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In vitro Gas Production and Stoichiometric Relationship Between Short Chain Fatty Acid and *in vitro* Gas Production of Semi-Arid Browses of North-Eastern Nigeria

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Abstract: The *in vitro* gas production of semi-arid browse species were evaluated. The relationship between *in vitro* gas measured on incubation of tannin-containing browses in buffered rumen fluid and calculated from short chain fatty acid (SCFA) production was investigated. Crude protein (CP) contents in the browses ranged from 13.23 to 18.31% Dry matter (DM). The NDF, ADF and ADL were 34.40 to 54.80, 16.55 to 33.40 and 9.70 to 67.17 g/100g DM respectively. The Crude fibre (CF), Ether Extract (EE) and (NFE) were in the range of 14.50 to 37.00, 2.00 to 6.00, 23.39 to 41.39% DM respectively. Total condensed tannin (TCT) ranged from 0.12 to 0.41 mg/g DM. The TCT was significantly correlated (P<0.05) with gas production (r = 0.95; P < 0.05). There as a weak correlation between phenolic content of the browses and gas production (R^2=0.44, n =4). A weak relationship (r = 0.06; P< 0.05) was observed between measured *in vitro* gas production and that calculated from SCFA. The relationship between *in vitro* gas measured on incubation of browse leaves and that calculated from SCFA allows prediction of SCFA from gas production. The study showed that the leaves of the browse forages had nutritive value and therefore, may serve as potential supplements for ruminants in Nigeria.

Key words: Forage · Tannin · Digestibility · Ruminant · Fermentation

INTRODUCTION

Browse plants play a significant role in nutrition of ruminant livestock in tropical regions. Browse species, because of their resistance to heat, drought, salinity, alkalinity, drifting sand, grazing and repeated cutting, are the major feed resources during the dry season [1]. In addition, a major advantage of browses over herbaceous legumes and grasses is their higher crude protein content. However, due to the presence of secondary plant metabolites (particularly tannins) in browses, digestibility of protein and organic matter in these feeds is low [2 - 4]. This limits the availability of nutrients for ruminant livestock. Tannins have both beneficial and adverse effects. Beneficial effects of tannins include suppression of bloat [5] and protection of dietary proteins in the rumen [6]. The adverse effects of tannins are associated with their ability to bind with dietary proteins, carbohydrates and minerals [7].

The *in vitro* gas production method is widely used to evaluate the nutritive value of different classes of feeds [8] particularly to estimate energy value of straws [9], agro-industrial by-products [10], compound feeds [11] and various types of tropical feeds [12]. The gas production technique is more efficient than other *in vitro* techniques in determining the nutritive value of feeds containing tannins. The binding effect of tannins to macromolecules such as proteins and carbohydrates creates problems [13] in the use of the conventional *in vitro* method of Tilley and Terry [14] and nylon bag method of Mehrez, *et al.* [15]. Furthermore, the latter techniques are based on gravimetric determinations of residues, leading to solubilization of tannins which, although making no contributions to energy production in the system, are measured as dry matter digestibility. In the *in vitro* gas production method, the effects of tannins on rumen fermentation are reflected in the gas production. The technique has been used to assess actions of anti-nutritive factors on rumen fermentation of Mediterranean [16] and African browses [1,17].

MATERIALS AND METHODS

Forage Samples: Eight indigenous browse samples (leaves) commonly consumed by ruminants animals were used in this study. The species were: *Ficus polita,*

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Ficus thonningii, Batryospermum paradoxum, Kigalia africana, Celtis integuifolis, Khaya senegalensis, Leptadenia lancifolia and *Ziziphus abyssinica.* All forages were harvested from Gwoza local government area of Borno State Nigeria. The area is located at 11.05° North and 30.05° East and at an elevation of about 364 above sea level in the North Eastern part of Nigeria. The ambient temperature ranges between 30°C and 42°C being the hottest period (March to June) while its cold between November to February with temperatures ranging between 19-25°C. The browse forages were harvested from at least 10 trees per each specie selected at random in four locations with the study area at the end of the season. The harvested sample were then pooled for each individual tree species and then oven dried at 105°C for 24h to constant weight and ground to pass through a 1.0mm, sieve. The samples were then sub-sample to obtain three samples for each tree species and were sent for the laboratory analysis.

Chemical Analysis: Browse species were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF) and ash [20]. The leaves samples were analyzed for neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and cellulose [21]. Total condensed tannin was analyzed according to Polshettiwar *et al.* [22].

***In Vitro* Gas Production:** Rumen fluid was obtained from 3 WAD female sheep through suction tube before morning feed, these animals normally fed concentrate feed (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% dried brewers grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal. Incubation was carried out [23] using 120 ml calibrated syringes I n three batch incubation at 39°C. Into 200 mg sample (*n* = 8) in the syringe was introduced 30 ml inoculums containing cheese cloth strained rumen liquor and buffer ($NaHCO_3 + 3\ Na_2\ HPO_4 + KCl + NaCl + MgSO_4.\ 7H_2O + CaCl_2\ .2H_2O$) (1:4, v/v) under continuous flushing with CO_2. The gas production was measured at 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42 and 48h. The average of gas volume produced from the blanks was deducted from the volume of gas produced per sample.

Statistical Analysis: Metabolizable Energy (ME) was calculated as ME = 2.20 + 0.136GV + 0.057 CP + 0.0029 CF [24]. Organic matter digestibility (OMD%) was assess as OMD = 14.88 + 0.889 GV + 0.45 CP + 0.651 XA [24]. Short Chain Fatty Acids (SCFA) was also calculated [25] 0.0239 GV- 0.0601 , where GV, CP, CF and XA are total gas volume, crude protein, crude fibre and ash respectively. Data obtained were subjected to analysis of variance. Where significant differences occurred, the means were separated using Duncan multiple range F-test of the SAS [26] options.

RESULTS

Chemical Composition of Browse Forages: The chemical composition of the browse forages is presented in Table 1. The Dry matter, crude protein and crude fibre ranged from 94.00 in (*Sterculia setigera*) to 96.00% DM in *Ziziphus mauritiana*, 13.23 in *Olea hochstteteri* to 18.31% DM) in *Balanites aegytiaca* and 14.50 in *Balanites aegytiaca* to 37.00 in *Olea hochstteteri* respectively. *Olea hochstteteri* had the highest level of ether extract while *Balanites aegytiaca* and *Ficus sycomorus* were higher in ash content. The NFE was significantly higher (41.39% DM) in *Balanites aegyptiaca* than the other browse forages while the fibre fractions (NDF and ADF)

Table 1: Proximate composition of browses (% DM), TCT(mg/g DM), NDF, ADF, ADL (g/100g DM)

Browse species	DM	CP	CF	EE	Ash	NFE	NDF	ADF	ADL	TCT	PHE
Olea hochstteteri	94.8^{b}	13.23^{d}	37.00^{a}	6.00^{a}	14.00^{c}	23.39^{e}	40.52^{b}	30.42^{c}	13.12^{b}	0.12^{g}	0.24^{f}
Ziziphus mauritania	96.0^{a}	15.86^{bc}	24.00^{d}	2.00^{c}	12.00^{d}	30.73^{c}	38.67^{bc}	16.55^{f}	66.91^{a}	0.21^{d}	0.52^{d}
Ziziphus spinachisti	95.2^{a}	16.04^{b}	18.00^{e}	3.00^{b}	15.00^{b}	41.27^{a}	39.59^{b}	17.54	67.17^{a}	0.41^{a}	0.49^{e}
Pterocarpus erinceus	95.0^{a}	17.96^{a}	35.50^{a}	2.00^{c}	11.00^{a}	29.49^{c}	36.40^{d}	26.30^{d}	13.80^{b}	0.23^{c}	0.61^{c}
Sterculia setigera	94.0^{ab}	15.77^{bc}	28.50^{c}	2.00^{c}	15.00^{b}	33.45^{b}	34.40^{e}	32.10^{b}	12.60^{b}	0.34^{b}	0.48^{e}
Balanites aegyptiaca	94.2^{ab}	18.31^{a}	14.50^{f}	2.00^{c}	18.00^{a}	41.39^{a}	36.430^{d}	25.74^{de}	13.75^{b}	0.23^{c}	0.68^{b}
Ficus sycomorus	95.6^{a}	14.90^{c}	32.50^{b}	3.00^{b}	18.00^{a}	28.38^{cd}	54.80^{a}	33.40^{a}	12.60^{b}	0.17^{e}	0.81^{a}
Adansonia digitata	95.6^{a}	16.12^{b}	32.00^{b}	3.00^{b}	14.00^{c}	30.57^{c}	38.50^{bc}	27.20^{d}	9.70^{c}	0.13^{f}	0.50^{d}
MEANS	95.05	16.02	27.75	2.88	14.63	32.44	39.91	26.15	26.19	0.23	0.54
SEM	0.09	0.39	0.65	0.42	0.73	0.71	0.74	0.90	2.89	0.02	0.05

a, b, c, means in the same column with different superscript differ significantly ($P<0.05$). DM = Dry matter; CP = Crude Protein; CP=Crude Protein; EE = Ether Extract;CF = Crude fibre; NDF = Neutral detergent fibre; ADF = Acid detergent fibre; ADL=Acid detergent lignin; TCT = Total Condensed Tannin; PHE: Phenolics

Table 2: Net Gas Volume, Metabolizable Energy, Organic matter digestibility, Short Chain Fatty Acid of semi-arid browse forages

	Gas production parameters			
Browse Forages	NGV	ME	OMD	SCFA
Olea hochstteteri	7.66^{e}	4.86^{b}	36.73^{e}	0.11^{c}
Ziziphus mauritiana	10.83^{d}	4.62^{b}	46.53^{a}	0.20^{b}
Ziziphus spinachisti	8.16^{c}	4.24^{b}	38.72^{d}	0.12^{c}
Pterocarpus erinceus	15.16^{a}	5.35^{a}	43.34^{b}	0.30^{a}
Steculia setigera	6.83^{e}	4.12^{b}	37.58^{d}	0.10^{c}
Balanites aegyptiaca	2.83^{f}	3.62^{c}	37.02^{d}	0.007^{d}
Ficus sycomorus	13.16^{b}	4.91^{b}	44.97^{b}	0.25^{b}
Adansonia digitata	12.83^{b}	4.94^{b}	42.53^{bc}	0.25^{b}
MEANS	9.68	4.58	40.93	0.16
SEM	0.38	0.26	0.79	0.05

Net Gas Volume (NGV = ml/ 200 mg DM), Metabolizable Energy (ME=MJ/Kg DM), Organic matter digestibility (OMD=%), Short Chain Fatty Acids (mmol) of semi-arid browse forages

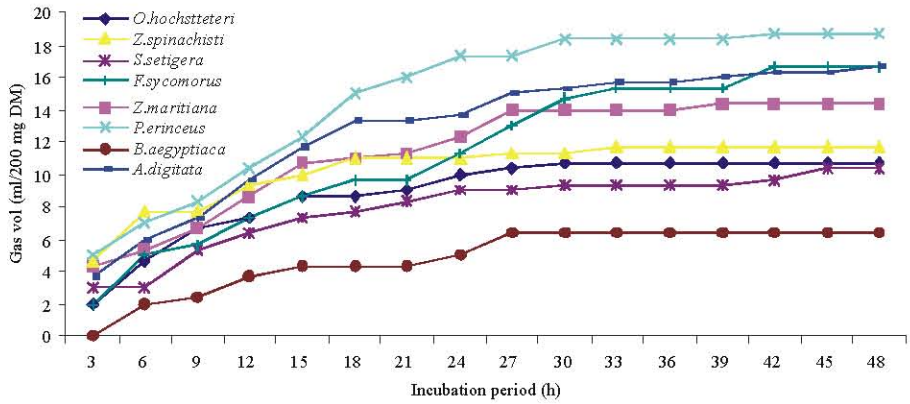

Fig. 1: A cumulative gas production of semi-arid browse forages of North-eastern Nigeria

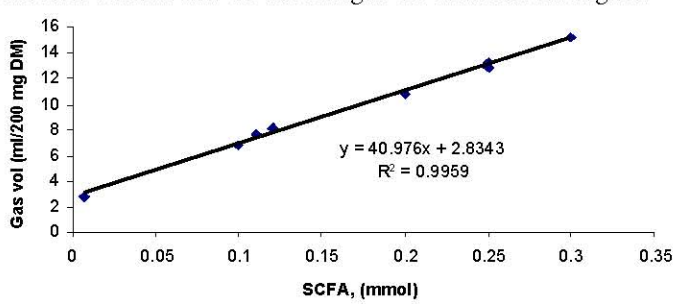

Fig. 2: Relationship between gas volume and SCFA of browses of North-eastern Nigeria

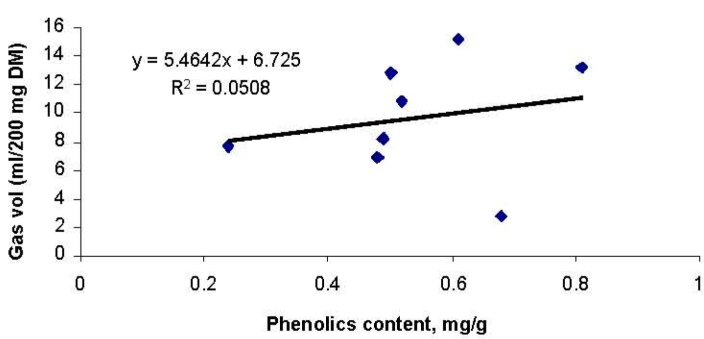

Fig. 3: Relationship between gas volume and phenolic of browses of North-eastern Nigeria

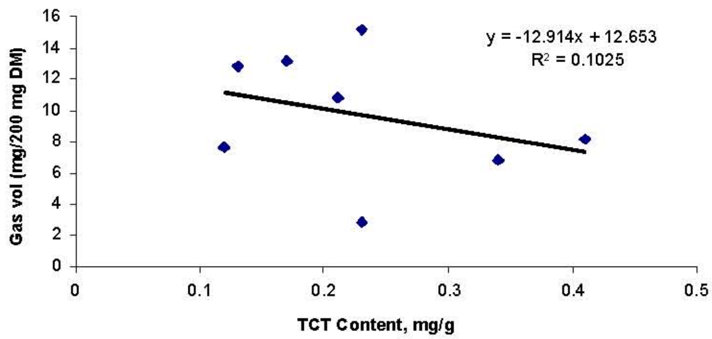

Fig. 4: Relationship between gas volume and TCT of browses of North-eastern Nigeria

was significantly higher (54.80 and 33.40g/100g DM) in *Ficus sycomorus*. The ADL was significantly higher (66.91g/100g DM) in *Ziziphus mauritiana* and lowest (9.70) in *Adansonia digitata*. The total condensed tannins and phenolics was observed to be higher 0.41 in *Ziziphus spinachisti* and 0.81mg/g DM in *Ficus sycomorus*.

***In Vitro* Gas Production Characteristics:** Figure 1 shows the *in vitro* gas fermentation of the browse forages. Net gas volume was higher in *Pterocarpus erinceus* and lowest in *Steculia setigera*. Gas production increased with increased incubation time and tend to be stable from 27 to 48 h of incubation except for *Ficus sycomorus*.

Metabolizable energy (ME), Organic matter digestibility (OMD) and Short chain fatty acids (SCFA) are shown in Table 2. The ME ranged between 3.62 MJ/Kg DM in *Balanites aegyptiaca* and 5.35 MJ/Kg DM in *Pterocarpus erinceus* There were significant (P<0.05) differences in the ME among the browse forages. The OMD was significantly (P<0.05) higher in *Ziziphus mauritiana* (46.53 MJ/Kg DM) lowest in *Olea hochstteteri* (36.73 MJ/Kg DM). The highest (0.30) and lowest (0.007) SCFA were found in *Pterocarpus erinceus* and *Balanites aegyptiaca* respectively.

There were negative relationship between short chain fatty acids and gas production (r=0.06, n=8). Highly significant (P<0.001) correlations were observed between gas production and TCT (r=0.95, n=8). The result also revealed a weak correlation between phenolic content of the browses and gas production (R^2=0.44, n =4); Total condensed tannin and gas production (R^2=0.05, n=8).

DISCUSSION

The cumulative gas production for the *Balanites aegyptiaca* was significantly low in comparison to other browse forages. The highest cumulative gas production was in the order of *Pterocarpus erinceus*> *Ficus sycomorus*> *Adansonia digitata*> *Ziziphus maritania*> *Ziziphus spinachisti*> *Olea hochstteteri*> *Sterculia setigera*>*Balanites aegyptiaca*. The variation in gas production and potential gas production between the browse species forages can be attributed to compositional differences of the browse forages, especially CP, fibre, NFE, nature and concentration of polyphenolics and may be other anti-nutritional components. These factors influence the amount of substrate OM that is fermented and the short chain fatty acids (SCFAs) produced upon fermentation. Other reason may be due to low NFE content for browses which has a positive correlation with gas production. On the other hand, cell wall content (NDF and ADF) were negatively correlated with gas production at all incubation times and estimated parameters. This may tend to reduce the microbial activity through increasing the adverse environmental conditions as incubation time progress. This is consistent with De Biover, *et al.* [27], who reported that gas production was negatively related with NDF content and positively with starch. Also, the relatively high level of ADL in the browses in this study explained in part the limited *in vitro* degradation and therefore the lower amount of gas produced. However, since gas production on incubation of feeds in buffered rumen fluid is associated with feed fermentation and carbohydrate fractions, the low gas production from *Balanites aegyptiaca* could be related to low feeding value of these feeds. Incubation of feedstuff with buffered rumen fluid *in vitro*, the carbohydrates are fermented to short chain fatty acids (SCFA), gases, mainly CO_2 and CH_4 and microbial cells. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate [28,29] and substantial changes in carbohydrate fractions were reflected by total gas produced [30]. Gas production from protein fermentation is relatively small as compared to carbohydrate fermentation while, contribution of fat to gas production is negligible [28,31]. The correlation between *in vitro* gas productions measured after 24 h incubation of tropical browses and that calculated from SCFA was similar to that reported for conventional feeds [32]. About 94% of the variation in the *in vitro* gas production on incubation of browse leaves was explained by SCFA produced, which mainly comes from carbohydrate fermentation. These results suggest that from browses with a similar range of CP contents, the SCFA production from sources other than carbohydrates is negligible. The use of different proportions of casein and carbohydrate sources (glucose and starch) with a resultant poor correlation between gas measured and calculated from SCFA has been reported [33]. These poor correlations could be due to the highly fermentable carbohydrate sources that drastically changed the molar proportions of SCFA, indicating the pattern of fermentation of pure substrate does not reflect the normal fermentation pattern that occurs in the rumen.

The results of the relationship between gas volume calculated from the SCFA and measured using the *in vitro* gas method of Menke and Steingass, [24] confirm the close relationship between SCFA production and gas volume liberated on fermentation of browse species with a wide range of CP (77 to 300 g/kg) and phenolic contents (TP from 17 to 250 g/kg DM and T from 7 to 214 g/kg DM respectively). From the results observed in the present study, SCFA production could be predicted from *in vitro* gas production. Attempts have been made to predict the SCFA production using mathematical models [33]. However, such models involve several variables and variations in these variables could affect the prediction of SCFA. The close association between the *in vitro* gas and SCFA production would allow the determination of the amount of apparently fermented substrate (substrate used for SCFA, CO_2, CH_4 and H_2O production) for tannin-containing browses from the stoichiometrical relationship between *in vitro* gas and SCFA using the approach outlined by Getachew, *et al.* [8] and Cone and Van Gelder [34].

CONCLUSION

In conclusion, the close association between SCFA and gas production may allow the use of the relationship between SCFA and gas production to estimate the SCFA production from gas values, which is an indicator of energy availability to the animal.

REFERENCES

1. Fagg, C.W. and J.L. Stewart, 1994. The value of *Acacia* and *Prosopis* in arid and semi-arid environments. J. Arid Environment, 27: 3-25.
2. Terill, T.H., G.B. Douglass, A.G. Foote, R.W. Purchas, G.F. Wilson and T.N. Barry, 1992. Effect of condensed tannins upon body growth, wool growth and rumen metabolism in sheep grazing sulla (*Hedysarum coronarium*) and perennial pasture. J. Agric. Sci. Cambridge, 119: 265-273.
3. Silanikove, N., N. Gilboa and Z. Nitsan, 1997. Interactions among tannins, supplementation and polyethylene glycol in goats given oak leaves: effects on digestion and food intake. Animal Sci., 64: 479-483.
4. Waghorn, G.C. and I.D. Shelton, 1997. Effect of condensed tannins in *Lotus corniculatus* on the nutritive value of pasture for sheep. J. Agric. Sci. Cambridge, 128: 365-372.
5. Jones, W.T., B. Anderson and M.D. Ross, 1973. Bloat in cattle: detection of protein precipitants (flavolans) in legumes. New Zealand J. Agric. Res., 16: 441-446.
6. Waghorn, G.C., I.D. Shelton, W.C. McNabb and S.N. Mccutcheon, 1994. Effect of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 2. Nitrogenous aspects. J. Agric. Sci. Cambridge, 123: 109-119.
7. McSweeney, C.S., B. Palmer, D. M. McNeill and D.O. Krause, 2001. Microbial interactions with tannins: nutritional consequences for ruminants. Animal Feed Sci. and Technol., 91: 83-93.
8. Getachew, G., M. Blummel, H.P.S. Makkar and K. Becker, 1998. *In vitro* gas measuring techniques for assessment of nutritional quality of feeds: a review. Animal Feed Sci. and Technol., 72: 261-281.
9. Makkar, H.P.S., E.M. Aregheore and K. Becker, 1999. Effect of saponins and plant extracts containing saponins on the recovery of ammonia during urea-ammoniation of wheat straw and fermentation kinetics of the treated straw. J. Agric. Sci. Cambridge, 132: 313-321.
10. Krishna, G. and K.D. Gunther, 1987. The usability of Hohenheim gas test for evaluating *in vitro* organic matter digestibility and protein degradability at rumen level of some agro industrial by-products and wastes used as livestock feeds. Landwirtschaftlich Forschung, 40: 281-286.

11. Aiple, K.P., H. Steingass and W. Drochner, 1996. Prediction of the net energy content of raw materials and compound feeds for ruminants by different laboratory methods. Archives of Animal Nutrition, 49: 213-220.
12. Krishnamoorthy, U., H. Soller, H. Steingass and K.H. Menke, 1995. Energy and protein evaluation of tropical feedstuffs for whole tract and ruminal digestion by chemical analysis and rumen inoculum studies *in vitro*. Animal Feed Science and Technol., 52: 177-188.
13. Makkar, H.P.S., M. Blummel and K. Becker, 1995. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins and their implications in gas production and true digestibility in *in vitro* techniques. British J. Nutirtion, 73: 897-913.
14. Tilley, J.M. and R.A. Terry, 1963. A two-stage technique for the in vitro digestion of forage crops. British J. Nutrition, 18: 104-111.
15. Mehrez, A.Z. and E.R. Ørskov, 1977. A study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. J. Agric. Sci. Cambridge, 88: 645-650.
16. Khazaal, K.A., J. Boza and E.R. Ørskov, 1994. Assessment of phenolics-related anti-nutritive effects in Mediterranean browse: a comparison between the use of the in vitro gas production technique with or without insoluble polyvinylpolypyrrolidone or nylon bag. Animal Feed Sci. and Technol., 49: 133-149.
17. Siaw, D.E.K.A., P.O. Osuji and I.V. Nsahlai, 1993. Evaluation of multipurpose tree germplasm: the use of gas production and rumen degradation characteristics. J. Agric. Sci. Cambridge, 120: 319-330.
18. Nsahlai, I.V., D.E.K.A. Siaw and P.O. Osuji, 1994. The relationship between gas production and chemical composition of 23 browses of genus *Sesbania*. J. the Sci. of Food and Agric., 65: 13-20.
19. Bonsi, M.L.K., P.O. Osuji and A.K. Tuah, 1995. Effect of supplementing teff straw with different levels of leucaena or sesbania leaves on the degradabilities of teff straw, sesbania, leucaena, tagasaste and vernonia and on certain rumen and blood metabolites in Ethiopian Menz sheep. Animal Feed Sci. and Technol., 52: 101-129.
20. AOAC, 2005. Official Methods of Analysis of the Official Analytical Chemists, 18th ed. (Horwitz, W. eds.), Association of Official Analytical Chemists, Washington DC.
21. Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary neutral detergent fibre and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.
22. Polshettiwar, S.A., R.O. Ganjiwale, S.J. Wadher, P.G. Yeole, 2007. Spectrophotometric estimation of total tannins in some ayurvedic eye drops. Indian J. Pharmaceutical Sci. 69(4): 574-576.
23. Fievez, V., O.J. Babayemi and D. Demeyer, 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 Sci. and Technol., 123-124: 197-210.
24. Menke, K.H. and H. Steingass, 1988. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. Animal Research and Development, 28: 7-55.
25. Getachew, G., H.P.S. Makkar and K. Becker, 1999. Stoichiometric relationship between short chain fatty acid and *in vitro* gas production in presence and absence of polyethylene glycol for tannin contaning browses, EAAP Satelite Symposium, Gas production: fermentation kinetics for feed evaluation and to assess microbial activity, 18 - 19 August, Wageningen, The Netherlands.
26. Statistical Analysis system Institute Inc., 1988. SASSTAT Programme, Cary, NC: SAS Institute Inc.
27. De Biover, J.L., J.M. Aerts, J.M. Vanacker and D.L. De Brabander, 2005. Evaluation of the nutritive value of maize silages using a gas production technique. Animal Feed Sci. Technol., 255: 123-124.
28. Wollin, M.J., 1960. Theoretical rumen fermentation balance. J. Dairy Sci., 43: 1452-1459.
29. Steingass, H. and K.H. Menke, 1986. Schatzung des energetischen Futterwertes aus der in vitro mit Pansensaft bestimmten Gasbildung und der chemischen Analyse. Tierernahrung, 14: 251-271.
30. Deaville, E.R. and D.I. Givens, 2001. Use of automated gas production technique to determine the fermentation kinetics of carbohydrate fractions in maize silage. Animal Feed Sci. Technol., 93: 205-210.
31. Sallam, S.M.A., M.E.A. Nasser, A.M. El-Waziry, I.C.S. Bueno and A. L. Abdalla, 2007. Use of an *in vitro* Rumen Gas Production Technique to Evaluate Some Ruminant Feedstuffs. J. Applied Sci. Res., 3(1): 34-41.
32. Blümmel, M., K.P. Aiple, H. Steingass and K. Becker, 1999. A note on the Stoichiometrical relationship of short chain fatty acid production and gas evolution *in vitro* in feedstuffs of widely differing quality. J. Animal Physiology and Animal Nutrition, 81: 157-167.

33. Pitt, R.E., J.S. Van Kessel, D.G. Fox, A.N. Pell, M.C. Barry and P.J. Van Soest, 1999. Prediction of ruminal volatile fatty acids and pH within the net carbohydrate and protein system. J. Animal Sci., 74: 226-244.
34. Cone, J.W. and A.H. Van Gelder, 1999. Influence of protein fermentation on gas production profiles. Animal Feed Sci. and Technol., 76: 251-264.
35. Blümmel, M., H.P.S. Makkar, G. Chisanga, J. Mtimuni and K. Becker, 1997. The prediction of dry matter intake of temperate and tropical roughages from *in vitro* digestibility/gas-production of African roughages in relation to ruminant liveweight gain. Animal Feed Sci. and Technol., 69: 131-141.