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Non-starch polysaccharide composition and in vitro fermentability of tropical forage legumes varying in phenolic content

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Abstract

The fermentation kinetics of leaves of eleven tropical forage legumes varying in phenolic and condensed tannin content were investigated. The leaf samples were from *Acacia cyanophylla, Chamaecytisus palmensis, Calliandra sp., Dioclea guianensis, Flemingia macrophylla, Leucaena leucocephala, Sesbania sesban, S. goetzei* and three accessions of *Tadehagi* sp. (accession numbers 13269, 13275 and 23227). The phenolic, condensed tannin (bound condensed tannin and extractable condensed tannin) and non-starch polysaccharide (NSP ≡ to dietary fibre) contents of leaves were determined. Leaves were incubated at 39 °C with rumen micro-organisms in a habitat-simulating medium and the fermentation kinetics investigated using a pressure transducer. The NSP content of leaves both pre-and post-incubation was quantified and proportional losses of the NSP constituents calculated. The NSP content of leaves ranged from 124–252 mg g^{-1} dry matter, the major components in each case being cellulose-derived glucose and the uronic acids. In all samples, the most degradable NSP constituent was the uronic acids, closely followed by arabinose; cellulose-derived glucose and xylose were the least degradable NSP components. Samples ranked in terms of asymptotic gas-pool in descending order as follows: *L. leucocephala* = *C. palmensis* > *D. guianensis* > *S. sesban* > *A. cyanophylla* > *S. goetzei* > *Tadehagi* sp., (23227) > *Tadehagi* sp., (13269) > *F. macrophylla* > *Calliandra* sp. > *Tadehagi* sp., (13275). There were significant relationships between gas accumulation throughout the incubation and the NSP loss (NSP_L) values at the end of the incubation. Moreover, the gas pool at the end of the incubation period and NSP_L were significantly correlated ($r = 0.90$) with a yield constant (ml gas

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g^{-1} NSP loss) of 200 ml. Furthermore, there were significant ($P \leq 0.05$) negative relationships between both gas accumulation and NSP_L and the phenolic and condensed tannin content of leaves.

Keywords: Legumes; Gas production; Fermentability; Polysaccharides; Phenolics; Polyphenolics; Tannin

1. Introduction

Dietary fibre in forage legumes in the tropics is an important source of energy for farm livestock. The potentially energy-yielding fraction of dietary fibre consists of non-starch polysaccharides (NSP) which are largely derived from the structural polysaccharides of the plant cell wall. The major constituents of NSP are arabinose, galactose, mannose, rhamnose and xylose (from the hemicellulose fraction), glucose (mainly from cellulose) and the uronic acids (from the pectic fraction). Gravimetric methods for determining dietary fibre (as ADF and NDF) result in variable losses of one or more of the NSP components, the extent of which depends upon the botanical origin of the sample (Low, 1985). The pectic fraction in forage legumes, for example, can account for as much as 120 mg g^{-1} dry matter (Chesson and Munro, 1982) and is particularly sensitive to underestimation using the above gravimetric procedures (Van Soest, 1982). However, techniques are now available for routine use which measure the individual constituent monomers of the NSP fraction of plant cell walls, the sum of which represents the total NSP content of the sample. Unlike the gravimetric techniques, these methods define NSP composition without loss of constituent groups of polysaccharides, a crucial factor when evaluating the nutritive value of feeds (Longland and Low, 1989).

Many tropical forage legumes contain tannins, compounds with large numbers of phenolic groups which can form stable complexes with proteins and/or cell wall carbohydrates (Bate-Smith and Lerner, 1954; Mueller-Harvey et al., 1988). Thus, tannins can reduce the fermentability of plant cell walls either by forming indigestible complexes with plant proteins or cell wall polysaccharides, or by precipitating microbially-produced cell wall degrading enzymes (Barry et al., 1986). Tannins may also have bactericidal or bacteriostatic effects on the rumen or hind-gut microflora, further compromising the fermentation of feedstuffs (Mueller-Harvey et al., 1988).

The aim of the present study was to relate the fermentation kinetics of leaves (in vitro) of eleven tropical forage legumes varying in tannin content, as determined using the pressure transducer technique of Theodorou et al. (1994) to the loss of NSP (NSP_L) and its constituent monomers.

2. Methods and materials

2.1. Plant biomass

The forage legumes were from germplasm banks where they had been chosen for potential use as ruminant feedstuffs. Randomly selected leaves were harvested from

mature plants and midribs and petioles of single and multi-pinnate forms were included in the samples. Leaves of eleven tropical forage legumes were used: six were supplied by Dr. Carlos Lascano of Centro Internacional de Agricultura Tropical (CIAT), Colombia, and five by Dr. Jean Hanson of the International Livestock Centre for Africa (ILCA), Addis Ababa, Ethiopia. The samples from Colombia were *Calliandra* sp., *Dioclea guianensis, Flemingia macrophylla, Tadehagi* sp. (13269), *Tadehagi* sp. (13275) and *Tadehagi* sp. (23227). The samples from Ethiopia were *Acacia cyanophylla, Chamaecytisus palmensis, Leucaena leucocephala, Sesbania sesban* and *S. goetzei*. The CIAT samples were lyophilised and ground in Colombia, whereas those from ILCA were brought to the UK in the freshly-frozen state and lyophilised and ground to pass through a 1 mm dry mesh screen at the Institute of Grassland and Environmental Research (IGER), Aberystwyth, UK.

2.2. Microbial inoculum

Digesta were taken as grab-samples from a rumen-fistulated cow given grass silage *ad libitum* with 2.5 kg day^{-1} of a concentrate containing 150 g kg^{-1} crude protein (in two feeds) and transported to the laboratory in a vacuum flask. The microbial inoculum was prepared as described by Theodorou et al. (1994) with the following modifications: digesta were squeezed through two layers of muslin and homogenized in medium D (prepared without the leaf samples; see below).

2.3. Culture medium

Cultures were grown in medium D, a habitat-simulating medium prepared such that any contaminating micro-organisms from leaf samples were of no consequence in gas accumulation studies. The composition of medium D was similar to that of the digestibility medium of Tilley and Terry (1963). The medium was composed of a basal solution, prepared by mixing together (in numerical order) the following components: [1] Trypticase peptone (0.2 g) (Becton Dickinson Microbiology Systems, Cockeysville, MD 21030, USA), [2] micromineral solution (0.1 ml), [3] buffer solution (200 ml), [4] macromineral solution (200 ml), [5] resazurin solution (1 ml), [6] distilled water (500 ml). The solutions had the following composition. Micromineral solution (g l^{-1}) $CaCl_2 \cdot 2H_2O$ (132), $MnCl_2 \cdot 4H_2O$ (100), $CoCl_2 \cdot 6H_2O$ (10), $FeCl_3 \cdot 6H_2O$ (80). Macromineral solution (g l^{-1}) $Na_2HPO_4 \cdot 12H_2O$ (9.45), KH_2PO_4 (6.20), $MgSO_4 \cdot 7H_2O$ (0.60). Buffer solution (g l^{-1}) $(NH_4)_2HCO_3$ (4), $NaHCO_3$ (35). The resazurin solution contained resazurin as the redox indicator at a concentration of 1 g l^{-1}. Each solution was prepared using glass distilled water and kept in the dark at 4 °C until required. The basal solution was pre-reduced by bubbling a stream of oxygen-free CO_2 through the medium for ca. 2 h.

To complete the medium, a reducing agent (4 ml), the leaf sample to be fermented (1 g $\pm$ 0.5%) and 90 ml of basal solution were added together (5 replicates per sample) using anaerobic procedures in serum bottles (Phase Separations Ltd., Clwyd, UK; nominally of ca. 160 ml capacity, but retailed as 125 ml bottles) sealed with butyl rubber stoppers and aluminium crimp seals (Bellco Glass Inc., Vineland, NJ, USA). The

reducing agent was freshly prepared prior to use as follows. Cysteine HCl (6.25 mg) was added to distilled water (95 ml) to which was added 1M NaOH (4 ml) and $Na_2S \cdot 9H_2O$ (6.25 mg).

Sealed bottles were chilled to 4 °C (for not longer than 24 h prior to inoculation), warmed to 39 °C and inoculated with 10 ml of microbial inoculum using a 10 ml syringe fitted with a 23 gauge × 1.5 in needle (Sabre International Products Ltd., UK). Bottles were incubated without agitation at 39 °C until the end of the fermentation period.

2.4. Gas accumulation measurements

Digestion of leaf samples was quantified relative to control blanks, containing inoculated medium but no leaf sample by measuring gas evolution according to the pressure transducer technique of Theodorou et al. (1994). After the final reading, bottles were refrigerated at 4 °C to arrest fermentation. Thereafter, culture fluid was separated from residual plant particles and adherent microbial biomass by filtration through pre-weighed sintered glass funnels (porosity 1) under reduced pressure and the residue rinsed with two volumes (ca. 65 ml) of distilled water. The washed residues were then lyophilised to constant weight, lightly ground with a pestle and mortar and analyzed for NSP content and composition.

2.5. Chemical analyses

Plant cell wall carbohydrates were measured as NSP. Neutral sugars in NSP were determined by the method of Englyst and Cummings (1984), whereby alditol acetate derivatives of carbohydrate monomers derived from acid hydrolysates of washed, polymeric, de-starched samples were quantified by gas chromatography. A Varian 3400 chromatograph fitted with an automatic sampler (Varian 8000) and a flame-ionisation detector, linked to a Dell PC with Dionex A1-450 integration software, was used for NSP quantification. The uronic acid content of the hydrolysates was determined by the colorimetric method of Scott (1979). Bound and acetone extractable condensed tannins were determined by the method of Terrill et al. (1992). Phenolics were determined by the modified Prussian blue method of Price and Butler (1977).

2.6. Statistics and curve fitting

A spread-sheet programme (Quatro-Pro, Version 2.1; Borland Int. Inc) was used for data handling and processing of gas accumulation data (Theodorou et al., 1994) and to determine relationships between gas accumulation, NSP_L and the phenolic and tannin content of leaf samples. A micro Vax 3600 computer (Digital Equipment Corp., Marlborough, MA, USA) with Genstat 5 Committee (1987) or MLP (Ross, 1987) was used to fit curves to experimentally derived gas accumulation profiles using the model of France et al. (1993):

$$y = A - BQ^tZ^{\sqrt{t}}$$

where $Q = e^{-b}$, $Z = e^{-c}$, and $B = e^{bT+c\sqrt{T}}$. Here, y denotes cumulative gas production (ml), t is incubation time (h), A is the (predicted) asymptotic value for gas pool size (ml), T is the lag time (h) and b (h^{-1}) and c ($h^{-0.5}$) are rate constants. Estimated values of the four parameters, A, T, Q and Z, were determined after subtraction of the mean control profiles for gas produced in inoculated cultures incubated in the absence of substrate.

3. Results

3.1. Leaf NSP and phenolic content and composition

The range of NSP content varied from 124–252 mg g^{-1}, leaf samples from CIAT generally containing greater levels of NSP than those from ILCA, with mean values being 205 and 161 mg g^{-1}, respectively (Table 1). These differences in NSP content could be largely accounted for by the CIAT samples having a higher glucose content than those from ILCA (Table 1). In both cases the NSP glucose was predominantly from the cellulosic fraction as negligible amounts of non-cellulosic glucose were detected in any of the samples.

The major cell wall carbohydrate constituent in all leaf samples was glucose, which accounted for ca. 380–670 mg g^{-1} of the NSP, with the uronic acids being the next most abundant fraction, accounting for ca. 130–340 mg g^{-1} of the NSP. The pentoses, arabinose and xylose were generally present in near-equal proportions in *Calliandra* sp.

Table 1
Non-starch polysaccharide (NSP) content and composition of leaves from eleven tropical forage legumes (mg g^{-1} dry matter)

Site/sample	NSP components							Total NSP
	Arabinose	Glucose	Galactose	Mannose	Rhamnose	Xylose	UAC	
International Livestock Centre for Africa (ILCA)								
A. cyanophylla	10.2	74.5	4.3	8.3	2.0	12.4	32.3	144.0
C. palmensis	26.4	99.9	8.0	11.9	6.4	8.4	63.7	224.7
L. leucocephala	12.9	53.8	6.2	7.0	6.4	11.5	42.9	140.6
S. goetzei	6.2	90.1	5.5	16.0	3.7	5.8	43.5	170.9
S. sesban	10.9	48.2	4.4	7.7	2.5	7.5	42.3	123.6
Centro Internacional de Agricultura Tropical (CIAT)								
Calliandra sp.	5.8	105.2	5.1	10.4	1.7	6.2	31.1	165.4
D. guianensis	9.5	136.3	6.4	28.1	3.4	15.5	43.4	242.6
F. macrophylla	10.1	168.3	6.4	15.5	2.0	16.5	33.6	252.5
Tadehagi sp., (13269)	9.1	121.5	6.1	15.1	1.7	17.7	35.8	207.0
Tadehagi sp., (13275)	9.1	100.6	5.1	12.2	1.4	12.6	33.0	174.0
Tadehagi sp., (23227)	9.3	114.0	4.4	12.9	1.7	15.2	31.4	188.9

NSP components (neutral sugars) were determined by gas chromatography from alditol acetate derivatives of acid hydrolysates of de-starched samples as described by Englyst and Cummings (1984). Uronic acids (UAC) were determined by the colorimetric method of Scott (1979). Total NSP was calculated by summing neutral sugars and uronic acids. Five figure numbers in parentheses are accession numbers.

Table 2
Total phenolic and condensed tannin content of leaves from eleven tropical forage legumes

Site/sample	Total phenolics (Abs g^{-1})	Bound condensed tannins (mg g^{-1})	Extractable condensed tannins (mg g^{-1})	Total condensed tannins (mg g^{-1})
International Livestock Centre for Africa (ILCA)				
A. cyanophylla	544	8.2	62.6	70.9
C. palmensis	79	0	0	0
L. leucocephala	257	7.8	22.0	29.9
S. goetzei	482	14.6	142.0	156.7
S. sesban	337	11.9	75.9	87.8
Centro Internacional de Agricultura Tropical (CIAT)				
Calliandra sp.	1078	22.5	103.1	125.7
D. guianensis	450	12.5	120.2	132.7
F. macrophylla	692	31.9	134.9	166.8
Tadehagi sp., (13269)	758	15.3	147.6	161.9
Tadehagi sp., (13275)	894	16.8	158.2	175.2
Tadehagi sp., (23227)	611	9.7	136.3	146.0

Total phenolics were determined by a modification of the Prussian blue method as described by Price and Butler (1977). Condensed tannins were determined by the method of Terrill et al. (1992). Five figure numbers in parentheses are accession numbers.

(from CIAT) and all of the ILCA samples, except *C. palmensis* where the arabinose content was ca. 3-fold that of xylose. In the remaining samples from CIAT, the xylose content averaged 1.6-fold that of arabinose. In both CIAT and ILCA samples, mannose represented ca. 60–120 mg g^{-1} of NSP and at < 50 mg g^{-1}, the deoxy-hexose sugar, rhamnose, and the hexose, galactose, were relatively minor components in all cases.

Phenolics varied from 79 (*C. palmensis*) to 1078 (*Calliandra* sp.) Abs g^{-1}, CIAT samples generally containing greater levels of phenolics than those from ILCA, with mean values of 747 and 340 Abs g^{-1}, respectively (Table 2). A similar difference between sites was also observed for condensed tannins, with *Tadehagi* sp., (13275) (CIAT) containing the highest levels, whereas none were found in *C. palmensis* (ILCA), the mean values being ca. 70 and 150 mg g^{-1}, respectively (Table 2). Of the 10 leaf samples which contained condensed tannins, levels ranged from 29.9 (*L. leucocephala*) to 175.2 mg g^{-1} (*Tadehagi* sp., 13275) (Table 2). Fractionation of the condensed tannins into bound and extractable components showed that the majority (74–93%) were extractable, the remainder being fibre-or protein-bound. Extractable condensed tannins ranged from 22 (*L. leucocephala*) to 175 mg g^{-1} (*Tadehagi* sp., 13275) and bound condensed tannins ranged from 7.8 (*L. leucocephala*) to 31.9 mg g^{-1} (*F. macrophylla*) (Table 2).

3.2. Loss of NSP (NSP_L) and gas accumulation

There were considerable differences in NSP_L from the eleven samples, ranging from 147 mg g^{-1} (*F. macrophylla*) to 784 mg g^{-1} (*L. leucocephala*); losses from the ILCA samples generally being greater than 2-fold those from CIAT (Table 3). With the exception of *A. cyanophylla*, where NSP_L was 534 mg g^{-1}, NSP_L from the remaining

Table 3
Loss of non-starch polysaccharide components (NSP_L) from leaves of eleven tropical forage legumes (mg g NSP or NSP constituents)

Site/sample	NSP component loss							Total NSP_L
	Arabinose	Glucose	Galactose	Mannose	Rhamnose	Xylose	UAC	
International Livestock Centre for Africa (ILCA)								
A. cyanophylla	696	412	649	591	628	226	846	534
C. palmensis	958	571	888	868	847	694	934	759
L. leucocephala	885	700	871	814	904	444	917	784
S. goetzei	837	617	717	819	682	420	912	717
S. sesban	867	553	720	721	606	495	960	734
Centro Internacional de Agricultura Tropical (CIAT)								
Calliandra sp.	233	139	173	28	419	−302	641	217
D. guianensis	748	632	734	320	795	499	942	652
F. macrophylla	16	110	207	154	251	−278	561	147
Tadehagi sp., (13269)	582	170	617	501	478	−77	787	313
Tadehagi sp., (13275)	506	166	607	433	477	−129	813	319
Tadehagi sp., (23227)	576	197	632	529	586	−27	748	326

NSP components (neutral sugars and uronic acids (UAC)) were determined by gas chromatography and colorimetry (see legend to Table 1) both pre-and post-incubation with rumen micro-organisms in batch culture. NSP_L and NSP constituent losses were determined by subtracting the amounts remaining in the residue after 168 h of incubation from those initially present prior to incubation. Five figure numbers in parentheses are accession numbers.

ILCA samples averaged 750 (±2.75) mg g^{-1}. By contrast, loss from the CIAT samples was more varied, with NSP_L from *D. guianensis* being 652 mg g^{-1}, which was ca. double that of the *Tadehagi* leaves and ca. 3-and 4-fold the values obtained from *Calliandra* and *F. macrophylla*, respectively (Table 3). This difference in NSP_L between leaves from ILCA and CIAT generally reflected poorer digestion of each of the NSP constituent sugars present in the CIAT samples as compared with those from ILCA. This was particularly noticeable for the glucose and xylose fractions, their loss from CIAT samples, with the exception of *D. guianensis*, was < 200 mg g^{-1} for glucose, with the xylose being non-fermentable. For the ILCA samples, however, loss of glucose ranged from 412–700 mg g^{-1} and that of xylose ranged from 226–694 mg g^{-1} (Table 3). The most abundant NSP constituent after glucose was the uronic acids, the loss of which from ILCA samples was high (generally in excess of 900 mg g^{-1} NSP). With the exception of *D. guianensis*, the uronic acid fraction of the CIAT samples was much less fermentable (561–813 mg g^{-1} NSP; Table 3)). Likewise, average losses of the minor NSP components, rhamnose, mannose and galactose, from the CIAT samples were respectively 69, 52 and 42% of those from ILCA. Xylose was the least digestible NSP constituent in all samples.

Gas accumulation profiles for each of the eleven leaf samples are shown in Fig. 1; estimates from fitted curves for parameter values and derived quantities were determined as described in Methods and Materials according to France et al. (1993). There were notable differences between gas accumulation profiles, with leaves of *C. palmensis* and *L. leucocephala* producing the most gas and *Tadehagi* sp., (13275) and *Calliandra* sp.

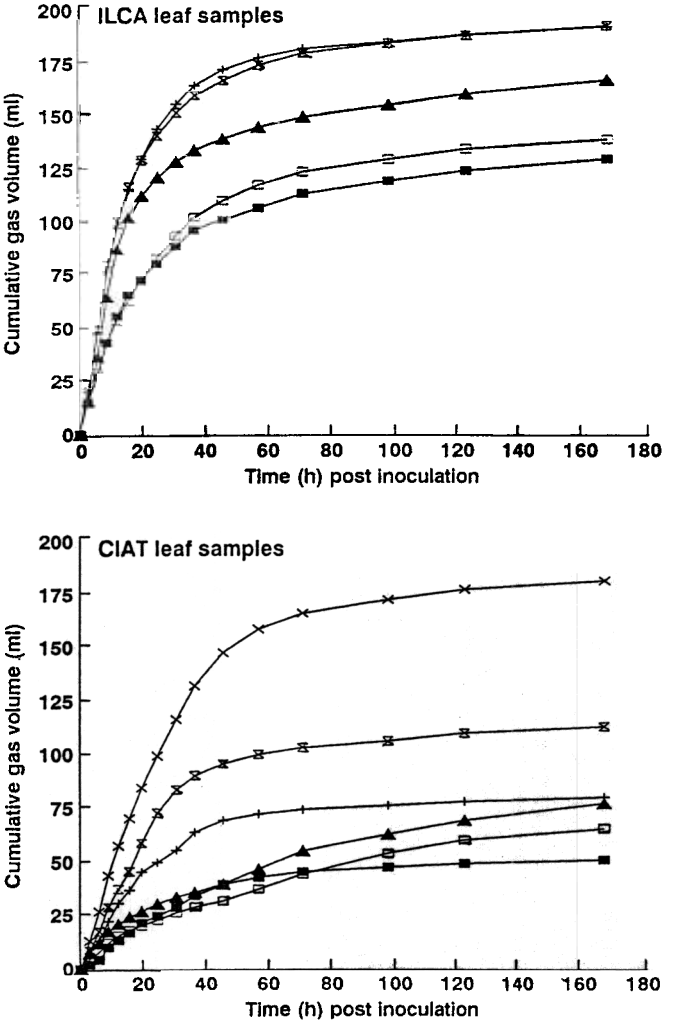

Fig. 1. Cumulative gas production profiles from the fermentation of eleven samples of tropical forage legume varying in polyphenolic content. Five were from the International Livestock Centre for Africa (ILCA), Ethiopia (*Acacia cyanophylla*, □; *Chamaecytisus palmensis*, +; *Leucaena leucocephala*, △; *Sesbania goetzei*, ■; *S. sesban*, ▲) and six were from the Centro Internacional de Agricultura Tropical (CIAT), Colombia (*Calliandra* sp., △; *Dioclea guianensis*, ×; *Flemingia macrophylla*, ▲; *Tadehagi* sp., (13269), +; *Tadehagi* sp., (13275), ■; *Tadehagi* sp., (23227), □). The results were obtained using 125 ml serum bottles (five bottles per sample) containing 100 ml culture medium plus inoculum. Five figure numbers in parentheses are accession numbers.

producing the least gas during the 168 h incubation. In general, the ILCA samples had higher gas accumulation rates and greater gas pools than those from CIAT (Fig. 1). Intra-site variation in the rate and extent of gas accumulation was considerable, the largest differences being seen for the leaves from CIAT where the estimated (asymptotic) gas pool from *Tadehagi* sp., (13275) was only 28% of that from *D. guianensis*.

For the ILCA samples, however, the lowest estimated gas pool was from *S. goetzei* being 67% of that produced by the highest gas producer, *L. leucocephala* (Fig. 1). In all but two cases, ≥ 50% of the estimated gas pool was produced within the first 25 h of fermentation, with gas accumulation from the ILCA samples being generally faster (ranging from 60–75% within 25 h) than those from CIAT (ranging from 35–64% within 25 h). With *Calliandra* sp. and *F. macrophylla*, only 35 and 39% respectively, of the estimated gas pool was produced within the first 25 h of fermentation. Despite rapid gas accumulation during the initial fermentation period, gas continued to be produced, albeit slowly, throughout the entire 168 h incubation period. According to the fitted model, all but two of the samples (*Tadehagi* sp., 13275 and *Calliandra* sp.) reached a predicted asymptote within the 168 h incubation period. In a subsequent experiment, not reported herein, 6 of the 11 leaf samples were fermented by rumen micro-organisms and it was found that gas accumulation rates and gas pool sizes from the corresponding samples ranked in the same order.

3.3. Relationships between NSP_L, gas accumulation and phenolics

The relationships for gas accumulation at 12, 25, 46 and 168 h post-inoculation and NSP_L at 168 h are shown in Table 4. Throughout the incubation, gas accumulation was proportional to NSP_L ($r \geq 0.90$; $P < 0.001$). The equation for this relationship at 168 h gave a yield constant of 200 ml gas g^{-1} NSP_L after subtraction of the gas volume at the intercept, which was assumed to represent gas evolution from non-NSP fermentable components of the leaves (Fig. 2). The strongest relationships between gas accumulation throughout the incubation and NSP constituent losses at 168 h were for the loss of xylose ($r \geq 0.91$; $P < 0.001$) and glucose ($r \geq 0.87$; $P < 0.001$) (Table 4). Lesser relationships were obtained between gas accumulation and the loss of arabinose ($r \geq 0.80$; $P < 0.01$), uronic acids ($r \geq 0.78$; $P < 0.01$) and mannose ($r \geq 0.66$, $P < 0.05$).

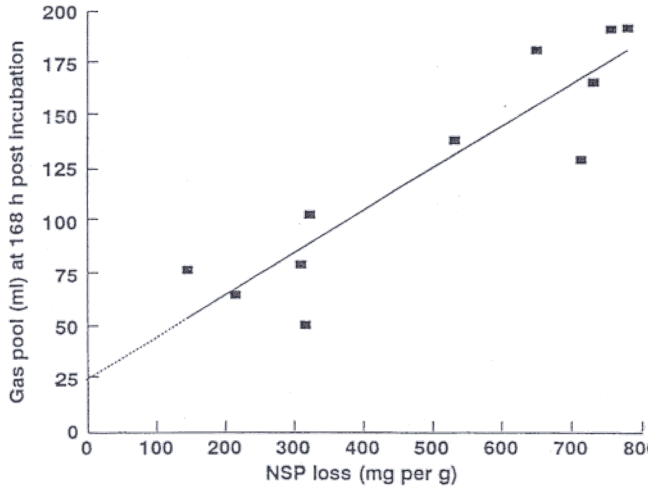

Fig. 2. Relationship between NSP_L and gas pool at 168 h post-inoculation ($r = 0.90$, $P < 0.001$). The regression equation describing this relationship is shown in Table 4.

Table 4
Linear regression relationships between gas accumulation and loss of NSP and constituent monomers (mg g^{-1}) at various times post-inoculation

NSP_L and constituent monomer losses at 168 h post-inoculation	Gas pool at 12 h post-inoculation	Gas pool at 25 h post-inoculation	Gas pool at 46 h post-inoculation	Gas pool at 168 h post-inoculation
NSP_L	$GP = 9.52 + 0.121NSP_L$ $r = 0.92$, $P < 0.001$	$GP = 5.66 + 0.169NSP_L$ $r = 0.91$, $P < 0.001$	$GP = 3.76 + 0.194NSP_L$ $r = 0.91$, $P < 0.001$	$GP = 22.6 + 0.197NSP_L$ $r = 0.90$, $P < 0.001$
Arabinose loss (A_L)	$GP = -5.8 + 0.092A_L$ $r = 0.83$, $P < 0.01$	$GP = -3.4 + 0.13A_L$ $r = 0.86$, $P < 0.001$	$GP = -5.8 + 0.092A_L$ $r = 0.86$, $P < 0.001$	$GP = 34.6 + 0.145A_L$ $r = 0.80$, $P < 0.01$
Glucose loss (G_L)	$GP = 5.01 + 0.119G_L$ $r = 0.88$, $P < 0.01$	$GP = 14.2 + 0.167G_L$ $r = 0.87$, $P < 0.001$	$GP = 24.4 + 0.198G_L$ $r = 0.89$, $P < 0.001$	$GP = 51.67 + 0.196G_L$ $r = 0.91$, $P < 0.001$
Mannose loss (M_L)	$GP = 5.84 + 0.083M_L$ $r = 0.79$, $P < 0.01$	$GP = 12.88 + 0.126M_L$ $r = 0.79$, $P < 0.01$	$GP = 24.6 - 0.126M_L$ $r = 0.85$, $P < 0.01$	$GP = 59.9 + 0.126M_L$ $r = 0.66$, $P < 0.05$
Xylose loss (X_L)	$GP = 36.8 + 0.082X_L$ $r = 0.91$, $P < 0.001$	$GP = 58.0 + 0.116X_L$ $r = 0.93$, $P < 0.001$	$GP = 36.8 + 0.082X_L$ $r = 0.94$, $P < 0.001$	$GP = 101.3 + 0.139X_L$ $r = 0.93$, $P < 0.001$
Uronic acid loss (U_L)	$GP = -104 + 0.19U_L$ $r = 0.78$, $P < 0.01$	$GP = -156 + 0.283U_L$ $r = 0.82$, $P < 0.01$	$GP = -167 + 0.326U_L$ $r = 0.83$, $P < 0.01$	$GP = -133.2 + 0.315U_L$ $r = 0.78$, $P < 0.01$

NSP components (neutral sugars and uronic acids [UAC]) were determined by gas chromatography and colorimetry (see legend to Table 1) both pre-and post-incubation with rumen micro-organisms in batch culture. NSP_L and NSP constituent losses were determined by subtracting the amounts remaining in the residue after 168 h of incubation from those initially present prior to incubation.

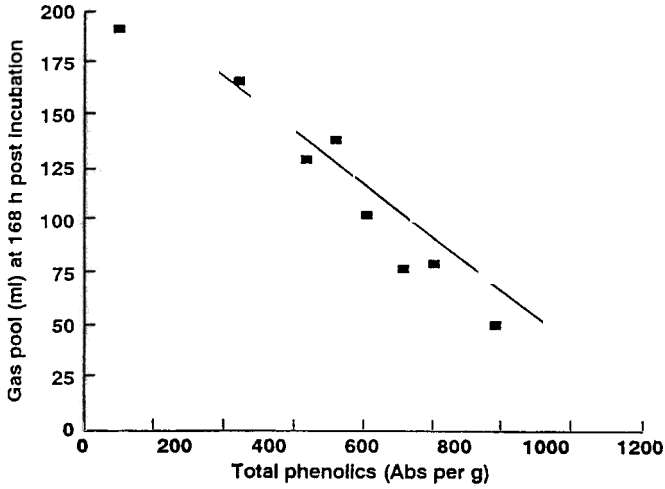

Fig. 3. Relationship between gas pool at 168 h post-inoculation and the phenolic content of the initial samples ($r = -0.92$, $P < 0.001$). The regression equation describing this relationship is shown in Table 5.

There were significant inverse relationships between gas accumulation at 12, 25, 46 and 168 h post-inoculation and the phenolic ($r \geq -0.92$; $P < 0.001$) and tannin ($r \geq -0.72$; $P < 0.02$) contents of the original samples (Table 5; Fig. 3); for simplicity, data for 168 h only are tabulated. However, the gradients of the regression lines for these data sets were broadly similar to those obtained at 168 h and the y-axis intercepts can be calculated from the corresponding regression equations shown in Table 4. Additionally, NSP_L at 168 h and the initial phenolic content of the samples were significantly inversely correlated ($r = -0.86$; $P > 0.001$) (Table 5). Of the individual NSP constituents, the most robust relationship was between xylose loss and phenolic content ($r = -0.91$; $P > 0.001$) with lesser, albeit significant ($P > 0.05$) relationships between losses of all the other major constituents and phenolic content ($r \leq -0.83$, ≥ -0.73). The regression lines describing these relationships are shown in Table 5.

NSP_L had a stronger inverse relationship with bound condensed tannins ($r = -0.73$; $P < 0.01$) than with extractable condensed tannins or total condensed tannins. Of the NSP constituents, r-values for monosaccharide losses ranged from -0.89 to -0.64 for arabinose ($P > 0.001$) and glucose ($P > 0.05$), respectively. Relationships between NSP_L, condensed and extractable condensed tannins were generally less significant, with the strongest inverse relationships being for xylose ($r \geq -0.67$), whereas correlations between uronic acid loss and condensed and extractable condensed tannins were not significant (Table 5).

4. Discussion

The gas accumulation profiles from leaves of the eleven tropical forage legumes differed over a wide range in both their rate and extent of fermentation. A common

Table 5
Linear regression relationships between gas accumulation, NSP_L and constituent monomer losses at 168 h post-inoculation and the initial phenolic and tannin contents of the leaf samples

Gas pool, NSP_L and constituent monomer losses post-inoculation	Phenolics (tp) (Abs g^{-1})	Condensed tannins (ct) (mg g^{-1})	Extractable condensed tannins (ect) (mg g^{-1})	Bound condensed tannins (bct) (mg g^{-1})
Gas pool (ml)	GP = 220 − 0.16tp $r = -0.92$, $P < 0.001$	GP = 204.4 − 0.69ct $r = -0.77$, $P < 0.01$	GP = 203 − 0.86ect $r = -0.73$, $P < 0.02$	GP = 188.5 − 4.49bct $r = -0.72$, $P < 0.02$
NSP_L (mg g^{-1})	NSP_L = 903.7 − 0.07tp $r = -0.86$, $P < 0.01$	NSP_L = 815 − 0.27ct $r = -0.67$, $P < 0.05$	NSP_L = 788 − 0.28ect $r = -0.64$, $P < 0.05$	NSP_L = 792.5 − 2.09bct $r = -0.73$, $P < 0.02$
Arabinose loss (A_L) (mg g^{-1})	A_L = 1073 − 0.79tp $r = -0.79$, $P < 0.01$	A_L = 973 − 3.03ct $r = -0.62$, $P < 0.05$	A_L = 924 − 2.96ect $r = -0.54$, NS	A_L = 1055 − 31.1bct $r = -0.89$, $P < 0.001$
Glucose loss (G_L) (mg g^{-1})	G_L = 764.9 − 0.67tp $r = -0.83$, $P < 0.01$	G_L = 669 − 2.47ct $r = -0.62$, $P < 0.05$	G_L = 648 − 2.59ect $r = -0.59$, NS	G_L = 632.9 − 17.82bct $r = -0.64$, $P < 0.05$
Mannose loss (M_L) (mg g^{-1})	M_L = 958.0 − 0.77tp $r = -0.80$, $P < 0.01$	M_L = 835.7 − 2.72ct $r = -0.58$, $P < 0.05$	M_L = 796 − 2.7ect $r = -0.51$, NS	M_L = 879.9 − 25.9bct $r = -0.78$, $P < 0.01$
Xylose loss (X_L) (mg g^{-1})	X_L = 805.9 − 1.12tp $r = -0.91$, $P < 0.001$	X_L = 645.6 − 4.09ct $r = -0.68$, $P < 0.05$	X_L = 602 − 4.22ect $r = -0.64$, $P < 0.05$	X_L = 627.6 − 32.66bct $r = -0.77$, $P < 0.01$
Uronic acid loss (U_L) (mg g^{-1})	U_L = 1010 − 0.33tp $r = -0.73$, $P < 0.05$	U_L = 954.9 − 1.15ct $r = -0.52$, NS	U_L = 933 − 1.1ect $r = -0.44$, NS	U_L = 994.9 − 12.42bct $r = -0.79$, $P < 0.01$

NSP components (neutral sugars and uronic acids (UAC)) were determined by gas chromatography and colorimetry (see legend to Table 1) both pre-and post-incubation with rumen micro-organisms in batch culture. NSP_L and NSP constituent losses were determined by subtracting the amounts remaining in the residue after 168 h of incubation from those initially present prior to incubation. NS = not significant ($P > 0.05$).

feature of the gas accumulation profiles was the presence of a slight sigmoidal trend which rendered the use of a simple exponential form, such as that advocated by Ørskov and McDonald (1979), inappropriate for their description. The model of France et al. (1993), which incorporates two exponential forms, was used to fit data in the current study and this generally showed an improved fit ($P < 0.05$) when compared with the single exponential form. Most but not all of the gas was produced from leaves within the first 48 h post-inoculation, although with two of the CIAT samples (*Calliandra* sp. and *F. macrophylla*) a discernable asymptote was not reached within the 168 h incubation period; these two legumes also showed the least NSP_L. According to Beuvink and Spoelstra (1992), gas accumulation during the initial incubation period was representative of the sum of gas output from two main sources: (1) gaseous end-products of fermentable substrate (including NSP) and (2) the release of CO_2 from the bicarbonate buffer in the medium by way of volatile fatty acid neutralisation. The amount of gas produced as an end-product from fermentation by rumen micro-organisms is not constant, but varies with the composition of the volatile fatty acids produced which in turn is substrate dependent (Beuvink and Spoelstra, 1992). For example, for the fermentation of 1 mM glucose, maximum and minimum amounts of gas would be evolved when acetate and propionate respectively, were the fermentation end-products. However, from the literature (Champ et al., 1989,1991) it would appear that major variations in volatile fatty acid ratios resulting from the fermentation of legumes may be uncommon; indeed a very close relationship exists between volatile fatty acid and gas accumulation (O'Hara and Ohki, 1973; Naga and Harmeyer, 1975; McBurney et al., 1990). During the latter incubation period, on depletion of available substrate, moribund micro-organisms may autolyse releasing small amounts of utilizable substrates which can then be fermented by the remaining viable micro-organisms and contribute small quantities of gas to the accumulating gas pool. This is a feature of gas accumulation profiles that is not often commented on; these small quantities of gas, produced over a variable length of time, dependent upon the primary fermentation period, can have a significant influence on the values estimated from curve fitting models. Their effects could be removed, however, by incorporating a stop function in the model. Tangent skimming to stop within 5° of the gas pool horizon could be a simple way of incorporating such a function.

The monosaccharide composition of the cell walls of many tropical forages has not been evaluated and similarly there is little information in the literature on the cell wall content of tropical forage legumes. Nevertheless, the monosaccharide composition of the NSP fraction of leaves of *L. leucocephala* has been determined (Vadilevoo and Fadel, 1992) and was similar to that reported herein. Likewise, information on NSP_L is also sparse. In the current study, the uronic acids (originating from the pectic fraction) were the most degradable fraction of each of the leaf samples and this was consistent with the findings of Dekker et al. (1972). Although pectins may be cross-linked to phenolics (Minor, 1982), Chesson and Munro (1982) suggested that the rate of uronic acid loss from ingested clover and lucerne was related to physical damage rather than the intrinsic chemical nature of the uronic acids. Thus, the damage caused by milling may be crucial in determining uronic acid loss from finely ground samples, particularly as the pectolytic rumen bacteria may require the surface layers of cells to be extensively abraded prior to

colonizing the pectic fraction (Howarth et al., 1978). Loss of pectins from the CIAT samples was lower than that from the ILCA samples. On visual inspection, the size of particles between leaf samples appeared to differ and were therefore subjected to computer-assisted particle size analysis. The median area (μm^2) of particles from ILCA samples was larger (1072) than that of those from CIAT (925), although these differences were not statistically significant (data not presented). However, leaf particles from the two sites were ground on different mills, possibly resulting in varying degrees of cell damage.

Xylose was a relatively minor component of the leaf samples and the least degradable of the NSP constituents, as has been found for lucerne (Nordkvist and Åman, 1986) and red clover (Åman and Nordkvist, 1983). The high degree of xylan substitution with phenolic acid constituents (Chesson et al., 1983) and to a lesser extent, arabinose residues (Brice and Morrison, 1982) have been implicated in reducing xylose degradation. Given that xylose was not detectable in rumen micro-organisms (Longland and Low, 1989), its accumulation during fermentation of most of the CIAT samples probably originated from carry-over of partially degraded graminaceous biomass in the rumen fluid inoculum.

Cellulose-derived glucose was the second most poorly fermented component (after xylose), as has been reported for a variety of feeds (Graham and Åman, 1987, Longland and Low, 1989). One of the restrictions to cellulose degradation is the hydrogen bonding between parallel microfibrils as these must be disrupted before degradation can occur (Hatfield, 1989); hydrogen bonding may also occur between cellulose and other wall polysaccharides, limiting access of cellulases to the cellulose core (Selvendran, 1983).

Arabinose loss was generally high, particularly for the ILCA samples and this has also been observed for clover and lucerne by Gaillard (1962). Losses of the minor constituents, galactose and mannose, were generally intermediate between the uronic acids and arabinose on the one hand and xylose and cellulose on the other and this has also been reported for other species (Albrecht et al., 1987).

There is no information in the literature on the relationship between gas accumulation and NSP_L. In this study, gas accumulation at recording-times throughout the fermentation was proportional to NSP_L at 168 h post-inoculation. Thus, irrespective of the model used for quantification, the fermentation kinetics of leaf samples as measured by gas accumulation was determined by the kinetics of NSP_L and was therefore a worthwhile predictor of cell wall carbohydrate losses. Given that linear relationships were obtained in correlations involving all samples, it would appear that constraints with similar net effects on the fermentation of leaves of each of the forage legumes were in force. The gradients of the regression lines shown in Table 4 for the relationships between gas accumulation at 12, 25, 46 and 168 h post-inoculation and NSP constituent losses were broadly similar for a given NSP constituent at each time point. Differences in gradients between NSP constituent losses and gas accumulation reflect the relative rates at which each of the constituent sugars were lost from the NSP fraction and the line equations represent the yield of gas (ml) g^{-1} of NSP constituent loss. According to Fig. 2, gas yields were constant at 200 ml gas g^{-1} NSP_L, with a Y-axis intercept value of 25 ml. The Y-axis intercept may be interpreted as representing fermentation of soluble components of the leaf samples, whereas the regression line was from the fermentation of NSP.

Although the gas yields are compromised, representing gas from both direct and indirect sources, these results suggest similarities between the leaves in the composition of their cell walls and cell contents. However, the NSP content g^{-1} of leaf of different forage legume leaves varied over a near two-fold range, indicating that by difference they contained varying amounts of other potentially fermentable constituents; this is reflected in the scatter of the points about the line in Fig. 2.

In the current study, gas accumulation, NSP_L and NSP constituent losses, were inversely related to the phenolic and tannin contents of leaf samples (Fig. 3, Table 5). The correlations between fermentation parameters (gas accumulation, NSP_L) and the phenolic content of leaf samples were greater than between fermentation parameters and any of the condensed tannin fractions. This suggests that the phenolic fraction contained components other than condensed tannins that had detrimental effects on the fermentation; these could include hydrolysable tannins and/or simple phenolics. Carbohydrate-phenolic acid complexes have been isolated from plant cell walls and have also been found free in rumen fluid (Gaillard and Richards, 1975). Binding of phenolics to the arabinose and xylose fractions of hemicellulose may reduce degradation, whilst free phenolic acids (such as p-coumaric and ferulic acids) have been shown to be toxic to rumen micro-organisms thus limiting cellulose digestion (Akin and Rigsby, 1985; Theodorou et al., 1987). In the current study, loss of xylose was highly correlated with the phenolic content of the initial sample and to a lesser extent with condensed tannin content. This may suggest that the xylose residues in these samples were highly substituted with phenolics in a manner similar to that described for clover by Chesson et al. (1983). Of the condensed tannins, the bound fraction had the highest inverse relationship with NSP_L and particularly arabinose loss. The observation that arabinose loss from clover and immature grasses was inversely proportional to its degree of polyphenolic substitution (Chesson et al., 1983) may be of interest in this context. Mechanisms by which bound condensed tannins influence cell wall digestion could involve the masking of potential binding sites for micro-organisms or cell-wall degrading enzymes (Martin and Martin, 1983), while extractable condensed tannins might mediate their effects by complexing with microbially produced extra-cellular enzymes (McCleod, 1974).

Components other than NSP make up a considerable proportion of the sample dry matter, but these were not characterized in this study. However, significant relationships were found across species between NSP_L, phenolic components and digestion characteristics. Although soluble components may affect digestion, they are likely to vary between species and not all of them are necessarily fermentable. In this study their influence was not sufficient to compromise the relationships obtained with NSP_L. Further investigations on the nature of the relationship between phenolics, NSP_L, loss of other components and gas accumulation are being conducted in our laboratories.

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