Discussion

The reduction in *C. vulgaris* cover with increased sheep density confirms previous findings (Rawes & Hobbs, 1979), as does the reduction in *E. vaginatum* (Grant *et al.*, 1985). *C. vulgaris* removal may result in an increased Sitka spruce growth rate in the plots (Weatherall, 1953).

The high percentage of dead herbage in October supports the conclusion of Forrest & Smith (1975), who highlighted a negative relationship between vascular plant productivity and increased wetness of the site. Few of the October clips had more than 20% dry weight. The increased senescence rate coupled with reduced productivity likely increased sensitivity to grazing. It is unsure at what point the sheep move to the trees as a source of nutrition.

The recovery period required for many bog species to exhibit regrowth needs further examination although a long term study would be unrealistic on this site as it appears that high density grazing (12 sheep ha^{-1} for 4 months) has caused long term damage to the vegetation. Stocking densities above the lowest density tested (4 sheep ha^{-1}) are likely to irreversibly alter the ecology of the ecosystem and even at this level, repeated grazing may have a major impact on the vegetation.

At levels of sheep grazing above c. 1 sheep ha^{-1} the impact on vegetation can be substantial. The removal of 50-60% of *C. vulgaris* green shoot biomass, and higher levels for sedges and grasses, indicated that it is unlikely that the experimental area could be grazed for a second field season. Overgrazing in 1992, to establish levels of tree damage by sheep grazing, has resulted in the intermediate and high density plots being unable to carry further stock. However the lower density plots will continue to be grazed to a pre-determined sward surface height level so that sheep grazing days at a range of grazing densities can be evaluated and long term decisions on the likely impact on subsequent tree growth made.

Once the data have been fully analysed, the implications for re-spacing Sitka spruce or for replanting Sitka spruce at wide spacing on a blanket bog to incorporate into agroforestry systems can be better evaluated.

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Evaluation of Bolivian tree leaves as fodders by an *in vitro* fermentation technique

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Abstract

An *in vitro* fermentation method which measures the production of gas by rumen microbes has been developed to estimate the nutritive value of feeds to ruminants. The method may also be indicative of the relative digestion characteristics of tree leaves when used as manures and mulches. The method was used to evaluate differences in digestion of leaves from three sites and from two species (*Leucaena leucocephala* and *Flemingia congesta*) and to rank 19 species which, according to local farmers, ranged from being highly palatable to completely unpalatable for cattle. Cumulative gas production after 52 and 166 hours were used as indices of digestibility to allow comparisons to be made and these, together with apparent digestibility (measured by recovery of insoluble residue), were used for ranking.

The extent of digestion of tree leaves varied significantly ($P<0.001$) between sites for both species. The sites which were regarded as having the most fertile soil had the more degradable leaves. For *L. leucocephala* and *F. congesta* between species differences were much greater than site related differences with *L. leucocephala* leaves being the more degradable.

There was no apparent relationship between palatability and digestion. This indicated that both palatability and digestibility must be investigated to assess the nutritive value of fodder trees. While knowledge of which trees animals eat is of vital importance, by itself it is not a good indicator of nutritive value.

Introduction

Ruminant species such as sheep, goats and cattle differ from monogastrics such as pigs and poultry in that they have a very specialised gut which allows feeds to be extensively digested by micro-organisms before they are subjected to the gastric and intestinal enzymes of the animal itself. The rumen acts as a fermentation vessel containing a complex mixture of bacteria, protozoa and fungi which are capable of digesting feed components such as cellulose. Animals themselves are generally unable to produce the enzymes required to digest cellulose. Ruminants are, therefore, able to extract nutrients from roughages such as straw which have high cellulose contents, although the feeds must contain sufficient nutrients to sustain both the rumen microbes and the animal itself. Poor quality feeds may be poorly digested even by rumen microbes while many roughages, if fed alone, contain insufficient nitrogen (protein) to enable the microbes to grow and fully digest the potentially digestible feed components.

In many tropical countries the shortage of fodder, particularly during the dry season, is a major constraint to animal production. In the tropical regions of Bolivia cattle frequently suffer weight losses during the dry season as fodder is not only limited in supply but is also of poor nutritive quality (Paterson *et al.*, 1979). Indigenous fodder trees are used as feed in some regions of the country but there is considerable potential to increase their contribution through the introduction of species which produce a higher yield of biomass.

The selection of suitable species requires assessment of agronomic and nutritional characteristics. Assessment of nutritive value is more complex than relying on conventional analytical methods due to the presence of anti-nutritive factors in many tree leaves (reviewed by Makkar, 1993). Leaves of leguminous species in particular are rich in protein, but much of this protein may be indigestible in species which contain high levels of tannins.

Theodorou *et al.* (1991) developed an *in vitro* gas production method to provide an estimate of nutritive value. The method gives information on the availability of nutrients in feedstuffs (related to their digestibility), nutritive value depending on the levels of nutrients and their availability. The higher the gas production the higher the digestibility. The objective of this work was to investigate the variability in digestibility of leaves from genetically similar trees growing at different sites (experiment 1) and to rank tree leaves from different species according to their ease of digestion as judged by *in vitro* gas production (experiment 2). The tree species used were classified by local farmers on the basis of their palatability to cattle and the rankings by gas production characteristics were compared with these classifications.

Methods

Sampling procedure and preparation

Experiment 1: Comparison between species requires an estimate of the variability within species. To assess this samples of *Leucaena leucocephala* (LL) and *Flemingia congesta* (FC) were taken in duplicate from each of three trees at three environmentally distinct sites. The three sites, all in the province of Santa Cruz, Bolivia, were Yapacani, San Pedro and Saavedra. These sites, respectively, have slightly acidic soils of low fertility, fertile alluvial soils and light to medium textured soils of medium fertility. All three sites had similar altitudes (300 to 500 m above sea level). Yapacani had higher rainfall than the other two sites, although for several months before the samples were taken all 3 sites had received atypically high rainfall. For both species, trees originated from single genetic sources.

Experiment 2: Different tree species were classified by local farmers and forestry experts as being palatable to cattle (ie were regularly eaten), of medium palatability (eaten occasionally when there was a shortage of more palatable feed) or unpalatable (never eaten). The species analysed were then selected to include several species within each classification: the species and palatability classification are given in Table 1.

Table 1: Bolivian tree species samples for evaluation by the *in vitro* gas production technique

Local name	Palatibility Class	Scientific name	Species Code
Kare	H	not identified	K
Melendre	H	not identified	M
Quine	H	*Acacia* sp.	Q
Yareta	H	not identified	Y
Choroquete	H	not identified	C
Chamba	H	*Leucaena leucocephala*	LL
not identified	H	*Gliricidia sepium*	GS
not identified	H	*Erythrina poeppigiana*	EP
not identified	M	*Fleminigia congesta* (=F *macrophylla*)	FC
not identified	M	*Inga marginata*	IM
not identified	M	*Inga ingoides*	II
Pica pica	M	not identified	PP
not identified	M	*Erythrina fusca*	EF
Leche leche	U	not identified	LE
Jorori	U	*Schwrtzia jorori*	SJ
Bibosi	U	*Ficus* sp.	B
Curupau	U	*Piptadenia macrocarpa*	PM
Ajuano	U	*Pterogyne nitens*	PN
not identified	U	*Erythrina olei*	EO

H = High, M = Medium, U = Unpalatable

Individual samples of fresh leaves were taken from different parts of the tree. For tall trees branches were cut down from different parts of the tree otherwise, if access was possible from the ground, leaves were stripped off directly. Leaves were taken such that the sample represented the leaves as a whole, that is it included leaves of different ages, diseased and healthy, and leaves from different positions. Large stalks were removed from those species which contained this type of material. The process was then repeated so that duplicate representative samples were obtained. Samples were transported to the laboratory and the leaves dried in an oven at 50°C for 16 - 24 hours. Drying started within 48 hours of the fresh leaves being harvested. Dried samples were ground to pass through a 1 mm sieve.

The gas production method

The method of Theodorou *et al.* (1991) involves the fermentation of a dried, ground feed sample with rumen microbes. 1 g of feed was transferred under anaerobic conditions into 125 ml glass serum bottles together with 90 ml of a buffered microbiological medium and the bottles were sealed. The mixture was inoculated with microbes prepared from fresh rumen fluid and was fermented anaerobically at 39°C. At predetermined times, initially every 3 hours then at gradually lengthening intervals as the rate of fermentation slows, the gas pressure in the bottles was measured using a pressure transducer. The gas pressure was adjusted to atmospheric pressure by removing the gas produced with a syringe. The volume of gas was recorded. Each fermentation was performed in triplicate and gas production monitored for a total duration of 166 hours by which time the fermentations had been largely completed.

The samples were autoclaved at the end of the experiment and the residual dry matter was estimated by filtering each sample into preweighed filter crucibles (porosity P160). The particulate material was washed with distilled water, oven dried at 100°C and weighed.

Computation of data and statistical analysis

Cumulative gas production data were corrected to a common basis of 1 g dry substrate, Figure 1 illustrates some curves obtained during this study. Apparent digestibility (%) was estimated assuming that all of the residual dry matter after 166 hrs fermentation was unfermented substrate. Apparent digestibility was correlated against cumulative gas produced after 166 hrs (CG166) using a linear equation (Statgraphics).

Gas production data were analysed by comparing values obtained for cumulative gas production after 52, 166 hours (CG52 and CG166 respectively) together with apparent digestibility. Earlier studies (Prasad, Wood and Sampath, unpublished data) indicated that *in vitro* digestibilities calculated from gas production data were similar to in vivo digestibilities at about 52 hours.

Genstat was used for nested analysis of variance to examine between tree, site, species, sampling and analytical variance in experiment 1; sampling and analytical variance in experiment 2.

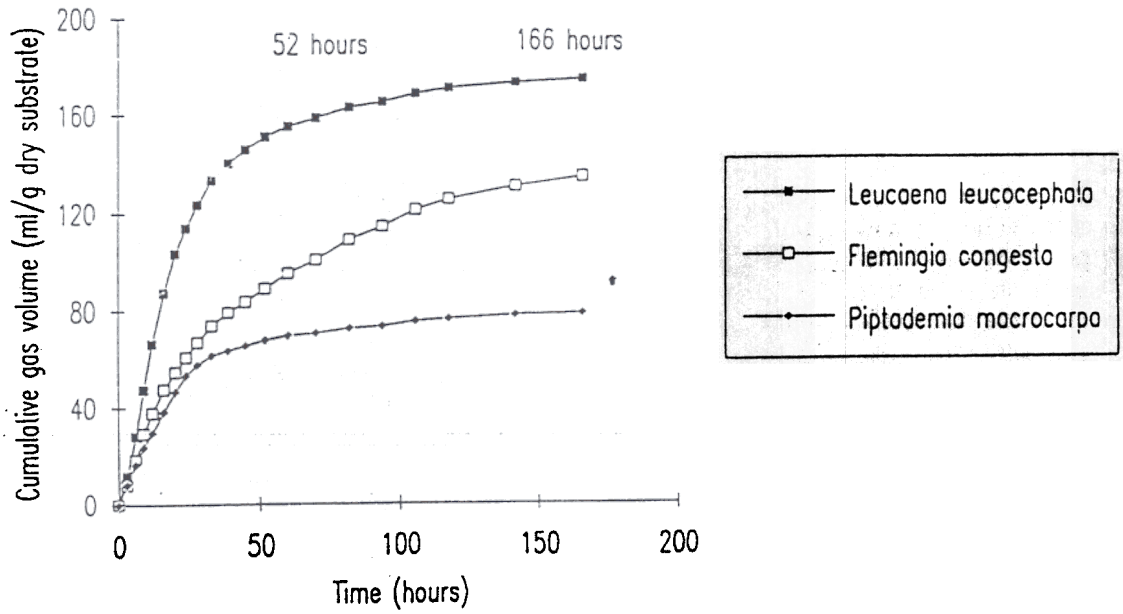

Figure 1 Gas production curves for some Bolivian tree leaves. *Leucaena leucocephala* is a highly digestible fodder, *Flemingia congesta* a fodder of low digestibility while *Piptademia macrocarpa* is not used as a fodder but is used as a source of tannins.

Table 2: Influence of site on cumulative gas production after 52 and 166 hours from the *in vitro* gas production technique using samples of *Leucaena leucocephala* and *Flemingia congesta* leaves.

Tree species	*Leucaena leucocephala*			*Flemingia congesta*			
Site	San Pedro	Yapacani	Saavedra	San Pedro	Yapacani	Saavedra	standard error
Cumulative gas production after 52 hours, CG52 (ml)	167.5	134.9	155.9	90.6	82.4	97.5	4.38
Cumulative gas production after 166 hours, CG166 (ml)	197.1	158.0	186.0	144.2	109.7	130.3	4.85

Results and Discussion

Site to site differences

Table 2 presents mean data for CG52 and CG166 for three trees of species LL and FC grown at three different sites. Between species differences were greatest but for both species there were highly significant ($P<0.01$) site to site differences. Trees of a particular species at San Pedro were generally more readily fermented than those from Saavedra (exceptionally CG52 for FC was higher for Saavedra than San Pedro, but difference not statistically significant $P>0.05$), and in all cases these were more readily fermented than those from Yapacani.

There was an apparent relationship between soil fertility and gas production with the infertile soils of Yapacani giving leaves of lower digestibility. Yapacani also has a higher rainfall than the other two sites which could have been a factor, although at the time when the samples were taken all three sites had received atypically high rainfall for several months. Soil analysis was not undertaken during this study so it is assumed that the general site description applies to the specific sites where the trees were sampled. It is perhaps to be expected that soils with sub-optimal nutrient availability will lead to fodders of reduced nutrient content. Also, levels of tannins may be increased when trees are grown in unfertilised acidic soils (see Barry & Forss, 1983, with *Lotus pendunculatus*).

Differences between species

Mean data, together with their standard errors for the various parameters used for comparison are given in Table 3. The palatability classes as perceived by local farmers are also indicated for the different species. For all parameters a wide spread of mean values was observed. The different parameters were used to rank the species in descending order of digestibility (that is in descending order of the parameters). Rankings are given in Table 4 together with the palatability classes of the species ranked according to CG52. The ranking orders given by CG52 and CG166 were very similar.

Table 3: Parameters obtained using the *in vitro* gas production technique on leaf samples from 19 Bolivian tree species

Species code	Palatibility class	Parameters from *in vitro* gas production		
		CG52 (ml)	CG166 (ml)	Apparent digestibility (%)
K	H	55	75	41.41
M	H	146	192	66.01
Q	H	94	127	42.11
Y	H	119	147	74.7
C	H	31	38	39.5
LL-1[1]	H	132	157	52.78
LL-2[1]	H	152	179	61.13
GS	H	193	220	64.43
EP	H	118	140	50.25
FC	M	94	134	48.16
IM	M	64	82	29.8
II	M	46	63	24.36
PP	M	134	169	58.97
EF	M	109	130	44.02
LE	U	196	223	75.13
SJ	U	83	98	35.6
B	U	118	148	51.51
PM	U	64	73	43.16
PN	U	90	119	44.63
EO	U	126	156	53.59
sd[2]		5.65	7.37	1.82

[1] LL-1 and LL-2 were two separately prepared samples
[2] standard error

Ranking by apparent digestibility (by filtration) showed less agreement particularly for 4 species (K, Y, C and PM) which deviated from the general correlation between apparent digestibility and gas production. These were more highly ranked by the former, considerably so for species Y (2nd by apparent digestibility, 8th or 9th by gas production).

Lack of correlation between gas production and apparent digestibility could be due to the presence of soluble material which is not digested by rumen micro-organisms or the production of soluble end products rather than gas. Soluble, indigestible material would not be retained by filtration, hence would be considered to be digested even though the gas production method indicates otherwise. More work is required to explain the above observations.

Table 4: Ranking of 19 species of Bolivian trees in descending order of digestibility from *in vitro* gas production

Rankings	Palatibility class (CG52)	CG52	CG166	Apparent digestibility
1	U	LE	LE	LE
2	H	GS	GS	Y
3	H	LL-2	M	M
4	H	M	LL-2	GS
5	M	PP	PP	LL-2
6	H	LL-1	LL-1	PP
7	U	EO	EO	EO
8	H	Y	B	LL-1
9	U	B	Y	B
10	H	EP	EP	EP
11	M	EF	FC	FC
12	H	Q	EF	ON
13	M	FC	Q	EF
14	U	PN	PN	PM
15	U	SJ	SJ	Q
16	U	PM	IM	K
17	M	IM	K	C
18	H	K	PM	SJ
19	M	II	II	IM
20	H	C	C	II

Large differences between species were observed with CG166, for example, ranging between 38 ml g^{-1} to 223 ml g^{-1}. This was a much wider range than that found for between site differences (158 to 191 ml g^{-1} CG166 for LL, 109 to 155 ml g^{-1} CG166 for FC).

While rankings would vary slightly depending on site (and also parameter used), the species rankings achieved were apparently a reasonably robust indicator of their relative *in vitro* digestibilities. GS and LL, both regarded as fodders of high nutritive value (Glover, 1989; Pound & Martinez Cairo, 1983), were highly ranked by all the parameters studied. FC which was low in the rankings has been reported to have a low *in vitro* dry matter digestibility (Thomas & Schultze-Kraft, 1990).

Species rankings with respect to palatability

There was no apparent relationship between any of the digestibility rankings and palatability. This is illustrated in Table 4 using cumulative gas production after 52 hours fermentation (CG52), a similar picture being obtained using the other parameters.

Although the palatability classifications were obtained from brief, non-systematic interviews it is considered unlikely that the classifications were so inaccurate as to conceal relationships with gas production. Species of low digestibility could be readily eaten, while others of high digestibility were not consumed at all.

Wilson (1977) also found that there was no correspondence between digestibility and organic matter intake for 8 shrub and tree species from New South Wales, Australia. It must, however, be noted that in the Santa Cruz region tree leaf fodders are commonly browsed in an uncontrolled way and farmers may be unaware of general levels of intake or performance of animals eating these materials. Further, palatability can be affected by how a feed is presented to the animal and if the animal is adapted to the particular feed. For example fresh GS can be of low palatability to animals which are not used to eating it, possibly due to the presence of volatile substances. Wilting before feeding, feeding with other feeds and gradually adapting animals to eat GS have all been reported to increase its palatability (Glover, 1989).

Data on intake, digestibility and composition are essential for the selection of suitable fodder species. The data can be used to select fodder tree species of high palatibility, digestibility and nutrient content as part of an initial screening process. The gas production

method appears to be a useful indicator of nutritive value although this must be confirmed by comparison with animal performance. In the longer term these data may be calibrated against animal performance to develop an index of nutritive value particularly for diets which contain anti-nutritive factors.

Conclusions

The extent of digestion of tree leaves varied significantly ($P<0.001$) between sites for both LL and FC. The sites which were regarded as having the most fertile soil had the more digestible leaves. Between species differences were much greater than the extent of within species variability observed for LL and FC leaves. LL leaves were more digestible than those of FC.

There was no apparent relationship between palatability and digestibility as measured by any parameter investigated. This indicates that both palatability (that is some indicator of voluntary intake) and digestibility must be investigated to assess the nutritive value of fodder trees. While knowledge of which trees animals select is of vital importance, on its own it is not a good indicator of the value of the leaves as an animal feed.

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