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Effect of *Moringa oleifera* Leave Meal on Digestibility and Haematological Parameters, and Health Status of Doe Rabbits

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ABSTRACT The study involved the use of the *Moringa oleifera* leaf meal (MOLM) to feed doe rabbits and determine its effect on their nutrient digestibility and haematological indices. Five (5) treatment diets were formulated to contain MOLM inclusion levels of 0% (control), 25%, 50%, 75% and 100%. Forty five (45) rabbits of same sex were distributed randomly into five groups of nine animals and replicated three times per treatment having between 24 – 28 weeks of age with initial weight of about 1800 – 2400 g per animal. The does were provided with feed and water throughout the experimental period which lasted eight weeks. Analysis of the *Moringa leaf* meal (MOLM) on DM basis indicated that the leaves contained 92.15% Dry Matter, 12.85% Crude Protein, 5.10% Ether Extract, 6.57% Crude Fibre, 11.20% Ash, 64.28% Nitrogen Free Extract. The apparent digestibility of CP, CF, EE and NFE were affected ($P<0.05$) by the graded levels of MOLM inclusion except for the dry matter. The haematological parameters like packed cell volume, white blood cell, lymphocyte, neutrophil and monocyte vary significantly ($p<0.05$) between treatments. MOLM could be included in rabbit feed at an inclusion level of 25% without any adverse effect on the digestibility performance while blood indices of doe rabbits improved with increasing levels of *Moringa oleifera*.

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The strong demographic growth in the last recent years has created an imbalance between demand and supply of animal protein. Malnutrition has been installed and the consequences can be very serious especially for children (Nworgu*et al.*, 1999; Jahoor*et al.*, 2008). Given the poverty in most developing countries and lack of space for livestock farming especially in areas of high population density, the development of the mini-farm is becoming a key solution to fight against insufficient protein intake and malnutrition. This includes the rearing of micro-livestock such as grass cutter and rabbit farming. Rabbit farming is widely practiced especially in the southern part of Nigeria. This activity is very promising given the fact that rabbit farming provides a good quality meat and requires a small capital investment. Despite the dynamism of this activity, many problems remain unsolved. In rabbit farming, digestive system diseases are very common in young rabbit and are responsible for significant economic losses (mortality, treatment costs) and some of the solutions to prevent these diseases are development of health and medical prophylaxis, adjustment of feeding programs, mastering of good breeding practices (renewal of breeders, breeding rate etc.) (Cornelius *et al.*, 2019; Scarpelli *et al.*, 2018).Of all these prevailing factors, feeding is of greater interest to rabbit farmers. As a matter of fact, the fundamental function of the feed is to provide the body with necessary nutrients for its maintenance, growth and eventually production needs (lactation, pregnancy etc.). A well balanced feed must maintain the rabbit health status. Moreover, the

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immune system is regulated by numerous hormones at a certain level of secretion depends upon nutritional factors (Klasing and Leshchinsky, 2000).The idea of improving the performance of rabbit has compelled researchers to introduce various plant extracts in the feed of rabbit. Decreases in the oxidative stress and a resistance to endotoxaemia induced by lipopolysaccharides have been shown in rabbit receiving extract (Ben-Shaul*et al.,* 2000). Many plants present antibacterial and anti-inflammatory activities (Ben-Shaul *et al.,* 2000). Other researchers have highlighted the positive effects of these plants upon coccidiosis (Allen *et al.,* 1998; Youn, and Noh, 2001; Khan *et al.,* 2021).The use of *Moringa oleifera* Lam (Brassicales: Moringaceae) in the present study may help in the strategy of reducing incidents of disease rabbit farm. According to Ahemen *et al.* (2013), *M. oleifera* has the potential of being an inexpensive source of protein. It has carotene, ascorbic acid, iron, methionine and cysteine which are generally deficient in other feedstuff. Additionally, the presence of some functional bioactive phytochemical compounds such as saponins and flavonoids explained the medicinal action of the plant as encountered in its therapeutic uses (Bamishaiye*et al*., 2011; Saini *et al*., 2016). Also alkaloids, tannins, phenolics, saponins, flavonoids and steroids were detected by Bamishaiye *et al*. (2011) in an aqueous extract *of M. oleifera*. The research therefore aimed at studying the effect of *Moringa oleifera* leave meal on the digestibility and haematological parameters of doe rabbits.

MATERIALS AND METHODS

Forty five (45) doe rabbits of 24 – 28 weeks of age and weighing between 1.80 – 2.40 kg were used in this experiment. They were acquired from the Teaching and Research Farm, Department of Animal Science, Faculty of Agricultural Production and Management, Osun State University, Osogbo, Osun State, Nigeria. The procedure for the production of and use of rabbit as an experimental animal model (Mapara *et al*., 2012) was approved by the Animal Ethics Committee of the Department of Animal Science, Faculty of Agricultural Production and Management, Osun State University, Nigeria. The experimental animals were housed in cage of galvanized iron wire mesh of 76.2 x 50.8 x 76.2 cm 3 with 2.54 x 5.08 cm 2 mesh for the top and sides of the cage, while the floor was made of 1.27 x 2.54 cm 2 mesh to allow free movement. Each cage was provided with clean water in a watering trough and feed in feeding trough on daily basis without restriction; both troughs were fixed to the corners of the cage to avoid spillage. The floor of cages was constructed to facilitate easy drainage of urine and removal of faeces, and does did not have body contact with these waste products. Animals were randomly divided into five treatment groups, replicated three times with three rabbits per replicate. Each replicate was housed in a separate hutch raised from the ground. The animals were provided with a feeder and a drinker in each hutch. The leaves of *M. oleifera* were harvested from a tree at the Moringa Plantation of the Teaching and Research Farm, College of Agriculture, Osun State University, Ejigbo Campus, Ejigbo, Osun State, Nigeria. MOLM was prepared by destalking each leaf, washed and air-dried at room temperature of 31 ± 4ºC and relative humidity of 75 ± 6% to a constant weight. Continuous turning of the leaves was done to avert fungal growth for two week (Bamishaiye *et al*., 2011). They were kept away from high temperatures and direct sun light to avoid destroying active compounds. The dried leaves were crushed using a mortar and pestle to semi-fine powder.

Five experimental diets were formulated and were as follows: control (T1) was served with the basal diet (no MOLM), while T2, T3 and T4 contained an inclusion of MOLM at 0.25, 0.50 and 0.75 g/kg of basal diet respectively, and the T5 contained 1.0 g of MOLM in 1 kg of basal diet. Each rabbit was fed an assigned diet which lasted for eight weeks. The proximate composition of the dietary ingredients as well as the formulated basal diet was done according to the methods of AOAC (1990). The calculated digestible energy, crude protein and crude fibre were 2540.59kcal/kg, 16.34% and 10.51% respectively (Table 1).

Digestibility study was carried out during the last week of the feeding trial, which lasted for 7 days. Total feaces voided per treatment was collected daily and oven dried to determine its moisture content. The amount of water was determined by subtracting the dry weight from the initial weight, and the moisture content is then calculated as the amount of water divided by the dry weight or total weight of the feacal sample.

Representative samples of dried feaces were taken to the laboratory for proximate analysis. The apparent digestibility (AD) values for dry matter (DM), crude protein (CP), ether extract (EE) and crude fibre (CF) were calculated following the formula presented by Machado *et al*. (2012).

$$AD = \frac{Nutrient\ in\ feed - Nutrient\ in\ feaces}{Nutrient\ in\ feed} x100$$

Where AD = Digestibility values

At the end of the feeding period, the animals were starved of feed overnight before blood samples were collected from each of the replicates for

haematological analysis. Blood sample (2 ml) were collected from wing vein after conclusion of feeding trial, using sterile syringes and needles one per cock into EDTA test tubes. The 2 ml EDTA blood was used for haematology. Haematological parameter assayed in all the treatments were pack cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), platelets, lymphocytes, neutrophil, monocyte and eosinophil counts. The procedure for haematology determination as described by Jain (1986) was followed. Red blood cell and White blood cell were determined by the simple haematocytometer method, the PCV by micro-haematocrit method and the haemoglobin (Hb) concentration by cyanmethaemoglobin method.

Data Analysis: The data collected was subjected to analysis of variance using XLStat analytical software (XLStat, 2014). The significant differences at $p<0.05$ were separated using Tukey's Highest Significant Different of the same software.

RESULTS AND DISCUSSION

The gross composition of the diet fed to the rabbit was presented in Table 1, shown the calculated digestible energy, crude protein, and crude fibre as 2540.59kcal/kg, 16.34%, and 10.51%respectively. Proximate composition of the treatment diets were represented in Table 2 below. It revealed that all the parameters considered; dry matter, crude protein, crude fibre, ether extract, ash, and Nitrogen free extract were significantly affected ($P<0.05$) by the treatment diets. For the Dry matter, T2 and T4 were significantly higher than the other treatments having the values 92.99 and 92.97 respectively. Ash composition of T2 (12.85%), Ether extract composition of T1 (6.24) and Crude fibre composition of T5 (6.51) was higher than that of the other treatments. Crude protein was also significantly affected with T4 and T2 having the highest values of 19.31 and 19.20 respectively. The crude protein values are between 12.79% and 19.31% which differs from 15-17% recommended for rabbits by Fielding [10] and (Ograin, 2011). The result reveals that the Crude protein level of T5 (whole *Moringaoleifera* leaf meal) however, was low compared to that of diets T4, T2, T3 and T1 having the value 12.79%. This was low compared to values of 27.1% and 27.51% MOLM reported by Booth and Wickens (1988) and Odeyinka *et al*.(2008) respectively. The Crude fibre composition of T5 (100% MOLM) was higher than that of the other treatment diets with the value 6.51% which was lower than 19.20% reported by Booth and Wickens (1988). The variations in the nutrients could be attributed to the age of cutting or harvesting, climatic conditions, edaphic factors, agronomic practices as well as methods of processing and analysis of *M. oleifera* (Fuglie, 2001). The differences observed in the diet composition can be attributed to the differences in the inclusion level of *Moringa oleifera* to each of the treatment diets. Table 3 below shows the apparent digestibility of doe rabbits fed diets containing varying level of *Moringa oleifera* leaf meal (MOLM). There were significant differences ($p<0.05$) in the digestibility level of crude protein, crude fibre, ether extract and Nitrogen free extract (NFE) across the treatment diets. In the Table 3, crude protein digestibility increased with increased level of *Moringa oleifera* inclusion at T2, though there were no differences in the values in T1, T2, T3 and T4. This could be due to the fact that rabbit are animals that for their caecotrophic activities, increase the digestibility of protein, presenting higher values of digestibility when compared with other animals (Machado *et al*., 2012), and this result correlate with the findings of Fahey *et al*. (2001) who reported that *Moringa oleifera* is an outstanding indigenous source of highly digestible protein but the digestibility level begins to decline at T5. The Crude fibre digestibility was higher on treatment 5 (100% MOLM) compared to the other treatment diets which could be due to the higher digestible fibre content of the *Moringa oleifera* leaf meal compared to the fibre content of the control diet. Also there were significant difference ($P<0.05$) in the ether extract digestibility of T2, T4, T1, T3 compared to T5 with T2 having the highest ether extract digestibility of 47.37%. Nitrogen free extract digestibility on the treatment 5 (100% MOLM) was higher than that of the other dietary treatments.

Table 1: Gross composition of basal diet (Control)

Ingredients	% Inclusion
Maize	42.00
Rice husk	25.00
Soya bean meal	20.00
Wheat bran	10.25
Common salt	0.25
Premix	0.25
Dicalcium	2.00
Methionine	0.13
Lysine	0.12
Total	100

Calculated Nutrients; Digestible Energy= 2540.59kcal/kg; Crude Protein = 16.34%; Crude Fibre = 10.51%

The increased digestibility in some of the parameters may be due to the low content of acid detergent fibre, thus presenting a lower rate of passage, which aids digestion and absorption, with longer retention in the gastrointestinal tract. The results on the haematological components of the experimental rabbits fed the *Moringa oleifera* diets are presented in Table 4 below. There were no significant ($P>0.05$) differences in the values recorded on Haemoglobin (Hb), Red Blood Cell (RBC), Platelet and Eosinophil

for the does on the control diet and those on the test diets. All other haematological parameters (that is, PCV, WBC, Lymphocyte, Neutrophil and monocyte) were significantly affected by the treatment diets (P>0.05).This is contrary to the report of Adu and Egbunike (2010) who observed no significant difference in all the blood indices considered except haemoglobin of growing rabbit.

Table 2: Proximate Composition of the Diets

Parameters	T1	T2	T3	T4	T5	SEM	Significance
DM	92.56^{b}	92.99^{a}	92.66^{b}	92.97^{a}	92.09^{c}	0.05	***
CP	15.34^{c}	19.20^{a}	18.79^{b}	19.31^{a}	12.79^{d}	0.05	***
ASH	10.64^{d}	12.85^{a}	11.69 ^{b}	10.41^{e}	11.14^{c}	0.05	***
EE	6.24^{a}	5.65^{b}	5.48^{c}	5.75^{b}	5.04^{d}	0.05	***
CF	5.90^{b}	5.45^{c}	5.59^{c}	5.66^{c}	6.51^{a}	0.05	***

^{abcd}least means square along the column with different superscript are significantly different.

*SEM = Standard Error of Means, ***=highly significantly different, T1=100% Concentrate, T2=75% concentrate: 25% MOLM, T3=50% Concentrate: 50% MOLM, T4=25% Concentrate: 75% MOLM, T5= 100% MOLM, MOLM= Moringaoleifera leaf meal, DM=Dry Matter, CP=Crude Protein, EE=Ether Extract, CF=Crude Fibre.*

Table 3: Apparent nutrient digestibility of rabbits fed *Moringaoleifera* leaf meal diets

PARAMETERS	TREATMENT						
	T1	T2	T3	T4	T5	SEM	Significance
Dry Matter (DM)	56.69	56.28	56.62	56.76	56.97	0.47	NS
Crude Protein (CP)	70.43^{a}	72.83^{a}	66.56^{a}	66.02^{a}	36.13^{b}	1.92	***
Crude Fibre (CF)	50.51^{a}	42.09^{b}	51.23^{a}	44.69^{b}	51.29^{a}	1.01	***
Ether Extract (EE)	41.27^{ab}	47.37^{a}	34.42^{b}	42.03^{ab}	23.86^{c}	1.77	***
NFE	64.64 ^{c}	59.97^{c}	66.15^{c}	70.33^{b}	80.99^{a}	0.85	***

^{abc}least means square along the column with different superscript are significantly different.

*SEM = Standard Error of Means, ***=highly significantly different, NS= Not Significant, T1=100% Concentrate, T2=75% concentrate: 25% MOLM, T3=50% Concentrate: 50% MOLM, T4=25% Concentrate: 75% MOLM, T5= 100% MOLM, MOLM= Moringaoleifera leaf meal.*

Table 4: Effect of treatment on the haematological parameters of doe rabbit

PARAMETERS	TREATMENT						
	TI	**T2**	**T3**	**T4**	**T5**	**SEM**	Significance
PCV (%)	24.50^{ab}	22.00^{b}	25.00^{ab}	23.00^{ab}	29.50^{a}	1.52	*****
HB (g/dl)	8.10	7.50	7.85	7.70	8.50	0.54	**NS**
RBC ($\times10^{6}$ /µl)	3.73	3.69	3.71	3.75	4.13	0.25	**NS**
WBC ($\times10^{3}$ /dl)	3.50^{b}	2.95^{b}	1.85^{c}	4.78 ^{a}	1.43^{c}	230.40	*******
Platelets (10^{4})	7.65	5.00	6.45	6.20	5.55	7065.17	**NS**
Lymphocyte (%)	57.00^{b}	61.50^{ab}	69.50 ^{a}	53.50^{b}	71.50 ^{a}	2.41	*******
Neutrophil (%)	37.00^{ab}	38.50^{ab}	23.50^{c}	42.00 ^{a}	29.00^{bc}	2.17	*******
Monochyte (%)	3.00 ^{a}	1.50^{b}	2.00^{ab}	2.00^{ab}	1.00^{b}	0.29	******
Eosinophil (%)	3.00 ^{a}	2.00^{b}	3.50 ^{a}	2.50^{ab}	3.50 ^{a}	0.43	**NS**

^{abc} least means square along the column with different superscript are significantly different.

*SEM = Standard Error of Means, * = significantly different (P<0.05), **= more significantly different and ***=Highly significantly different, T1=100% Concentrate, T2=75% concentrate: 25% MOLM, T3=50% Concentrate: 50% MOLM, T4=25% Concentrate: 75% MOLM, T5= 100% MOLM, MOLM= Moringa oleifera leaf meal*

The values recorded on the PCV for the experimental rabbits ranged from 22.00% to 29.50%. Does on the 100% *Moringa oleifera* meal recorded the highest value (29.50%) which was higher than the control diet. The Packed Cell Volume levels observed for the does on the T5 diets being generally higher than those on the other treatment diets is an indication that the *Moringa oleifera* meal at 100% improved the packed cell volume of the blood of the rabbit which implies that the red blood cell percentage in the animals' blood increased, thereby improving the oxygen carrying capacity of the does and did not in any way render the does anaemic as red blood cells are cells which carries oxygen from the lungs to the other organs in the animals' body and carry carbon dioxide away. Packed cell volume (PCV) is a measure of the relative mass blood, and the result obtained in this study showed that PCV of doe rabbit was within the normal range reported by Fudge (1999) who considered the normal PCV of a healthy rabbit to be between 30 – 50%.The values recorded on the WBC for the experimental rabbits ranged from 1.43x10^{3}/L to 4.78x10^{3}/L. Does on the 75% *Moringa oleifera* meal recorded the highest value (4.78 x10^{3}/L) which was significantly higher than those on the control diet (T1) and the other treatment diets. The white blood cell levels observed for the rabbits on the T4 diet being generally higher than those on the other diet is an indication that the *Moringa oleifera* meal inclusion at 75% improved the immunity level of the animals as white blood cell in the blood acts as the first responder for the immunity system and get rid of dead or dying blood cells as well

as foreign materials thereby building the animals' resistance to diseases (Ahemen *et al.*, 2013). The values recorded on the Lymphocyte level for the experimental rabbits ranged from 53.50% -71.50**%**. Does on the 100% *Moringa oleifera* (T5) meal recorded the highest value (71.50**%**) which was significantly (P<0.05) higher than those on the control diet (T1) and the other treatment diets. This implies that the *Moringa oleifera* meal fed at 100% increased the lymphocyte level in the animal's blood thereby increasing the level of their immunity as lymphocyte is a type of white blood cell that functions as part of the immune system and respond to foreign invaders in the animal's body (Adu and Egbunike, 2010). The values recorded on the Neutrophil level for the experimental rabbits ranged from 23.50% to 42.00**%**. Does on the 75% *Moringa oleifera* meal (T4) recorded the highest value (42.00**%**) which was significantly (p<0.05) higher than those on the control diet (T1). The neutrophil levels observed for the rabbits on the T4 diet being generally higher than those on the other diet is an indication that the *Moringa oleifera* meal fed at 75% inclusion improved the Neutrophil level in the animal's blood thereby improving the animal's innate immune system and preventing the animals from infection. All the haematological parameters measured in the present experiment were within the normal physiological ranges reported for rabbits by Jenkin (1999) and Hillyer (1994) except for the Packed cell volume, White blood cell and Lymphocyte; Haemoglobin (8.0 – 17.5g/dl), Packed cell volume (30.0 – 50.0%), Red blood cell (4.0 – 8.0×10^6 /µl), White blood cell (5.0 – 12.0 × 10^3 /dl), Neutrophils (35.0 – 55.0%), Lymphocytes (25.0 – 50.0%) and Eosinophils (0.0 – 5.0%).

Conclusions: It is indicated that *Moringa oleifera* leaf meal (MOLM) incorporated at 25% and 100% inclusion levels into rabbit feed can improve dry matter (DM) and crude protein (CP) digestibility of rabbits. *Moringa oleifera* leaf meal at 100% level can improve the blood components of rabbits thereby improving the immune system and their resistance to various infections and diseases. MOLM can be used in rabbit diets as a supplementary protein source. In conclusion, MOLM can be included to formulate diet of rabbit at 20% to improve the digestibility, and at 100% inclusion to improve the blood components of rabbits.

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