ISSN 0189-0514
J. Anim. Prod. Res. (2016) 28(2):28-36

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INFLUENCE OF SOLE GRASSES AND GRASS-LEGUME MIXTURES ON QUALITY ESTIMATION USING *IN VITRO* GAS PRODUCTION TECHNIQUE

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ABSTRACT

The research was carried out to evaluate the effect of forage mixtures on the nutritive quality of *Stylosanthes hamata* legume, *Panicum maximum* and *Pennisetum purpureum* grasses. *Stylosanthes hamata* was harvested at five months after sowing while *P. maximum* and *P. purpureum* were harvested at eight weeks after planting. The experiment was a completely randomised design with forage proportion of 100% grass or legume and grass-legume mixture of 50%:50%, 75%:25%, 25%:75% replicated three times. There were significant ($P<0.05$) differences in all the parameters investigated. The crude protein content ranged from 8.94 % in sole *P. maximum* to 14.12 % in sole *S. hamata* while neutral detergent fibre ranged from 42.00 in sole *S. hamata* to 65.00% in sole *P. purpureum.* Sole *S. hamata* produced the highest gas at 48 h of incubation while the least gas volume was recorded for sole grasses. Increased gas production was observed in the mixtures than in sole grasses. Metabolisable energy value ranged from 6.20 MJ kg^{-1} DM^{-1} in sole *P. purpureum* to 9.09 MJ kg^{-1} DM^{-1} in sole *S. hamata*. Potential gas production from the insoluble fraction of the forage species ranged from 15.65 in sole *P. maximum* to 17.01 ml/200mgDM in sole *S. hamata*. From this study it can be concluded that grass-legume mixtures are the most appropriate feed for ruminants based on their higher nutritive qualities than in sole grasses.

Keywords: sole grasses, grass-legume mixtures, *in vitro* gas production, quality

INTRODUCTION

Forages are the major and cheapest sources of nutrients for ruminants and they continue to represent the most important feed resources for livestock in the developed and developing countries.

However, a shortage of affordable feeds of adequate quality and quantity, particularly during the dry season is a major obstacle to improving production. One of the logical approaches to alleviate forage scarcity is by pasture improvement vis-a-vis incorporation of forage legumes into native grass swards or grass / legume mixed pasture cultivation (Ajayi, *et al.*, 2007).

Grasses are most common forage species in the derived savannah region of Nigeria. Under good conditions, its nutritional value is high, having up to 12.5 % crude protein, total digestible nutrients (TDN) of 10.2 % and calcium, phosphorus and magnesium (McDonald *et al.*, 1988). However, during the dry season, the crude protein content can be as low as 4% which is too low for good animal performance.

Herbaceous forage legumes have been identified as potential protein supplements for ruminants since they contain high crude protein (150 - 300 g/kg DM), minerals and vitamins needed for the growth of ruminal microbes (Norton and Poppi, 1995). Protein supplementation of grass diets containing 70 g CP / kg DM or less has been reported to increase dry matter intake, dry matter digestibility and animal performance (Umuna *et al.*, 1995). Forage legumes can be grazed, harvested and fed fresh or stored as hay or silage (Harricharan *et al.*, 1988). Legumes can maintain their quality even to the dry season.

The low nitrogen (N) content of most mature grasses points to a need to combine them with forage with a high N content. The ideal N concentration in the rumen for efficient digestion has been variously estimated at 50-70 mg/litre (Satter and Slyter, 1974) and at 150-200 mg/litre (Krebs and Leng, 1984). A sustainable way of improving the feeding value of poor quality pastures is through supplementation with forage legumes (Andrea and Pablo, 1999).

The *in vitro* gas production method is a laboratory estimation of degraded feeds which are important in livestock nutrition. It is a method that is reproducible and parameters obtained correlate well with *in vivo* method. *In vitro* gas production method have the advantage of not only being less expensive and less time consuming, but the method allows experimental conditions more precisely than the *in vivo* method (Makkar, 2002).

The objective of this study was to determine the best legume-grass mixtures at different proportion as feed resources for ruminants during the dry season by evaluating the chemical composition and *in vitro* gas production technique.

MATERIALS AND METHODS

The experiment was conducted at the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The site is situated in the derived savanna agro-ecological zone of South Western Nigeria (latitude: 7°N, longitude 3.5°E, 75 m above sea level, average annual rainfall: 1037 mm). Mean monthly temperature ranges between 25.70 ^{0}C in July and 30.20ºC in February (Google Earth, 2012).

Stylosanthes hamata legume were harvested at five months after sowing while *Panicum maximum* and *Pennisetum purpureum* grasses were harvested at eight months after planting from 15cm above ground level, during the late rainy season of 2012. The forages were then mixed together at different proportions and sub sample of 300g where oven -dried at 65^{o}C until a constant weight was attained to determine the dry matter percentage. The dried samples were milled through a 1mm sieve and they were used for chemical composition determination and *in vitro* gas production.

Experimental Design

The experiment was a completely randomized design (CRD) and replicated three times. The treatments were as follows:

100% *Panicum maximum*

100% *Pennisetum purpureum*

100% *Stylosanthes hamata*

25% *Panicum maximum:* 75% *Stylosanthes hamata*

50% *Panicum maximum*: 50%*Stylosanthes hamata*

75% *Panicum maximum*: 25% *Stylosanthes hamata*

25% *Pennisetum purpureum:* 75% *Stylosanthes hamata*

50% *Pennisetum purpureum:* 50% *Stylosanthes hamata*

75% *Pennisetum purpureum:* 25% *Stylosanthes hamata*

Chemical analyses and *in vitro* gas production measurement

Proximate composition such as dry matter contents, crude protein, ether extract and ash of the dry samples were determined according to AOAC (2006) while non-fibre carbohydrate was calculated as NFC=100-CP-Ash-EE-NDF. Fibre fractions analyses of neutral detergent fibre, acid detergent fibre and acid detergent lignin of the forages were determined according to the procedure of Van Soest *et al*. (1991). Cellulose was calculated as the difference between acid detergent fibre and acid detergent lignin, while hemicellulose was calculated as the difference between neutral detergent fibre and acid detergent fibre. *In vitro gas* production was determined following the procedure of Menke and Steingass (1988).

Statistical analysis

Data collected were subjected to analysis of variance and the treatment means were separated using Duncan's Multiple Range Test (SAS, 2002) package.

RESULTS AND DISCUSSION

Table 1 showed the proximate composition of grass-legume mixtures at different proportions. There were significant differences ($P<0.05$) in all the parameters investigated. The crude protein (CP) contents of sole *S. hamata* legume was significantly ($P<0.05$) highest, followed by grass-legume mixtures and finally the sole grasses. The CP content of the sole *S. hamata, P. maximum* and *P. purpureum* and their different proportion were above the critical limit (60 g/kg) required by rumen microbes to build their body protein for effective digestion of forages by ruminants, below which intake of forages and rumen microbial activity would be adversely affected (Van Soest, 1994). The range of 65-80g/kg was also prescribed for optimum level for meeting the protein requirement of ruminants so as to generate a high level of ammonia in the rumen from degradable protein to ensure an efficient digestion process. The value of the CP reported for the sole *S. hamata was* lower than 18.05% in *S. guianensis* reported by Ajayi and Babayemi (2008). The CP content of *P. maximum* in this study, was higher than 7.02 g/100 g DM reported by Bamikole *et al*. (2003) and 8.50 g/100 g DM reported by Arigbede *et al*. (2005). The CP value for *P. purpureum* in this study was lower than 11.9% reported by Naginene and Abdullah (1992). Meanwhile, the CP contents of the sole legume and the grass-legume mixtures at different proportions were higher than CP contents in the sole grasses. This agreed with the report of Zemenchik *et al.* (2002) that improvement in forage nutritive values were substantially greater for mixtures than with the grass monoculture. The differences recorded in this study with reports

of authors elsewhere, could be as a result of different ages of the forages, climatic data and the soil types of the study areas.

Table 1: Effect of grass-legume mixtures on the proximate composition of *Stylosanthes hamata, Panicum maximum* and *Pennisetum purpureum*

Factors	DM	CP	EE	ASH	NFC
	←		(%)		→
25% *S. hamata*+ 75% *P. maximum*	95.50^{ab}	9.91^{de}	8.50^{a}	5.50^{a}	20.90^{f}
50% *S. hamata*+ 50% *P. maximum*	95.50^{ab}	10.63^{d}	9.00^{b}	4.00^{b}	24.37^{b}
75% *S. hamata*+ 25% *P. maximum*	92.50^{d}	12.11^{b}	7.00^{ab}	6.50^{a}	20.39^{g}
25% *S. hamata*+ 75% *P .purpureum*	95.00^{abc}	10.42^{d}	7.53^{ab}	7.50^{b}	22.56^{d}
50% *S. hamata*+ 50% *P. purpureum*	96.50^{a}	10.81^{cd}	8.80^{a}	6.50^{a}	17.59^{h}
75% *S. hamata*+ 25% *P. purpureum*	94.50^{bc}	11.96^{bc}	8.90^{a}	7.00^{a}	22.14^{e}
100% *S. hamata*	92.50^{d}	14.12^{a}	9.11^{a}	8.50^{a}	26.27^{a}
100% *P. maximum*	95.00^{abc}	8.94^{e}	4.90^{c}	3.50^{b}	23.66^{c}
100% *P. purpureum*	93.50^{cd}	9.10^{e}	6.00^{bc}	4.50^{b}	14.59^{i}
SEM	0.30	0.32	0.33	0.35	1.48

^{a,b,c,d,e,f,g,h} means with different superscript are significantly different (P<0.05)
DM: Dry matter, CP: Crude protein, EE: Ether extract, NFC: Non fibre carbohydrate,
SEM: Standard Error of Mean

The non fibre carbohydrate (NFC) recorded in this study for all the forage mixtures as well as their sole, fell within the range (127-259 g/kg) reported by Anele *et al*. (2009). The NFC content of the forages were high enough which indicate that they can be easily degraded or fermented as NFC is a crude estimate of the carbohydrate pool that differ in digestibility from NDF.

Table 2 showed the fibre composition of grass-legume mixtures at different proportion. Highest significant (P<0.05) NDF content was recorded for sole *P. purpureum* grass (65.00 %), followed by the grass/legume mixtures and the least value was recorded for sole *S. hamata* legume. The range of NDF for these forages were not above the range of 600-650g/kgDM suggested as the limit above which intake of tropical feeds by ruminants would be limited (Van Soest *et al*., 1991*).* The NDF values were however, within the range of 24 – 61 reported for tropical forages (Topps, 1992) except in sole *P. purpureum* grass. Akkasaeng *et al*. (1989) reported similar value of 42.10 % NDF for *E. cyclocarpum* a leguminous plant which was comparable with 42 % of NDF recorded for sole *S. hamata* in this study. All the forage mixtures and the legumes were richer in soluble carbohydrate (NDF) than in sole grasses. Sole *S. hamata* legume and forage mixtures of 75% *S. hamata*+ 25% *P. purpureum* recorded significant (P<0.05) lower ADF values compared with sole grasses and other grass/legume mixtures. The implication of this is that, higher energy will be supplied to animals that are fed with forages that contain lower ADF. Slater (1991) reported that ADF of forages and silages should be within 22-50 %. The ADF contents of forages in this study were within the range, indicating that the diets have potentials to supply needed energy to animals during dry season. Ibrahim and Olaloku (2000) reported that higher cellulose content is undesirable because rumen micro-organisms are unable to degrade it. The values of cellulose in this study for various sole forages and their mixtures are around the values of 30 to 40 % that are adequate for the maintenance requirement of ruminant livestock.

Table 2: Effect of grass-legume mixtures on the fibre composition of *Stylosanthes hamata, Panicum maximum* and *Pennisetum purpureum*

Factors	NDF	ADF	ADL	Hemicellulose	Cellulose
	←		(%)		→
25% *S. hamata*+ 75% *P. maximum*	56.00^{c}	37.00^{ab}	7.14^{cd}	19.00^{d}	29.86^{b}
50% *S. hamata*+ 50% *P. maximum*	52.00^{de}	36.00^{a}	6.00^{e}	16.00^{f}	30.00^{a}
75% *S. hamata*+ 25% *P. maximum*	54.00^{cd}	36.00^{a}	6.00^{e}	18.00^{e}	30.00^{a}
25% *S. hamata*+ 75% *P .purpureum*	52.00^{de}	34.00^{b}	7.00^{cd}	18.00^{e}	27.00^{f}
50% *S. hamata*+ 50% *P. purpureum*	56.30^{bc}	36.00^{ab}	7.23^{c}	20.30^{c}	28.17^{d}
75% *S. hamata*+ 25% *P. purpureum*	50.00^{e}	29.00^{c}	6.30^{de}	21.00^{b}	22.70^{g}
100% *S. hamata*	42.00^{f}	28.00^{c}	6.00^{e}	14.00^{g}	22.00^{h}
100% *P. maximum*	59.00^{b}	38.00^{a}	9.07^{b}	21.00^{b}	28.93^{c}
100% *P. purpureum*	65.00^{a}	39.00^{a}	10.43^{a}	26.00^{a}	28.57^{e}
SEM	1.19	0.76	0.30	0.64	0.57

^{a,b,c,d,e} means with different superscript are significantly different (P<0.05)
NDF: Neutral detergent fibre, ADF: Acid detergent fibre, ADL: Acid detergent lignin,
SEM: Standard Error of Mean

Sole legumes as well as grass-legume mixtures in this study recorded higher values of nutrients which makes them to be positively correlated to crude protein concentration and negatively correlated to NDF concentration (Zemenchik *et al*., 2002).

Figure 1 showed the *in vitro* gas production of grass-legume mixtures and their soles. *Stylosanthes hamata* recorded highest gas production followed by the mixtures, while the least gas was from the sole grasses. Feeding of legume forages generally lead to higher intakes and animal production than grass silages of comparable digestibility (Dewhurst *et al*., 2003). Rumen microbes require a source of fermentable nitrogen, usually as ammonia (Russell and Baldwin, 1978) which must have been supplied better by the legumes, hence the highest gas production. Moreover, higher gas production could have been as a result of higher CP contents of the sole legume and the mixtures above the sole grass. Digestibility has been reported to be synonymous to *in vitro* gas production (Fievez *et al*., 2005) so that the higher the gas produced, the higher the digestibility.

It was observed that, sole *P. maximum* and *P. purpureum* had the lowest gas production compared to sole *S. hamata* and grass/legume mixtures. This might be as a result of higher fibre contents in them. Higher gas production of *P. maximum* above *P. purpureum* could be because it tissue is principally made up of carbohydrate which can be degraded easily by microbes compared with *P. purpureum* grass.

However, it has been reported that the high rates of release of soluble protein and breakdown to small particles from herbaceous legumes is associated with susceptibility of ruminants to bloat (Dewhurst *et al*., 2009). Feeding of legume species alone to the ruminants is not advisable because of the problem of bloat. Moreover, it has been reported that, the efficiency of N used declined with increasing proportion of legumes in animal diets. So, higher legume proportion in ruminants diets should be discouraged because it leads to waste of nitrogen (Dewhurst *et al*., 2009). The low nitrogen (N) content of most mature grasses points to a need to combine them with forage with a high N content. The ideal N concentration in the rumen for efficient digestion

has been estimated at 50-70 mg/litre (Satter and Slyter, 1974) and at 150-200 mg/litre (Krebs and Leng, 1984).

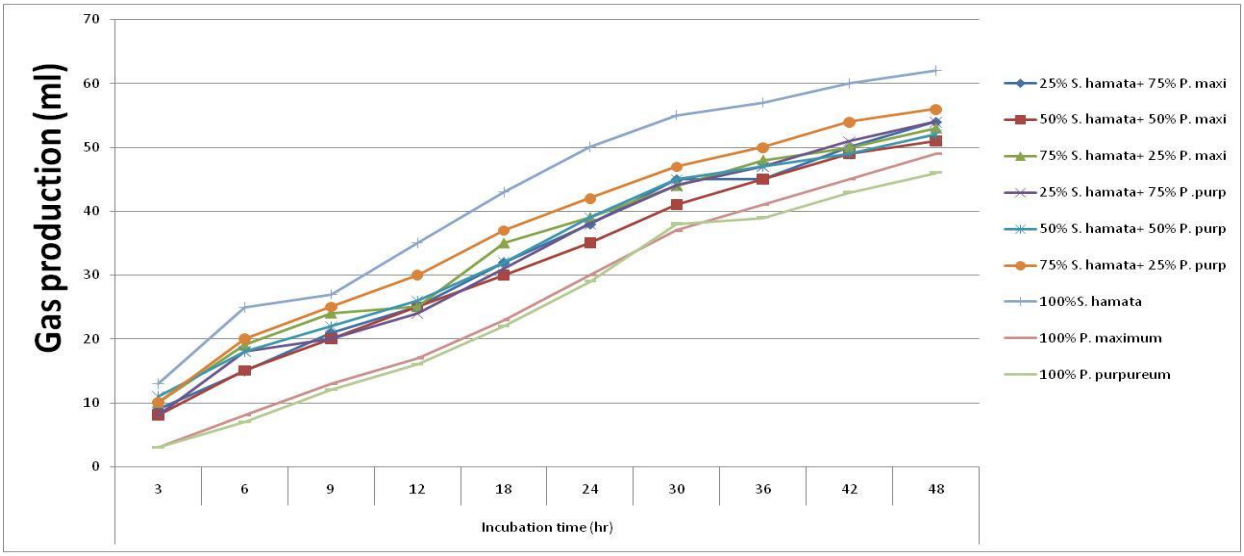


Fig. 1: *In vitro* gas production of grass-legume mixtures at different proportions and incubation time.

Table 3 showed the fermentative characteristics of grass-legume mixtures at different proportions. There were significant differences ($P<0.05$) in all the parameters investigated. Metabolizable energy (ME) and Organic matter digestibility (OMD) of the grass-legume mixtures were better than in sole grasses. This observation was in line with the report of Babayemi and Bamikole (2006). The low ME and OMD observed in the grasses could be as a result of the higher NDF they contained. The ME and OMD mean values obtained in this study, were higher than ranges of 2.99-4.75 MJ/kg DM and 21.46-33.80 %, respectively reported by Babayemi and Bamikole (2006).

The grass-legume mixtures had higher short chain fatty acids contents in this study compared to the sole grasses. This implies that more energy would be made available to animals when grass is supplemented with legumes in the diet of ruminants.

Intake of a feed is mostly explained by the fractional rate of gas production which affects the rate of passage of feed through the rumen. The relatively higher value for sole *S. hamata* legume and the grass/legume mixtures above the sole grasses for fractional rate of gas production may be attributed to higher nutrient composition of the legume over the grasses (Jessop and Herrero, 1996) while Ajayi and Babayemi (2008) reported higher values for grass-legume mixtures compared to sole legumes which was not in agreement with the results obtained in this study.

Table 3: Effects of grass-legume mixtures on the *in vitro* fermentative characteristics of *Stylosanthes hamata, Panicum maximum* and *Pennisetum purpureum*

Treatments	b(ml/200mgDM)	c(ml/hr)	SCFA(µmol)	ME(MJKg^{-1})	OMD(%)
25% *S. hamata*+ 75% *P. maximum*	16.21^{b}	0.73^{cd}	0.85^{c}	7.44^{c}	56.36^{bc}
50% *S. hamata*+ 50% *P. maximum*	15.82^{c}	0.65^{d}	0.78^{d}	7.03^{d}	62.17^{ab}
75% *S. hamata*+ 25% *P. maximum*	15.75^{c}	0.84^{bc}	0.87^{c}	7.56^{c}	59.23^{b}
25% *S. hamata*+ 75% *P .purpureum*	15.71^{c}	0.70^{d}	0.85^{c}	7.43^{c}	58.23^{b}
50% *S. hamata*+ 50% *P. purpureum*	15.68^{c}	0.88^{b}	0.87^{c}	7.57^{c}	58.65^{b}
75% *S. hamata*+ 25% *P. purpureum*	15.66^{c}	0.86^{b}	0.94^{b}	7.99^{b}	62.81^{ab}
100% *S. hamata*	17.01^{a}	1.03^{a}	1.14^{a}	9.09^{a}	71.22^{a}
100% *P. maximum*	15.65^{c}	0.02^{e}	0.66^{e}	6.36^{e}	47.85^{c}
100% *P. purpureum*	15.95^{c}	0.03^{e}	0.63^{e}	6.20^{e}	48.05^{c}
SEM	4.39	0.07	0.03	0.16	1.60

^{a,b,c,d} means with different superscript are significantly different (P<0.05)

b: Volume of gas produced in time (t), c: Fractional rate of gas production, SCFA:Short chain fatty acid, ME: Metabolizable energy, OMD: Organic matter digestibility, SEM: Standard Error of Mean

CONCLUSION

1. Higher nutritive values of grass-legume mixtures in this study indicated that it will help to improve ruminants productivity and performance when they are fed.
2. Mixtures of grasses and herbaceous legumes can be harvested during the rainy season, conserved and utilized as supplements for ruminants feeding during the dry season so as to maintain their body weight.

REFERENCES

Ajayi, F.T. and Babayemi, O.J. (2008). Comparative *in vitro* evaluation of mixtures of *Panicum maximum* cv Ntchisi with stylo (*Stylosanthes guianensis*), Lablab (*Lablab purpureus*), Centro (*Centrosema pubescens*). *Livestock Research for Rural Development* 20 (6) 83. http://www.lrrd.org/lrrd20/6/ajay20083.htm

Ajayi, F.T., Babayemi, O.J. and Taiwo, A.A. (2007). Effects of *Stylosanthes guianensis* and *Aeschynomene histrix* on the yield, proximate composition and *in situ* dry matter and crude protein degradation of *Panicum maximum* (Ntchisi). *Livestock Research for Rural Development* 19 (32). http://www.lrrd.org/lrrd19/3/ajay19032.htm

Akkasaeng, R., Gutteridge, R.C. and Wanapat, M. (1989). Evaluation of trees and shrubs for forage and fuelwood in Northeast Thailand. *International Tree Crops Journal 5,* 209-220.

Andrea M. M. and Pablo E. C. (1999). A tropical forage solution to poor quality ruminant diets: A review of *Lablab purpureus*. *Livestock Research for Rural Development* http://www.lrrd.org/lrrd11/2/colu112.htm

Anele, U. Y., Arigbede, O. M., Südekum, K.-H., Oni, A. O., Jolaosho, A. O., Olanite, J. A., Adeosun, A. I., Dele, P. A., Ike, K. A. and Akinola, O. B. (2009). Seasonal chemical composition, *in vitro* fermentation and *in sacco* dry matter degradation of four indigenous multipurpose tree species in Nigeria. *Animal Feed Science and Technology* 154: 47–57.

AOAC (2006). Official Methods of Analysis of the Association of Official Agricultural Chemists, 18th ed. (1st revision). AOAC International, Gaithersburg, MD, USA.

Arigbede O. M., Olanite J. A. and Bamikole M. A. (2005). Intake, performance and digestibility of West African dwarf goats supplemented with graded levels of *Grewia pubescens* and *Panicum maximum*. *Nigerian Journal of Animal Production,* 32: 293-300.

Babayemi O. J. and Bamikole M. A. (2006). Effects of *Tephrosia candida* DC Leaf and its mixtures with Guinea grass on the *in vitro* fermentation changes as feed for ruminants in Nigeria. *Pakistan Journal of Nutrition*, 5 (1): 14-18.

Bamikole M. A., Ikhatua U. J., Anota B. and Omaduvie F. (2003). Feed intake, feed utilization and weight gain of West African dwarf goats fed varying proportions of *Ficus religosia* and *Panicum maximum*. Proceedings of the 28th Annual Conference of the Nigerian Society of Animal Production (NSAP), held in I.A.R.&T, Obafemi Awolowo University, Moor Plantation, Ibadan, March 16-20, 2003. Pp 274 ¨C 278.

Dewhurst R. J., Fisher W. J., Tweed J. K. S. and Wilkins R. J. (2003). Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. *Journal of Dairy Science*, 86(8), 2598-2611.

Dewhurst, R.J., L. Delaby, A. Moloney, T. Boland and E. Lewis. (2009). Nutritive value of forage legumes used for grazing and silage. *Irish Journal of Agricultural and Food Research*, 48: 167–187.

Fievez V., Babayemi O. J. and Demeyer D. (2005). Estimation of direct and indirect gas production in syringes: a tool to estimate short chain fatty acid production requiring minimal laboratory facilities. *Animal Feed Science and Technology* 123-124: 197-210

Google Earth (2012). http://www.google.earth

Harricharan H. J., Morris J. and Devers C. (1988). Mineral content of some tropical forage legumes. *Tropical Agriculture* (Trinidad) 65, (2): 132-136.

Ibrahim, H. and Olaloku, E. (2000). Improving cattle for milk, meat and traction. ILRI Manual 4. Nutrient requirements and feed resources. ILRI publication. Nairobi, Kenya. 135pp.

Jessop N.S. and Herrero M. (1996). Influence of soluble components on parameter estimation using *in vitro* gas production technique. *Journal of Animal Science*, 62:627-627.

Krebs G. and Leng R. A. (1984). The effect of supplementation with molasses/urea blocks on ruminal digestion. *Proceedings of the Australian Society of Animal Production* 15:704.

Makkar H. P. S. (2002). Application of the *in vitro* gas method in the evaluation of feed resources and enhancement of nutritional value of Tannin-rich tree/browse leaves and agro-industrial by-products. Proceedings of the final review meeting of an IAEA Technical Co-operation Regional AFRA Project organized by the joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture held on 25-29 November 2000. Cairo, Egypt. Pp23-40.

McDonald P., Edwards R. A., and Greenhalgh J. F. D. (1988). *Animal Nutrition* (4th edition). Longman Publisher. Pp 200-250.

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

Naginene A.C. and O. S. Abdullah (1992). Chemical Composition of some fodder grass of the Dares Salaam, *Tropical Agriculture* (Trinidad) 60 (3): 152 - 153.

Norton B. W. and Poppi D. P. (1995). Composition and nutritional attributes of pasture legumes. In: J P F D'Mello and C Devendra (Eds). Tropical legumes in Animal nutrition (CAB International) Wallingford, UK, 23-46.

Russell J.B. and Baldwin R.L. (1978). Substrate preferences in rumen bacteria: Evidence of catabolite regulatory mechanisms. *Applied Environmental Microbiology* 36:319-329.

SAS, (2002). SAS/STAT User's guide, Version 6, 4th edn. Cary, NC, USA: SAS Institute..

Satter L.D. and Slyter L.L., (1974). Effect of rumen ammonia concentration on rumen microbial production *in vitro. British Journal of Nutrition* 34: 199-208.

Slater, K. (1991). *The Principles of Dairy Farming, 11th Edition*. Farmers' Press. Ipswich, UK. 359 p.

Topps J. H. (1992) Potential composition and use of legume shrubs and trees as fodder for livestock in the tropics. *Journal of Agricultural Science* (*Cambridge*), 118: 1-18

Umuna N. N., Osuji P. O., Nsahlai I. V., Khalili H. and Mohammed-Saleem M. A. (1995). Effect of supplementing oat hay with lablab, sesbania, tagasaste and wheat middlings on voluntary intake, N-utilization and weight gain of Ethiopia Menz sheep. *Small Ruminant Research,* 18, 113-120.

Van Soest P.J., Roberson 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. http//jds.fass.org/cgi/reprint/74/10/3583.pdf

Van Soest, P.J. (1994). *Nutritional ecology of ruminants*. 2nd edition. Cornell University Press.

Zemenchik R. A., Albrecht K. A. and Shaver R. D. (2002). Improved nutritive value of kura clover- and birdsfoot trefoil-grass mixtures compared with grass monocultures. *Agronomy Journal,* 94(5), 1131-1138.