Nigerian J. Anim. Sci. 2021, 23 (1): 219-228

Irrigation and manure application influence on *in vitro* digestibility of herbaceous legumes

***Ojo, V. O. A., Rafiu, M. A., Odunaye, B. T., [1]Adelusi, O. O., Adetokunbo, G. A., Adeyemi, T. A. and [1]Olagoke, K. O.**

Department of Pasture and Range Management and [1]Department of Animal Nutrition, Federal University of Agriculture, Abeokuta, P. M. B. 2240, Abeokuta, Nigeria.

***Corresponding Author:** *ojovoa@funaab.edu.ng*, **Telephone Number:** *2348037178044*

***Target Audience:** *Forage Scientists, Ruminant Nutritionists*

Abstract

The study was conducted to investigate the effects of swine manure rates (0, 5, 10 tonha^{-1}) and irrigation frequencies (2 and 4 days interval) on in vitro fermentation characteristics of three herbaceous forage legumes (Lablab purpureus, Mucuna pruriens and Vigna unguiculata). The experiment was a factorial design with three replicates. The legumes were harvested 12 weeks after sowing. The results showed no significant ($p > 0.05$) difference in crude protein content for all the parameters determined while lowest neutral detergent fibre content was 450.00 g kg^{-1} in legumes fertilized with 10tonha^{-1} manure and the highest was 482.78 g kg^{-1} in unfertilized legumes. Tannin contents ranged ($p < 0.05$) from 3.60 g kg^{-1} in V. unguiculata to 6.60 g kg^{-1} in M. pruriens. Application of 10 tonha^{-1} manure caused ($P<0.05$) higher total anaerobic bacteria count (TABC) (1.65 x10^{6} cfu/ml), dry matter (63.98%) and crude protein (59.5 %) digestibility to be produced by the legumes compared to other manure rates. Lablab purpureus had highest TABC (1.13 x10^{6} cfu/ml) while M. pruriens recorded highest DMD (62.22%) and CPD (58.00%). Legumes irrigated at 2 days intervals had higher TABC content (1.13 x10^{6} cfu/ml) while legumes irrigated at 4 days interval had higher DMD (57.91%) and CPD (52.50%). In conclusion, application of 10 tonha^{-1} manure rate at 4 days irrigation frequency of the legumes will go a long way to improve the digestibility and availability of nutrients to ruminants.

***Keywords:** *irrigation, manure, nutrient digestibility, rumen fermentation and volatile fatty acids.*

Description of Problem

Ruminant productivity in tropical smallholder farming systems faces serious constraints, since majority are raised on natural pastures which decline rapidly in quality and quantity during dry season. Often the most limiting nutrient is protein (**1**). This results to irregularities in growth and seasonal variations in weights of animals. Poor condition of livestock in the tropics during this period is more likely as a result of inefficient digestion in the rumen and utilization of nutrients absorbed from low quality feeds (**2**). However, when ruminal fermentation is limited by a deficiency in degradable N compounds, a reduced protein degradation in the rumen may have detrimental effects on carbohydrate fermentation (**3**). Availability of high-quality sown forage such as leguminous crops is important since it supplies the nutrients that are lacking in grassland pastures (**4**).

The feed shortage by the ruminants can be met by deliberate cultivation of forages. However, many grasslands are characterized by low productivity (5). To maintain the quality of the legumes, provision of irrigation and manure for the plants will go a long way to supply the needed nutrients throughout the dry season. Organic manures are noted to improve the soil nutrient status thereby providing plants with better nutrient uptake that is eventually beneficial to animals that feed on such plants for body maintenance and production. Such a profitable use will help in addressing the problem of manure management and disposal (**6**). Water stress affects plant growth in different ways such as causing impaired germination and poor stand establishment (7). Drought-induced reduction in leaf area is ascribed to suppression of leaf expansion through reduction in photosynthesis (8). A common adverse effect of water stress on plants is the reduction in fresh and dry biomass production (9). In order to alleviate the scarcity of feeds for ruminants especially during the dry season, there is need to supply water artificially (irrigation) and nutrients to the soil for easy plant uptake.

The rumen ecosystem consists of a consortium of anaerobic micro-organisms which includes the bacteria, fungi and protozoa. These microbes are responsible for the initial fermentative digestion of feeds consumed by ruminants to produce simpler constituents from which the animal derives vital nutrients. These microbes survive in the rumen under different constraints which may be either natural or feed associated as some of the feeds contain a significant amount of anti-nutritional factors, which sometimes limit the growth of some of these natural microbial inhabitants (10).

The objective of this study therefore, is to determine fermentation characteristics, microbial contents and digestibility of *Lablab purpureus*, *Vigna unguiculata* and *Mucuna pruriens* as influenced by irrigation frequency and manure application rates using the gas production technique.

Materials and Methods

Field experiment and plant materials

The experiment was carried out at the Pasture Unit of College of Animal Science and Livestock Production farm, Federal University of Agriculture, Abeokuta (FUNAAB), Nigeria in November, 2014. This lies between the savannah agro-ecological zone of Western Nigeria (Latitude 7°, 12^{1} N, Longitude 3°, 20^{1}E, average annual rainfall of 1037mm). The climatic data for the experimental field during the study was presented in Figures 1. Prior to planting, soil samples of the experimental area were randomly collected from the plots at the depth of 0-15 cm using soil auger to determine the nutrient content of the soil.

Experimental design and procedures

The experiment was arranged in a 3 x 3 x 2 factorial in a randomized complete block design with plots measuring $12m^2$ and replicated three times. The factors were three herbaceous legume species (*Lablab purpureus*, *Mucuna pruriens* and *Vigna unguiculata*), three swine manure application rates (0, 5, 10 $tonha^{-1}$) and two irrigation frequencies (2 days and 4 days interval).

The land was cleared and fine seed plots prepared. Swine manure was collected from the Piggery Section of the Directorate of University Farms, FUNAAB and air dried under shed for two weeks and sub-sample taken from the manure and analyzed prior to application to determine the nutrient composition. The manure was later applied by broadcasting to individual plot according to treatment in one application. The plots were left for two weeks to rest before sowing of the legumes. The analyzed result reveals that the manure contained Calcium (116.54 mg/l), Magnesium (65.58 mg/l), Potassium (19.1 mg/l), Sodium (20.47 mg/l), Nitrogen (1.66 mg/l), Phosphorus (45.47 mg/l), Manganese (2.77 mg/l), Iron (45.68 mg/l), Copper (0.65 mg/l) and Zinc (2.13 mg/l).

The herbaceous legumes seeds were sourced from National Animal Production Research Institute, Zaria. A germination test was carried out so as to know the viability of the seeds. The seed rates were 20kg ha^{-1} for *L. purpureus*, 20 kg ha^{-1} for *V. unguiculata* and 40 kg ha^{-1} for *M. pruriens*. Spot planting method at 0.5m spacing was used. Water was applied at field capacity at every irrigation frequency (2 and 4 days interval) with average volume of 120,000 litre/ha throughout the period of the experiment.

Chemical analyses

Forages were harvested 5 cm above the ground level twelve weeks after sowing and sub samples were taken, weighed, oven-dried at 65 °C to constant weight and milled through a 1 mm sieve for laboratory analyses of proximate composition (11), fibre fractions (12) and tannin contents (13).

The *in vitro* gas production was determined following the procedure of 14. Two hundred (200) mg of the milled leaf samples of each legume were measured into fibre bags, tied and put into 100 ml glass syringes fitted with plungers. Each sample was replicated ten times. After 24 h of incubation, the fibre bags were washed under running tap water to stop the fermentation. Residues were oven-dried at 105 °C for 24 hours. The dry residues were weighed and dry matter digestibility calculated while crude protein and neutral detergent fibre contents of the residues of the legumes after the *in vitro* were determined according to 11 to get the nutrients digestibility of the legumes.

The pH of the *in vitro* supernatant was determined using Hanna pH meter, model 211. Thirty (30) ml of the supernatants were decanted into separate plastic bottles and sub-fractioned into two sets for the determination of ammonia nitrogen and volatile fatty acids (VFA) concentration using 15 and 16 procedures respectively. The second sub-portion was fixed with 10% formalin solution in a sterilized 0.9% saline solution for the enumeration of total bacteria, protozoa and fungal zoospore according to the method of 17.

Statistical analysis

All data obtained were subjected to the analysis of variance (ANOVA). Means were separated using Duncan's Multiple Range Test, 18 package.

Results and Discussion

The physico-chemical characteristics of the composite soil sample taken from 0 to 15 cm depths of the experimental site contained 0.95% total nitrogen, 0.75% organic carbon, and 2.85 mg/kg phosphorus. Typically, with tropical soils, the soil of the experimental site was low in both total N and available P. Low levels of these elements in most tropical soils have been the major cause of low dry-matter yields of tropical forages and have prompted attention to the use of animal manures so as to enhance soil quality to improve forage productivity.

Table 1 shows the effect of manure rate and irrigation frequency on the chemical composition of *M. puriens*, *L. purpureus* and *V. unguiculata* (g kg^{-1}). Legumes fertilized with manure recorded higher CP contents than unfertilized ones. The CP content recorded for legumes in this study surpassed the threshold of 6.0% (60 g kg^{-1}) required by rumen microbes to build their body protein (19). High protein in the diet and especially in the forage should be desired as it largely determines the intake and digestibility (20). The higher CP content of the legumes was supported by 21 who reported that one of the greatest contributions of legumes, is their potential to fix atmospheric nitrogen, resulting in forage of better nutritional quality and improvement of soil fertility.

Table 1: Effect of swine manure rates and irrigation frequencies on the chemical composition of *M. pruriens, L. purpureus* and *V. unguiculata*

Factors	DM	CP	EE	Ash	NFC	NDF	Tannin
	g kg^{-1}						
Manure rates (tonha^{-1})							
0	937.50	134.91	95.58^{b}	99.17^{b}	192.87	482.78^{a}	4.30^{b}
5	942.50	143.21	96.83^{ab}	118.33^{b}	184.57	476.67^{a}	5.00^{a}
10	948.33	154.90	98.58^{a}	160.00^{a}	174.37	450.00^{b}	5.10^{a}
SEM	5.99	7.13	0.68	10.04	9.57	5.97	0.40
Species							
Mucuna pruriens	936.67^{b}	133.95	97.83^{a}	93.33^{c}	197.38^{a}	477.50	6.60^{a}
Lablab purpureus	958.33^{a}	150.38	97.58^{ab}	154.17^{a}	175.53^{b}	461.94	4.10^{b}
Vigna unguiculata	933.33^{b}	148.68	95.58^{a}	130.00^{b}	179.12^{b}	470.00	3.60^{b}
SEM	5.51	7.19	0.73	10.53	9.71	7.04	0.20
Irrigation frequencies							
2	941.11	150.84	97.00	103.33^{b}	169.94^{b}	478.89^{a}	4.90
4	944.44	137.84	97.00	148.33^{a}	197.95^{a}	460.74^{b}	4.90
SEM	4.96	5.65	0.64	8.80	7.57	5.59	0.40
P- value							
Manure	0.3966	0.1405	0.0227	<.0001	0.3773	0.0007	0.0562
Species	0.0051	0.2021	0.0743	<.0001	0.2098	0.1963	<.0001
Irrigation frequencies	0.6076	0.1157	1.0000	<.0001	0.0119	0.0117	0.4518
Species x manure rates	<.0001	0.0071	0.0460	<.0001	0.0126	0.0071	0.5423
Species x irrigation	0.0227	0.1720	0.4449	<.0001	0.0218	0.0432	0..2089
Manure rates x irrigation	0.3896	0.0003	<.0001	<.0001	0.0629	<.0001	0.2916
Species x manure rates x Irrigation	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0039

[a,b,c] Means on the same column having different superscripts are significantly different (P<0.05), SEM- Standard error of mean

The NDF content of legumes obtained in this study was higher than that of *Phaseolus calcaratus* hay (42.5 %) as reported by 22. The differences could be attributed to the age of plant because the amount of NDF in forage increased linearly with the age of plant. Increased in NDF and ADF in stems indicated the formation of tannin– fibre complexes that are not solubilized in the acid detergent solution (23).

Tannins are phenolic compounds that form complexes particularly with proteins and thus may act as anti-nutritional agent at higher concentrations (>50 g/kg plant DM) and reduce voluntary feed intake (24). Low condensed tannin concentrations (3.60–6.60 g kg^{-1}) obtained in this study may have no negative effect upon voluntary feed intake nor may not reduce protein solubility (22).

Table 2 shows the effects of irrigation frequency and manure rate on the *in vitro* nutrients digestibility of herbaceous legume species. Digestibility of DM and CP was highest (P<0.05) in legumes fertilized with 10 tonha^{-1} manure while it was lowest in NDF content. Highest CP content of legumes fertilized with 10 tonha^{-1}of manure played significant role in improving digestibility of protein as crude protein digestibility (CPD) was highest in legumes fertilized with 10 tonha^{-1}of manure. This result was in line with the report by 25 who stated that concentration of CP in a feedstuff affect its digestibility. The values for CPD in this study ranges from 42.5% – 59.5% which compares with the report by 26 for *Dolichos lablab* (56.5%). Highest CPD could be attributed to high uptake of nitrogen from soil rich in manure which makes the nutrient more available in legume parts for ruminant utilization.

Table 2: Effect of swine manure rates and irrigation frequencies on the CPD, DMD and NDFD of *M. pruriens, L. purpureus* and *V. unguiculata* using *in vitro* gas production technique

Factors	DMD (%)	CPD (%)	NDFD (%)
Manure (tonha^{-1})			
0	52.98^{b}	42.50^{c}	45.36^{a}
5	52.99^{b}	50.50^{b}	42.42^{b}
10	63.98^{a}	59.50^{a}	41.59^{c}
SEM	4.70	0.56	1.33
Species			
Mucuna pruriens	62.22^{a}	58.00^{a}	48.56^{a}
Lablab purpureus	45.32^{b}	51.00^{b}	42.11^{b}
Vigna unguiculata	62.22^{a}	44.50^{c}	38.69^{c}
SEM	4.33	0.55	0.94
Irrigation frequencies			
2	55.39^{b}	49.50^{b}	43.79^{a}
4	57.91^{a}	52.50^{a}	42.45^{b}
SEM	0.05	0.49	1.11
P-value			
Manure	<.0001	<.0001	<.0001
Species	<.0001	<.0001	<.0001
Irrigation frequencies	<.0001	<.0001	<.0001
Species x manure rates	<.0001	<.0001	<.0001
Species x irrigation	<.0001	<.0001	<.0001
Manure rates x irrigation	<.0001	<.0001	<.0001
Species x manure rates x Irrigation	<.0001	<.0001	<.0001

^{abc}: Means in same column with different superscripts are significantly ($p<0.05$) different
SEM = Standard Error of Mean; CPD = Crude protein digestibility; DMD = Dry matter digestibility; NDFD = Neutral detergent fibre digestibility

The total volatile fatty acids obtained in this study (Table 3) were higher than values of 80.9 to 93.4 mmol L^{-1} reported in ruminal fluid of cattle fed cassava foliage by 27. It represents a good profile with values between a minimum of 2mg/100ml^{-1} (28) and a maximum of 30mg 100ml^{-1} (29) suggested for maximum microbial growth in the rumen. The pH range (6.3 to 6.6) obtained across treatments were within the normal range (pH 6 to 7) reported as optimal for microbial digestion of fibre and protein (30, 31).

Acetic and propionic acid contents obtained in the *in vitro* supernatant was higher than the values 66.35-70.85 mol/100mol and 20.75-23.4 mol/100mol for acetic and propionic acids respectively reported by 32 when sheep were fed different tropical roughages. The variation observed could be as a result of higher nutrients in the legumes compared with the roughages.

Table 3: Effects of swine manure rate and irrigation frequencies on the fermentative characteristics of *M. pruriens, L. purpureus* and *V. Unguiculata* using *in vitro* gas production technique

Factors	Acetic acid (%)	Butyric acid (%)	Propionic acid (%)	Volatile fatty acid (%)	Ammonia N (%)	Temp (^{0}C)	pH
Manure (tonha^{-1})							
0	1.41^a	0.14^a	0.94^a	112.0^c	7.85^b	24.55^a	6.64^a
5	0.93^b	0.09^b	0.61^b	163.0^a	7.12^c	24.52^{ab}	6.42^b
10	1.08^{ab}	0.10^{ab}	0.72^{ab}	139.0^b	7.97^a	24.49^b	6.38^c
SEM	0.16	0.02	0.01	11.0	0.26	0.06	0.04
Species							
Mucuna pruriens	1.53^a	0.15^a	1.02^a	129.0^b	6.66^c	24.50^b	6.53^b
Lablab purpureus	0.95^b	0.09^b	0.63^b	142.0^a	8.19^a	24.33^c	6.59^a
Vigna unguiculata	0.94^b	0.09^b	0.63^b	141.0^a	8.08^b	24.72^a	6.32^c
SEM	0.14	0.02	0.09	22.0	0.21	0.04	0.04
Irrigation frequencies							
2	1.04	0.10	0.69	155.0	7.73^a	24.60^a	6.45^b
4	1.25	0.12	0.83	187.0	7.56^b	24.44^b	6.52^a
SEM	0.13	0.01	0.09	19.00	0.22	0.04	0.05
P-value							
Manure	0.5623	0.5623	0.5623	0.5623	<.0001	0.0030	<.0001
Species	0.0842	0.0842	0.0842	0.0842	<.0001	<.0001	<.0001
Irrigation frequencies	0.5087	0.5087	0.5087	0.5087	<.0001	<.0001	<.0001
Species x manure rates	0.0064	0.0064	0.0064	0.0064	<.0001	<.0001	<.0001
Species x irrigation	0.0773	0.0773	0.0773	0.0773	<.0001	<.0001	<.0001
Manure rates x irrigation	0.9387	0.9387	0.9387	0.9387	<.0001	<.0001	<.0001
Species x manure rates x irrigation	0.1353	0.1353	0.1353	0.1353	<.0001	<.0001	<.0001

[a, b]: Means in same column with different superscripts are significantly ($p<0.05$) different
SEM = Standard Error of Mean

Manure rate and irrigation frequency showed ($P<0.05$) effect on the microbial contents of the selected herbaceous legumes were significant ($P<0.05$) (Table 4). Highest total anaerobic bacteria count (TABC) ($P<0.05$) value ($1.65x10^6$ CFC/ml) was recorded for legumes fertilized with 10tonha $^{-1}$ manure rate while the least value ($0.33x10^6$ CFC/ml) was found in legumes fertilized with 5tonha^{-1} of manure. Protozoa population was highest in *V. unguiculata* ($2.91x10^3$ cell/ml), unfertilized legumes (3.36 $x10^3$ cell/ml) and legumes with 2 days irrigation frequency ($3.11x10^3$ cell/ml) ($P<0.05$). It is possible that high condensed tannins (CT) contained in *M. pruriens* forage with lower Protozoa content ($2.13x10^3$ cell/ml) may play an important role in decreasing Protozoa population. In general, CT could lower methanogenesis through reductions of numbers of protozoa and methanogens (33). Consequently, reduction of protozoa and methanogens population could decrease greenhouse gases. 34 had reported that condensed tannin altered rumen ecology and increased microbial protein synthesis.

Table 4: Effect of swine manure rates and irrigation frequencies on the microbial contents of *M. pruriens*, *L. purpureus* and *V. Unguiculata* using *in vitro* gas production technique

Factors	TABC (x10^6 CFU/ml)	TFC (x10^6 CFU/ml)	TPC (x10^3 cell/ml)
Manure (tonha^{-1})			
0	0.78^b	0.03^b	3.36^a
5	0.33^c	0.07^a	1.90^c
10	1.65^a	0.01^c	2.33^b
SEM	0.15	0.01	0.39
P-value	0.00	0.00	0.00
Species			
Mucuna pruriens	0.68^c	0.00^c	2.13^c
Lablab purpureus	1.13^a	0.03^b	2.55^b
Vigna unguiculata	0.95^b	0.09^a	2.91^a
SEM	0.21	0.01	0.42
P-value	0.00	0.00	0.00
Irrigation frequencies			
2	1.13^a	0.06^a	3.11^a
4	0.53^b	0.02^b	1.95^b
SEM	0.14	0.01	0.32
P-value			
Manure (tonha^{-1})	<.0001	<.0001	<.0001
Species	<.0001	<.0001	<.0001
Irrigation frequencies	<.0001	<.0001	<.0001
Species x manure rates	<.0001	<.0001	<.0001
Species x irrigation	<.0001	<.0001	<.0001
Manure rates x irrigation	<.0001	<.0001	<.0001
Species x manure rates x Irrigation	<.0001	<.0001	<.0001

[a,b,c]: Means in same column with different superscripts are significantly ($p<0.05$) different
SEM= Standard Error of Mean; TABC - Total anaerobic bacteria count; TFC-Total fungi count; TPC – Total protozoa count.

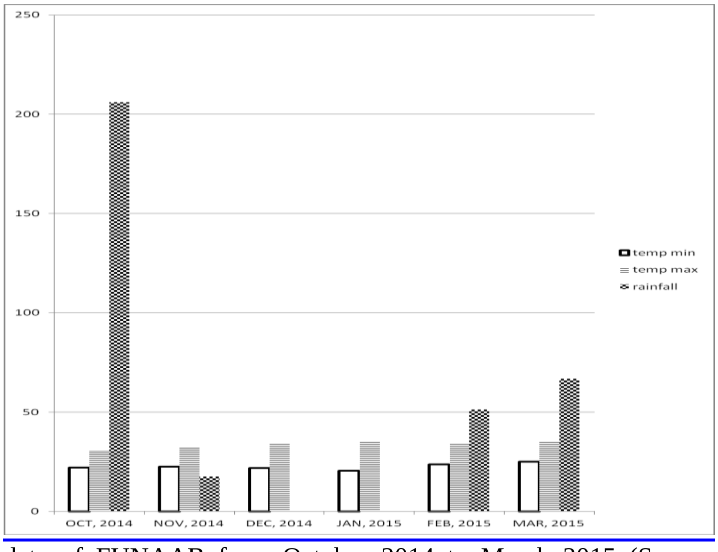

Figure 1: Climatic data of FUNAAB from October 2014 to March 2015 (Source: Agro meteorology Department, FUNAAB)

The low fungi count obtained in the digested rumen supernatant of this study could be due to low fibre content of these legumes since 35 showed that fungi were more prevalent in ruminants fed high fibre diets than in those fed less fibrous ones.

Conclusion and Applications

1. It can be concluded that different manure application rates and irrigation frequencies have significant effect on the digestibility and microbial contents of herbaceous legumes.
2. Legumes fertilised with 10 $tonha^{-1}$ manure and irrigated at 4days interval recorded higher nutrients digestibility as well as microbial contents.

Conflict of interest: The authors declare that they have no conflict of interest.

References

1. Minson, D. J. 1990. Forage in ruminant nutrition. Academic Press Inc., London, UK., 483.
2. Leng, R. A. 1997. Tree foliage in ruminant nutrition. FAO, Animal production and health paper 139, FAO Rome, Italy.
3. Hess, H. D., Monsalve L, M., Lascano, C. E., Carulla, J. E., Dıaz, T. E. and Kreuzer, M. 2003. Supplementation of a tropical grass diet with forage legumes and Sapindus saponaria fruits: effects of *in vitro* ruminal nitrogen turnover and methanogenesis. *Australian. Journal of Agricultural Research,* 54: 703–713.
4. Bamikole, M. A., Akinsoyinu, A. O., Ezenwa I., Babayemi O.J. Akinlade J. and Adewumi M. K. 2004. Effect of six-weekly harvests on the yield, chemical composition and dry matter degradability of *Panicum maximum* and *Stylosanthes hamata* in Nigeria. *Grass and Forage Science,* 59: 357.
5. Adeyemi, T. A., Adeoye, S. A., Ogunyemi, T. J., Adedeji, E. A., Oluyemi, B. and Ojo, V. O. A. (2021). Comparisons of nutrient solutions from organic and chemical fertilizer sources on herbage yield and quality of hydroponically produced maize fodder, *Journal of Plant Nutrition,* 44:9, 1258-1267, DOI:10.1080/01904167.2020.1845382
6. Ojo, V. O. A., Dele, P. A., Olanite, J. A., Adeoye, S. A., Ajayi, S. O. and Idowu, O. M. O. 2013. Influence of manure type on the productivity of *Pennisetum purpureum* under different planting method and age. *Journal of Organic Agriculture and Environment* 1 (1):71 - 77.
7. Odunaye, B. T., Ojo, V.O.A., Adetokunbo, G. A., Okukenu, O. A., Rafiu, M. A., Adeniran, M. M., Adeoye, S. A., Amodu, J. T., Arigbede, O.M. and Busari, M. A. 2015. Effects of animal manure rates and irrigation frequencies on the growth responses and dry matter yield of selected herbaceous legumes. In: J. A. Olanite, O. S. Onifade and A. O. Jolaosho (eds.). Towards s sustainable utilization of forage and grassland resources for improved livestock production in Nigeria. *Proceedings of the 1st Biennial Conference of Society for Grassland Research and Development in Nigeria*, Abeokuta, 6th – 9th 2015, pg 91 – 94.
8. Rucker, K. S., Kvien, C. K., Holbrook, C. C., and Hook, J. E. (1995). Identification of peanut genotypes with improved drought avoidance traits. *Peanut Science,* 24: 14-18.
9. Zhao, T. J., Sun, S., Liu, Y., Liu, J. M., Liu, Q., Yan, Y. B and Zhou, H. M. (2006). Regulating the drought-

responsive element (DRE)-mediated signaling pathway by synergic functions of trans-active and trans inactive DRE binding factors in *Brassica napus*. *Journal of. Biological Chemistry,* 281: 10752-10759.

10. Kamra, D. N. 2005. Rumen Microbial Ecosystem. *Current Science,* 89 (1): 124-135).
11. AOAC. 1995. Official Methods of Analysis of the Association of Official Agricultural Chemists, 16th ed. AOAC, Arlington, VA. USA.
12. Van Soest, P. J., Robertson, J. B. and Lewis, B. A. 1991. Methods for dietary fibre and Nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74: 3583–3597.
13. Price, M. L. and Butler, L. G. 1977. Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grains. *Journal of Agricultural and Food Chemistry,* 25:1268-1273.
14. 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 and Development,* 28: 7-55.
15. AOAC. 1990. Association of Official Analytical Chemists. Official methods of Analyses. 15th edn. Arlington, VA.
16. Samuel, M, Sagathewan. S, Thomas, J. and Mathen, G. 1997. An HPLC method for estimation of volatile fatty acids of ruminal fluid. *Indian Journal of Animal Sciences* 69: 805–07.
17. Baker, F. J. and Breech, M. R. 1986. Total and viable counts, Miles and Mistra Method. Medical Microbiological Techniques. page 419– 23.
18. SAS. 1999. User's Guide: Statistics, Version 5 Edition. SAS. Inst. Cary, NC.
19. Van Soest, P. J. 1994. *Nutritional ecology of the ruminant,* 2nd edn. Ithaca, NY USA: Comstock Publishing Associates/Cornell University Press. 476pp.
20. Babayemi, O. J., Daniel, I. O., Bankole, M. A., Ogungbesan, A. and Oduguwa, B. O. 2003. Preliminary studies on Tephrosia species: effect of seed treatments on germination. *Nigeria Journal of Animal Production* 30(2):209-216.
21. Miranda, C. H. B., Vieira, A. and Cadisch, G. 2003. Determinação da fixação biológica de nitrogênio no amendoim forrageiro (Arachis spp.) por intermédio da abundância natural de ^{15}N. *Revista Brasileira de Zootecnia*, 32(6) 1859-1865.
22. Foiklang, S., Wanapat, M. and Toburan, W. 2011. Effects of various plant protein sources in high-quality feed block on feed intake, rumen fermentation, and microbial population in swamp buffalo. *Tropical Animal Health and Production,* 43:1517–1524. DOI 10.1007/s11250-011-9836-y
23. Gatachew, G., Gravetto, G. M. and Fondevelia, M. 2001. Laboratory variation of 24 hour *in vitro* gas production and estimated metabolizable energy value of ruminant feed. *Animal Feed Science and Technology,* 102: 169-172.
24. Barahona, R., Lascano, C. E., Cochran, R., Morill, J. and Titgemeyer, E. C. 1997. Intake, digestion and nitrogen utilization by sheep fed tropical legumes with contrasting tannin concentration and astringency. *Journal of Animal Science*, 75 (6): 1633-1640.
25. McDonald, P., Edwards, R. A. and Greenhailgh, J. P. D. 1987. Animal Nutrition. pp. 154–176. 4th edn. Longman Scientific and technical

Company, England.
26. Jaffe, W. G. 1950. Toxicity of raw kidney beans. Experientia, 5: 81.
27. Khang, D. N. 2004. Cassava foliage as a protein source for cattle in Vietnam. Doctoral thesis. Agraria 471. Swedish University Agric. Sci. Uppsala, Sweden ISSBN 1401-6249 ISBN 91-576-6752-7.
28. Satter, L. D. and Slyter, L. L. 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. *The British Journal of Nutrition*, 32, 199-208.
29. Srinivas, B. and Gupta, B. 1997. Rumen fermentation, bacterial and total volatile fatty acids production rates in cattle fed on urea-molasses-mineral block licks supplements. *Animal Feed Science and Technology*, 65(1-49): 275-286.
30. Firkins, J. L. 1996. Maximizing microbial protein-synthesis in the rumen. *Journal of Nutrition,* 126: 1347–54.
31. Wanapat, M. 1999. Feeding of Ruminants in the Tropics Based on Local Feed Resources. P. 236. Khon Kaen Publishing Company Ltd., Khon Kaen, Thailand.
32. Akinbode, R. M., Isah, O. A., Oni, A. O., Adewumi, O. O. and Omoniyi, L. A. 2014. Effect of different tropical roughages on nutrient digestibility and rumen fermentation parameters of West African Dwarf sheep during dry season. *Indian Journal of Animal Sciences*, 84 (10): 1105–1108.
33. Patra, A. K. and Saxena, J. 2009. Dietary phytochemicals as rumen modifiers: a review of the effects on microbial populations. *Antonie van Leeuwenhoek*, 96, 363–375.
34. Makkar, H. P. S., Blummel, M., Beckar, K. 1995. *In vitro* effect of and interaction between tannins and saponins and intake of tannin in rumen. *Journal of Science and Food Agriculture*. 69: 841 – 93.
35. Bauchop, T. 1979. Rumen anaerobic fungi of cattle and sheep. *Applied Environmental Microbiology*, 38:148.