

GROWTH PERFORMANCE, HAEMATOLOGICAL INDICES AND COST BENEFIT ANALYSIS OF GROWING RABBITS FED DIETS CONTAINING MEXICAN SUNFLOWER (*Tithonia diversifolia*)

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ABSTRACT

This study was carried out at the Teaching and Research Farm of Ekiti State University, Ado Ekiti. Twenty-four growing rabbits of about thirteen weeks of age with an initial average body weight of 650 - 700g were used to evaluate performance of rabbits fed varying levels of *Tithonia diversifolia* leaf meal extract/fibre residue as well as cost benefits for a period of 70 days, using a completely randomized design. The *Tithonia diversifolia* was processed and incorporated into the diet at 0%, 1.5%, 3.0% and 4.5% inclusion levels replacing groundnut cake at four treatments of 0%, 5%, 10% and 15% dietary inclusion for 100kg feed formulation, three replicates of unsexed two rabbits each per treatment. The average feed intake of the rabbits was significantly ($P < 0.05$) affected by the inclusion of *Tithonia diversifolia* leaf meal extract residue protein. The average daily weight gain of rabbits fed control 0 and 5% inclusion levels, had the highest value of 61.67g and 58.67g, while at 15% inclusion level, rabbits had the lowest mean value of 51.67g. The feed conversion ratio also followed the same trend. The protein efficiency ratio was also significantly influenced ($P < 0.05$). Rabbits on 5% inclusion of *Tithonia diversifolia* leaf meal extract/fibre residue had the best protein efficiency ratio which indicated a better utilization. All the haematological parameters were not significantly ($P > 0.05$) affected. *Tithonia diversifolia* leaf meal extract/fibre residue can be incorporated into the diets of growing rabbits up to 5% without showing any adverse effect on the feed intake, daily weight gain, feed conversion ratio and haematological parameters of the growing rabbit.

Keywords: Performance, haematology, Cost, Rabbit, *Tithonia diversifolia*.

INTRODUCTION

The inadequacy of protein in human diets in developing countries especially Nigeria is as a result of high cost of animal feeds. Inadequate protein intake as recommended by Food and Agriculture Organization (FAO, 2002), is more rampant in developing countries which has adverse effect on their citizens (Schonfeldt and Hall, 2012). Nigeria being part of the developing countries of lower income has suffered from the absence of the recommended protein intake levels. Today, the world is suffering from a serious shortage of feed ingredients such as wheat, maize and soya beans because of rapid increase in human population. Therefore, provision must be made to solve the problem of increase in population and food availability quickly. The competition between man and animal for available grains has been established with animals always at a losing end (Rijal *et al.*, 2009).

Majorly, the pre-occupation of animal nutritionists has been to research alternative feed sources for animals and their implication on production, health and management. This has yielded results in terms of competitive sources of ingredients for energy and protein in animal ration formulation (Agbede and Aletor, 2004; Ahamefule *et al.*, 2006; Dairo, 2008). Reduction in dependence on importation of livestock feed in Nigeria has led to increase in the demand and cost of conventional feedstuff (Togun *et al.*, 2006). Hence, the need to explore the use of non-conventional feed sources that have the capacity to yield the same output as conventional feeds, and perhaps at cheaper cost.

Rabbit industry has an important role to play in the world as a most economical source of protein with low cholesterol level. The most important aspect of rabbit production is the reduction of feed cost using cheaper and unconventional feed resources (Bhatt and Sharma, 2001; Muriu *et al.*, 2002). However,

scarcity of feed resource has been the main limitation in the production of livestock products to meet the animal protein requirements of human and other industrial needs. The conventional cereals and vegetable protein sources being used in animal feed formulations are under pressure of competition. Feed cost is about 75% in non-ruminant production out of which protein accounts for about 45% (Agbede and Aletor, 2004). It is therefore, necessary that some efforts are made towards improving rabbit production through the use of relatively less expensive and newly improved ingredients, these can be used as replacement for most of the expensive conventional feed ingredients.

Tithonia diversifolia is a wild plant found in fallowed plot or land in the forest zones of Nigeria. The type that is used for agricultural research is the maturing ones and the leaves are the needed part of the plant. However, anti-nutrients present in the leaves which include: tannins (0.39%), phytic acid (79.1%), flavonoids (0.87%), oxalate (1.76%), alkaloids(1.23%) and saponins (2.36%) makes the leaves bitter thus hindering the acceptability of the leave among different species of animals (Fasuyi *et al.*, 2010).

Assessment on the growth performance, haematological parameters and cost benefits of rabbit fed *Tithonia diversifolia* can enhance the inclusion of the forage among alternative feeds and feeding stuff for rabbit production hence, the focus on the *Tithonia diversifolia* leaf meal extract/fibre residue is been harnessed.

MATERIALS AND METHODS

Experimental site

The study was carried out in the rabbit unit of the Teaching and Research Farm, Faculty of Agricultural Sciences, Ekiti State University, Ado-Ekiti, Nigeria. Ekiti State is situated entirely within the tropics. It is located between longitudes 40° 51' and 50° 451' East of the Greenwich meridian and latitudes 70° 151' and 80° 511' North of the Equator. It lies south of Kwara and Kogi State, East of Osun State and bounded by Ondo State in the East and in the South. The State enjoys a tropical climate with two distinct seasons; the rainy season (April - October) and the dry season (November - March). Temperature ranges between 21°C and 28°C with high humidity.

Site Preparation

The hutches were properly cleaned and washed with detergent, Lysol and Izal and then fumigated adequately with 40% formaldehyde in water solution and allowed one week to destroy disease causing microbes. Feeding and drinking trough were also washed and cleaned, then provided and arranged in the labelled hutches.

Collection of Experimental Feed Ingredients

Maturing *Tithonia diversifolia* leaves were harvested prior the flowering stage, from the university teaching and research farm premises. The leaves were chopped into smaller portions to reduce the surface area of the leaves for a faster grinding process. The leaves were mixed with water to form a pulp and sieved, leaf meal extract/fibre residue from the mixture was sun-dried to crispy touch, milled to a powdery form and later mixed thoroughly with other conventional feed ingredients using commercial hammer mill.

Experimental Animals and their management

The experimental diets were prepared by replacing a protein source groundnut cake meal at varying levels of leaf meal extract/fibre residue of *Tithonia diversifolia* formulated growing rabbit ration at 0, 5, 10 and 15% inclusion levels of 100kg formulated diet. These gave four treatment diets with diet one as the control; they were designated as D1, D2, D3 and D4. Twenty-four growing rabbits of 13 weeks old and average weight of 650 - 700g were randomly allotted to the treatment groups replicated three time two per replicate with different sexes. The experiment lasted for 70 days. The rabbits were allowed to acclimatize and quarantined for a period of 7 days, so as to adjust them to the experimental diets and the environment. They were later fed the experimental diets for ten weeks. The average feed consumed by the rabbits was collected over a period of ten weeks. Water and experimental diets were given to the rabbits *ad libitum*.

Experimental Diet

Composition of experimental diets is shown in table 1, feed was formulated to meet up the nutritional requirement of the rabbits, were all necessary ingredients were supplied at appropriate inclusion level except *Tithonia diversifolia* leaf meal extract/fibre residue and groundnut cake, which were supplied at varying inclusion level at 0, 5%, 10% and 15%.

Table 1: Composition of experimental diets

| Ingredients | % Inclusion of TDLMEFR | | | |
|------------------|------------------------|------|-----|------|
| | 0% | 5% | 10% | 15% |
| TDLMEFR | - | 1.5 | 3.0 | 4.5 |
| Maize | 40 | 40 | 40 | 40 |
| Groundnut cake | 15 | 13.5 | 12 | 10.5 |
| Wheat offal | 12 | 12 | 12 | 12 |
| Palm kernel cake | 20 | 20 | 20 | 20 |
| Rice bran | 10 | 10 | 10 | 10 |

| | | | | |
|-----------------------------|------|--------|--------|--------|
| Starch | 2.3 | 2.3 | 2.3 | 2.3 |
| Methionine | 0.1 | 0.1 | 0.1 | 0.1 |
| Lysine | 0.1 | 0.1 | 0.1 | 0.1 |
| Salt | 0.25 | 0.25 | 0.25 | 0.25 |
| Premix | 0.25 | 0.25 | 0.25 | 0.25 |
| Total | 100 | 100 | 100 | 100 |
| Calculated Analysis | | | | |
| Crude Protein | 17.0 | 16.84 | 16.60 | 16.37 |
| Crude Fibre | 6.12 | 6.34 | 6.51 | 6.70 |
| Metabolize Energy (kcal/kg) | 2800 | 2789.1 | 2778.8 | 2767.3 |
| Ether Extract (%) | 5.87 | 5.80 | 5.72 | 5.64 |

TDLMEFR: *Tithonia diversifolia* leaf meal extract/fibre residue.

Data Collection

Feed Intake

The rabbits were fed twice daily in the morning and in the evening. The feed given were weighed and leftover were collected and also weighed.

Feed intake per day was obtained as the difference of the initial quantity of feed weighed and the leftover.

Feed Intake = Initial weight of feed (g) - Weight of leftover feed (g)

Feed Conversion Ratio

The feed conversion ratio was computed using this formula;

$$\text{Feed Conversion Ratio} = \frac{\text{Average Feed Intake/Day (g)}}{\text{Average body weight gain/Day (g)}}$$

Protein Efficiency Ratio

Protein efficiency ratio was calculated using the formula below;

$$\text{Protein Efficiency Ratio} = \frac{\text{Gain in body weight (g)}}{\text{protein intake (g)}}$$

Body Weight

The body weights of the rabbits were measured on arrival before they were distributed into the experimental pens. They were therefore, weighed on weekly basis throughout the experimental period.

Body weight gain = final weight - initial weight

Collection of Blood Samples

Blood samples of each rabbit in all the replicates were collected at the 8th week of the experiment through the marginal ear vein of the rabbits in sample bottles containing EDTA anticoagulant which were taken to the laboratory to determine the Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC) and Haemoglobin (HB), Neutrophils (N), Lymphocyte (L), Monocyte (M), Eosinophils (E).

Cost Benefit Analysis

Cost of a kg weight gain = feed conversion ratio × cost of a kg of feed (total cost of other ingredients not including *Tithonia diversifolia* per kg)

Cost of feed consumed = total feed intake in kg × cost of a kg of feed

Experimental Design/Statistical Analysis

The experimental design used was Completely Randomized Design (CRD).

The data collected were subjected to Analysis of Variance (ANOVA) according to Steel and Torrie (1980). The means were separated using Duncan Multiple Range Technique (DMRT) as described by Duncan (1955).

RESULTS

Proximate Analysis of *Tithoniadiversifolia*

Table 2 below shows the proximate composition of *Tithoniadiversifolia* leaf protein fibre residue. The crude protein (CP) value obtained for the proximate analysis was high which could be as a result of the pulping and processing procedures which tend to concentrate the nutrients in the leaf meal extract/fibre residue.

The value of 12.42% crude fibre was recorded; this was due to the fibrous nature of *Tithoniadiversifolia* leaf meal extract/fibre residue. All other parameters are still within the requirement of rabbits.

Table 3 shows the effect of *Tithoniadiversifolia* leaf meal fibre residue on performance characteristics of growing rabbits. There were significant ($p < 0.05$) differences among the treatments for daily feed intake. Rabbits fed D1 & D2 inclusion levels of *Tithoniadiversifolia* leaf protein fibre residue had similar and higher (97.67 g and 97.33 g) mean values, compared to rabbits on D3 & D4 replacement of groundnut cake.

The same trend was followed for the body weight gain and feed conversion ratio. However, the rabbits on 5% inclusion of *Tithoniadiversifolia* leaf protein fibre residue had the best protein efficiency ratio. The body weight gain is lowest in 15% inclusion level of *Tithonia diversifolia* leaf protein fibre residue.

There was significant ($p < 0.05$) difference on the protein efficiency ratio, as treatment 0%, 5% and 10% inclusion levels of *Tithoniadiversifolia* leaf protein fibre residue recorded similar mean values, while treatment four (15%) had the least value.

Table 4 showed results on haematological indices were found to be significant ($p < 0.05$) only on Eosinophil. Rabbits fed D2 were significantly ($p < 0.05$) higher among treatment levels.

Economics of feeding *Tithonia diversifolia* leaf meal extract/fibre residue to growing rabbits.

Table 4 below shows the economics of feeding TDLMEFR to growing rabbits. There were significant ($p < 0.05$) differences on the economics indices of the treatments. The cost of feed per kilogram (N) expressly decreased as the inclusion levels of TDLMEFR increased. However, cost of total feed consumed and cost per kilogram flesh gained were both not significantly ($p > 0.05$) affected by the treatments.

DISCUSSION

The decrease from (97.67g – 96.00g) in daily feed intake (Table 2) as TDLMEFR increased in the diets of the rabbits is in agreement with the report of Adedeji *et al.*, (2012) and Foku, (2014), who reported a reduction in feed consumed by rabbits when *T. diversifolia* leaves were incorporated into their diets. This decrease was associated to low palatability of the diets resulting from the bitter taste of the leaves accompanied by the presence of anti-nutritional factors which includes alkaloids, tannins, and sesquiterpene lactones as well as the bulkiness of the feed which tend to increase with the inclusion levels of TDLMEFR in the diets, the reduced feed intake may also be linked to the report of Bhatt and Swain (2003) that feed consumption of livestock tends to reduce with the presence of anti-nutrients in feed meal as well as the non-palatability of the feed ingredients affecting the rate of feed intake. However, the poor feed intake associated with feeding *T. diversifolia* was attributed to the low palatability as a result of tannin (Togun *et al.*, 2006; Fasuyi and Ibitayo, 2011).

The daily weight gain of the rabbits significantly reduced as the level of *T. diversifolia* increased in the rabbits' diets which was also reported by Fasuyi and Afolabi (2013), who fed pigs with molasses ensiled *T. diversifolia* which resulted in drastic reduced weight gain at the highest inclusion level. However, daily weight gain of 58.67g – 51.67g observed in this study was higher than the report of Foku *et al.*, (2019) who fed treated *T. diversifolia* leaf meal to rabbit. Okorie (2003) and Bhatt and Swain (2003) reported a lower value of 24-30g for daily weight gain of rabbits. This higher value could be attributed to the washing in water to form a pulp before sun drying which would have reduced the bitterness of the meal hence aiding the consumption rate which led to a higher DWG of the rabbit.

The FCR of the control (1.59%) is not different from 1.66% at 5% inclusion level of *T. diversifolia* which indicated that the rabbits were able to convert the feed to meat at the same rate in the 0% and 5% inclusion levels. This is similar to the report of Foku *et al.*, (2019) who also fed rabbits with boiled *T. diversifolia* leaves at varying inclusion rate. However, Fakolade and Adetomiwa (2018) reported that rabbits performs best when *T. Diversifolia* leaves is

incorporated with conventional feed ingredient as diet for rabbits due to the inherent nutrients present. Table 4 shows the effect of *Tithonia diversifolia* leaf protein fibre residue on haematological indices of growing rabbits. Report from literature showed that blood profile are important and crucial for the nutritive component of a given ration (Agbede and Aletor, 2004). All the haematological parameters were not significantly ($p > 0.05$) affected with the inclusion levels of *Tithonia diversifolia* leaf meal extract/fibre residue and the values falls within the recommended haematological range for growing rabbits (Jain, 1986). This could be inferred that all the experimental diets were equally utilized and did not jeopardize the health of the animals.

The cost benefits were cheap for all the experimental fed rabbits and this is because TDLMEFR is a low cost feed raw material when compared to groundnut cake and other protein sources.

CONCLUSION/RECOMMENDATIONS

From this study, TDLMEFR could be incorporate into the diets of growing rabbits up to 5% without showing any adverse effect on the feed intake, daily weight gain, feed conversion ratio and haematological parameters of the growing rabbit.

REFERENCES

- Adedeji, O.S., Amao S.R., Ameen S.A., Olayeni T.B., Ojedapo L.O. and Ayandiran T.A., (2012). The effects of graded levels of wild sunflower meal on growth and blood parameters in weaned rabbits. *Continental Journal of Animal and Veterinary Research*, 4(1): 22-26.
- Agbede, J.O. and Aletor, V.A. (2004). Comparative evaluation of weaning foods from *Glyricidia* and *Leucaena* leaf protein concentrates and some commercial brands in Nigeria. *Journal of Science and Agriculture*. 83: 21-30.
- Ahamefule, F.O., Eduok, G.O., Usman, A., Amaefule, K.U., Obua, B.E., Oguike, S.A., (2006). Blood biochemistry and haematology of weanerrabbits fed ensiled and fermented cassava peel based diets. *Pakistan Journal of Nutrition*, 5 (3) 248 – 253.
- Bhatt, R.S. and Sharma, S.R. (2001). Nutrient utilization and growth performance of broiler rabbit fed oat plant meal and tall fescue hay. *Asian-Australasian Journal of Animal Science*, 14: 1228-1232.
- Bhatt, R.S. and Swain, N., (2003). Effect of graded level of fat supplementation on the growth performance in the rabbits. *World Rabbit Science*, 11 (1): 33-40.

- Dairo, F.A.S. (2008). Performance and haematological evaluation of weaner rabbits fed loofah gourd seed meal *Luffa cylindrical*. *African Journal of Food Agriculture Nutrition and Development*, 8 (4): 451 – 463.
- Duncan, D.B., (1955). Multiple range and multiple F-tests. *Biometrics*. 11:(1), 1–42.
- FAO, (2002). *Production Year Book*. Food and Agricultural Organization, Rome, Italy.
- Fakolade, P.O. and Adetomiwa, A.A. (2018). Performance and carcass characteristics of weaner rabbit fed wild sun flower (*Tithonia diversifolia*) inclusion in their diets. *International Journal of development and sustainability*. 7(8): 2216 – 2226.
- Fasuyi, A.O. and Afolabi, A.A. (2013). Protein supplementation value of sundried sunflower (*Tithonia diversifolia*) in grower pigs. Growth, performance and nitrogen utilization. *African Journals of Science*. 7(9): 344 – 349.
- Fasuyi, A.O., Dairo, F.A.S. and Ibitayo, F.J. (2010). Ensiling wild sunflower (*Tithonia diversifolia*) leaves with sugarcane molasses. *Livestock Research for Rural Development*. 2 (42): 42-48
- Fasuyi, A.O. and Ibitayo, F.J. (2011). Nitrogen balance and morphometric traits of weanling pigs fed graded levels of wild sunflower (*Tithonia diversifolia*) leaf meal. *African Journal of Food and Agriculture, Nutrition and Development*. 11(5):15-20
- Foku, V. K., Defang, H. F., Kana, S. D. A., Amandine, M., Fonteh, F. A. A., Mube, K. H., and Ndukum, J. A. (2019). Effects of graded levels of boiled wild sunflower (*Tithonia diversifolia* Hemsl A. Gray) leaf meal on growth and carcass characteristics of rabbits. *Journal of Animal and Plant Sciences*. 41(2): 6940-6950.
- Foku, V. K., (2014). Growth performance, carcass characteristics, some haematological and bio-chemical parameters of male local rabbits fed graded levels of wild sun flower (*T. diversifolia* Hemsl A. Gray) leave meal. *Department of Animal Production*. PP: 42-62.
- Jain, N. C. (1986). *Schalms Veterinary Haematology (4th Edition)*. Washington square, Philadelphia, USA: Lea and Febiger, p. 600.
- Muriu, J.I., Njoka-Njiri, E.N., Tuitoek, J.N. and Nanua, J.N. (2002). Evaluation of sorghum (*Sorghum bicolor*) as replacement of maize in the diet of growing rabbit (*Oryctolagus cuniculus*) *Asian-Australasian Journal of animal science*. 15: 565-569.
- Okorie, K.C. (2003). The effect of palmitic acid fortified maize wet milling by-product on the performance of weaner rabbits. *Czech. Journal of Animal Science*. 48(9): 365-370.
- Rijal, T.B., Nepali, D.B., Sah, R.A. and Sharma, M.P. (2009). District livestock service office, Chitwan, Rampur. *Nepal Journal of Science and Technology*, 10: 29 – 35.
- Schonfeldt, H.C. and Hall, N.G. (2012). Dietary protein quality and malnutrition in Africa. *British Journal of Nutrition*, 108: S69 – S76.
- Steel, R.G.D. and Torrie J.H. (1980). Principles and procedures of statistics, a biometrical approach.
- Togun, V.A., Farinu, G.O. and Olabanji, R.O. (2006). “Effect of graded level of wild sunflower (*Tithonia diversifolia*). Morphometric characteristics of Genitalia and some organs of Isa Brown Cock at the pubertal age”, *World Applied Science Journal*, 1 (2): 115-121.

Table 2: Proximate composition of *Tithonia diversifolia* leaf meal extract/fibre residue.

| Parameter | Percentage (%) |
|--------------------------------|----------------|
| Dry Matter | 10.40 |
| Crude Fibre | 12.42 |
| Crude Protein | 30.81 |
| Ether Extract | 1.58 |
| Carbohydrate | 29.23 |
| Ash | 5.54 |
| Metabolizable Energy (kcal/kg) | 613.40 |

Table 3: Performance of growing rabbits fed *Tithonia diversifolia* leaf meal extract/fibre residue.

| Parameters | 0% | 5% | 10% | 15% | SEM |
|--------------------------|--------------------|---------------------|--------------------|--------------------|------|
| Daily Feed Intake (g) | 97.67 ^a | 97.33 ^a | 96.33 ^b | 96.00 ^b | 0.27 |
| Daily Weight (g) | 61.67 ^a | 58.67 ^{ab} | 56.33 ^b | 51.67 ^c | 1.34 |
| Feed Conversion Ratio | 1.59 ^a | 1.66 ^a | 1.72 ^b | 1.86 ^c | 0.03 |
| Protein Efficiency Ratio | 3.73 ^a | 3.63 ^{ab} | 3.57 ^{ab} | 3.37 ^c | 0.08 |

^{a,b,c} Means with different superscripts in the same row differs (p<0.05)

Table 4: Haematology indices of growing rabbits fed with *Tithonia diversifolia* leaf meal extract/fibre residue.

| Parameters | 0% | 5% | 10% | 15% | SEM |
|---|---------------------|---------------------|----------------------|----------------------|---------------------|
| PCV (%) | 33.33 | 37.00 | 37.00 | 34.00 | 1.97 |
| Hb (g/100ml) | 9.67 | 9.39 | 9.39 | 8.21 | 0.60 |
| RBC (10 ⁶ /mm ³) | 3.0 | 2.6 | 3.0 | 2.98 | 1.4×10 ⁵ |
| WBC 10 ³ /Cumm | 4.5×10 ³ | 5.4×10 ³ | 4.87×10 ³ | 4.87×10 ³ | 5.4×10 ² |
| MCHC (%) | 29.9 | 25.39 | 25.40 | 24.13 | 1.91 |
| MCH (g) | 0.31 | 0.36 | 0.31 | 0.27 | 0.027 |
| MCV (Um ³) | 1.07 | 1.42 | 1.23 | 1.14 | 0.11 |
| Neutrophil (%) | 35 | 46 | 42.7 | 46 | 6.11 |
| Lymphocyte (%) | 54.33 | 39.67 | 45.33 | 42.67 | 7.01 |
| Eosinophil (%) | 4.62 ^{ab} | 8.0 ^a | 4.0 ^b | 4.67 ^{ab} | 1.07 |
| Monocyte (%) | 5.33 | 6.0 | 8.0 | 6.67 | 1.23 |

^{abc} Means with different superscripts in the same row differs (p<0.05)

Table 5: Cost benefits of feeding *Tithonia diversifolia* leaf meal extract/fibre residue on growing rabbits.

| Parameters (₦) | 0% | 5% | 10% | 15% | SEM |
|---|--------------------|--------------------|--------------------|--------------------|------|
| Cost of feed per kilogram | 88.44 ^a | 87.18 ^b | 86.23 ^c | 84.34 ^d | 0.00 |
| Cost of total feed consumed | 74.15 | 73.56 | 73.99 | 74.09 | 0.36 |
| Cost of total feed per kilogram flesh gain | 140.6 | 144.7 | 148.3 | 156.8 | 10.4 |

^{a,b,c} Means with different superscripts in the same row differs (P<0.05).