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In vitro gas production of compounded ration containing graded levels of *Terminalia catappa* leaf meal

[1]Adedeji, O. Y., [1]Amusan, S. A., [1]Orimogunje, A. A., [2]Faniyi, T. O., [1]Saka, A. A. and [1]Popoola, M. A.

[1]*Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Oyo state, Nigeria.*

[2]*Department of Crop and Animal Science, Ajayi Crowther University, Oyo-Town, Oyo state, Nigeria.*

Corresponding Author: adedejiolayinka27@gmail.com

Abstract

This study investigated the in vitro gas production characteristics of compounded rations containing with graded levels of Terminalia catappa leaf meal (TCLM), to assess its potential as a feed ingredient in ruminant nutrition. Terminalia catappa (Indian almond) is a tropical tree valued for its nutritional potential as a non-convectional feed resource. Four compounded rations with varying levels of TCLM at 0%, 3%, 6% and 9% inclusion levels with other conventional feed ingredients to formulate treatment 1, 2, 3, and 4 respectively. These rations containing varying levels of TCLM were subjected to in vitro gas production analysis using rumen fluid obtained from healthy goats. Parameters such as fermentation rate, and total gas production were measured to assess the effect of TCLM inclusion on the rations. Results revealed that TCLM inclusion had influence on the chemical composition of the experimental diets. Significant differences ($p < 0.05$) in gas volume were observed at 3, 30, 36, 42, and 48 hours, with Treatment 2 (3% TCLM) showing the highest values (4.35, 33.67, 36.67, 39.00, and 42.00 mL/g DM), while the control diet produced the lowest gas volumes except at 3 hours. Carbon dioxide production ranged from 16 to 22 mL/200 mg DM, with the highest value in the control diet (0% TCLM) and the lowest in T_2 (3% TCLM). Methane production varied significantly among treatments, with the highest value observed in T_2 (3% TCLM) and the lowest in T1 (control). It can be concluded that TCLM at 3% inclusion optimizes crude protein content and gas production, indicating its potential as a valuable feed supplement for ruminants.

Keyword*: Terminalia catappa, ruminant, digestibility, gas production, incubation, fermentation, methane*

Introduction

Livestock productivity is often constrained by the use of low-quality feeds that lack the essential nutrients required for optimal rumen microbial fermentation (Birhan and Adugna, 2014). This challenge becomes even more critical during the dry season, when the scarcity of nutritious feed resources further limits animal growth and reproductive performance. To address these limitations, there is increasing interest in evaluating alternative feed ingredients, including tree leaves with promising nutritional and bioactive properties. In this context, the present study investigates the in vitro gas production characteristics of compounded rations formulated with graded levels of *Terminalia catappa* leaf meal, with the aim of assessing its potential as a supplementary feed resource for ruminants.

The dry season is a particularly stressful period for livestock, as limited rainfall results in inadequate pasture and feed availability (Lamidi and Ologbose, 2014). The negative consequences of this period are evident in weight loss, reduced milk production, and increased mortality among animals. Furthermore, malnutrition weakens the immune system, making animals more susceptible to disease outbreaks. In response, nomadic farmers often migrate long distances in search of greener pastures for their herds (Tugjamba *et al*., 2021). During these migrations, livestock are frequently exposed to harsh weather conditions and hazards such as snakebites, which further reduce the productivity of species like goats, cattle and sheep,

The high cost of establishing improved pastures, feed concentrates, and supplements to alleviate dry-season feed shortages is beyond the financial capacity of most smallholder farmers. Consequently, they rely heavily on roadside grazing, browse plants, crop residues, and agro-industrial by-products to sustain their animals during this period. Some of these alternative materials possess great potential as feed resources because they degrade rapidly in the rumen (Nagaraja, 2016), while others exhibit poor degradability, requiring some form of processing before inclusion in livestock diets (John *et al*., 2010).

The limited availability of raw materials for the feed industry has led to a steady rise in production costs, which in turn increases the unit cost of livestock products (Devendra and Leng, 2011). This situation highlights the urgent need to enhance livestock productivity and ensure the availability of affordable, high-quality animal products such as meat and milk. Achieving this goal requires adequate feed supplementation for small ruminants, particularly goats, with emphasis on energy, protein, and mineral sources.

According to Ahamefule *et al.* (2002), addressing the feed shortage in ruminant production calls for the exploration of non-conventional feed resources, especially those that remain available during the dry season. One such potential feed material is *Terminalia catappa*, commonly known as Indian almond or tropical almond. This tree, native to tropical regions, is a low-cost and readily available non-conventional feed resource. It thrives in coastal and moist tropical climates and remains green for much of the year. The leaves of *Terminalia catappa* have been used as feed ingredients due to their nutritional potential, containing appreciable amounts of crude protein, fibre, minerals, and beneficial secondary metabolites. However, studies on its use as livestock feed remain limited (Babayemi, 2007; Nwosu *et al*., 2008), mainly due to a lack of awareness regarding its feeding value and potential to enhance feed availability during the dry season.

The *in vitro* gas production technique is a laboratory method used to evaluate the fermentation characteristics and nutritional potential of feed ingredients or formulated diets (Tolera *et al*., 1997; Larbi *et al*., 1998). This method is cost-effective, time-efficient, and has been widely adopted to estimate dry matter intake (Blümmel and Ørskov, 1993), digestibility (Khazaal *et al*., 1993), and metabolizable energy (Babayemi and Bamikole, 2006). It also allows for more controlled experimental conditions compared to *in vivo* methods (Makkar, 2002). This study was conducted to evaluate the *in vitro* gas production characteristics, methane output, and chemical composition of compounded rations containing graded levels of *Terminalia catappa* leaf meal, with the aim of determining its optimal inclusion level for ruminant nutrition.

Materials And Methods

Source and Processing of *Terminalia catappa* Leaf

A total of 20 kg of fresh *Terminalia catappa* leaves was harvested within the premises of the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Oyo State, Nigeria. Only healthy, disease-free leaves were selected. The leaves were thoroughly washed to remove dirt and debris, then air-dried under shade to prevent loss of phytochemicals from direct sunlight. The dried leaves were subsequently ground into a fine powder to increase surface area. The dried *Terminalia catappa* leaf meal(TCLM) was then incorporated into the experimental diet at varying levels of 0, 3, 6 and 9 %inclusion level with other ingredients to formulate four dietary treatments1, 2, 3, and 4 respectively. The gross composition of the experimental diets is presented in Table 1

Microbiology Laboratory

The *in vitro* gas experiment was carried out at the Ruminant Microbiology Laboratory, Department of Animal Science, University of Ibadan, Ibadan, Oyo state, Nigeria. Freshly plucked *Terminalia catappa* leaves harvested within Federal College of Animal Health and Production Technology, Ibadan were cleaned to remove dirt, air dried for 15 days, milled and stored in an air tight container until when needed.

Collection of Rumen Liquor

Samples of ruminal content were obtained from growing West African Dwarf (WAD) rams placed on mixed ration diet under intensive management system, A representative sample of the rumen fluid from each animal was collected using suction method as described by Babayemi and Bamikole (2006). A soft rubber tube with an internal diameter of 1 cm was inserted through the oesophagus into the rumen, vacuum of 1.05 kg/m was applied to extract the rumen fluid.

Preparation of buffer solution

McDougall's buffer adopted by Babayemi (2007) which consisted of sodium bicarbonate ($NaHCO_3$), sodium phosphate dibasic ($NaHPO_4$), potassium chloride (KCl), sodium chloride (NaCl), and magnesium sulphate ($MgSO_4.7H_2O$) and calcium chloride ($CaCl_2.2H_2O$). The buffer solution was freshly prepared and stored in a dark bottle.

The following were the quantities of reagents used in preparing the buffer:
9.80g $NaHCO_3$ + 2.77g Na_2HPO_4 + 0.57g KCl + 0.47g NaCl + 2.16g $MgSO_4.7H_2O$ + 0.16g $CaCl_2.2H_2O$ + 1g Urea.

The reagents were dissolved in distilled water, calcium chloride was added only after the other reagents were completely in solution. Prior to use, a volume of buffer was warmed at 30^0Cand flushed with $CO_2$to remove Oxygen. During the warming and reducing step, urea was added to the McDougall's buffer at the rate of 1.0g/L

Preparation of liquor-buffer solution

The rumen liquor was strained through a four-layer cheese cloth. Rumen liquor and buffer were mixed together (1:2 v/v) in a 4 liter bucket as inoculum, all under continuous flushing with streams of CO_2.

Preparation of syringes for incubation

To each syringe containing 200 mg of the experimental diet, 30 mL of inoculum was added. Prior to use, the syringes were thoroughly washed, dried, and lubricated with Vaseline to ensure smooth movement of the plunger (Menke and Steingass, 1988). The syringes served as a simulation of the goat rumen environment. Using 50 mL capacity syringes, 30 mL of the inoculum was introduced into the substrate through a silicone tube, after which the plunger was gently pushed upward until the inoculum reached the syringe tip. The silicone tubing was then tightly secured with a Hoffman clip.

Blank syringes were also prepared by dispensing the same volume of the buffer–rumen liquor mixture into labeled syringes without any feed sample. Gas volume recorded from the blanks was deducted from the total gas produced by each sample to determine the net gas production. The gas volume generated from the incubation of 200 mg of feed dry matter over 48 hours was subsequently plotted against incubation time.

Table1: Gross Composition of Experimental Diets

	Inclusion Levels of TCLM			
Ingredients (Kg)	T_1 (0%)	T_2 (3%)	T_3 (6%)	T_4 (9%)
TCLM	0.00	1.59	3.18	4.77
Maize bran	53.00	51.41	49.82	48.23
PKC	5.00	5.00	5.00	5.00
Cassava peel	7.25	7.25	7.25	7.25
Wheat offal	30.00	30.00	30.00	30.00
Limestone	3.00	3.00	3.00	3.00
Salt	1.50	1.50	1.50	1.50
Premix	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00

PKC: Palm kernel cake TCLM: *Terminalia catappa* leaf meal

***In vitro* gas production study**

The rumen liquor was collected from animals using oesophageal technique, by using rubber tube through the mouth of the goat into the rumen and collecting the rumen liquor by suction as described by previous researchers (Babayemi and Bamikole, 2006;Babayemi, 2007). This was done in the morning before the goats were given feed. After collecting a sumptuous amount needed for the analysis, the liquor was poured into a thermo flask that has been preheated with hot water to maintain the temperature of the rumen liquor while taking it to the laboratory. And to remove the solids, the liquor was sieved through 4 layers of cheese cloth. Carbon dioxide gas was bubbled into the liquor through a thin tube from a gas cylinder. The carbon dioxide gas was introduced to maintain the anaerobic condition of the liquor to keep the needed microbes alive and to perform optimally by the total expulsion of oxygen gas. This is because methanogens cannot function in an oxygen environment and to also to avoid the death of the anaerobes in the liquor.

Duration of incubation

The syringes were incubated at 39 ± 1^0C for 48 hours. The volume of gas produced was determined by the measurement of the piston displacement level during the period of incubation. Gas readings were recorded at 3hoursinterval from0 to 48 h after incubation.

Estimation of methane gas produced

Methane production from the substrate was estimated by introducing 4 mL of 10 M NaOH immediately after the syringes were removed from the incubator, using a 5 mL capacity syringe. The content was inserted into the silicon tube, which was fastened to the 60 ml capacity syringe. The clip was then opened while the NaOH was gradually released. The content was shaken vigorously/agitated while the plunger began to shift position to occupy the vacuum created by the absorption of CO_2. The volume of methane was read on the calibration. To determine the actual gas produced, the average of the volume of gas produced from the blanks was deducted from the volume of gas produced per sample (Fievez *et al.*, 2005 and Babayemi, 2007).

Chemical analysis

Subsamples of the experimental diets were taken to the laboratory for proximate analysis according to AOAC (2002) while neutral detergent fibre, acid detergent fibre and acid detergent lignin were determined as reported by Van Soest *et al.* (1991).

Statistical analysis

Data obtained were subjected to one way analysis of variance (SAS, 2005), where significant means were separated using Duncan's multiple range test at 5% level of significance

Results

The chemical composition of the experimental diets was presented in Table 2. The Dry matter content ranged from 88.21to89.88%. Crude protein content increased as the levels of *Terminalia catappa* leaf meal increased in the diets. The Ether extract values ranged from 2.21 to 3.19%.

Table 2: Chemical Composition of Experimental Diet

Parameters (%)	Inclusion levels of TCLM			
	T_1 (0%)	T_2 (3%)	T_3 (6%)	T_4 (9%)
Dry matter	88.21	89.12	89.88	89.88
Crude protein	10.97	12.29	12.67	14.42
Ether extract	2.71	2.99	2.21	3.19
Ash	9.53	9.61	9.68	9.58
Nitrogen Free Extract	56.69	57.24	54.41	56.21
Neutral Detergent Fibre	66.00	64.00	64.67	66.67
Acid Detergent Fibre	31.67	34.00	26.00	29.33
Acid Detergent Lignin	6.67	6.33	4.67	6.67
Hemicellulose	34.33	30.33	38.67	37.34
Cellulose	25.00	27.67	21.33	22.66

TCLM: *Terminalia catappa* leaf meal

Gas production of compounded rations containing graded levels of *Terminalia catappa* leaf meal is presented in Figure 1. The carbon dioxide (ml/200mg/DM) production ranged from 16 to 22 among the dietary treatm ents. Methane output was highest in T2 (3% TCLM) and lowest in the control diet (T1, 0% TCLM).

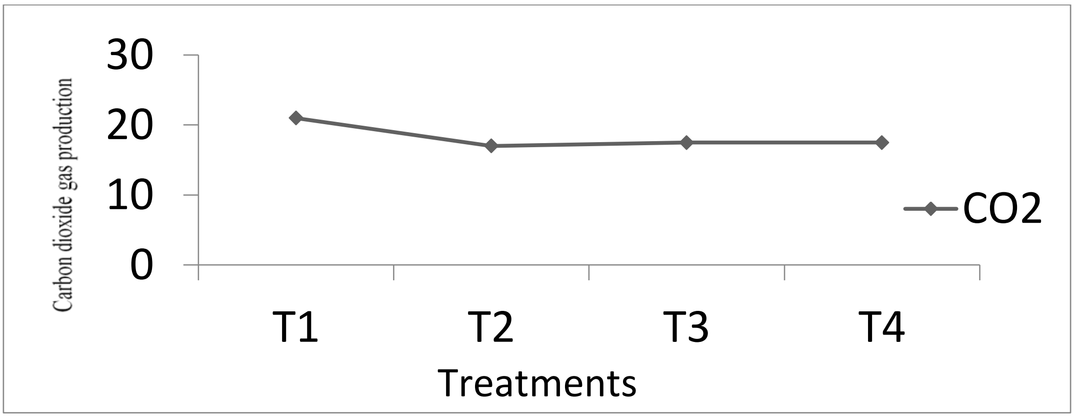

Fig 1: Carbon dioxide gas production of compounded ration containing graded levels of *Terminalia catappa* leafmeal

The Methane gas production of compounded ration containing graded levels of *Terminalia catappa* leaf meal is presented in Figure 2. The highest value recorded was in T_2 with 3% TCLM while the lowest value was obtained in the control diet T_1 (0% TCLM)

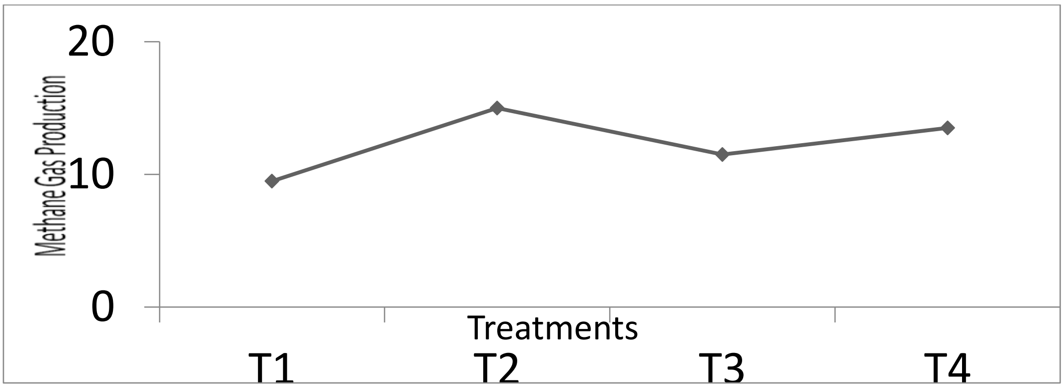

Fig 2: Methane gas production of compounded ration containing graded levels of TCLM

Gas production at different incubation timefor ration containing graded level o containing graded levels of *Terminalia catappa* leaf meal is presented in Table 3. The results at 3, 30, 36, 42, and 48 hours indicated that there were significant differences ($p<0.05$) with T_2 having the highest value of (4.35, 33.67, 36.67, 39.00 and 42.00) respectively and the control having the lowest value except at the first 3 hours. There were no significant differences among others parameters. However the cumulative gas volume at 48 hours of incubation ranged between 30.00 and 42.00M/g/DM.

Table 3: *In vitro* Gas Production of Compounded ration containing Graded levels of *Terminalia catappa* Leaf meal

	Inclusion level of TCLM				
Time (hrs)	T_1 (0%)	T_2 (3%)	T_3 (6%)	T_4 (9%)	SEM
3	3.65^{ab}	4.35^{a}	3.50^{ab}	3.10^{b}	0.19
6	6.70	8.55	5.97	6.63	0.44
9	12.50	13.00	9.33	10.93	0.66
12	14.00	16.67	13.00	14.00	0.77
15	16.67	21.00	17.17	18.33	1.06
18	18.33	24.67	21.00	22.00	1.46
21	20.33	27.67	24.17	25.17	1.44
24	22.33	30.33	27.00	27.00	1.53
30	23.67^{b}	33.67^{a}	30.00^{a}	30.00^{ab}	1.62
36	26.67^{b}	36.67^{a}	32.00^{ab}	32.00^{ab}	1.53
42	28.67^{b}	39.00^{a}	35.33^{ab}	35.33^{ab}	1.59
48	30.00^{b}	42.00^{a}	37.67^{ab}	37.67^{ab}	1.70

^{a,b} means along the same row with different superscripts are significantly different ($p<0.05$)

Discussion

The crude protein (CP) content of the diets in this study was found to be above 8%, which meets the minimum requirement necessary for efficient rumen function in ruminants. Norton (2003) emphasized that feeds containing less than 8% crude protein fail to provide sufficient ammonia for optimal microbial activity in the rumen. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) values of the concentrate diets recorded in this study were higher than those reported by Daodu and Babayemi (2009). The higher crude protein and ash content in T_2 may have contributed to the increased gas production observed in this treatment. Bamikole and Babayemi (2004) noted that the rate of gas production during fermentation largely depends on the nature and composition of the fibre fraction. Generally, feeds with lower fibre and lignin contents tend to have greater intake and digestibility, as excessive lignification hinders the breakdown of structural carbohydrates. According to Combe (1981), lignin forms a physical barrier that prevents enzymatic action and microbial attachment, possibly explaining the lower gas production observed in T_1. Furthermore, Okoruwa and Njidda (2012) highlighted that understanding the crude fibre composition of a diet is essential for evaluating its fibre fractions and predicting its overall nutritive value.

The *in vitro* gas production results showed that treatment T_2 recorded the highest gas volume, ranging from 4.35 to 42.00 mL, while the control diet (T_1) produced the lowest gas volume, ranging from 3.65 to 30.00 mL. Significant differences were observed at 3, 30, 36, 42, and 48 hours of incubation. Generally, feedstuffs with a high gas production capacity are often associated with increased methane generation. Methane production represents a loss of energy to the animal and has also been linked to environmental concerns, as many tropical feedstuffs are known to promote methanogenesis (Babayemi and Bamikole, 2006), thereby contributing to global warming.

Among the treatments, the control diet (T_1) exhibited the lowest methane production but the highest carbon dioxide output, whereas T_2 showed the highest methane production and the lowest carbon dioxide output. Additionally, it was observed that gas production increased progressively with incubation time, a trend consistent with the findings of Delgado *et al.* (2010).These findings indicate that TCLM can serve as a useful supplement in ruminant diets.

Conclusion

It can therefore be concluded that *Terminalia catappa* leaf meal (TCLM) can be supplemented at 3% inclusion level in compounded ration for ruminants, as it positively influenced crude protein content and gas production during *in vitro* fermentation.

Recommendation

It is hereby recommended that further research should be carried out on factors influencing methane production in diets containing *T. catappa* leaf meal and to explore nutritional or microbial strategies for its mitigation. In addition, in vivo feeding trials should be conducted to evaluate animal performance, nutrient utilization, and economic feasibility of TCLM inclusion in ruminant diets.

References

Association of Official Analytical Chemist (2002). AOAC official methods of Analysis. Appendix G: Guidelines for Collaborative study procedures to validate characteristics of a method of analysis. 12p.

Ahamefule, F. O., Beawchi, J. A. and Ajala, A. A. (2002). Intake, digestibility and nitrogen balance studies of potato peel yeast slurry diets by West African dwarf goats. Proc. of 27th *Ann. Conf. Nig. Soc. For Anim*. Prod. (NSAP). Federal University of Technology Akure, Nigeria.

Babayemi, O. J. (2007). *In vitro* fermentation characteristics and acceptability by West African dwarf goats of some dry season forages. *African Journal of Biotechnology*. 6(10): 1260-1265.

Babayemi, O. J. and Bamikole, M. A. (2006). Supplementary value of *Tephrosia bracteolta, Tephrosia candida, Leucaena leucocephela* and *Gliricida sepium* hay for West African dwarf goats kept on range. *Journal of Central Europe Agricultural,* 7(2):323-330.

Bamikole, M. A. and Babayemi. O. J (2004). Feeding goats with guinea grass-verano stylo and nitrogen fertilized grass with energy concentrate. *Arch zootech* 53; 13-23.

Birhan, M. and Adugna, T. (2014). Livestock feed resources assessment, constraints and improvement strategies in Ethiopia. *Middle-East Journal of Scientific Research,* 21(4): 616-622.

Blummel, M. and Orskov, E. R. (1993). Comparison of *in vitro* gas production and nylon bag degradability of roughages in predicting feed intake in cattle. *Animal Feed Science and Technology* 40; 109-119.

Coombe, J. B. (1981). Utilization of low quality residues in F.H.W. Morley, Grazing Animals. *World Ani. Sc.* Elsevier Scientific Publishing Company, Amsterdam, *Netherland*, 319-331.

Daodu, M. O. and Babayemi, O. J. (2009).Utilization of some hedges-Row plants as Forage in Nigeria. *Parkistan Journal of Nutrition,* 8:1269-1274.

Delgado, D. C., Gallindo, J., Gonzalez, R., Savon, L., Scull, I., Gozaleaz, N. and Marrero, Y. (2010). Potential of tropical plants to exert defaunating effects on the rumen and to reduce methane production. In: Odongo, N. E., Garcia, M., Viljoen, G. J. (Eds). Sustainable improvement of animal production and health. IAAEA/FAO. Rome, Italy.

Fievez, V., Babayemi, O. J. and Dameyer, D. (2005). Estimation of direct and indirect gas production in syringe, a tool to estimate short chain fatty acid production requiring minimal laboratory facilities. *Animal Feed Science and Technology* 123-124.

John, A. B., Yahaya M. S. and Julius, N. (2010). Effect of Sorrel Seed (Hibiscus Sabdariffa) Supplementation on the Performance of Yankasa Rams Fed *Brachiaria decumbens* as Basal Diet. *Journal of Agriculture and Veterinary Sciences.*2: 8-16

Khazal, K., Dentinbo M. T., Ribeiro, J. M. and Orskov, E. R. (1993). A comparison of gas production during incubation with rumen contents in *in vitro* digestibility and the voluntary intake of hays. *AnimalProduction.* 57; 105-112.

Lamidi, A. A. and Ologbose, F. I. (2014). Dry Season Feeds And Feeding: A Threat To Sustainable Ruminant Animal Production In Nigeria. *Journal of Agriculture and Social Research,* Vol. 14 (1): 17- 30

Larbi, A., Smith, J. W., kurdi, I. O., Raji, A. M. and Ladipo, D. O. (1998). Chemical composition, rumen degradation and gas production characteristics of some multipurpose fodder trees and shrubs during wet and dry seasons in the humid tropics. *Animal Feed Science Technology* 72(1-2): 81-96.

Makkar, H. P. S. (2002). Application of the *in vitro* gas method in the evaluation of feed resource and enhancement of nutritional value of Tannin rich tree/browse leaves and agro-industrial by-products. Proceding of the final review meeting of the IAEA. Technical Co-operation regional AFRA project organized by the joint FAO/IAEA division of nuclear technique in food and agriculture held on 25-29 November, 2000 Cairo, Egypt. Pp 23-40

Menke, K. H. and Steingass, H. (1988).Estimation of the energetic feed value from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research Development,* 28: 7-55.

Nagaraja, T. G. (2016). Microbiology of the rumen. In *Rumenology* (pp. 39-61). Cham: Springer International Publishing.

Norton, B. W. (2003).The nutrient value of tree legumes http.www.Fao.org/ag/agpc/doc/publicat/gult-shel/x555oj.htm.page1-10.

Nwosu, F. O., Dosumu, O. O. and Okocha, J. O. C. (2008)The potential of Terminalia catappa (Almond) and Hyphaene thebaica (Dum palm) fruits as raw materials for livestock feed. *African Journal of Biotechnology*. 7 (24): 4576-4580.

Okoruwa, M. I. and Njidda, A. A. (2012). Rumen characteristics and nitrogen utilization of West African Dwarf sheep as influenced by guinea grass and dried pineapple pulp. *Pakistan Journal of Nutrition*, 21:580-583.

Statistical Analysis System (SAS) Institute Incorporation (2005).SAS/STAT. Users guide Version 8. 3rd Ed. Cary. North Carolina, USA. Pp.944/

Tolera, A. and Said, A. N. (1997). *In vitro* gas production techniques were widely used to evaluate the nutritive value of forages used in ruminant nutrition. Pp: 56-61.

Tugjamba, N., Walkerden, G. and Miller, F. (2021). Adaptation strategies of nomadic herders in northeast Mongolia: climate, globalisation and traditional knowledge. *Local Environment*, *26*(4): 411-430.

Van Soest, P. J., Robertson, J. B. and Lewis B. A. (1991). Methods for dietary fibre, Neutral detergent fibre, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science.* 74(10): 3583 – 3597.