

Physiological response of rabbit does fed diets supplemented with *Moringa oleifera* leaf meal

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Target Audience: Rabbit farmers; Animal Scientist; Researchers

Abstract

An experiment was conducted to assess the effect of *Moringa oleifera* leaf meal (MoLM) on haematological and biochemical response of gestating does in a 168-days feeding trial. The rabbits were fed varied levels of MoLM at 0, 2.5, 5.0 and 7.5 % to obtain four diets. A total of 40 rabbit does aged 7-8 weeks old were allotted to four treatments in a completely randomized design with 10 replicates per treatment. Blood was sampled from the rabbits during the third trimester of the gestation period (23 weeks into the experiment) for haematological and serum biochemical analyses. Results showed that there were significant ($p < 0.05$) differences in the values obtained for Packed Cell Volume (PCV), haemoglobin (Hb) and erythrocytes in the gestating does among the treatments. Leucocyte counts were not significantly influenced by dietary treatments. The rabbits does fed diet 2 (2.5% MoLM) had the highest PCV (40.2%) and Hb (13.0 g/100mL) and erythrocyte ($6.79 \times 10^6/l$) which reduced in does fed 5.0 and 7.5 % MoLM dietary levels. The serum biochemical parameters assessed in the gestating does fed MoLM-based diets were not significantly different from those fed the control diet. Alanine amino Transferase was significantly ($p < 0.05$) influenced by dietary treatment in the does with the highest mean value recorded in does fed 7.5% MoLM diet (48.2 IU/L) compared to those fed diet 1 with 0% MoLM (19.2 IU/L). Findings from this study suggested that supplemental levels of MoLM up to 7.5% inclusion in rabbit diet may not have adverse effect on the haematology and biochemical response of the gestating does but liver damage may result from prolonged dietary supplementation.

Keywords: Haematology; Serum enzymes; Gestation; Diet Supplementation

Description of Problem

Rabbits can be considered as one of the several livestock species suitable for meat production. They are prolific and provide meat with high nutritive value which can provide an excellent economic source of animal protein for human consumption. Poor body condition and impaired health status are among the main factors that affect the reproductive performance of rabbit does (1). The practice of ceacotrophy in rabbits enhances its utilization of forages and legumes thus improving its

overall performance (2). There is a need to explore available feed resources such as leaf meals of tropical trees, readily available in our environment to feed livestock since most of the leaf meals are rich in micronutrients that can influence growth and development.

Moringa oleifera commonly called drumstick tree or horseradish tree, has been reported to have high nutritional, medicinal and therapeutic qualities (3). The potential of different morphological parts of *Moringa oleifera* tree as animal feed has been evaluated

(4), it has generally been used as a protein source for livestock and the leaves contain highly beneficial phytochemicals such as steroids, tocopherols and carotenoids (5,6). Hence, it can be used as a dietary supplement to boost performance of rabbit does. Feeding, stress and pregnancy are among several factors that can influence haematological and biochemical indices (7, 8). The influence of feed components on these indices cannot be underestimated as changes occur in the body during the various physiological phases. Haematological studies are important health status indicators because the blood is the major transport system of the body and evaluations of the haematological indices usually furnishes vital information on the body's response to injury of all forms, including toxic injury (9). Serum biochemical analysis is used to determine the level of heart, liver and kidney damages as well as to evaluate protein quality and amino acid levels in animals. Therefore, this study was conducted to evaluate the effect of dietary supplemental levels of *Moringa oleifera* leaf meal on the haematology and serum biochemistry of gestating rabbits does.

Materials and Methods

The study was conducted at the rabbitry unit of the Teaching and Research farm, University of Ibadan located at latitude 7°27'0N and longitude 3°54'0E, 200 – 300 meters above sea level. 40 rabbits does (New Zealand white × Chinchilla) aged 7-8 weeks old were used for the experiment with 10 does per group. The does were housed individually in wooden cages that were raised from the floor. The feeding trial lasted for 24 weeks after two weeks of acclimatization. Feed and water was provided *ad-libitum* throughout the experiment.

The *Moringa oleifera* leaf used for this experiment was harvested from an established orchard within Ibadan metropolis of Oyo State. The *Moringa oleifera* leaves were air-dried

under shade for 3- 5 days until it was crispy to touch while retaining its greenish colouration. The leaves was milled and stored in air tight containers until incorporation into the diet. The concentrate feed ingredient used for the experiment was purchased from a commercial feed supplier in Ibadan, Nigeria. The control diet was formulated and subjected to proximate analysis (Table 1). Four experimental diets were prepared, as T0 served as the control diet containing no *Moringa oleifera* leaf meal. Diets T2.5, T5.0 and T7.5 contained *Moringa oleifera* leaf meal at 2.5, 5.0 and 7.5 % inclusion levels respectively in replacement of the equivalent proportion of the control diet as presented below:

T0: 100% Control diet + 0% MoLM (Control)

T2.5: 97.5% Control diet + 2.5% MoLM

T5.0: 95.0% Control diet + 5.0% MoLM

T7.5: 92.5% Control diet + 7.5% MoLM

Blood was sampled from the rabbits does during the third trimester of gestation for haematological, serum biochemical analysis, Alanine Amino transferase and Aspartate amino transferase activities. Five (5) mls of blood was taken from the jugular vein of the rabbits into a set of sterile plastic bottles containing EDTA for haematological test and another into EDTA-free bottles which was allowed to clot for the separation of serum from the clot by decantation for serum biochemical tests. The blood sample containing the EDTA was mixed gently and drawn up into a micro-haematocrit capillary tube filled up to 2/3 of its length. The samples were centrifuged for 5 minutes at 10,000 rpm and the Packed Cell Volume (PCV) was read as a percentage using the haematocrit reader. The erythrocyte, the total leukocyte, the differential leukocyte counts, haemoglobin (Hb) concentrations total protein, albumin and

globulin were determined using methods described by (10). The serum samples were also assessed for serum cholesterol, glucose, urea and Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) which were evaluated using commercial test kit (Randox laboratories Ltd., UK) specified for each variable.

All data obtained were subjected to statistical analysis of variance (ANOVA) procedure using Statistical Analytical System (11). Treatment means were compared using Duncan multiple range test of the same software.

Results and Discussion

Haematological response of gestating does fed varied levels of *Moringa oleifera* Leaf Meal Table 2 shows the haematological variables of gestating rabbits fed supplemental levels of *Moringa oleifera* leaf meal. The

haematological parameter of gestating does fed *Moringa oleifera* leaf meal varied with the inclusion levels. The PCV, Hb and erythrocytes count in the gestating does were significantly ($p < 0.05$) influenced by the dietary treatments. The PCV of rabbits fed 2.5% MoLM was not significantly different from that of rabbits fed 0 and 5 % MoLM but significantly higher (40.2%) than that rabbits on 7.5 % MoLM (33.7%). The Hb concentration of rabbit on diets T0, T2.5 and T5.0 were statistically similar but significantly ($p < 0.05$) higher than that of rabbits fed diet T7.5. The erythrocytes count of does fed diets T0 and T2.5 were not significantly different from each other but significantly ($p < 0.05$) higher than that of rabbits fed diet T5.0 and T7.5. The leukocyte, platelet, neutrophils, lymphocytes, eosinophils and monocytes were not significantly different among the treatments.

Table 1: Proximate Composition of *Moringa oleifera* Leaf Meal and Experimental diets

NUTRIENTS	MoLM	T0	T2.5	T5.0	T7.5
Dry Matter (%)	88.00	90.50	90.44	90.38	90.31
Crude Fibre (%)	11.00	13.65	13.56	13.52	13.36
Ether Extract (%)	13.00	5.00	5.23	5.41	5.76
Crude Protein (%)	27.92	16.80	17.08	17.36	17.63
Ash (%)	11.50	10.00	10.04	10.08	10.11
Nitrogen Free Extract (%)	24.56	45.05	44.53	44.01	43.45

MoLM – *Moringa oleifera* Leaf Meal

Table 2: Haematological response of gestating does fed varied levels of *Moringa oleifera* Leaf Meal

PARAMETERS	T0	T2.5	T5.0	T7.5
Packed Cell Volume (%)	37.38±0.79 ^{ab}	40.17±1.64 ^a	37.00±1.00 ^{ab}	33.67±1.61 ^b
Haemoglobin (g/100ml)	12.35±0.31 ^a	13.00±0.45 ^a	11.84±0.35 ^a	10.57±0.51 ^b
Erythrocytes (x 10 ⁶ /l)	6.43±0.25 ^{ab}	6.79±0.16 ^a	6.08±0.16 ^{bc}	5.71±0.27 ^c
Leukocytes(x 10 ³ /l)	4.34±0.41	5.01±0.45	5.36±0.50	4.93±0.71
Thrombocytes(x10 ⁴ /l)	6.75±0.55	7.45±0.80	8.96±0.11	8.18±0.65
Neutrophils (%)	20.50±1.69	22.33±3.17	18.80±2.76	20.67±2.50
Lymphocytes (%)	69.33±1.61	69.33±3.06	70.60±2.58	70.50±1.43
Eosinophils (%)	6.33±0.80	6.67±1.41	8.60±1.99	6.67±1.48
Monocytes (%)	2.33±0.49	1.67±0.42	2.00±0.44	2.00±0.58

a, b, c - Means in the same row with different superscript are significantly different ($P < 0.05$)

It has been established that feed components and physiological state of an animal affects its blood constituents. Decreased value of haematological and serum biochemical parameters in pregnant does compared to non-pregnant have been reported (8). Also, dietary components of *M. oleifera* were reported to have measurable effect on blood constituents (12). The gestating does fed 2.5% MoLM had the highest PCV (40.2%), Hb (13.0 g/100mL) and erythrocyte ($6.79 \times 10^6/l$) while a reduced level of the parameters were recorded those fed 5.0 and 7.5 % MoLM. However, the values obtained for these parameters (PCV, Hb and erythrocytes) on all treatments in this study were within the reported physiological range for normal rabbits (13). This is an indication that there was efficient erythrocyte production in terms of the volume of red blood cells, its pigmentation and concentration. Since haemoglobin and PCV measurements can be used as an assay of nutritional anaemia, it can be interpreted that the dietary treatment did not cause any nutritional anaemia and can supply the needed nutrients for efficient reproduction to the gestating does rabbits.

The leukocyte, eosinophils and monocytes were not significantly different among the treatments. The leukocyte count was higher in some does fed MoLM diets but was not significantly different among the treatments. In this study, there was no record of abnormal rise leukocyte counts. The gestating does fed 5.0% MoLM had higher leucocyte count ($5.36 \times 10^3/l$) with the least value ($4.34 \times 10^3/l$) on the control. The platelet, neutrophils, lymphocytes, eosinophils and monocytes were not significantly different

among the treatments. The non-significant difference observed in some of the haematological parameters is an indication of efficient utilization of MoLM by the rabbits thus implies that the immunity level of the rabbits was not challenged.

Serum biochemical response of gestating does fed diets with varied inclusion levels of *Moringa oleifera* Leaf Meal

The biochemical parameters and enzyme activities of gestating does fed varied levels of *Moringa oleifera* leaf meal are shown in Table 3 and 4. All the serum parameters assessed were not significantly influenced by dietary treatments. Serum glucose apparently decreased at 2.5% level of MoLM inclusion in the diet but was not significantly different among the treatments. The serum cholesterol was apparently lower in rabbits fed diets T2.5, T5.0 and T7.5 (21.9, 35.3, 24.6 mg/dL respectively) compared to those fed diet 1 (37.7 mg/dL) but the mean values were not significantly different among the treatments. Total serum protein, albumin and globulin were not significantly different among the treatment. Alanine amino transferase (ALT) of the rabbits were significantly ($p < 0.05$) influenced by the inclusion levels of MoLM. ALT values obtained in rabbits fed 2.5, 5 and 7.5 % MoLM based diets were significantly ($P < 0.05$) higher (38.7 - 48.2 IU/L) than those fed 0% MoLM diet (19.2 IU/L). Aspartate amino transferase (AST) were not significantly influenced by the inclusion levels of the MoLM in the diets but AST of rabbits fed MoLM based diets were higher (30.1 – 39.6 IU/L) than those fed diet 1 (29.8 IU/L).

Table 3: Serum biochemical response of gestating does fed diets with varied inclusion levels of *Moringa oleifera* Leaf Meal.

PARAMETERS	T0	T2.5	T5.0	T7.5
Glucose (mg/dL)	93.11 ± 6.76	81.20 ± 8.11	94.30 ± 6.15	100.72 ± 7.65
Cholesterol (mg/dL)	37.73 ± 13.08	21.90 ± 7.79	35.29 ± 10.36	24.64 ± 2.92
Total Protein (g/dL)	5.57 ± 0.20	5.32 ± 0.14	6.05 ± 0.31	5.90 ± 0.19
Albumin (g/dL)	2.12 ± 0.03	2.14 ± 0.04	2.23 ± 0.04	2.15 ± 0.08
Globulin (g/dL)	2.52 ± 0.96	3.18 ± 0.10	3.82 ± 0.28	3.75 ± 0.14

Table 4: Serum enzymes in gestating does fed diets with varied inclusion levels of *Moringa oleifera* Leaf Meal.

PARAMETERS	T0	T2.5	T5.0	T7.5
Alanine amino Transferase (iu/L)	19.15 ± 2.00 ^b	38.66 ± 4.15 ^a	39.68 ± 6.57 ^a	48.24 ± 2.70 ^a
Aspartate amino Transferase (iu/L)	29.76 ± 5.95	33.19 ± 5.23	30.07 ± 13.71	39.58 ± 14.83

a,b -Means in the same row with different superscript are significantly different (P < 0.05)

Serum glucose level decreased in does fed diets supplemented with 2.5% MoLM (81.2 mg/dL) compared with those on the control (93.1 mg/dL) while that of does fed 5.0 and 7.5 % MoLM levels were higher (94.3 and 100 mg/dL respectively). It has been reported that *Moringa oleifera* leaf contains quercetin-3-glucoside which is a phytochemical present in the leaf meal possessing glucose-lowering properties (14). This component of *Moringa* did not have a significant effect on serum glucose level during gestation in this study.

The serum cholesterol level was lower in rabbits fed MoLM diets (21.9 - 35.3 mg/dL) compared to those fed the control diet (37.7 mg/dL). The decrease in the serum cholesterol value at these levels of MoLM inclusion, although not significant may be attributed to the presence of beta-sitosterol, a bioactive substance present in the leaf meal which acts by inhibiting the absorption of cholesterol thus reducing serum cholesterol and its deposition in the muscles. This observation agrees with the findings of (15, 16) who reported moringa leaves as a potent hypocholesterolemic agent.

This suggests that *Moringa* leaf meal diets are capable of reducing serum cholesterol thus preventing excessive deposition of fat in gestating does. Reduced serum cholesterol in a study with albino rat was reported when methanolic extract of *Moringa oleifera* administered at a relatively low dose (150 mg/Kg), with a high fat diet daily for 30 days (17).

Total serum protein, albumin and globulin were not adversely altered by dietary supplementation since no abnormal rise or decrease was recorded in this study. This implies that there was efficient utilization of protein. The blood protein levels were not negatively affected by dietary MoLM since all values obtained compared favourably with that of does fed diets with no MoLM. All the values recorded for protein, albumin and globulin were within the physiological range for normal rabbits (18, 13).

The mean values obtained for Alanine Amino transferase (ALT) significantly (p < 0.05) increased in gestating does as the level of MoLM in the diet increased. This implies that

gestating does fed MoLM based-diets may be adversely affected by prolonged consumption of MoLM since high ALT level indicates organ toxicity. This result contradicts the finding of (19) who reported that administration of crude moringa extract did not significantly affect serum enzyme activities of gestating and lactating rabbits compared with those on the control diet in a 8weeks trial. Presence of tannins and saponnins in *Moringa oleifera* leaf meaf although minimal (5) might have induced damages to the hepatocytes thus resulting in increased serum ALT. However, all the ALT values obtained were within the physiological range reported for normal rabbits, whereas the does fed the control diet had the lowest value. Aspartate amino transferase (AST) were not significantly influenced by the supplemental levels of MoLM but AST of rabbits fed MoLM which was higher than those fed the control diet might be an indication of the adverse effect of long term feeding of MoLM as a feed supplement on organs such as kidney, heart or liver. As it has been reported that rats that received methanolic extract of *Moringa Oleifera* at 200 and 400 mg/kg body weight for eight (8) weeks had a significantly increased serum ALT, AST, blood urea nitrogen and creatinine which pointed to hepatic and kidney damage (20).

Conclusions and Applications

1. This study has demonstrated that feeding gestating rabbit does with diets containing *Moringa oleifera* leaf meal as a supplement up to 7.5% may not adversely alter their haematological and serum biochemical parameters thus enhancing growth and development.
2. However, its prolonged supplementation may have adverse effect on organs based on the increase in level of some serum enzymes as reported in this study.

3. *Moringa oleifera* leaf meal may be recommended as a good feed supplement for gestation and lactating rabbits. To feed rabbit does MoLM for a long period, dietary inclusion level should not exceed 2.5%.

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