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Abstract

Five *Gliricidia sepium* provenances, comprising three central American native populations and two land races, were grown at five sites. Replicated leaf samples were evaluated by an in vitro gas production technique, estimation of crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF) and coumarin content. Differences between provenances in fermentation characteristics generally achieved statistical significance ($p<0.05$) at all five sites, but were small (13% range or less). Significant differences ($p<0.05$) were found between samples of young and old leaves, leaves from sunny and shady plots and between air-dried and freeze-dried samples. Differences due to site were highly significant for all fermentation characteristics, and much greater than differences due to provenance. The compositional analyses showed highly significant genetic variation in CP and ADF, but not in NDF or coumarin levels. Again, the range of values was small (<10%), and was dwarfed by site-related effects. The reasons for the site related differences were not identified. The narrow range of provenance means for all the traits measured, compared with the considerable site-related variability, suggested that intraspecific variation in nutritive value of these five provenances of *Gliricidia sepium* was unlikely to be of great practical importance to livestock keepers. © 1998 Elsevier Science B.V.

Keywords: *Gliricidia sepium*; Composition; Gas production; Leaves; Fodders

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1. Introduction

Shortage of good quality feeds is a major constraint to ruminant production, particularly in dry seasons when nitrogen deficient, fibrous native pastures and crop residues may be the only feeds available. The intake and digestibility of such feeds is generally poor but can be improved by supplementation. Concentrates are too expensive for widespread use by farmers in less-developed countries, but locally produced forage legumes can be a viable alternative (for reviews of tropical legumes in animal nutrition see Topps, 1992; D'Mello and Devendra, 1995).

Gliricidia sepium (Jacq.) Steud. has been widely researched and promoted (reviewed by Glover, 1989; Stewart et al., 1996) as a high quality feed for ruminants. It is a highly nutritious fodder and has been shown to be a suitable alternative to concentrates in forage-based diets (Richards et al., 1994). Genetic variation within the species has been investigated by the Oxford Forestry Institute (OFI) in a programme of germplasm exploration and collection in the native range (Hughes, 1987), followed by evaluation of variation among the provenances (seed sources) collected. First, growth and yield were evaluated in a network of field trials (Dunsdon and Simons, 1996). The three highest yielding provenances identified in these trials were then evaluated, in feeding trials with small ruminants at five livestock research institutions in Colombia, Costa Rica, Indonesia, Nigeria and Sri Lanka (Stewart et al., in press).

In conjunction with the feeding trials, leaf samples of each provenance were collected from the fodder blocks established at each of the sites. These were evaluated by an in vitro gas production technique which has shown promise for the evaluation of ruminant feeds (Wood et al., 1993; Theodorou et al., 1994). Crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF) and coumarin were also measured in the same samples. Coumarin has been proposed as a possible determinant of palatability in *G. sepium* (e.g. Merkel et al., 1994); varieties of sweet clover (*Melilotus alba*) which are high in coumarin show reduced palatability for ruminants (Bray, 1981). This paper reports the results of the gas production trials and the other compositional analyses, and compares the findings with those from the feeding trials (Stewart et al., in press).

2. Materials and methods

2.1. *Gliricidia sepium* leaf samples

Leaf samples of the following five native and exotic populations of *G. sepium* were evaluated:

Native populations:

Monterrico, Guatemala	124/91
Retalhuleu, Guatemala	125/91
Belen Rivas, Nicaragua	126/91

Land races (i.e. naturalized populations outside the native range):

Ibadan, Nigeria	4/92
Monteria, Colombia	32/92

In this paper, the term 'provenance' is used to include both types of population. The samples came from plots established for feeding trials by the following institutions:

International Livestock Research Institute (ILRI), Humid Zone Programme, Ibadan, Nigeria,
Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), Turrialba, Costa Rica,
Centro para la Investigación en Sistemas Sostenibles de Producción Agropecuaria (CIPAV), Cali, Colombia,
Balai Penelitian Ternak (BALITNAK), Ciawi, Bogor, Java, Indonesia,
University of Peradeniya (UP), Sri Lanka.

124/91, 125/91 and 126/91 are high leaf yielding provenances selected from earlier OFI trials (Dunsdon and Simons, 1996). 32/92 is a land race from Monteria, Colombia, which is considered by local farmers to be highly palatable for ruminants, while 4/92 is a land race from Ibadan, Nigeria, considered by local farmers not to be very palatable for ruminants. Provenance 124/91 was not grown in Indonesia.

The layouts of the fodder plots were different at the various sites, and there were also differences in the leaf samples taken. In Colombia, Sri Lanka and Nigeria, the trees were grown in a randomised complete block design. In Nigeria, there were three replications, all of which were sampled. In Colombia, each of the three replications were lopped at different times. Samples of young and old leaves were taken from two of the blocks; the 'young' leaves were just flushing (typically less than 1 week of leaf development), whilst the 'old' leaves were taken after about 3 months regrowth. In Sri Lanka there were five replicates, but only two of these were sampled: one growing in full sun, the other shaded by surrounding larger trees. The trees were grown as unreplicated single blocks in Costa Rica and Indonesia.

A summary of the number of samples included in each of the analyses is given in Table 1. A standard sampling procedure was used at all the sites. Samples of whole leaves (including the rachis but excluding all stem material) were taken from ten randomly selected trees in each plot, from different parts of the trees so as to give a sample representative of leaves from the plot as a whole. Replicate samples were obtained by repeating the sampling procedure.

Samples were air dried in the shade, and in Costa Rica the samples were divided into two identical lots, one lot being air dried and the other freeze dried. All dried samples were ground and passed through a 1 mm mesh size dry sieve. The samples from Costa Rica were used to assess the effect of drying method on in vitro fermentation and coumarin level.

2.2. *In vitro gas production*

The in vitro gas production method of Theodorou et al. (1994) was used. This involved the anaerobic fermentation of 1 g of dried ground substrate using an inoculum

Table 1
Number of *G. sepium* samples evaluated by (a) in vitro gas production and (b) compositional analyses

Country	Sample	Provenance									
		4/92		32/92		124/91		125/91		126/91	
		(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
Colombia	Old leaves									3	
	Young leaves									3	
Costa Rica	Air dried									4	
	Freeze dried									2	
Indonesia										4	
Nigeria	Rep. 1									2	
	Rep. 2									2	
	Rep. 3									2	
Sri Lanka	Grown in sun									4	
	Grown in shade									4	

[a] Coumarin analysis only.
n/a, not analyzed.

prepared from fresh rumen fluid. Inoculum donors were two rumen fistulated sheep fed on hay and concentrate diet (70:30 dry matter basis). A nitrogen-rich medium (containing 160 mg l^{-1} N, mainly as ammonium bicarbonate) was used and the incubations were terminated after 70 h at 39°C. Residues were recovered by filtration into pre-weighed sintered glass crucibles, porosity P160 (British Standard grade 1), dried and weighed. All sample replicates were fermented in duplicate using *G. sepium* alone as a substrate. Gas production was measured after 3, 6, 9, 12, 16, 20, 24, 28, 33, 39, 45, 52, 60 and 70 h incubation, together with dry matter disappearance after 70 h incubation (DMD70).

2.3. Leaf chemical composition

CP, ADF and NDF were determined by standard methods: CP by the Kjeldahl method (AOAC, 1990) and ADF and NDF by the method of Van Soest et al. (1991). For the Costa Rican samples, these three traits were only measured in the air-dried samples, for direct comparability with samples from other sites.

Coumarin was determined by high performance liquid chromatography (HPLC). The coumarin was extracted and purified using a modification of the method developed at CATIE (Kass et al., 1993). 0.1 g of dried leaf sample was suspended in 2 ml of deionized water and incubated in a heating block at 50°C for 1 h. Two 0.5 ml aliquots of the supernatant were transferred to duplicate columns containing 2.5 ml of Extrelut (Merck), from which the coumarin containing fraction was eluted in 10 ml of dichloromethane. Twenty μl of the eluate were injected on to an HPLC column (25×4.9 mm) packed with Spherisorb S5ODS1, maintained at 25°C in a water bath. The mobile phase was 60% aqueous methanol and the flow rate 0.7 ml min^{-1}. The absorption

of the effluent was monitored at 273 nm. Coumarin (Sigma) was used in both internal and direct standards.

2.4. Computation of data and statistical analysis

The significance of the effects of provenance, sample-type within site, and site were determined by analysis of variance, using the Statgraphics, Genstat and Minitab statistical programs. In the across-site analyses, only the air-dried samples from Costa Rica were included, since the freeze-dried ones were not directly comparable with those from other sites. Differences between means of more than twice the standard error of the difference (s.e.d.) were regarded as statistically significant ($p<0.05$). For the gas production data, cumulative gas production per g dry matter substrate after 12, 52 and 70 h incubation (CG12, CG52 and CG70, respectively) and DMD70 were selected for statistical analysis. CG12 was taken as an indicator of the initial fermentation, CG52 as an indicator of the relative extent of degradation which may occur in vivo (Prasad et al., 1994), CG70 and DMD70 as indicators the end-point of fermentation. The following mathematical model (France et al., 1993) was fitted to the average gas production data for each sample-type, provenance and site combination to illustrate the range of average fermentation rates found across provenances:

$$G = A(1 - e^{-b(t-T)-c(t-T)})$$

where G is gas production at time t, A is gas pool, b and c are rate constants, and T is lag time.

Percentage difference between provenances (between the highest and lowest gas production parameters) was defined as:

$$\text{differences}(\%) = \frac{\text{highest} - \text{lowest}}{\text{lowest}} \times 100$$

3. Results

The mean CG52 for each provenance at each site is given in Table 2. The mean model parameters for each provenance is given in Table 3, and mean DMD70 data in Table 4. The same analyses were also conducted on CG12 and CG70 data but are not shown. Overall, significant ($p<0.001$) provenance and site effects, and provenance×site interactions were observed in cumulative gas production at all the three times analyzed and in DMD70. Between provenance differences only failed to reach statistical significance ($p>0.05$) for CG12 for the trial in Colombia and in CG52 and CG70 for the trial in Indonesia. Significant ($p<0.05$) differences were also observed between provenances in all four of the model parameters.

Leaf chemical composition data (CP, ADF, NDF) are given in Tables 5–7. The overall effect of genotype across sites was highly significant ($p<0.001$) for CP and ADF but non-significant ($p>0.05$) for NDF. At individual sites, too, CP and ADF, but not NDF, varied

Table 2
Mean cumulative gas productions after 52 h incubation (CG52) for *G. sepium* provenances at five sites (ml per g dry matter)

Country	Sample-type	Accession					Site mean	s.e.d. means	F-prob.
		4/92	32/92	124/91	125/91	126/91			
Colombia	Old leaf	192	201	188	186	194	192	Prov: 2.80	Prov: <0.001
	Young leaf	191	206	169	182	189	187	Age: 1.77	Age: 0.005
	Mean	192	204	178	184	191	190		Prov×age: 0.002
Costa Rica	Air-dried	198	173	141	165	180	171	Prov: 5.72	Prov: <0.001
								Drying: 3.37	Drying: <0.001
	Freeze-dried	237	203	156	186	185	193		Prov×drying: 0.066
Indonesia	Mean	214	224	n/a	215	202	214	Prov: 8.07	Prov: 0.139
Nigeria	Mean	209	210	190	188	204	200	Prov: 2.60	Prov: <0.001
Sri Lanka	Sun	196	204	209	197	204	202	Prov: 3.77	Prov: <0.001
	Shade	188	207	201	200	193	198		light: <0.001
	Mean	192	206	205	198	198	200	Light: 2.13	Prov×Light: <0.001
All sites	Mean	199	204	186	190	196	Over-all mean: 195	Prov: 2.12	Prov: <0.001
								Site 2.26	Site: <0.001
									Prov×site: <0.001

Table 3
Average model[a] parameters for each of the five provenances of Gliricidia

France parameter	Provenance					Mean across provenances	Standard error of mean
	4/92	32/92	124/91	125/91	126/91		
Gas pool (A) ml g^{-1} DM	189	199	204	196	204	202	3.8
Rate constant (b) h^{-1}	0.0572	0.0541	0.0454	0.0413	0.0501	0.0499	0.00186
Rate constant (c) $h^{-1/2}$	−0.101	−0.088	−0.067	−0.067	−0.069	−0.080	0.0045
Lag time (T) h	2.86	2.34	2.08	2.59	2.84	2.52	0.0919

[a] France et al. (1993): $G = A(1 - e^{-b(t-T)-c(t-T)})$, where G is gas production at time t; A is gas pool; b and c are rate constants, and T is lag time.

significantly with provenance in most cases, though the opposite was true in the case of the Colombian samples, for which only NDF showed any provenance effect. Provenance 125/91 was highest in protein at all the sites where variation was significant. For ADF, 125/91 had the lowest levels overall, and was lowest or second lowest in ADF at every site.

Data on coumarin contents are presented in Table 8. Of the samples with high enough coumarin levels to be included, only the freeze-dried samples from Costa Rica showed a significant provenance effect (p=0.018). For coumarin, while the levels found in the freeze-dried samples from Costa Rica (5–8 g kg^{-1} in individual samples) were comparable with others in the literature (e.g. Griffiths, 1962), and with those reported by López (1995) for samples of the same material at the same site, the air-dried samples from Costa Rica, as well as those from Colombia and Sri Lanka, had levels so much lower (<0.5 g kg^{-1}) that it is probable that most of the coumarin was lost during drying and storage. These data were therefore omitted from the analysis. Coumarin levels in the samples from Nigeria and Indonesia were also rather low, but not sufficiently so as to merit omission (0.5–3.5 and 0.7–5.5 g kg^{-1}, respectively in individual samples).

3.1. Genotype (provenance) effects

Overall, the two land races (32/92 from Colombia and 4/92 from southern Nigeria) were more fermentable than the three provenances from the native range, these being very similar to one another throughout the fermentation period. The model rate constant (b) also indicates that provenances 4/92 and 32/92 were fermented more rapidly than the other provenances, although 4/92 also had a relatively low value for the gas pool (A) and an extended lag time (T). Provenances 4/92 and 32/92 were distinguished from the other three provenances by the rate constant (c) which was more negative for the two more fermentable provenances. Comparing overall provenance means for gas production variables at each time, the percentage differences between the highest and lowest value were 13%, 11%, 10% and 9% for CG12, CG52, CG70 and DMD70, respectively. This indicated that the range of mean values was fairly narrow and tended to become more so at the later incubation times.

Table 4
Mean dry matter disappearance after 70 h incubation (DMD70) for *G. sepium* provenances at five sites (expressed as proportion of dry matter)

Country	Sample-type	Accession					Site mean	s.e.d. means	F-prob.
		4/92	32/92	124/91	125/91	126/91			
Colombia	Old leaf	0.61	0.64	0.54	0.54	0.60	0.59	Prov: 0.028	Prov: 0.006
	Young leaf	0.58	0.68	0.63	0.57	0.61	0.61	Age: 0.018	Age: 0.122
	Mean	0.59	0.66	0.59	0.55	0.60	0.60		Prov×age: 0.304
Costa Rica	Air-dried	0.73	0.64	0.63	0.70	0.68	0.68	Prov: 0.014	Prov: <0.001
								Drying: 0.008	Drying: <0.001
	Freeze-dried	0.75	0.67	0.72	0.74	0.76	0.73		Prov×drying: 0.088
Indonesia	Mean	0.74	0.75	n/a	0.68	0.77	0.73	Prov: 0.018	Prov: 0.003
Nigeria	Mean	0.70	0.72	0.65	0.70	0.69	0.69	Prov: 0.008	Prov: <0.001
Sri lanka	Sun	0.68	0.71	0.65	0.69	0.70	0.69	Prov: 0.013	Prov: <0.001
	Shade	0.70	0.73	0.68	0.75	0.72	0.72	Light: 0.007	Light: <0.001
	Mean	0.69	0.72	0.66	0.72	0.71	0.70		Prov×light: 0.414
All sites	Mean	0.68	0.70	0.64	0.67	0.68	Over-all mean: 0.67	Prov: 0.009	Prov: <0.001
								Site: 0.010	Site: <0.001
									Prov×site: <0.001

Table 5
Mean CP content (g per kg dry matter) in leaf samples from five *G. sepium* provenances at five sites

Country	Sample-type	Accession					Site mean	s.e.d. means	F-prob.
		4/92	32/92	124/91	125/91	126/91			
Colombia	Old leaf	252	252	229	226	240	240	Prov: 1.038	Prov: 0.941
	Young leaf	251	270	283	288	272	273	Age: 0.659	Age: <0.001
	Mean	252	261	256	257	256	256		Prov×age: 0.038
Costa Rica	Air-dried	369	330	356	370	353	355	Prov: 0.564	Prov: <0.001
Indonesia	Air-dried	247	247	n/a	255	242	247	Prov: 0.444	Prov: <0.001
Nigeria	Mean	229	220	211	237	220	224	Prov: 0.509	Prov: <0.001
Sri Lanka	Sun	259	273	241	275	271	264	Prov: 0.719	Prov: 0.008
	Shade	271	246	257	270	263	261	Light: 0.415	Light: 0.325
	Mean	265	260	249	272	267	263		Prov×light: 0.002
All sites	Mean	272	266	263	276	268	Over-all mean: 269	Prov: 0.372	Prov: <0.001
								Site: 0.466	Site: <0.001
									Prov×site: 0.546

Table 6
Mean ADF content (g per kg dry matter) in leaf samples from five *G. sepium* provenances at five sites

Country	Sample-type	Accession					Site mean	s.e.d. means	F-prob.
						126/91			
Colombia	Old leaf	238	228	253	258	240	243	Prov: 1.060	Prov: 0.158
	Young leaf	233	188	200	196	214	206	Age: 0.671	Age: <0.001
	Mean	235	208	226	227	227	224		Prov×age: 0.099
Costa Rica	Air-dried	223	232	227	195	215	218	Prov: 0.975	Prov: 0.018
Indonesia	Air-dried	214	199	n/a	186	208	204	Prov: 0.963	Prov: <0.001
Nigeria	Mean	197	184	195	182	199	191	Prov: 0.426	Prov: 0.001
Sri lanka	Sun	244	227	248	238	233	238	Prov: 0.859	Prov: <0.001
	Shade	282	221	258	226	249	247	Light: 0.496	Light: 0.079
	Mean	263	224	253	232	241	242		Prov×light: 0.095
All sites	Mean	226	208	221	207	218	Over-all mean: 216	Prov: 0.467	Prov: <0.001
								Site: 0.507	Site: <0.001
									Prov×site: 0.412

Table 7
Mean NDF content (g per kg dry matter) in leaf samples from five *G. sepium* provenances at five sites

Country	Sample-type	Accession					Site mean	s.e.d. means	F-prob.
		4/92	32/92	124/91	125/91	126/91			
Colombia	Old leaf	335	327	342	391	386		Prov: 1.612	Prov: 0.010
	Young leaf	369	316	289	311	360		Age: 1.019	Age: 0.015
	Mean	352	321	315	351	373	344		Prov×age: 0.023
Costa Rica	Air-dried	389	419	424	386	417	405	Prov: 3.461	Prov: 0.796
Indonesia	Air-dried	311	291	n/a	308	339	318	Prov: 2.178	Prov: 0.145
Nigeria	Mean	343	346	408	388	388	375	Prov: 2.176	Prov: 0.018
Sri Lanka	Sun	397	387	434	417	374	402	Prov: 2.957	Prov: 0.910
	Shade	466	447	410	393	426	428	Light: 1.751	Light: 0.308
	Mean	432	417	422	405	400	413		Prov×light: 0.305
All sites	Mean	369	367	373	371	374	Over-all mean: 371	Prov: 0.542	Prov: 0.264
								Site: 1.344	Site: <0.001
									Prov×site: 0.266

five *G. sepium* provenances at five sites

Country	Sample-type	Accession					Site mean	s.e.d. means
					126/91			
Costa Rica	Freeze-dried	6.11	7.53	7.13	7.72	6.85	7.07	Prov: 0.018
Indonesia	Air-dried	3.36	3.45	n/a	3.91	3.98	3.67	Prov: 0.668
Nigeria	Mean	1.70	1.71	2.24	1.39	1.77	1.76	Prov: 0.166
								Prov: 0.485
								Prov: 0.618
								Prov: 0.328

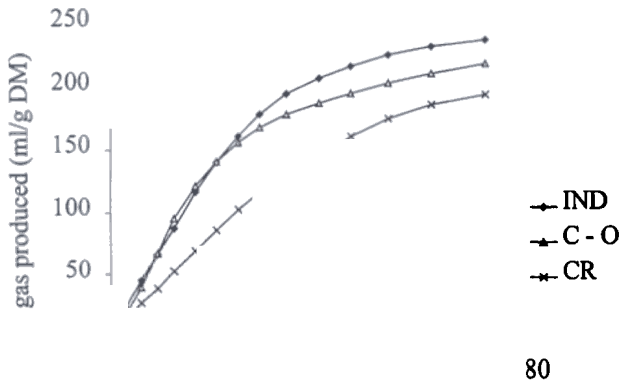

Fig. 1. In vitro gas production characteristics of *Gliricidia sepium* accession 32/92: samples from Indonesia (IND), Colombia 'old leaf' (CO) and Costa Rica air-dried (CR).

3.3. Genotype–environment interactions

Provenance×site interactions were highly significant in the fermentation experiments for all four response variables ($p<0.001$), although these interactions did not greatly affect the ranking of the provenances at most sites. For example, provenance 124/91, the lowest overall in vitro DMD, was the lowest at three of the four sites where it was grown whilst provenance 32/92, with the highest DMD, was the highest at three of the five sites. None of the compositional variables showed significant ($p>0.05$) genotype×environment interactions.

3.4. Effect of sample-type and preparation

In the *in vitro* fermentation of material from Colombia, CG12 values for old (mature) leaf samples were significantly ($p<0.001$) higher than the corresponding values for young leaves. The difference between the young and old leaves became less as the fermentation proceeded, although it remained statistically significant ($p<0.05$). DMD70, however, was not affected by leaf age. ADF and NDF were significantly ($p<0.001$ and $p<0.05$, respectively) higher in mature leaves, and CP was significantly ($p<0.001$) higher in young leaves.

The effect of shade was highly significant ($p<0.001$) for all the four fermentation variables, although there was no effect on CP, ADF or NDF ($p>0.05$). Provenances 124/91, 126/91 and 4/92 gave significantly higher gas production throughout the fermentation ($p<0.05$) when grown in full sunlight. Differences were not significant for the other two provenances ($p>0.05$). DMD, however, was significantly higher in the shade-grown samples for all the provenances ($p<0.05$).

In the in vitro fermentation, the freeze-dried samples from Costa Rica gave consistently higher values than the corresponding air-dried samples for all four variables, and this difference was highly significant ($p<0.001$). There was a significant ($p<0.05$) interaction between provenance and drying method for CG70 but rankings of provenances by gas production variables were largely unaffected by sample preparation method. CP, ADF and NDF were not measured in the freeze-dried samples. Coumarin concentration was up to 100 times higher in the freeze-dried samples than in the air-dried, indicating that most of the coumarin had been lost on air drying.

4. Discussion

4.1. Genotype (provenance) effects

In the fermentation experiments, leaves from the Colombian land race of *Gliricidia sepium*, 32/92, were generally the most degradable, followed by 4/92, the Nigerian land race. The three central American provenances 124/91, 125/91 and 126/91 had very similar characteristics to each other. Despite a high degree of statistical significance the overall differences between *G. sepium* provenances in the fermentation experiments were small and not always consistent, as reflected in the highly significant interactions between provenance and site.

While the differences and trends observed in the cumulative gas production data were also evident in the parameters obtained by fitting the France model, particularly in rate constant (c), the picture was not a clear one. This appeared to be in part due to some misfitting of the model and apparent artefacts arising from this. For example, there was an inverse relationship between the rate constant (b) and gas pool (A). However, in the cumulative gas production data CG12 tended to be positively related to CG70 and there did not appear to be a tendency for the rate to be inversely related to the final end point of fermentation. Possibly the model fitting would have been improved by extended incubation times. However, the model did not appear to cope well with all of the different shapes of curves obtained from samples from different sites.

4.2. Comparisons with in vivo studies (Stewart et al., in press)

In feeding trials carried out with the same five provenances of *Gliricidia sepium*, grown on the same five sites, much greater relative differences between provenances were observed in intake and live weight gain, although owing to the high level of variation between animals these differences were generally not statistically significant. Moreover, most of the gas production data relate to *G. sepium* fermented alone, whereas in practice it is almost always used in a mixture with a low-protein basal feed. Although differences between provenances were observed in vitro for the Colombian samples when the *G. sepium* was fermented alone, when the diet used in the feeding trials was mimicked the differences in fermentation were no longer statistically significant (Vargas, 1995). This is consistent with the lack of significant differences observed in the feeding trials.

Even when fed as the sole feed, no differences were detected between provenances in terms of in vivo (whole tract) dry matter digestibility in Nigeria and Sri Lanka, though in sacco studies in Colombia and Nigeria did reveal significant provenance variation. The in sacco study in Colombia gave similar results to the DMD70 data for that country (Table 4), with 32/92 showing the highest dry matter disappearance and 125/91 the lowest. The in sacco results for Nigeria, however, were not consistent with those from the in vitro fermentation of the Nigerian samples.

4.3. Site effects

Differences between sites were generally greater than differences between provenances, for both the in vitro fermentation data and the compositional data. These differences could have been due to a number of factors including edaphic and/or climatic effects, phenological state, and management aspects such as cutting frequency.

Site-related differences in gas production characteristics have been observed in *Leucaena leucocephala* and *Flemingia macrophylla* grown at three locations with different soil fertilities in Bolivia; the more fertile the soil the more fermentable the tree leaves (Wood et al., 1993). Environment can affect levels of tannins and hence digestibility (Barry and Forss, 1983; Mueller-Harvey and Dhanoa, 1991). Although *G. sepium* is very low in extractable tannins, there is a considerable amount of protein-bound tannin in the cell wall fraction (Jackson et al., 1996).

4.4. Effect of sample-type and preparation

It is possible, for all the variables measured, that an element of the apparent variation between samples from different sites was in fact due to differences in sample-type and preparation. Sample preparation was standardized as far as possible between the sites, with samples from all sites air dried in the shade, but differences in drying rate caused by factors such as temperature, relative humidity and wind could have had an effect.

Air drying was found to reduce significantly both gas production and DMD relative to freeze drying, in the Costa Rican samples. Siaw et al. (1993) have noted that oven drying at 60°C for 48 h can affect gas production characteristics compared with fresh samples, although their study did not include *G. sepium*. Mahyuddin et al. (1988) reported that the degradation characteristics (as determined by various end-point methods) of *Gliricidia sepium* were not affected by oven drying at temperatures as high as 100°C, although reduced degradabilities were observed in other species and freeze drying was recommended where possible. This study indicates that *G. sepium* may also have its degradability reduced by air drying, though it is also possible that the higher gas production from freeze-dried samples could have arisen from fermentation of the extra coumarin in these samples. Coumarin is known to be degraded in the rumen (Wina et al., 1994), and almost all the coumarin was lost from the air-dried samples.

It appears that differences in illumination between the two blocks sampled in Sri Lanka induced differences in the fermentation characteristics of provenances 124/91, 126/91 and 4/92. These all gave significantly higher gas production when grown in full sunlight. These differences could have been due to various factors such as the production of fermentable sugars due to increased photosynthesis in the sunlight and/or the production of anti-nutritive factors in shaded leaves. Local farmers indicated that *G. sepium* from sunny locations is preferred over that from shady locations. This may be consistent with the reduced gas production from shade-grown samples. Conversely, however, a feeding trial with goats, using the same material, showed significantly higher intake of shade-grown material (Stewart et al., in press), although this analysis used data pooled across all provenances.

The age of the leaves was also found to affect composition and fermentation characteristics of the samples from Colombia. As expected young leaves had higher protein and lower fibre contents than old leaves. Perhaps surprisingly the gas production from the young leaves was less than from the old leaves, particularly at shorter incubation times. This may have been the result of anti-nutritive factors inhibiting rumen microbes. The gas production technique has been shown to be inhibited by tannins (Wood and Plumb, 1995) and presumably may also be susceptible to inhibition by other factors. The higher protein content of the young leaves would also tend to reduce the gas produced as gas production predominantly reflects the fermentation of carbohydrate (Menke et al., 1979).

5. Conclusions

Provenance-related differences were found in the in vitro fermentation characteristics, CP and ADF of the leaf samples. No significant differences between provenances were found in NDF and coumarin contents. However, site-related differences were generally much greater than provenance-related differences. The reasons for the site related differences were not identified but could have been related, at least in part, to the nature of the samples taken, sample preparation as well as differences due to the different environments at the various sites. Nutritive value is only one of many factors, such as yield of leaves, resistance to disease, ease of cultivation, which determine which provenances are likely to be most favoured by farmers. Given the relatively high level of site-related variability, from whatever cause, all of the provenances tested would probably be of similar practical merit to farmers in terms of their nutritive value for ruminants. This conclusion was supported by the parallel feeding trials conducted by Stewart et al. (in press).

The gas production technique appeared to be a rapid and sensitive tool for the early screening of fodders such as tree leaves. Differences in vitro gas production of about 10% usually achieved statistical significance. Such differences may require large in vivo trials to detect. The gas production method appeared to be more than sensitive enough to detect differences of a magnitude likely to be of practical importance to farmers in less developed countries. More work is required before parameters derived from this technique can be used to provide reliable indicators of animal performance. However, the method could usefully help to rank and characterize feeds and assist in the design of appropriate animal feeding trials.

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