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CHEMICAL COMPOSITION, *IN VITRO* GAS AND METHANE PRODUCTION AND DRY MATTER DEGRADABILITY OF SOME BROWSE PLANTS IN SEMI-ARID ZONE OF NORTH EASTERN NIGERIA

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ABSTRACT

Leaves from seven browse plants namely: *Cassia siaberiana, Acacia seberiana, Buahinea nufescens, Diospyrus mispliformis, Analgeousus leocarpus, Combretum leati* and *Gerdena sokotensis* were analysed to estimate the chemical composition, methane production and *in vitro* dry matter degradability at 24, 48, 72, and 96 hours. Crude protein (CP) content ranged from 11.49 to 15.60% DM with *Accacia sieberiana* having the highest protein content. NDF, ADF and ADL ranged from 49.31 to 58.05,21.85 to 25.38 and 9.37 to 16.90 g/100g DM respectively. Total condensed tannin (TCT) ranged from 0.09 to 021 mg/g DM. in viro methane emission ranged from 1 to 9, 2 to 10, 4 to 15 and 7 to 25ml/200 ml DM at 24,48,72 and 96h incubation periods with *Garderna sokotensis* and *Cassia sieberiana* having the highest gas production (22.66 ml) at all incubation periods. The lowest methane value was obtained from *Accacia siebriana* at 24, 48, 72 and 96 hours while *Buahenia nufescens* had the highest methane production at all incubation periods. The fermentation characteristic showed significant difference ($P<0.05$) for all the parameters. All the gas production parameters (a, b, a+b) were highest for *Buahenia nufescens* while the highest rate constant of gas production 'c' (0.054) was observed for *Acacia sieberiana* based on chemical composition and *in vitro* results. It was concluded that the browse forages studied can serve as supplements to ruminant animals with short incubation period.

Keywords: Browse; *in vitro*; methane; forages; fermentation characteristics

INTRODUCTION

In vitro ruminal evaluations of browse fodders are not only applicable to *in vitro* gas production kinetics because partitions of energy released from deterged substrate contribute both to short chain fatty acids (SCFA) production and for microbial protein synthesis (Blummel *et al.*, 1994). The concept of partitioning fermentation production products (Partitioning factor; PF) was introduced to express the conversion of energy form truly degraded substrate required to yield 1ml of gas (Blummel and Becker, 1997). The effect of tannins on PF values of browse plants has not been extensively investigated. This is largely due to the difficulty in quantifying the amount of substance deterred *in vitro*. It is important

to determine the PF values for browse plants because most of these plants contain condensed tannins, which may cause harmful effects on rumen microbial fermentation such as reduction of the VFA concentration and ruminal microorganisms' toxicity (Jones *et al*., 1994).
Livestock are one of the largest sources of methane emission with 80 to 115 million tons produced per year, equivalent to 15% to 20% of total anthropogenic methane (IPCC, 2001). The global cattle population is responsible for 73% of methane emissions of all livestock, and methane produced during ruminal fermentation represent a loss of 2 to 15% of gross energy intake and may contribute to global warming (Johnson and Johnson, 1995). Methane has a global warming potential 23 times more potent than carbon dioxide, which makes methane one of the most important greenhouse gases (Wuesbbles and Hayheo, 2002). The microbes producing methane, methanogenic *archaea* (i.e. methanogens), complete with bacteria in the rumen for substrates such as hydrogen (Zinder, 1993). Furthermore, during fermentation, hydrogen is produced, and the removal of hydrogen is important for the efficiency of rumen fermentation (Stewart *et al.*, 1997). Many researches carried out to find ways to lower the methanogenic *arcnaea* was described by Finlay *et al.* (1994), and selective suppression of the rumen protozoa had been suggested to be a promising approach to reduce methane release (Moss *et al.*, 2000). Therefore, extensive research interests have been focused on methods of reducing methane production in the rumen. The methane production (*in vitro*) of some semi-arid browses of northern Nigeria is also an area that need to be exploited.

MATERIALS AND METHODS

Forage Samples: Collection, Preparation and Analysis

Seven indigenous browse plants leaf samples commonly consumed by ruminant animals were used in this study. The species were: *Cassia sieberiana, Acacia seberiana, Buahinea nufescens, Diospyrus mispliformis, Analgeousus leocarpus, Combretum leati and Garderna sokotensis*. All the forage samples were harvested from Gwoza local government area of Borno state. The harvested samples were then pooled for each individual tree species, oven dried at 105^{0}C for 24 hours to a constant weight and ground to pass through a 1.0mm sieve. Fine sample of the browse plants leaves were then analysed for dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) according to Van Soest *et al.* (1991). Total condensed tannin was determined by butanol-HCL method (Porter *et al.*, 1986).

In vitro gas production study

Rumen fluid was obtained from 3 WAD female sheep through suction tube before morning feed, normally fed with concentrate feed (40% corn, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soya bean meal, 10% dried brewers' grain, 1% common salt, 3.75% oyster shell and 0.25% fish meal. Incubation was carried out as reported by Fievez *et al.* (2005) using 120 ml calibrated syringes in three batch incubation at 39^{0}C into 200 mg sample (n=10) in the syringes was introduced 30 ml inoculums containing cheese cloth strained rumen liquor and buffer (9.8g $NaHCO_3$ +2.77g Na_2 HPO4 + 0.57gKCL+0.47g NaCl +02g MgSO4.7H_2O+0.16g $CaCL_2$ 2H_2O) (1:4, v/v) under continuous flushing with CO_2. The gas production was measured at 3, 6, 12, 24, 48, 72 and 96 h. Total gas volume was corrected for blank incubation. Cumulative gas production data were fitted to the model of Ørskoy and McDonald (1979)

G = a+b (1-e),

Where:
G = is the gas production (ml) at time t
a = is the gas production from the immediately soluble fraction (ml),
b= is the gas production from the insoluble but degradable fraction (ml),
a+b= is the potential gas production (ml),
c = is the rate constant of gas production (fraction/h).

Methane Production

In order to estimate methane production by the substrate and immediately after evacuation from the incubator, 4 ml of NaOH (10M) was introduced using 5 ml capacity syringe as reported by fievez *et al.* (2005). The content was inserted into the silicon tube, which was fastened to the 120ml capacity syringe. The clip was then opened while the NaOH was gradually released. The content was 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.

Statistical Analysis

Data obtained was subjected to analysis of variance where significant differences occurred, the means were separated using Duncan multiple range F-test of the SAS (1988) options.

RESULTS

Chemical Composition of the Browse Leaves

The chemical composition of the browse forage leaves determined in this study is presented in Table 1. Dry matter content ranged from 953.30 g kg^{-1} DM in *C. sieberiana* to 984.46 g kg-1 DM in *A. sieberiana.* The examined browse plant leaves generally had high crude protein content values, ranging from 114.90 g kg^{-1} DM in *B. nufescens* to 156.60 g kg-1 DM in *A. sieberiana.* Ash content of the browse forages range from 110.00 g kg-1 DM in *A. sieberiana* to 216.60 g kg-1 DM in *C. sieberiana*

The highest neutral detergent fibre content of 580.50 g kg^{-1} DM was recorded for *A. sieberiana* while *B. nufescens* had the lowest value of 493.10 g kg-1 DM. The acid detergent fibre levels ranged from 218.50 g kg^{-1} DM in *C. sieberiana* to 253.80 g kg^{-1} DM in *A. sieberiana*. The lowest lignin content of 93.70 g kg^{-1} DM was recorded in *B. nufescens* while *A. sieberiana* had the highest value of 169.00 g kg^{-1} DM.

Table 1: Chemical composition DM, CP, Ash, NDF, ADF, ADL (g Kg^{-1}DM TCT (mg/g DM)

Browse forages	DM	CP	Ash	NDF	ADF	ADL	TCT
Cassia sieberiana	953.00	125.00^{b}	216.60^{a}	531.00^{c}	218.50^{bc}	116.80^{bc}	0.21
Acacia sieberiana	984.60	156.00^{a}	111.00^{c}	580.50^{a}	253.80^{a}	169.00^{a}	0.09
Buahinea nufescens	975.30	114.90^{bc}	161.00^{c}	493.10^{d}	231.40^{b}	93.70^{d}	0.11
Diospyrus mispliformis	975.60	150.80^{a}	112.30^{c}	569.80^{b}	251.30^{a}	134.40^{b}	0.15
Analgeousus leocarpus	973.60	150.70^{a}	125.30^{d}	542.10^{c}	241.80^{a}	130.00^{b}	0.17
Combretum leati	978.00	135.40^{b}	121.30^{d}	567.70^{b}	221.80^{b}	111.80^{bc}	0.10
Garderna sokotensis	983.00	151.40^{a}	181.40^{b}	544.20^{c}	219.30^{bc}	121.30^{b}	0.12
SEM	0.14NS	0.32	0.65	0.86	0.32	0.55	0.02^{ns}

a, b, c, means in the same column with different superscript differ significantly (P<0.05); SEM=Standard error of means; DM = Dry matter; CP = Crude protein; NDF = Neutral detergent fibre: ADF = Acid detergent fibre; ADL = Acid detergent lignin; TCT = Total condensed tannin.

In vitro gas production

The gas production from the selected browse forage plants is shown in Table 2. The gas production and fermentation characteristics of the forages differed significantly (P<0.05). *B. nufescens* produced higher (P<0.05) gas volume (29.66 ml/200 mg DM) throughout the incubation period from the 3 h to 96 h while *A. sieberiana* produced the least gas volume of 10.33 ml/200 mg DM at 96 h.

Table 2: *In Vitro* gas production (ml/200 mg DM)

Browse	3 h	6 h	12 h	24 h	48 h	72 h	96 h
Cassia sieberiana	3.00^{b}	5.33^{c}	7.66^{c}	14.00^{c}	18.66^{c}	22.66^{c}	22.66^{c}
Acacia sieberiana	2.00^{c}	3.33^{d}	5.00^{c}	8.00^{g}	9.33^{g}	10.33^{g}	10.33^{b}
Buahinea nufescens	3.33^{b}	5.00^{c}	9.00^{b}	17.00^{b}	25.66^{b}	29.33^{b}	29.33^{b}
Diospyrus mispliformis	1.66^{d}	3.00^{e}	5.33^{c}	11.00^{c}	15.33^{e}	17.66^{e}	22.33^{c}
Analgeousus leocarpus	2.00^{c}	3.66^{d}	5.66^{e}	7.66^{g}	11.33^{f}	14.00^{f}	14.00^{f}
Combretum leati	2.33^{c}	5.33^{c}	8.00^{c}	12.33	15.33^{e}	18.00^{e}	18.00^{f}
Garderna sokotensis	3.00^{b}	6.33^{b}	8.00^{c}	13.66^{c}	17.33^{d}	22.66^{c}	22.66^{c}
SEM	0.14	0.03	0.04	0.09	0.11	0.04	0.02

a, b, c, means in the same column with different superscript differ significantly (P<0.05); SEM=Standard error of means; h=hour

In Vitro Fermentation Characteristics

The *in vitro* fermentation characteristics of the browse plants are shown in Table 3. The immediately soluble fraction 'a' was generally low for all the browse forages with values ranging from 1.67 in *D. mispliformis* to 3.33 ml in *B. nufescens.* The fermentation of the insoluble but degradable fraction 'b' was highest in *B. nufescens* (26.00 ml) and lowest in *A. sieberiana* (8.00 ml). The potential gas production 'a+b' was low for all the browse forages with the highest value of (29.33 ml) found in *B. nufescens* and lowest value of (10.33 ml) recorded for *A. sieberiana*. The gas production rate 'Y' at time 't' ranged from 0.026 for *D. mispliformis* to 0.054 for *A. sieberiana.*

Table 3: Fermentative characteristics of semi-arid browse forages

Browse	A	b	a+b	C
Cassia sieberiana	3.00^{c}	19.67^{c}	22.67^{c}	0.035^{c}
Acacia sieberiana	2.33^{d}	8.00^{g}	10.33^{g}	0.054^{a}
Buahinea nufescens	3.33^{b}	26.00^{a}	29.33^{a}	0.027^{d}
Diospyrus mispliformis	1.67^{e}	20.67^{b}	22.33^{c}	0.026^{d}
Analgeousus leocarpus	2.33^{d}	14.00^{e}	11.67^{f}	0.029^{d}
Combretum leati	2.33^{d}	18.00^{d}	15.67^{e}	0.050^{a}
Garderna sokotensis	3.00^{c}	20.67^{b}	23.67^{c}	0.043^{b}

Methane Production

Methane (ml/200 mg DM) production (Figure 1) showed that the least and the highest were obtained from *Buahenia nufescens* (26 ml/200 mg DM) and *Acassia sieberiana* (3 ml/200 mg DM) respectively.

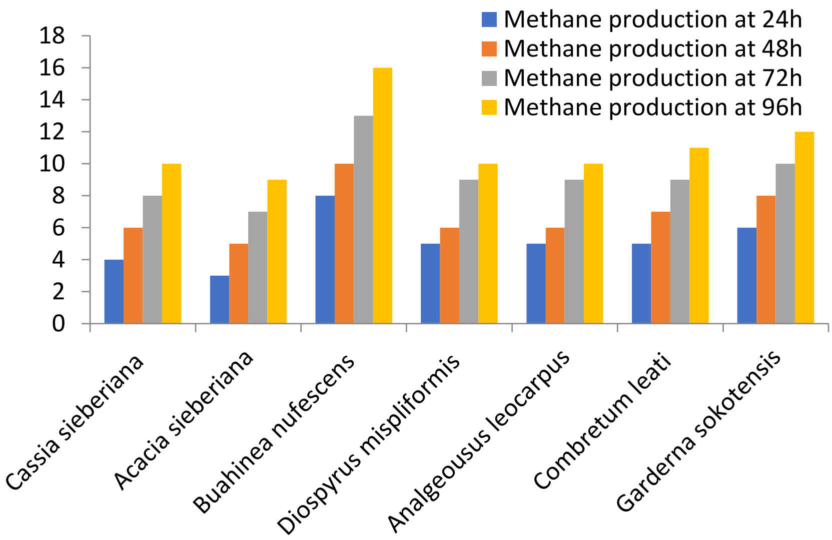

Figure 1: *In vitro* Methane production of semi-arid browse forages

DISCUSSION

The result of the chemical composition of the browse forages were shown in Table 1. The crude protein (CP) content ranges from 11.49 to 15.60 % of DM which is adequate to meet the protein requirement of ruminant animals. The NDF and ADF values (49.31 to 58.05) and (21.85 to 25.38 g/100g) per Kg DM were higher compared to other values similar to the report of Bakshi and Wadhwa (2004). This might affect DM intake and DM digestibility of the forages. The ADL values (which ranges from 9.37 to 16.90g/100g DM) were similar to those reported by Njidda (2010) for semi-arid browses. Moore and Jung, (2001) reported that lignin is generally higher in browse plants than in herbaceous plants and that the content varies according to specie, age and part of the plant. The total condensed tannins (TCT) (which ranged from 0.09 mg/g to 0.21mg/g DM) is lower than a range of 60 to100g Kg DM, a level that tend to depress feed intake and growth (Barry and Duncan, 1984). However, in ruminants, dietary condensed tannins of between 2 to 3% have been shown to have beneficial effects of reducing protein degradation in the rumen by formation of a protein-tannin complex (Barry, 1987). The phenolic content of the browse (0.24 to 0.65mg/g DM) was adequate for ruminants.

The gas production from the browse forages (Table 2) were within the range reported earlier for browse forages from North eastern Nigeria by Njidda, (2011) (*n*-37). Plant species, plant morphological fraction, environmental factors, and stage of maturity largely affect chemical composition and *in vitro* fermentation and digestibility (Chikagwa-Malunga *et al.*, 2009). These factors influence the amount of substrate OM that is fermented and the short chain fatty acids (SCFAs) produced upon fermentation. This is because gas production results from fermentation of the feed OM and CO_2 produced from the buffering of the SCFAs by the bicarbonate buffer. One of the main reasons for low degradability is the presence of lignin which protects carbohydrates from attack by rumen microbes. During 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 (Wolin,1960; Steingass and Menke, 1986; Njidda, 2012) and substantial changes in carbohydrate fractions were reflected by total gas produced (Deaville and Givens, 2001).

Gas production from protein fermentation is relatively small compared to carbohydrate fermentation while, contribution of fat to gas production was negligible (Wolin, 1960). However, some other prospective and novel plant genera with desirable agronomic and nutritive profiles, such as *Acacia*, (Dynes and Schlink, 2002), had potent inhibitory effects on gas production and VFA. Other researchers have reported similar findings with plants that are known to contain plant secondary compound (PSC) that can affect rumen microbes when examined *in vitro* (Tefera *et al.*, 2008). While legumes are reported to contain tannins that can reduce fermentation parameters (Tefera *et al.*, 2008), for others, such as the genus *Leptadenia*, the effect may be related to different classes of bioactive PSC (Ghisalberti, 1994). On the other hand, cell wall contents (NDF and ADF) were negatively correlated with gas production at all incubation times and the estimated parameters. This may tend to reduce the microbial growth and enzyme activity (McSweeny *et al.*., 2001) or intestinal bacterial activity (Salem *et al.*., 2004). Getachew *et al.* (2000) and Salem *et al.* (2007) reported a decrease in rate and extent of gas production of some shrubs due to high contents of lignin and tannins caused by increasing adverse environmental conditions as incubation time progressed. This is consistent with the findings of De Boever *et al.* (2005) who reported that gas production was negatively related to NDF content and positively to starch. Also, the relatively high level of ADL in the browse forages studied explained in part the limited *in vitro* degradation and the lower amount of gas produced. Similar observations were reported by Nordheim-Viken and Volden (2009) and Camacho *et al.* (2010). McAllister *et al.* (1994) also observed that higher NDF and lignification and/or higher ADF/NDF proportion and free-CT contents can reduce attachment of rumen microbes to feed particles and can as well inhibit microbial growth and enzyme activity (McSweeny *et al.*, 2001) or intestinal bacterial activity (Salem *et al.*, 2004) by free-condensed tannins and lead to lower gas production.

Kinetics of gas production obtained from the exponential model (Table 3) - rate constants b and c showed significant differences ($P<0.05$) among browse forages with higher extent of gas volumes (*a+b*) for *Buahinea nufescens* than the other browse forages under study. Khazaal *et al.* (1995) indicated that the intake of a feed is mostly explained by the rate of gas production (*c*) which affects the rate of passage of the feed through the rumen, whereas the potential gas production (*a + b*), is associated with the degradability of the feed. Thus, the higher values obtained for the (*c*) and (a + b) parameters in the browse forages may indicate a better nutrient availability for rumen micro-organisms in animals grazing vegetative species in semi-arid areas.

Methane production is an energy loss to the animal and, when accumulates in the rumen, it results in bloat (Babayemi, 2006). A reduction in methane production is expected when the residence time of feed in the rumen is reduced since ruminal digestion decreases since mehanogenic bacteria are less able to compete in such conditions (Moss *et al.*., 2000). Furthermore, a rapid passage rate favours propionate production and the relevant H use. According to Kennedy and Milligan (1978) and Okine *et al.* (1989), a 30% decline in methane production is observed when the ruminal passage rate of liquid and solid phase increased by 54 to 68%. Mean retention time was shown to explain 28% of the variation in methane emissions (Okine *et al.*., 1989). An increase in feeding level induces lower methane losses as a percentage of daily energy intakes (Moss *et al.*., 1995). Methane losses expressed as the proportion of gross energy intake declined by 1.6 percentage units for each multiple of intake as reported by Johnson and Johnson (1995). The major effect of feeding level is explained by its consequences on passage of feed particles out of the rumen (Owen and Goetch, 1986). There are several methods proposed as a means of reducing methane production in the rumen, and some of these examples are: proportion of concentrate in the diet (Lee *et al*., 2003), processing of forages (Santoso *et al*.., 2003) and some methane inhibitors such as halogenated compounds (Martin and Macy, 1985), ionophores (Van Nevel and Demeryer, 1988), Organic acids (Martin, 1998), sarsaponin (Lila *et al*.., 2003), and unsaturated fatty acids (Moss *et al*., 2000).

CONCLUSION

It was concluded that the leaves of the selected browse forages have a good nutritional potential to supply highly digestible feed suitable for ruminants. The various methods of reducing methane production should be used to minimize its production so as to avoid digestive disorders.

REFERENCES

AOAC (2002). Official Methods of Analysis of the Official Analytical Chemist (AOAC), 18th ed. (Howitz, W. ed.). association of official analytical chemists, Washington DC.

Babayemi, O.J., (2006). Anti-nutritional factors, nutritive value and in vitro gas production of foliage and fruit of Enterolobium cylocarpum. *World Journal Zoology,* 1 (2): 113-117.

Bakashi, M.P.S. and Wadhwa, M. (2004). Evaluation of forest leaves of Semi-hilly arid regions as livestock feed. *Asian-Australasian Journal Animal Science*, 95:93-104.

Barry, T. N. and Duncan, S. J. (1984). The role of condenced tannins in the nutritional value of *Lotus pedunculatus* for sheep. I. Voluntary intake. *Journal of Association of Official Analytical Chemists* 65: 496-497.

Blümmel, M. and Becker, K. (1997). The degradability characteristics of fifty-four roughages and roughage neutral-detergent fibres as described by *in vitro* gas production and their relationship to voluntary feed intake. *British Journal of Nutrition* 77: 757-768.

Blümmel, M., Steingass, H. and Becker, K. (1994). The partitioning of *in vitro* fermentation products and its bearing for the prediction of voluntary feed intake. Proceedings of the *British Society for Nutrition and Physiology* 3: 123

Camacho, L. M., R. Rojoa, A. Z. M. Salema, G. D. Mendozac, D. Lópeza, J. L. Tinocoa, B. Albarrána, and O.D. Monta˜nez-Valdezd (2010). *In vitro* ruminal fermentation kinetics and energy utilization of three Mexican tree fodder species during the rainy and dry period. *Animal Feed Science and Technology* 160: 110–120.

Chikagwa-Malunga, S.K., A.T. Adesogan, M.B. Salawu, N.J. Szabo, R.C. Littell, S.C. Kim, and S.C. Phatak, (2009). Nutritional characterization of Mucuna pruiriens. 2. *In vitro* ruminal fluid fermentability of Mucuna pruriens, Mucuna l-dopa and soybean meal incubated with or without l-dopa. *Animal Feed science and Technology,* 148: 51-67.

De Boever, 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 Science and Technology,* 123:124-255.

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 Science and Technology*, 93: 205-211.

Dynes, R.A. and A.C. Schlink (2002). Livestock potential of Australian species of *Acacia*. *Conservation Science of Western Australia*, 4: 117–124.

Fievez, V., Babeyemi, 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.

Finlay, B.J., Esteben, G., Clarke, K.J., Williams, A.G., Embley, T.M., Hirt, R.P. (1994). Some rumen ciliates have endosymbiotic methanogens. *FEMS Microbiology Letters*, 117: 157-162.

Getachew, G., M. Blümmel, H.P.S. Makkar and K. Becker (2000). Tannins in tropical browses: effects of tannins on *in vitro* microbial fermentation and microbial protein synthesis in medium containing different amounts of nitrogen. *Journal of Agriculture and Food Chemistry*, 48: 3581-3588.

Ghisalberti, E. L., (1994). The ethnopharmacology and phytochemistry of Eremophila species (*Myoporaceae*). *Journal of Ethnopharmacology*, 44: 1–9.

IPCC (Intergoverment Panel on Climate Change). Climate change 2001. *The Scientific Basis*. Cambridge UK: Cambridge University Press.

Johnson, K.A and Johnson, D.E. (1995). Methane emissions from cattle. *Journal Animal Science* 73:2483-2492.

Jones, G.A.., McAllister, T.A., Muir, A.D., Cheng, J. (1994). Effects of sainfoin (*Onobrychis viciifolia* Scoop) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. *Applied Environmental Microbiology*, 60: 1374-1378.

Khazaal, K., Dentino M.T., Ribeiro J.M. and Orskov E.R. (1995). Prediction of apparent digestibility and voluntary intake of hays fed to sheep: comparison between using fibre compo-nents, *in vitro* digestibility or characteristics of gas production or nylon bag degradation. *Anim. Sci.* 61, 527-538.

Kenney P.M., Milligan L.P., Lee, H.J., Lee, S.C., Kim, B.K., Kim, C.W. and Kim, K.J. (2003). Methane production potential of feed ingredients as measured by *in vitro* gas test. *Asian-Australasian Journal of Animal Science*, 16(8): 1143-1150.

Lee, H.J., Lee, S.C., Kim, J.D., Oh, Y.G., Kim, B.K., Kim, C.W. and Kim, K.J. (2003). Methane production potential of feed ingredients as measured by *in vitro* gas test. *Asian-Australasian Journal of Animal Science*, 16 (8): 1143-1150.

Lila, Z.A., Mohammed, N. Kanda, S. Kamada, T. and itabashi, H. (2003). Effect of sarsaponin on ruminal fermentation with particular reference to methane production *in vitro Journal of Dairy Science*, 86(10): 3330-3336.

Martin, S.A (1998). Manipulation of ruminal fermentation with organic acids: a review. *Journal of Animal Science* 76(12): 3123-23.

Martin, S.A. and J. M. Macy. (1985). Effects of monesin, pyromellitic diimide and 2-bromoethanesulfonic acid on rumen fermentation *in vitro*. *Journal of Animal Science* 60:544-550.

McAllister, T.A., H. D. Bae, G. A. Jones, K. J. Cheng, (1994). Microbial attachment and feed digestion in the rumen. *Journal of Animal Science* 72: 3004–3018.

McSweeny, C.S., B. Palmer, D. M. McNeill, D. O. Krause (2001). Microbial interactions with tannins: nutritional consequences for ruminants. *Animal Feed Science and Technology* 91: 83–93.

Moore, K.J and Jung, H.J.G (2001). Lignin and fibre digestion. *Journal.range management* 54, 420-430.

Moss, A.R. (1993). Methane: Global warning and production by animals. Chalcombe publications, Kingston, UK.

Moss, A.R., Jouany, J.P. and newbold, J. (2000). Methane production by ruminants: its contribution to global waning. *Annal Zootechnology*. 49:231-253.

Moss A.R., Givens D.I., and Garnsworthy P.C., (1995). The effect of supplementing grass silage with barley on digestibility, in sacco degradability, rumen fermentation and methane production in sheep at two levels of intake. *Animal Feed Science Technology* 55: 933.

Njidda. A.A. (2010). 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. *Global Veterineria* 4(3): 292-298.

Njidda, A.A. (2011). Evaluation of the potential nutritive value of browse forages of semi-arid region of Nigeria. Ph D Thesis submitted to the Department of Animal Science, Ambrose Alli University, Ekpoma, Nigeria. 219pp

Njidda A.A. (2012). Mineral Profile and *in vitro* gas Production of Leaves of Four *Ziziphus* Species Used as Fodder for Ruminants in the Semi-arid Zone of Nigeria. *Journal of Agriculture, Biotechnology and Ecology*, 5(2), 1-19.

Nordheim-Viken, H. and H. Volden (2009). Effect of maturity stage, nitrogen fertilization and seasonal variation on ruminal degradation characteristics of neutral detergent fibre in timothy (Phleum pratense L.). *Animal Feed Science and Technology* 149: 30–59.

Okine E.K., Mathison G.W and Hardin R.T. (1989). Effects of changes in frequency of reticular contractions of fluid and particulate passage rates in cattle. *Journal of Animal Science* 67(12): 3388-3396

Ørskov E. R. and McDonald P. (1979). The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. *J. Agricultural Science*, 92: 499-503.
Owens F.N. and Goetsch A.L. (1986). Digesta passage and microbial protein synthesis in: Milligan L.P., Grovum W.L Dobson A. (Eds.). *Control of Digestion and Metabolism in Ruminants*, prentice hall, Englewood cliffs. New jersey. USA. Pp. 196226.
SAS Statistical Analysis System Institute Inc., (1988). SASSTAT Programme, Cary, NC: SAS Institute Inc.
Salem, A. Z. M., Y. M. Gohar, M. M. El-Adawy and M. Z. M. Salem (2004). Growth-inhibitory effect of some antinutritional factors extracted from Acacia saligna leaves on intestinal bacteria activity in sheep. In: Proceedings of the 12th Scientific Conference of the Egyptian Society of Animal Production (ESAP), Mansoura, Egypt, pp. 283–300.
Santoso, B., Kume, S., Nonaka, K., Kimura, K., Mizukoshi, H., Gamo, Y. and Tahahashi, J. (2003). Methane emission, nutrient digestibility, energy metabolism and blood metabolites in dairy cows fed silages with and without galacto-oligosaccharides supplementation. *Asian – Australasian Journal of Animal Science* 16(4): 534-540.
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.
Stewart, C.S., H.J Flint, and Bryant, M.P. (1997). The rumen bacteria. In: P.N. Hobson and C.S Sterwart, eds. *Rumen Microbial Ecosystem* Blackie acad., London, UK. 1072pp
Tefera, S., V. Mlamboa, B. J. Dlamini, A. M. Dlamini, K. D. N. Koralagama, F. L. Mould (2008). Chemical composition and *in vitro* ruminal fermentation of common tree forages in the semi-arid rangelands of Swaziland. *Animal Feed Science and Technology* 142: 99– 110.
Van Nevel, C.J. and Demeyer D. I (1988). Manipulation of rumen fermentation in the rumen microbial ecosystem (P.N. Hobson, ed) pp. 387-443. Elsevier science publishers, new york, USA.
Van Soest, P.J., Robertson, J. B. and Lewis, B.A. (1991). Methods for dietary neutral detergent fibre and non starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74:3583-3597.
Wolin, M. J. (1960). A theoretical rumen fermentation balance. *Journal of Dairy Science*. 43: 1452-1459.
Wuebbles, D.J., and K. Hayhoe. (2002). Atmospeheric methane and global change. *Earth-Science Review*. 57:117210.
Zinder, S.H. (1993). Physiological ecology of methanagenesis. Ecology of methanogens. In: J.G. Ferry (ed) Methanogenesis. Ecology, Physiology. Biochemistry and Genetics, J.G. Ferry, ed. Chapman and Hall, New York, NY.128206 pp.