

**EFFECT OF DIETARY SUPPLEMENTATION
OF *MORINGA OLEIFERA* LEAF MEAL ON BLOOD
PROFILE, HAEMAGGLUTINATION POTENTIAL
AND TESTICULAR ACTIVITIES OF RABBITS**

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Abstract

Forty-eight unsexed weaner rabbits of mixed breeds, with initial live weight of 710–780 g were randomly allocated into four experimental dietary treatments (0%, 15%, 30% and 45% *Moringa oleifera* leaf meal (MOLM)) and replicated three times with four rabbits per replicate for ten weeks. Data were collected on blood profile (haematology: packed cell volume – PCV, red blood cell counts – RBC, white blood cell counts – WBC, haemoglobin – Hb, lymphocytes, mean corpuscular and haemoglobin concentration – MCHC; serum biochemical indices: total serum protein, albumin, globulin, glucose, cholesterol, alkaline phosphate – ALP and aspartate transferase – AST) and testicular activities while haemagglutination assay was also carried out. All data generated were subjected to one-way analysis of variance in a completely randomized design. White blood cell concentration, alkaline phosphatase, aspartate aminotransferase and glucose were significantly ($p < 0.05$) influenced by MOLM. Highest titre was obtained at 45% MOLM while spermatogenic activities were supported by Moringa supplementation at 15% and 30% inclusion level. The study concluded that supplementation of MOLM in the diets of weaner rabbits up to 45% boost the immune systems of the rabbits as shown by increased in haemagglutination titre and normal range of blood values but decreased testicular activities.

Introduction

Livestock producers are facing much difficulty with availability and higher prices of feed ingredients (KHATUN et al. 2003). Feeding ingredients are getting expensive and scarce due to high competition between human and animals resulting in the escalating cost of these ingredients. The cost of feed constitutes the major proportion of between 60–75% of the total cost of livestock production and protein cost account for over 15% of the total feed cost in livestock farming (BHUIYAN 1998, AWONIYI et al. 2004, OJEWOLA et al. 2005). The price of conventional protein feeds resources such as groundnut cake, fish meal and soybean meal, is on the high side and cannot permit profit maximization in livestock ventures. In view of this, current research interest in the livestock industry is aimed at finding alternatives to this elusive feed ingredient. One of such unconventional animal feed ingredient worth considering is moringa leaves. *Moringa oleifera* leaf meal (MOLM) is promising as a food source in the tropics because the tree is covered with green leaves during the dry season when other foods are scarce (MELESSE et al. 2009). However, ODURO et al. (2008) gave details of the composition of this plant to be very rich in crude protein among others. There is no doubt that it can be used as rabbit feed.

The production of rabbits imposes no competition directly with man for most conventional cereals and legumes. It has ability to utilize diverse forages and is also favoured because of its high fecundity, short generation interval, as well as low cost of investment (TAIWO et al. 2004). However, it is imperative to give consideration to the health status of the animals subjected to this feeding trial; this is because the overall deficiency of a feed can be determined by examining the haematological component of the blood and this would provide a way to prevent nutrient deficient feed to farm animals. ISAAC et al. (2013) stated that haematological components are valuable in monitoring feed toxicity, especially with feed constituents that affect the blood as well as the health status of the animal. Serum protein had been used as an indirect measure of protein utilization. TERZUN-GWE et al. (2013) reported that MOLM can be included in weaner rabbits' diets at up to 15% dietary level without adverse effect on the haematological and serum biochemical indices of rabbits EWUOLA et al. (2012) reported that the chemical composition of MOLM contains 27.53% crude protein – CP, 9.93% ether extract – EE, 14.05% crude fibre – CF, 7.98% ash and 40.51% nitrogen free extract – NFE. It was also documented to have multiple antioxidants with high levels such as phenolic acids (ellagic, chlorogenic, gallic and ferulic acid), glucosinolate and flavonoids such as kaempferol, quercetin and rutin (MBIKAY 2012). Hence, this study was carried

out with the aim of evaluating the effect of MOLM at higher inclusion levels on the haematological, biochemical indices, haemagglutination potential of erythrocytes and testicular activities of weaner rabbits.

Materials and Methods

The experiment was carried out at the Directorate of University Farms, Federal University of Agriculture, Abeokuta. It is located in 76 m above sea level and falls within Latitude 7°N and Longitudes 3°E. The climate is humid and located in the forest zone of South Western Nigeria. The mean precipitation and the temperature are 1,112.7 mm and 23.5°C respectively. Relative humidity averaged 81.5% throughout the year. Seasonal distribution of rainfall is approximately 110.9 mm (9.97%) in the late dry season (January – March), 462.1 mm (41.53%) in the early wet season (April – June), 376.6 mm (33.85) in the late wet season (July – September) and 163.1 mm (14.66%) in the early dry season (October – December) (ORBDA 2011).

The study protocol was approved and conducted in line with the Animal Ethics Committee guidelines of Federal University of Agriculture, Abeokuta, Nigeria (FUNAAB 2013). The experiment was carried out using 48 weaner rabbits, and these were divided into 4 dietary treatments having 12 rabbits per treatment. Each treatment was replicated three times containing 4 rabbits per replicate. The experiment was carried out for a period of 10 weeks. Weaner rabbits, weighing between 710–780 grams were procured from a reliable farm in Abeokuta metropolis. The rabbits were fed with experimental diet after a week of adaptation supplemented with forages. Fresh water was provided for them in the morning and late in the evening. All other routine management such as disinfection of the hutches and stable, cleaning of feeders and drinkers, etc. were observed.

Moringa oleifera leaves were harvested by hand-plucking the leaves from the trees within Abeokuta metropolis. The leaves were air-dried and milled to form leaf meal while other feed ingredients were sourced from a reputable feed mill. After this, experimental diets were formulated such that MOLM were included at 0% (control), 15%, 30% and 45% levels as a replacement of soybean for diets T_1 , T_2 , T_3 and T_4 respectively as shown on Table 1.

Table 1

Percentage composition of experimental diets

Specification	T_1	T_2	T_3	T_4
Level of inclusion of MOLM [%]				
Ingredients	0	15	30	45
Maize	39.00	39.00	39.00	39.00
Rice bran	20.00	20.00	20.00	20.00
MOLM	0.00	1.50	3.00	4.50
Soya bean meal	10.0	8.50	7.00	5.50
GNC	10.0	10.00	10.00	10.00
PKC	10.0	10.00	10.00	10.00
Wheat offal	4.80	4.80	4.80	4.80
Bone meal	3.00	3.00	3.00	3.00
Oyster shell	2.00	2.00	2.00	2.00
Salt	0.30	0.30	0.30	0.30
Vit. prix	0.30	0.30	0.30	0.30
Lysine	0.30	0.30	0.30	0.30
Methionine	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00
Calculated analysis				
ME [MJ/kg]	2507	2533	2559	2585
Crude protein [%]	17.60	17.35	17.10	16.86
Fibre [%]	12.19	12.36	12.54	12.72

At the 10th week of the experiment, 2.5 ml of blood sample was collected from 3 rabbits per treatment into ethylene diamine tetra acetic acid (EDTA) bottle for haematological parameters which include PCV, RBC, WBC, Hb and were determined as described by JAIN (1986). The procedure of CAMPBELL (2012) was used in calculating the mean corpuscular volume, mean corpuscular Hb and mean corpuscular Hb concentration. For the determination of biochemical constituents of the blood, plain bottle was used for blood sample collection. Total serum protein was determined using the Biuret method as described by KOHN and ALLEN (1995). Albumin was determined using Bromocresol Green (BCG) method while globulin was estimated by subtracting albumin values from total protein. Serum cholesterol was determined spectrophotometrically using commercial Bio-La-Tests, and serum glucose was estimated using a commercial glucose colorimetric assay kit.

Haemolymph was collected from the mantle (oxygenated) cavity region of the snail (*Archachatina marginata*), and temporarily stored in universal bottle before the commencement of haemagglutination assay for agglutinin evaluation.

Three millilitres of blood was collected aseptically from 3 rabbits per treatment into EDTA bottles. Blood was centrifuged at 900 g for 5 minutes to harvest erythrocytes. Erythrocytes were washed three times in phosphate buffer saline (PBS), diluted to 2%v/v and stored at 4°C.

Serial dilution of haemolymph was prepared using 0.85% Phosphate Buffer Saline (PBS – pH 7.2). Diluted haemolymph was aliquoted into wells of microtitre plates at 100 µl per well. Equal volumes of rabbits' red-blood cells suspension were then added. The plates were covered, mixed gently and incubated at 30, 60, 90, 120, 150 and 180 minutes at room temperature to determine the reaction time, after which titre values were recorded. Each test consisted of eight replicates. Red-blood cell in PBS served as the control.

At the end of ten weeks, testes were harvested from three bucks that were randomly selected per treatment for morphometric analysis and histology. The length and weight of each testis were measured with vernier caliper and electronic scale, respectively. The testes were then fixed in 10% formalin, dehydrated in series of alcohol (70%, 90%, 100%), cleared in xylene, embedded in paraffin wax after which the tissue were sectioned (5 µm) and stained with haematoxylin and eosin (HE).

Data generated were subjected to least square analysis of variance in a completely randomized design using the statistical package (SAS 2007) while the significant means among treatments were separated using Duncan Multiple Range Test of the statistical package.

$$Y_{ij} = \mu + M_i + \sum_{ij}$$

where:

Y_{ij} – dependent variables

μ – population mean

M_i – Effect of i^{th} concentrate with MOLM supplementation at different levels ($j = 0\%$, 15%, 30%, 45%)

\sum_{ij} – residual error.

Results

The effect of MOLM on the haematological parameters of weaner rabbits is presented in Table 2. No significant effect ($p > 0.05$) was found in all the parameters measured except WBC which was significantly ($p < 0.05$) influenced by the experimental diets. The WBC values at 0% and 30% inclusion levels of MOLM were statistically the same while 15% MOLM recorded the least value.

Table 2
Haematological parameters of weaner rabbits fed *Moringa oleifera* leaf meal diets

Parameter	0%	15%	30%	45%	SEM
Pack Cell Volume [%]	40.33	36.00	40.00	31.33	3.09
Haemoglobin [g/dl]	13.43	12.00	13.33	10.43	1.03
Red Blood Cell ($\cdot 10^{12}/L$)	3.63	3.16	3.60	2.82	0.26
MCH [pg]	37.03	37.16	37.01	36.99	0.10
MCHC [g/dl]	33.30	33.32	33.34	33.30	0.41
MCV [fL]	11.12	11.35	11.10	11.11	0.09
White Blood Cell [$\cdot 10^3/l$]	7.33 ^a	4.27 ^b	6.53 ^a	5.40 ^{ab}	0.65
Neutrophil [%]	41.33	42.00	38.67	43.00	5.19
Lymphocyte [%]	55.00	54.33	58.00	53.67	5.47
Eosinophil [%]	3.33	3.00	3.00	2.67	0.24
Monocytes [%]	0.67	0.67	1.00	0.67	0.29
Basophil [%]	0.00	0.00	0.00	0.00	0.00

Explanation: ^{a,b} means with different superscripts along the same row are significantly ($p < 0.05$) different; MCH – corpuscular haemoglobin; MCHC – corpuscular haemoglobin concentration, MCV – corpuscular volume

The result obtained on the effect of MOLM on the serum component of rabbit is shown on Table 3 . Significant effect of diet was not observed on all the parameters studied except serum glucose, AST and ALP. The values of ALP were significantly ($p < 0.05$) increased in rabbits fed 0%, 30% and 45% dietary supplementation of MOLM but decreased in those fed diet containing 15% dietary supplementation of MOLM.

Table 3
Serum biochemical indices of Weaner Rabbits fed *Moringa oleifera* leaf meal diets

Level of MOLM	0%	15%	30%	45%	SEM
Parameter					
Total protein [g/dl]	6.20	6.40	6.50	6.63	0.45
Albumin [g/dl]	3.37	3.87	3.93	4.07	0.20
Globulin [g/dl]	2.83	2.53	2.67	2.67	0.35
Alakaline phosphate [U/L]	143.00 ^a	102.33 ^b	141.33 ^a	140.67 ^a	10.63
Alkaline aminotransefrase [U/L]	55.33	55.33	74.00	70.67	6.78
Aspartate aminotransferase [U/L]	41.67 ^b	23.00 ^c	43.00 ^b	66.67 ^a	4.54
Cholesterol [Mg/dl]	77.87	84.17	82.03	84.33	7.07
Glucose [Mg/dl]	129.73 ^a	99.17 ^b	78.37 ^b	81.20 ^b	6.51

Explanation: ^{a, b, c;} means with different superscripts along the same row are significantly ($p < 0.05$) different

The haemagglutination potential of *Moringa oleifera* leaf meal on the erythrocytes of the experimental animals is shown on Table 4. The result shows that the best haemagglutination titre was obtained at 45% level of MOLM inclusion while the titre values were statistically similar at 0% and 15% MOLM levels of inclusion.

Table 4
Haemagglutination potential of *Moringa oleifera* leaf meal on erythrocytes of weaner rabbits

Treatment	Titre
T_1 (0%)	8.50±0.290 ^b
T_2 (15%)	8.67±0.290 ^b
T_3 (30%)	9.33±0.290 ^{ab}
T_4 (45%)	9.83±0.290 ^a

Table 5 shows the effect of timing on haemagglutination titre. At reaction time of 30, 60 and 90 minutes, haemagglutination titres were not significantly ($p > 0.05$) different for the four levels of inclusions of MOLM. However, there was significant ($p < 0.05$) variation observed at 120, 150 and 180 minutes with 45% inclusion level of MOLM recording the highest haemagglutination titre (9.00±0.204) at 180 minutes.

Table 5
Effect of timing on haemagglutination titre of erythrocytes of weaner rabbits fed diets containing graded levels of *Moringa oleifera* leaf meal

Reaction time (minute)	Treatment (titre)			
	T_1 (0%)	T_2 (15%)	T_3 (30%)	T_4 (45%)
30	10.00± 0.204	10.00±0.204	10.00±0.204	10.00±0.204
60	10.00±0.204	10.00±0.204	10.00±0.204	10.00 ±0.204
90	10.00±0.204	10.00±0.204	10.00±0.204	10.00±0.204
120	10.00±0.204 ^a	9.00±0.204 ^b	10.00±0.204 ^a	10.00±0.204 ^a
150	6.00±0.204 ^c	9.00±0.204 ^b	10.00±0.204 ^a	10.00±0.204 ^a
180	5.00±0.204 ^c	4.00±0.204 ^d	6.00±0.204 ^b	9.00±0.204 ^a

Explanation: *a, b, c, d* means along the same row with different superscript differs significantly ($p < 0.05$)

Table 6 shows the result obtained on the effect of MOLM on the testicular and epididymal weight of rabbit. Significant effect of diet was only observed in T_3 (30% MOLM) where the weight of the testis and epididymis was significantly reduced.

Table 6

Least-square means of testicular weight of weaner rabbits fed diets containing graded levels of *Moringa oleifera* leaf meal

Treatment	Testis + Epididymis [g]	
	left	right
T_1 (0%)	2.385±0.87 ^a	2.310±0.523
T_2 (15%)	2.100±0.87 ^a	1.925±0.523
T_3 (30%)	0.890±0.87 ^b	1.495±0.523
T_4 (45%)	2.170±0.87 ^a	2.305±0.523

Explanation: ^a, ^b means on the same column with different superscript differs significantly ($p < 0.05$)

HE stained sections of testes tissue of rabbit bucks fed dietary supplementation of MOLM at 0%, 15%, 30% and 45% (T_1 , T_2 , T_3 and T_4 , respectively) showing the histological appearance are shown in Figure 1.

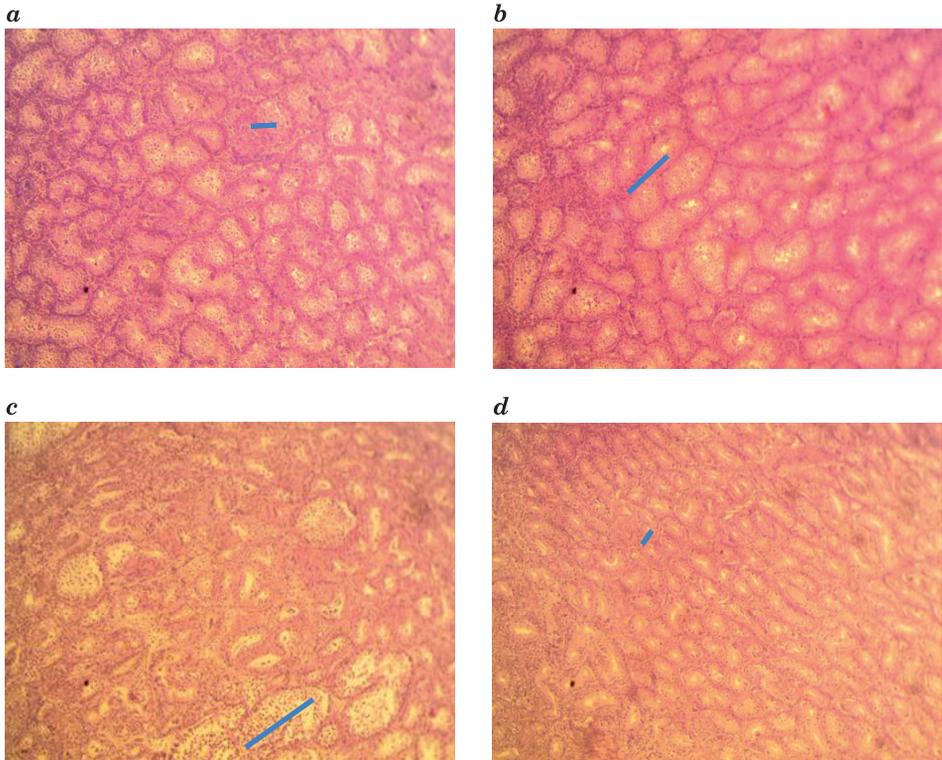


Fig. 1. Photomicrographs of testes tissue sections of rabbit bucks: *a* – T_1 : control rabbit showing normal testes architecture; *b* – T_2 : treated with 15% *Moringa oleifera* leaf meal; *c* – T_3 : treated with 30% *Moringa oleifera* leaf meal; *d* – T_4 : treated with 45% *Moringa oleifera* leaf meal. Magn. × 400

Discussion

Some of the haematological parameters measured in the present experiment were within the normal physiological ranges reported for rabbits most especially haemoglobin, MCH, MCV, MCHC, neutrophils, lymphocytes and eosinophils. BAKER and SILVERTON (1982) reported that a measure of the relative mass of blood is referred to as Packed cell volume (PCV). The observed PCV of rabbits which ranged from 31.33–40.33% in this study were within the normal range reported by MEDIRABBIT (2011) who considered the normal PCV of a healthy rabbit to be between 30–50%. These values suggested adequate nutritional status of the rabbits (CHURCH et al. 1984). This result is also in agreement with the findings of OKEKE et al. (2009) who observed no significant ($p > 0.05$) effect of feeding *Moringa oleifera* leaf meal in the diet on PCV of Rabbit. The values of RBC obtained in this study were in line with report of OKEKE et al. (2009), who found no significant influence of diet on RBC of rabbit fed *Moringa oleifera* leaf meal and reported lower values ($2.82\text{--}3.63 \cdot 10^6 \text{ mm}^3$). The values of haemoglobin, MCV and MCHC obtained suggest that the animals responded positively to the test diet. The lower ($p < 0.05$) value of WBC observed in 15% MOLM ($4.27 \cdot 10^3/l$) shows that these animals were predisposed to high risk of disease infection; however, the immunity levels of the rabbits were not challenged as the values still fell within the recommended normal range reported by MITRUKA and RAWNSLEY (1977). This is however contrary to the report of TERZUNGWE et al. (2013) who reported no significant mean value in WBC of rabbits fed 15% moringa leaf meal diet.

The total protein values obtained fall within the normal range (6.00–8.30 g/dl) recommended by MITRUKA and RAWNSLEY (1977). Since total protein, albumin and globulin are generally influenced by the quality and quantity of protein intake (ONIFADE and TEWE 1993), the values obtained in the study indicates nutritional adequacy of the dietary protein. Rabbits fed the control diet had higher ($p < 0.05$) mean serum glucose level than those on the test diets. The reduced serum glucose values in the test diets gave an indication that the control will supply more energy for the animals than the test diets. It is also promising that moringa inclusion into the diets may decrease excess glucose thus preventing the animal of being diabetic. The serum ALP value of the rabbits fed T_2 was significantly ($p < 0.05$) lower than the mean serum ALP of those fed the control diet (T_1), which was significantly ($p > 0.05$) similar to the serum ALP of those on T_3 and T_4 , while the serum AST activities of the rabbits fed the T_1 and T_3 were significantly ($p < 0.05$) lower than those on T_4 but significantly higher than those on T_2 . CHAMPE et al. (2007) stated that aminotransferases are normally intracel-

lular enzymes, with low levels found in the plasma representing the release of cellular contents during normal cell turnover. The serum ALT and AST levels, according to CHAMPE et al. (2007) are elevated in nearly all liver diseases and are particularly high in conditions that cause extensive cell necrosis, including severe viral hepatitis or toxic liver injury.

The result on haemagglutination potential of *Moringa oleifera* showed that the highest haemagglutination titre was at 45% MOLM inclusion, which indicated that this inclusion level boost the immune system best. This result corroborates the findings of ABIONA et al. (2012) which reported higher haemagglutination titre for layer birds raised on legumes compared to concentrate feeding. Haemagglutination titres were not significantly ($p > 0.05$) different for the four levels of inclusions of MOLM; however, the significant ($p < 0.05$) variation observed at 120, 150 and 180 minutes with 45% inclusion level of MOLM recording the highest haemagglutination titre (9.00 ± 0.204) at 180 minutes is an indication that inclusion at this level modulates the immune function most. This observation is in line with the work of NUHU (2010), where MOLM was used to achieve similar result at 25% level of inclusion in rabbit's feed. The reason for this observation may be as a result of over-reactivity at these levels. On the overall basis, immune boosting effect of *Moringa oleifera* may be as a result of the presence of lectins which are found mostly in legumes.

The significant reduction observed in the weight of the testis and epididymis at 30% inclusion level of MOLM is contrary to the works of ABIONA et al. (2012) and NUHU (2010) who reported insignificant effect of MOLM on the morphometry performances of the male reproductive organs in the diets fed to African giant snails and rabbits respectively.

Figure 1 shows the photomicrographs of testis tissue sections of rabbit bucks fed different levels of MOLM. Considering the testicular architecture, seminiferous epitheliums were seen to be intact in treatments 1, 2, and 3. Tubular diameter of the seminiferous tubule were also seen to consistently increase in treatments 2 and 3, but decreased in treatment 4. This observation is an indication that spermatogenic activities were supported by *Moringa* inclusion at 15% and 30% inclusion level since spermatozoa produced are flushed via the lumen. Decreased in seminiferous diameter is an indication of arrest of spermatogenesis as shown by 45% supplementation of MOLM. This further showed this amount is in excess and could affect the normal spermatogenesis. The supportive role of this plant may be attributed to the fact that it contains some substances that could stimulate testicular androgenesis that are responsible for testicular differentiation, integrity and steroidogenic functions (DAWSON et al. 1990, LUCK 1995, EL-MISSIRY 1999, GHOSH et al. 2002, KUJO 2004).

Conclusion

The supplementation of *Moringa oleifera* leaf meal in the diets of weaner rabbits up to 45% boost the immune systems of the rabbits as shown by increased in haemagglutination titre and normal range of blood values but decreased testicular activities.

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