



## Growth Response of Rabbits fed Multi-Enzyme Treated *Moringa oleifera* Leaf Meal

Wilberforce O ADUGUBA<sup>1</sup>, Raphael OBENG<sup>1</sup>, Matthew TIETA AH<sup>1</sup>, Lydia QUANSAH<sup>1</sup> and Terry ANSAH<sup>2\*</sup>

<sup>1</sup>Department of Biotechnology, Faculty of Biosciences, University for Development Studies, Tamale, Ghana,

<sup>2</sup>Department of Animal Science, Faculty of Agriculture, Food and Consumer Sciences, University for Development Studies, Tamale, Ghana,

\*Corresponding author: [tansah@uds.edu.gh](mailto:tansah@uds.edu.gh)

### ABSTRACT

The effect of multi-enzyme (Kemzyme plus P Dry) treated moringa oleifera leaf meal on the growth performance, haematological parameters, nutrient digestibility and carcass characteristics of weaned rabbits were evaluated. Twenty New Zealand White rabbits with an average initial weight of  $908.75 \pm 135.3$  g were divided into four groups and allocated four test diets in a completely randomized design for a period of 49 days after 14-days of adaptation. The experimental diets comprised of T0 (formulated concentrate), T1 (T0 + *M. oleifera* leaf), T2 (T0 + *M. oleifera* leaf + multi-enzyme) and T3 (T0 + multi-enzyme). Feed intake and final body weight gain were significantly ( $p < 0.05$ ) influenced by test diets. Rabbits fed T2 and T1 had higher feed intake ( $p > 0.05$ ) and weight gain ( $p < 0.05$ ). Apparent digestibility of Crude protein and Neutral detergent fibre ( $p < 0.05$ ) were found to be higher in rabbits fed with T2. Dressing percentage, hot and cold carcass weight were not significantly ( $p > 0.05$ ) affected. White blood cell, Haemoglobin and Packed cell volume indices significantly ( $p < 0.05$ ) differed with superior performance recorded among the rabbits fed with T2. Incorporating *M. oleifera* leaf meal at 40% in concentrate diet with 0.03 mg multi-enzyme could enhance growth of rabbits without any detrimental effect.

**Keywords:** Rabbit, digestibility, Multi-enzyme, *Moringa oleifera*, Haematology

### INTRODUCTION

Daily dietary intake of animal protein (3.24 g) in most low and middle- income countries falls grossly short of the suggested 27 g animal protein per caput/day (Ajayi *et al.*, 2007). There is therefore the need to increase the level of animal production in a manner that is consistent with scarce resource to augment animal protein intake in human.

Rabbits are a group of farm animals that have high production characteristics; early maturity rate and potential for genetic selection, efficient use of feed and land, limited competition with humans for similar foods and high-quality nutritious meat (Adam *et al.*, 2015). The small-body size of matured rabbits relative to ruminants takes away the problem of finding space to store the meat after slaughter. Though they are monogastrics, they have the benefit of a well-

developed caecum where a good proportion of the structural carbohydrates in roughages are digested and absorbed in the form of volatile fatty acids (Liu *et al.*, 2018).

Feeding rabbits with concentrates without any herbaceous roughage may have a negative effect on the efficient functioning of the digestive system. Herbaceous roughages contribute significantly to the fiber composition of the feed which helps to maintain gut motility (Dorota *et al.*, 2018). It has been suggested that feeding rabbits with a diet low in structural carbohydrate and high in energy from finely ground concentrate diet containing more of soluble carbohydrates can result in high mortality due to intestinal disorders, such as enterotoxemia (Maertens *et al.*, 2002). Adding herbaceous roughage as a source of structural carbohydrate to the diet of rabbits can significantly reduce the cost of feeding as most carbohydrate concentrates are relatively costly (McCroskey, 2000).

*Moringa oleifera* leaf meal (MOL) have been found to contain appreciable levels of digestible nutrients for livestock production (Adam, 2013; Alagbe & Oluwafemi, 2019). *Moringa* leaves, have protein concentration ranging from 15% to over 30% dry matter (DM), depending on maturity stage and fraction considered (Reyes Sanchez, 2004; Price, 2007). Compared to non-forage-based concentrate, MOL contains higher (28.3%) levels of neutral detergent fiber. They also contain some phytochemicals, especially, condensed tannins (8%) which may inhibit microbial fermentation in the caecum (Amaglo *et al.*, 2010). Unlike ruminants, rabbits have a small holding capacity for microbial fermentation of fiber which may lead to a faster passage of digesta resulting in poor digestibility of fiber (Gidenne, 2003).

Addition of multi-enzyme to feed has led to some improvement in digestibility of nutrients and enhanced growth of farm

animals (Saleh *et al.*, 2010; Raach-moujahed *et al.*, 2017). Kemzyme<sup>®</sup> Plus P Dry is a multi-enzyme feed additive made of both exogenous and endogenous enzymes with the potential of improving the digestion of non-starch polysaccharide and complex substrates to increase the amount of energy and amino acids available to the animal. Its use has been approved in avians (EFSA, 2015). However, it is unclear how the enzyme will influence the digestion of nutrients and growth of rabbits fed herbaceous roughage as a source of fiber.

The objective of the study was to determine the growth response of weaner rabbits fed Kemzyme Plus dry treated *Moringa oleifera* leaf meal.

## MATERIALS AND METHODS

### Study Area

The study was carried out at the Nyankpala Campus of the University for Development Studies, Tamale, Ghana. Nyankpala is about 18 km west of Tamale in the Tolon District. It is located on latitude 9° 25' 41" N and longitude 0° 58' 42" W at an altitude of 183 m above sea level (SARI, 2001). The area is in the Guinea Savanna Zone characterized by a unimodal rainfall pattern. Rains begin in April, rising to a peak in August–September and ending in October or November. Rainfall averages 1060 mm per annum. Temperatures range from as low as 15°C in January when the weather is under the influence of the North Easterly (Harmattan) winds and as high as 42°C around the end of the dry season in March (SARI, 2001).

### Experimental design and treatments

Twenty (20) weaned New Zealand White (NZW), both male and female rabbits (6-8 weeks) with initial mean weight of 908.75±135.3 g were sourced from the University of Ghana farms, Accra. Completely

Randomized Design (CRD) was used in grouping the rabbits and placed on four different diets. The feeding trial lasted for 49 days. Each dietary group had 5 replicates. The experimental diets comprised of T0 (formulated concentrate only), T1 (T0 + *Moringa olerifera* leaf meal (MOL) without multi-enzyme), T2 (T0 + MOL + multi-enzyme) and T3 (T0 + multi-enzyme without MOL).

### **Feed ingredients, inclusion level and nutrient analysis**

The ingredients used and inclusion levels are shown on Table 1. MOL was used as a partial replacement for maize with 0.03 g Kemzyme added. The experimental diets were analyzed for crude protein (CP), ash and DM content (AOAC, 2000). The CP content was calculated from nitrogen (N) content of the feed, determined by the Kjeldahl technique. Ash was determined according to the procedure of AOAC (2000). The NDF and ADF were analysed using the detergent method described by Van Soest *et al.* (1991) using the Ankom<sup>200</sup> fiber analyser.

### **Management of the experimental animals**

The rabbits were housed in wire mesh cages each raised 0.5 m above the ground. The dimension of the hutch is 50 cm × 60 cm × 60 cm. Each cage was provided with two bowls made of clay for water and feed. Each animal was given 100 g of feed per day for the 49 days. Water was given *ad libitum*. Medication was given as and when an animal was diagnosed with a disease. The following data were collected during the experiment: dry matter feed intake, weekly body weight, and carcass characteristics. Blood (5 ml) was sampled from jugular vein with the help of a

syringe into a test tube containing ethylene diamine tetra-acetic acid (EDTA) as anticoagulant (Radostits *et al.*, 1994) for the haematological assay.

### **Data Analysis**

Data collected on feed intake, nutrient digestibility, growth, haematology and carcass characteristics of rabbits were analysed by analysis of variance using Genstat 18<sup>th</sup> edition. Means were separated using Duncan's multiple range test at 5% level of significance.

## **RESULTS AND DISCUSSION**

The dry matter (DM) was in the range of 862 to 894 g/kg<sup>-1</sup> with T2 (T0 + MOL + multi-enzyme) having the highest (Table 2). The crude protein (CP) followed a similar trend. Both NDF and ADF were relatively higher in T1 (T0 + MOL) and T2 (T0 + MOL + multi-enzyme) suggesting a corresponding higher concentration of soluble carbohydrate in T0 (formulated concentrate only), and T3 (T0 + multi-enzyme). The CP content of the test diets in this study was higher than the 150-160 g/kg DM recommended for growing rabbits (Obinne & Okorie, 2008). It is apparent from the nutrient composition results that the high CP, NDF and ADF were due to the addition of MOL. *Moringa oleifera* is a legume with a higher concentration of crude protein (24.3%) and a relatively higher fiber content than the maize bran used in the control (Nuhu, 2010).

TABLE 1. Inclusion levels and chemical composition (gkg<sup>-1</sup> DM) of the various ingredients

Ingredients (%)	Test Diets			
	T <sub>0</sub>	T1	T2	T3
Maize	50	10	10	50
Maize Bran	20	20	20	20
Soy Bean	29	29	29	29
Salt	0.25	0.25	0.25	0.25
Di calcium phosphate	0.25	0.25	0.25	0.25
Vitamin premix	0.5	0.5	0.5	0.5
MOL	-	40	40	-
Kemzyme plus P dry	-	-	0.03g (30mg)	0.03g (30mg)
Total	100	100	100.03	100.03
Chemical composition (gkg <sup>-1</sup> DM)				
Dry matter	877	862	894	886
Crude protein	349.2	380.5	395.1	363.9
Neutral detergent fiber	246.91	412.76	489.72	271.9
Acid detergent fiber	38.80	78.83	77.89	39.3
Ash	47.50	70	52.50	47.50

\*premix composition (g/kg): vitamin A, 12, 500 IU; vitamin D3, 2500 IU; vitamin E, 50.00 mg; vitamin K3, 2.50 mg; vitamin B1, 3.00 mg; vitamin B2, 6.00mg; vitamin B6, 6.00 mg; niacin, 400 mg; calcium pantothenate, 10mg; biotin, 0.8 mg; vitamin B12, 0.25 mg; folic acid, 1.00 mg; chlorinechloride, 300 mg; manganese, 100 mg; iron, 50 mg; zinc, 45 mg; copper, 2.00 mg; iodine, 1.55 mg; cobalt, 0.25 mg; selenium, 0.10 mg; antioxidant, 200 mg. MOL: *Moringa oleifera* leaf; Kemzyme plus P dry (endo1,3(4)-beta-glucanase, endo-1,4-beta-glucanase, alpha-amylase, bacillolysin and endo-1,4-beta-xylanase)

In terms of acid detergent fibre (ADF), diets containing MOL (T1 and T2) had higher values compared to T3 and T0. The NDF and ADF levels in T0 and T3 were found to be below the levels recommended (NDF; < 300 gkg<sup>-1</sup> DM and ADF < 100 gkg<sup>-1</sup> DM) for growing rabbits (Sauvant *et al.*, 2002).

Rabbits fed diets T2 (4660 g: T0 + MOL + multi-enzyme) and T1 (4353 g: T0 + multi-enzyme without MOL) had higher (P < 0.05) total DM intake than the control (2966 g) and T1 (2817 g: T0 + MOL without multi-enzyme).

The high feed intake recorded in rabbits fed MOL, despite the relatively higher NDF and ADF is an indication of effective functioning

of the digestive system, particularly, microbial fermentation in the caecum. Fermentation of fibrous carbohydrates in the caecum produces volatile fatty acids which adds up to the total energy absorbed (Mooser & van Kempen, 2002; Abdel-Khalek 2011). Abdel-Khalek (2011) reported that rabbits derive about 10-20% of their energy expenditure from volatile fatty acids. Rabbits are monogastric herbivores with a well-developed caecum which serves as a habitation for fiber degrading micorbes. The performance of the rabbits relative to feed intake suggest that rabbits will consume more of a fibrous diet than those with less fiber concentration.

**TABLE 2. Growth Performance, Apparent digestibility coefficient (%) of Test diets, Haematological parameters and Carcass Characteristics of Rabbits**

Parameters	Growth Performance				SED	P- value
	Test ingredients					
	T0	T1	T2	T3		
Total feed intake (g)	2966 <sup>a</sup>	4353 <sup>b</sup>	4660 <sup>b</sup>	2817 <sup>a</sup>	440.9	<.001
Average daily feed Intake (g)	51.32 <sup>a</sup>	72.72 <sup>ab</sup>	81.66 <sup>b</sup>	50.94 <sup>a</sup>	7.58	0.001
Final weight (g/rabbit)	1363 <sup>a</sup>	1738 <sup>ab</sup>	1980 <sup>b</sup>	1548 <sup>ab</sup>	181.7	0.022
Total weight gain (g/rabbit)	536	780	1024	654	169.3	0.059
Average daily weight gain g	10.9	15.9	20.9	13.3	3.46	0.059
Feed conversion ratio	5.193	4.592	4.774	3.948	1.167	0.759
Apparent nutrient digestibility coefficient (%)						
DM	70.33	78.60	75.38	68.35	3.690	0.051
CP	75.67 <sup>a</sup>	83.58 <sup>ab</sup>	85.74 <sup>b</sup>	77.42 <sup>a</sup>	2.745	0.005
NDF	27.78 <sup>c</sup>	73.55 <sup>a</sup>	79.31 <sup>a</sup>	49.19 <sup>b</sup>	5.84	<.001
ADF	17.16	38.06	37.27	20.94	8.81	0.060
Haematological parameters						
WBC( $\times 10^9$ /L)	6.73 <sup>a</sup>	8.93 <sup>b</sup>	9.27 <sup>b</sup>	6.67 <sup>a</sup>	0.622	0.004
PCV (%)	41.33 <sup>b</sup>	35.93 <sup>a</sup>	37.87 <sup>ab</sup>	35.77 <sup>a</sup>	1.129	0.004
Lymphocytes (%)	68.7	44.4	48.9	46.1	12.98	0.287
Monocytes (%)	8.7	10.7	11.7	13	13	0.606
Neutrophils (%)	20.08	33.54	31.88	34.02	14.49	0.747
RBC( $\times 10^{12}$ /L)	5.35	5.17	5.88	5.39	0.449	0.463
Hemoglobin (g/dL)	13.37 <sup>b</sup>	11.57 <sup>a</sup>	12.40 <sup>ab</sup>	11.93 <sup>a</sup>	0.878	0.008
Carcass Characteristics of the Rabbits						
Dressing %	43.9	45.6	49.7	39	3.66	0.100
Cold Carcass (g)	661	786	944	691	123.3	0.174
Hot Carcass (g)	667	791	947.3	692.3	123.2	0.176

<sup>abc</sup> means with different superscript along rows differ significantly ( $p < 0.05$ ). DM= Dry matter, CP=Crude protein, NDF=Neutral Detergent Fibre, ADF=Acid Detergent Fibre. Abbreviations: WBC, white blood cell; monocyte and eosinophil; RBC, red blood cell; Hg, hemoglobin.

The apparent nutrient digestibility differed significantly with respect to CP and NDF. The highest CP digestibility was recorded in rabbits fed T2. The NDF digestibility did not differ between rabbits fed T1 and T2 but they were both significantly different from T0 and T3. The similarity of results suggest that the addition of Kemzyme plus P Dry did not significantly improve digestibility of fiber and other nutrient in the rabbits. However, the superiority of digestibility in rabbits fed T1 and T2, relative to CP and NDF suggest that MOL positively influenced the gut functionality. The supply of both degradable protein and fermentable carbohydrate in the caecum was not limited by the inclusion of MOL.

There was a significant difference in final weight of rabbits fed MOL with those fed with MOL with or without enzyme treatment having the highest. The control animals had the least final weight, suggesting that feeding rabbits with no herbaceous feed ingredients could compromise on their growth. The significantly higher final weight recorded in rabbits fed concentrate with enzyme treated MOL could be attributed partly to the addition of the enzyme which may have enhanced the amount of energy available for metabolism (Abdl-Rahman *et al.*, 2010). Additionally, further digestion of fermentable carbohydrate in the caecum supplied volatile fatty acid and microbial protein which may have been limited in the

control diet and T3 due to the absence of the MOL.

The final weight gain, average daily weight gain and feed conversion ratio did not differ significantly among the treatments. The lack of difference among rabbits fed MOL and concentrate diets could minimize or eliminate the competition between animals and humans for the use of maize which is a major staple in the diet of humans, especially in developing countries.

There was no significant difference ( $P < 0.05$ ) among the rabbits for the haematological parameters, except WBC, PCV and hemoglobin. The rabbits fed with control diet had a higher haemoglobin concentration and PCV than those on the other treatments even though they all fell within the normal range for rabbits (Medirabbit, 2011). The WBC, even though was found to be higher in rabbits fed with the treated diets, was within the normal range of  $5-19 \times 10^6/L$  reported by Medirabbit, (2011). was however, lower in the control but higher in the treated diets. Generally, the haematological values reported in the present study were similar to what has been reported earlier by Ansah et al (2014) for rabbits fed with agro-industrial by products within the same agro-ecological zones.

There was no significant difference ( $P > 0.05$ ) in the carcass characteristics. The dressing percentage was in the range of 39-49.7 with T2 having the highest. The dressing percentage for T1 and T2 was in the same range of 48.9% to 55.3% reported for rabbits fed agro-industrial by product-based-feed (Ansah *et al.*, 2014). The similarity in carcass composition is an indication that maize can be replaced with MOL without negatively affecting carcass.

## CONCLUSION

The addition of *Moringa oleifera* to the diet enhanced the crude protein and fiber

composition. Rabbits fed with MOL had a better nutrient digestibility and weight gain with those fed the enzyme treated diets having twice the weight recorded for the control animals. Blood profile and carcass characteristics were not negatively affected by the treatments.

## Conflict of interest

The authors declare that they have no conflict of interest.

## REFERENCES

- Abdel-Khalek, A.E., Kalaba, Z.M., & El-Gogary, M.R. (2011). Functional, Anatomical and Histological Development of Caecum in Rabbits. *Current Research in Poultry Science*, 1: 54-65.
- Abdl-Rahman, M. A., Sawiress, F. A. R., & Saleh, S. Y. (2010). Effect of Kemzyme - Bentonite Co-supplementation on Cecal Fermentation and Metabolic Pattern in Rabbit. *Journal of Agricultural Science*, 2(3), 183-188.
- Adam, N. (2013). Digestibility and Growth Performance of Female Rabbit Fed *Tithonia Diversifolia* Leaf Meal. A dissertation submitted to the Department of Agroforestry, Faculty of Renewable Natural Resources, College of Agriculture and Natural Resources, Kwame Nkrumah University of Science and Technology.
- Adam, R. H., & Brock, G. (2015). Historical Archaeology. Late-Nineteenth-Century Foodways in the "Garden State" at the Woodruff House, Rahway, New Jersey: Insights from

- Small Faunal and Large Macrobotanical Samples. 49 (4), 12.
- Ajayi, A. F. Farinu, G. O., Ojebiyi, O. O. & Oayni, T. B. (2007). Performance evaluation of male rabbit fed diets containing graded levels of blood-wild sunflower leaf meal mixture. *World Journal of Agriculture Science*, 3(2), 250-255.
- Alagbe, O.J. & Oluwafemi, R.A. (2019). Growth Performance of Weaner Rabbits Fed Noni (*Morinda Citrifolia*) and *Moringa olifera* Leaf Meal Mixture as Partial Replacement of Soya Bean Meal. *International journal of Advanced Biological and Biomedical Research*, 7(2), 185-195.
- Amaglo, N. K., Bennett, R. N., Lo Curto, R. B., Rosa, E. A. S., Lo Turco, V., Giuffrida, A., Lo Curto, A., Crea, F., & Timpo, G. M. (2010). Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree *Moringa oleifera* L., grown in Ghana. *Food Chemistry*, 122 (4), 1047-1054.
- Ansah, T., Kamara, F., Ninkarb, S. J. & Takinam, R. K. (2014). Growth, carcass, and blood profile of rabbits fed agro-industrial by-products in Northern Ghana. *Ghana Journal of Science, Technology and Development*, 1(1), 1-8.
- AOAC. (2000). Official methods of analysis 16<sup>th</sup> edition. Association of Official Analytical Chemists. Arlington, Virginia, U.S.A.
- Dorota, M., Króliczewska, B., Pecka-Kiełb, E., Piekarska, J., Marounek, M., & Zawadzki, W. (2018). Comparative in vitro study of caecal microbial activity in brown hares and domestic rabbits which were offered the same diet, *Mammal Research*, 10.1007/s13364-018-0357-1, 63 (3) 285-296.
- EFSA FEEDAP Panel (EFSA Panel on Additives and Products or Substances used in Animal Feed), (2015). Scientific Opinion on the safety and efficacy of Kemzyme® Plus Dry (endo-1, 3(4)-beta-glucanase, endo-1, 4-beta-glucanase, alpha-amylase, bacillolysin and endo-1, 4-beta-xylanase) for poultry and piglets (weaned). *EFSA Journal*; 13(9), 4234, doi:10.2903/j.efsa.2015.4234.
- Gidenne, T. (2003). Fibres in rabbit feeding for digestive troubles prevention: respective role of low-digested and digestible fibre. *Livestock Production Science*, 81(2-3), 105-117.
- Liu, S., Yuan, M., Jin, D., Wang, Z., Zou, H., Wang, L., Bai, X., Wu, D., Tian, G., Cai, J., Yan, T., & Peng, Q. (2018). Effects of the particle of ground alfalfa hay on the growth performance, methane production and archaeal populations of rabbits. 13(9), <https://doi.org/10.1371/journal.pone.0203393>
- Maertens, L., Perez, J., Villamide, M., Cervera, C., Gidenne, T., & Xiccato, G. (2002). Nutritive Value of Raw Materials for Rabbits. *World Rabbit Science*. 10(4), 157-166. doi:10.4995/wrs.2002.488.
- McCroskey, R. (2000). Raising Rabbits in the Pacific Northwest. Canadian Centre for Rabbit Production Development. Surrey, BC Canada V4N 3T7.
- Medirabbit (2011). Complete blood count and biochemistry reference values in rabbits. Medirabbit.com/EN/Hematolo

- gy/blood\_chemistry.htm.  
Retrieved on the 18<sup>th</sup> of March, 2020.
- Moeser, A. J. & Van Kempen, T. A. (2002). Dietary fibre level and enzyme inclusion affect nutrient digestibility and excreta characteristics in grower pigs. *Journal Science Food Agriculture*, 82: 1606-1613.
- Nuhu, F., (2010). Effect of moringa leaf meal (MOLM) on nutrient digestibility, growth, carcass and blood indices of weaner rabbits. In: MSc. Thesis. Anim. Nutr., Kwame Nkrumah University, Kumasi (Ghana), pp.122.
- Obinne, J.I. & Okorie, A.U. (2008). Effect of different crude protein and digestible energy levels on the growth performance of rabbits in the tropics. *Nigeria Journal Animal Production*, 35, 210-216.
- Price, M. L. (2007). The Moringa Tree. ECHO technical 1 note. Revised edition, pp 11-12.
- Raach-Moujahed, A., Zouaghi, F. & Hajlaoui, K. (2017). Effects of 3 additives: Rovabio Exel; Kemzyme Plus Dry and Cibenza DP 100 on performances of broiler chickens. *Journal of new Sciences*, 37(2).
- Radostits, O. M., Blood, D. C. & Gay, C. C. (1994). *Veterinary Medicine, A textbook of the diseases of Cattle, Sheep, Pigs, Goats, and Horses*. Eighth edition. Baillere Tindall, London, pp 66.
- Reyes Sanchez, N. (2004). Marango: Cultivation and utilization in animal feeding. Guia tecnica n°5, Universidad Nacional Agraria, Por un desarrollo agrario integral y sostenible, 24 p.
- Saleh, Y. S., Attia, K. A., Fouad, M. A., & Nassar, M. M. (2010). Effects of Multi-enzyme Feed Additive “Kemzyme” or/and Sodium Bentonite “as a Feed Binder” on Sexual Activity and Some Fertility Parameters of Rabbit Bucks. *Journal of Agricultural Science*, 2, (4).
- SARI. (2001). Savanna Agriculture Research Institute, Agro-metrological Station, Nyankpala Station. pp 10.
- Sauvant, D., Perez, J.M. & Tran, G. (2002). Tables de composition et de valeur nutritive des matières premières destinées aux animaux d'élevage. INRA Editions Paris, France
- Van Soest, P.J., Robertson, J.B. & Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74(10), 3583-3597.