibeawuchi JA, Okoye FC. Blood biochemistry and haematology of West African Dwarf (WAD) bucks fed pigeon pea-cassava peel based diets. Journal Anim Vet Adv. 2005;4(12):1016-1020.
16. Ugwuene MC. Effect of dietary palm kernel meal for maize on the haematological and serum chemistry of broiler turkey. Nigerian Journal of Animal Science. 2011;13:93-103.
17. Jiwuba PC, Ugwu DO, Onyekwere MU, Ogbuewu PI, Okechukwu SO, Olabode AD. Haematological response of waener rabbits fed dietary levels of *Gmelina arborea* leaf meal; in Proceeding of 41st Annual Conference for Nigeria Society for Animal Production (NSAP). 2016;129-132.
18. Research Animal Resource (RAR). Reference values for laboratory animals: Normal haemotological values. RAR websites, RAR, University of Minnesota; 2009. Available:http://www.ahe.umn.edu.rar.refvalues.html
19. Jiwuba PC. Performance blood profile and apparent nutrient digestibility of West African Dwarf Does fed dietary levels of *Moringa oleifera* leaf meal. M.Sc. Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria; 2014.
20. Burke J. Clinical care and medicine of pet rabbit, in: Proceedings of the Michigan Veterinary Conference. 1994;49-77.
21. Annon. Guide to care and use of experimental animal. Canadian Council of Animal Care. Ottowa Ontario, Canada. 1980;1:185-190.
22. Apata DF. Biochemical, nutritional and toxicological assessment of some tropical legume seeds. PhD Thesis, University of Ibadan, Nigeria; 1990.
23. Ghasi S, Nwobodo E, Ofili JO. Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high fat diet fed wistar rats. Journal of Ethnopharmacology. 2000; 69(1):21-25.
24. Jenkins JR. Rabbits in: Jenkins, J. R and Brown, S.A (ed) practioner"s guide to Rabbits and ferrets American Animal Hospital Association lake wood, U.S.A. 1993;1-42.
25. Eggun BO. Blood Urea - measurement as a technique for assessing protein quality. British Journal of Nutrition. 1970;24:985-988.
26. Oduye OO, Adadevoh BK. Biochemical values in apparently normal Nigerian goats. J. Nig. Vet. Med. Assoc. 1976;5:51-55.

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