PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
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Pakistan Journal of Nutrition 9 (7): 637-642, 2010
ISSN 1680-5194

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) content in the browses ranged from 13.96-21.421% Dry Matter (DM). The NDF and ADF were 33.31-58.81 and 21.16-31.39 g/100 g DM respectively. The ash content of the browses ranged from 10.76-17.76 (% DM). The content of phenolic and Saponin were 0.32-0.48 and 2.02-2.78 mg/g DM. Total Condensed Tannin (TCT) ranged from 0.32-2.96 mg/g DM. The TCT was significantly correlated ($p<0.05$) with gas production ($r = 0.95$; $p<0.05$). The Metabolizable Energy (ME) and Organic Matter Digestibility (OMD) were 3.31-6.23 (MJ/Kg DM) and 30.64-55.44 (% DM). A good relationship ($R^2 = 0.99$; $p<0.05$) 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: Stoichiometric, *in vitro*, browse, tannins, short chain fatty acids, phenolic

INTRODUCTION

Browsable plants, beside grass, constitute one of the cheapest sources of feed for ruminants. The diversity and distribution of browse plants in Nigeria have received early attention in studies carried out for the north (Saleem *et al*., 1979), southwest (Carew *et al*., 1980) and middle belt (Ibeawuchi *et al*., 2002) Nigeria. Tropical browse have been shown to contain varying quantities of condensed tannin and other anti-nutritional substances in their biomass that affect their optimal utilization by animals (Odenyo *et al*., 1999). The tree and shrub legume forages are rich in most essential nutrients such as proteins and minerals and tend to be more digestible than the grasses and crop residues. This necessitates the evaluation of the nutritional characteristics of the forages in order to maximize their use in ruminant diets.

The *in vitro* gas production technique as modified by Menke and Steingass (1988) is widely used to evaluate the nutritive value of feeds resources consumed by ruminants especially tree and shrub legume forages, particularly to estimate energy value of straws (Makkar *et al*., 1999), agro industrial by-products (Krishna and Gunther, 1987), compound feeds (Aiple *et al*., 1996) and various types of tropical feeds (Krishnamoorthy *et al*., 1995). The gas produced on incubation of cereal straws (Blummel and Ørskov, 1993), cereal grains (Opatparakit *et al*., 1994) and different classes of feed (Blummel *et al*., 1999) in buffered rumen fluid was closely related to the production of Short Chain Fatty Acids (SCFA) calculated using the stoichiometry outlined by Wolin (1960), which was based on carbohydrate fermentation. Little work has been done to investigate the effect of proteins and fats on stoichiometry of gas production. Cone and Van Gelder (1999) reported a poor correlation between measured and calculated gas volume on incubation of starch and glucose with increasing levels of casein. The objective of the present study was to assess the contents of phenolic compounds, *in vitro* gas production and stoichiometrical relationship between measured gas production and that calculated from SCFA production on incubating tropical browse species.

MATERIALS AND METHODS

Forage samples: Four indigenous browse samples (leaves) commonly consumed by ruminants animals were used in this study. The species were: *Ficus polita, 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 24 h to constant weight and ground to pass through a 1.0 mm, sieve. The samples were then sub-sample to obtain three samples for each tree species and used for the laboratory analysis.

Chemical analysis: Browse species were analyzed for Dry Matter (DM), Crude Protein (CP), Ether Extract (EE), Crue Fibre (CF) and ash according to AOAC (2005). The leaves samples were also analyzed for Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), according to Van Soest *et al*. (1991). Phenolic was determine using Folin Ciocalteu as describe by (Makkar, 2000). Saponins and total condensed tannin will be determined as reported by (Babayemi *et al*., 2004). Total condensed tannin was determined as reported by (Polshettiwar *et al*., 2007).

***In vitro* gas production study:** *In vitro* incubation was carried out using the method of Menke and Steingass (1988) in 30 ml buffered rumen fluid. Samples (200 mg DM) were incubated in triplicate. Rumen fluid was taken before the morning feed from three non lactating, non-pregnant West African Dwarf (WAD) female sheep through sunction method. The animals were fed with (*Panicum maximum*) *ad libitum* and 40% concentrate. Rumen fluid was collected into a pre-warmed insulated bottle, homogenized in a laboratory blender, strained using Cheese cloth with a pore size of 100 µm and then filtered through glass wool. All handling was carried out with continuous flushing by CO_2. The well-mixed and CO_2 flushed rumen fluid was added to the buffered mineral solution, which was maintained in a water bath at 39°C and mixed. Buffered rumen fluid (30 ml) was dispensed into each syringe containing browse samples. The syringes were immediately placed in a water bath at 39°C. Three syringes containing 30 ml inoculum served as blanks.

Statistical analysis: Metabolizable Energy (ME) was calculated as ME = 2.20 + 0.136GV + 0.057 CP + 0.0029 CF (Menke and Steingass, 1988). Organic Matter Digestibility (OMD%) was assess as OMD = 14.88 + 0.889 GV + 0.45 CP + 0.651 XA (Menke and Steingass, 1988). Short Chain Fatty Acids (SCFA) as 0.0239 GV-0.0601 (Getachew *et al*., 1999) was also obtained, 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 (1988) options.

RESULTS

Chemical composition of browse forages: The chemical composition and fibre fraction of the browse species are presented in Table 1. There was wide variation in the chemical composition of the roughages, with CP ranging from 13.96-21.42% DM, Ash from 10.70-17.96 %DM, NDF from 33.31-58.81 g/100 g DM, ADF from 21.16-31.39 g/100 g DM, TCT, PHE and SAP have these values of 0.25-2.96 mg/g DM, 0.32-0.48 mg/g DM and 2.02-2.78 mg/g DM respectively.

***In vitro* gas production:** Figure 1 shows the *in vitro* gas fermentation of the selected browses respectively. Net gas production was highest in *Z. mucronata* and tend to increase with increase in incubation time up to 48 h. It was however observe that gas production pattern of *A. tortilis*, *L. leucocephala* and *M. oleifera* were similar. Metabolizable Energy (ME), Organic Matter Digestibility (OMD), Short Chain Fatty Acids (SCFA) are shown in Table 2. The ME ranged between 3.31 MJ/Kg DM in *L. leucocephala* and 6.23 MJ/Kg DM in *Z. mucronata*. There was significant differences ($p<0.05$) in the ME among the browse forages. *Z. mucronata* was significantly ($p<0.05$) higher in ME than the other browse forages. The ME in *A. tortilis* and *L. leucocephala* was similar ($p>0.05$) below the value of *Z. mucronata*. The OMD ranged from 30.64% in *L .leucocephala* to 55.44% in *Z. mucronata*. The OMD increased significantly ($p<0.05$) with increase in gas production. Highly significant ($p<0.001$) correlations were observed between gas production and TCT (r = 0.93, n = 4). There were negative relationship between saponin and gas production (r = 0.10, n = 4); short chain fatty acids and gas production (r = 0.01, n = 4). 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 = 0.12, n = 4).

Table 1: Proximate composition of some selected browse species forages of semi-arid (% DM); NDF, ADF (g/100 g DM); TCT (mg/g)

Browse forages	DM	CP	Ash	NDF	ADF	TCT	PHE	SAP
Acacia tortilis	72.33^c	13.96^c	10.76^c	48.62^b	21.16^c	0.32^b	0.48	2.02
Leucaena leucocephala	89.63^b	19.42^b	17.96^a	58.81^a	25.52^b	2.96^a	0.37	2.02
Moringa oleifera	88.22^b	21.42^a	17.60^a	33.31^c	31.39^a	0.25^b	0.45	2.16
Ziziphus mucronata	92.26^a	19.23^b	15.43^b	58.67^a	22.89^c	0.72^b	0.32	2.78
Means	85.61	18.51	15.44	49.85	25.24	1.06	0.41	2.25
SEM	2.19	0.73	0.95	3.42	0.97	0.55	0.09	0.98

[a,b,c]Means in the same column with different superscript differ significantly ($p<0.05$). Dry matter; CP = Crude Protein; NDF = Neutral Detergent Fibre; ADF = Acid Detergent Fibre; TCT = Total Condensed Tannin; PHE = Phenolic; SAP = Saponin

Table 2: Net gas volume, metabolizable energy, organic matter digestibility, short chain fatty acid of semi-arid browse forages

Browse forages	NGV	ME	OMD	SCFA
Acacia tortilis	2.83^{c}	3.47^{c}	30.68^{b}	0.01^{c}
Leucaena leucocephala	1.16^{c}	3.31^{c}	30.64^{b}	-0.03^{d}
Moringa oleifera	8.16^{b}	4.33^{b}	40.94^{a}	0.13^{b}
Ziziphus mucronata	25.50^{a}	6.23^{a}	55.44^{a}	0.55^{a}
Means	9.41	4.33	39.42	0.17
SEM	2.43	0.28	3.35	0.06

Net Gas Volume (NGV = ml/200 mg DM), Metabolizable Energy (ME = MJ/Kg DM), Organic Matter Digestibility (OMD = %), Short Chain Fatty Acids (mmol)

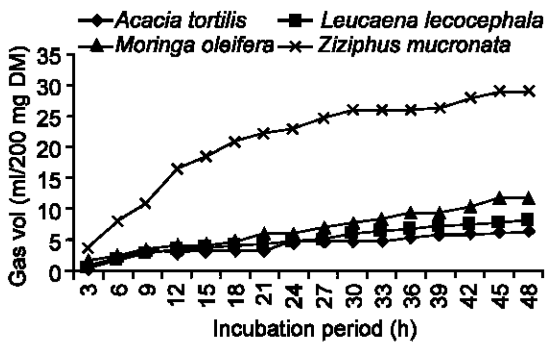

Fig. 1: Gas production of semi-arid browses

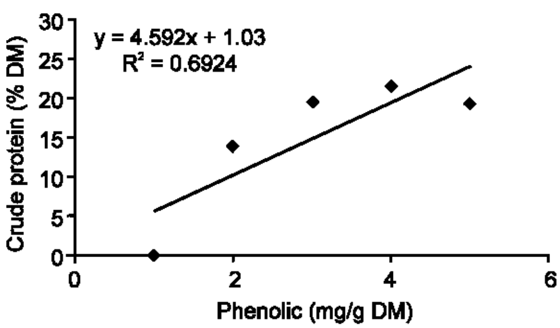

Fig. 2: Relationship between crude protein phenolic of semi-arid browses

DISCUSSION

The CP contents of the browses studied had a similar range as those from West Africa (Rittner and Reed, 1992). The results of the current study, those of Rittner and Reed (1992) and Njidda *et al.* (2009) indicate that most tropical browse species are high in CP and can be used to supplement poor quality roughages to increase productivity of ruminant livestock in tropical regions. The inverse relationship between CP and phenolic compounds indicates that considerable attention should be given in germplasm evaluation programmes to avoid selection against materials of high CP content. The result is similar (r = 0.87, n = 37) to the findings of Getachew *et al.* (2002) who further suggested that studies are required to understand the physiological mechanisms of plants that lead to the inverse

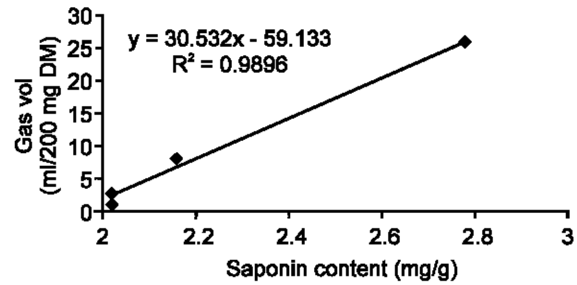

Fig. 3: Relationship between gas production and saponin of semi-arid browses

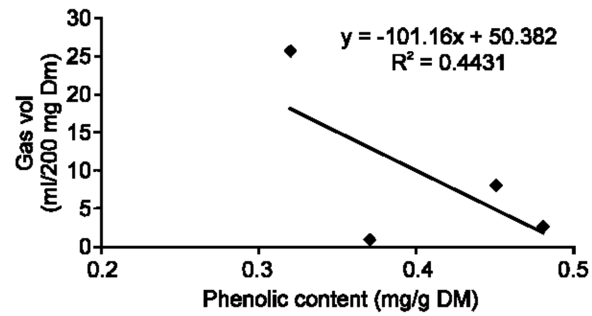

Fig. 4: Relationship between gas production and phenolic of semi-arid-browses

relationship between contents of CP and phenolic compounds and hence to make decisions in plant selection and screening programmes. Though a positive correlation was observed between CP and *in vitro* dry matter digestibility of the same browses (Njidda and Nasiru, 2010). The correlation between the change in gas production in the presence of tannin binding agent and phenolic contents of browses was consistent with those of (Khazaal *et al.*, 1994; Tolera *et al.*, 1997; Wood and Plumb, 1995). The relatively weak correlation between CT and gas production (R^2 = 0.41, n = 4), observed in the present study and that reported by others (Wood and Plumb, 1995; Abdulrazak *et al.*, 2000) could be due to the variation in structural and biological activity of tannins. The condensed tannins values by butanol-HCl method do not appear to reflect the biological activity. From the relationships between phenolic components and gas production observed in this study (Fig. 4), it can be concluded that tropical browses with less than approximately 45 and 20 g/kg of total phenol and tannin respectively are not likely to produce significant adverse effects on ruminant livestock. There was a positive correlation between metabolizable energy calculated from *in vitro* gas production together with content with metabolizable energy value of conventional feeds measured *in vivo* (Menke and Steingass, 1988). The equation of Menke and Steingass (1988) used to predict the metabolizable energy value of feeds from *in vitro* gas production has

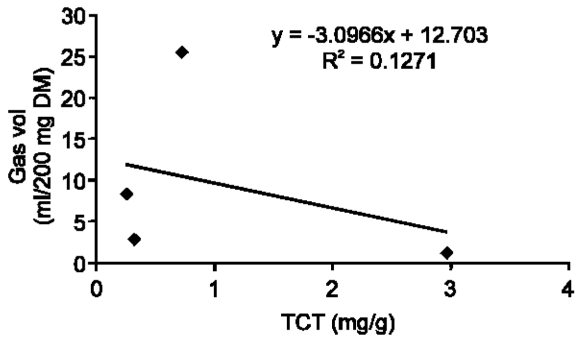

Fig. 5: Relationship between gas production TCT of semi-arid browses

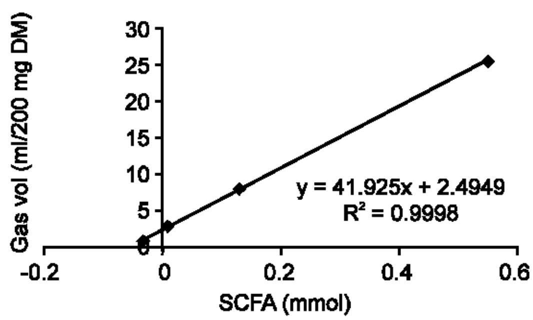

Fig. 6: Relationship between gas production SCFA of semi-arid browses

not been validated for tannin-containing tropical browses. However, since gas production on incubation of feeds in buffered rumen fluid is associated with feed fermentation, the low gas production from browses could be related to low feeding value of these feeds. 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 (Blummel *et al.*, 1999). 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 the SCFA production from sources other than carbohydrates is negligible. The negative relationship (r = 0.001, n = 4) observed in the study is similar to the report of Cone and Van Gelder (1999) who used different proportions of casein and carbohydrate sources (glucose and starch) and reported a poor correlation between gas measured and calculated from SCFA. 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, 1988) confirm the close relationship between SCFA production and gas volume liberated on fermentation of browse species with wide range of CP (77-300 g/Kg) and phenolic contents (Phenolic from 17-250 g/Kg DM and TP from 7-214 g/Kg DM respectively). From the results observed in the present study, SCFA production could be predicted from *in vitro* gas production. This relationship indicates that the presence of tannins does not influence the prediction of SCFA from *in vitro* gas production and that Wolin's stoichiometry also holds good for tannin-containing browses. Attempts have been made to predict the SCFA production using mathematical models (Pitt *et al.*, 1999). 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 in Getachew *et al.* (1998).

Conclusion: Semi-arid browses contain considerable amounts of phenolic compounds that reduced *in vitro* gas production. 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. Since SCFA measurement is important for relating feed composition to production parameters and to net energy values of diets, prediction of SCFA from *in vitro* gas measurement will be increasingly important in developing countries where laboratories are seldom equipped with modern equipment to measure SCFA.

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