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

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**ABSTRACT** 

The effect of multi-enzyme (Kenzyme 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±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 rumianats 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® 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 Kemzyne 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 oleifera* 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 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 testube 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 18th 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 gkg⁻¹ 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 (g kg\(^{-1}\) DM) of the various ingredients

| Ingredients (%) | T\(_0\) | T\(_1\) | T\(_2\) | T\(_3\) |
|-----------------|--------|--------|--------|--------|
| 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 (g kg\(^{-1}\) 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  | 78.73  | 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; chlorine chloride, 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, endo1,4-beta-glucanase, alpha-amylase, bacillolysin and endo1,4-beta-xylanase)

In terms of acid detergent fibre (ADF), diets containing MOL (T\(_1\) and T\(_2\)) had higher values compared to T\(_3\) and T\(_0\). The NDF and ADF levels in T\(_0\) and T\(_3\) were found to be below the levels recommended (NDF; < 300 g kg\(^{-1}\) DM and ADF < 100 g kg\(^{-1}\) DM) for growing rabbits (Sauvant \textit{et al.}, 2002). Rabbits fed diets T\(_2\) (4660 g: T\(_0\) + MOL + multi-enzyme) and T\(_1\) (4353 g: T\(_0\) + multi-enzyme without MOL) had higher (P < 0.05) total DM intake than the control (2966 g) and T\(_1\) (2817 g: T\(_0\) + 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 (Moeser & 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 microbes. 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.
| Parameters                          | Growth Performance | Test ingredients | SED | P-value |
|------------------------------------|--------------------|------------------|-----|---------|
|                                    |                    | T0               | T1  | T2     | T3     |       |
| Total feed intake (g)              | 2966              | 4353             | 4660| 2817   | 440.9  | <.001 |
| Average daily feed intake (g)      | 51.32             | 72.72            | 81.66| 50.94  | 7.58   | 0.001 |
| Final weight (g/rabbit)            | 1363              | 1738             | 1980| 1548   | 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              |                   |                  |     |        | 1.167  | 0.759 |

| Parameters                          | Test ingredients | SED | P-value |
|------------------------------------|------------------|-----|---------|
|                                    | T0               | T1  | T2     | T3     |
| DM                                 | 70.33            | 78.60| 75.38  | 68.35  | 3.690  | 0.051 |
| CP                                 | 75.67            | 83.58| 85.74  | 77.42  | 2.745  | 0.005 |
| NDF                                | 27.78            | 73.55| 79.31  | 49.19  | 5.84   | <.001 |
| ADF                                | 17.16            | 38.06| 37.27  | 20.94  | 8.81   | 0.060 |
| WBC(×10^9/L)                       | 6.73             | 8.93 | 9.27   | 6.67   | 0.622  | 0.004 |
| PCV (%)                            | 41.33            | 35.93| 37.87  | 35.77  | 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(×10^12/L)                      | 5.35             | 5.17 | 5.88   | 5.39   | 0.449  | 0.463 |
| Hemoglobin (g/dL)                  | 13.37            | 11.57| 12.40  | 11.93  | 0.878  | 0.008 |

| Parameters                        | Test ingredients |       |
|-----------------------------------|------------------|-------|
|                                    | T0               |       |
| Dresing %                         | 43.9             |       |
| Cold carcass (g)                  | 661              |       |
| Hot carcass (g)                   | 667              |       |

abc 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 Kemzyrne plus P Dry did not significantly improve digestibility of fiber and other nutrient in the rabbits. However, the superiroty of digestibility in rabbits fed T1 and T2, relative to CP and NDF suggest that MOL positively influenced the gut functionability. 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 eventhough they all fell within the normal range for rabbits (Medirabbit, 2011). The WBC, eventhough was found to be higher in rabbits fed with the treated diets, was within the normal range of 5-19 * 10^3/L reported by Medirabbit, (2011). This was however, lower in the control but higher in the treated diets. Generally, the haematological values reproteted in the present study were similar to what has been reported earlier by Ansah et al (2014) for rabbits fed 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 reaplced 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.

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