Report on a project on the analysis of tree leaves and assessment of their use as feed, green manure and mulch.
12 October 1992-31 March 1993

Dr C D Wood

Project No: Q0010

OBJECTIVES

1. The immediate objectives of this project (Q0010) were to:

a) introduce and adapt NRI's techniques for feed nutritive value assessment to the Centro de Investigacion Agricola Tropical (CIAT), Santa Cruz, Bolivia.

b) evaluate methods to assess the value of tree leaves from the most important species for feeds, green manure and mulch.

The wider objectives of the project were to improve the living standards of poor livestock owners by improving techniques for assessing the nutritive value of feeds which will assist in the introduction of more productive, environmentally sound and sustainable livestock feeding systems.

SUMMARY AND CONCLUSIONS

2. The techniques considered for introduction to CIAT were the gas production technique and the radial diffusion assay. The gas production technique involves the measurement of gases produced during the *in vitro* fermentation of feedstuffs by rumen micro-organisms. It gives information on the availability of nutrients in feedstuffs (related to their digestibility), nutritive value depending on the levels of nutrients and their availability. This is particularly important for feedstuffs such as tree leaf fodders which can contain high levels of anti-nutritive factors which reduce the availability of nutrients to the animals which consume them. The radial diffusion assay gives a measure of the most widespread anti-nutritional factor, tannins. Both assays can be conducted with only limited laboratory facilities and have been used in less developed countries.

3. CIAT were unable to obtain all the equipment required for the gas production technique so the programme was altered to perform gas production experiments at NRI using samples prepared at CIAT. The radial diffusion assay was conducted in Bolivia and counterparts trained to use it but at the time of the visit it was unclear whether CIAT would be able to sustain work on the nutritive value of feeds. This was because although there was considerable interest in nutritive value assessment, there was no livestock nutrition programme at CIAT or BTAM nor were the resources available to establish such a programme. Nevertheless the assay was being used to support work on the degradation of leaf litter as part of the on-going agroforestry project.

4. Work towards objective 2 was expanded to evaluate between tree and site differences (experiment 1 below). Very little information existed on animal performance on diets which include tree fodders in Bolivia. Local farmers and forestry experts were, however, aware of which tree

species cattle ate and which were avoided. Some tree leaves not consumed by cattle as well as the more important fodder species were ranked using the gas production technique (experiment 2 below). This gave a wider range of species for investigating relationships between feed palatability and nutritive value as judged by gas production. Evaluation of the effects of sample preparation on tannin levels was conducted in Bolivia. Activities in Bolivia are reported in the visit report attached as Appendix 1 and the project as a whole is reported as a draft scientific paper attached as Appendix 2.

5. Experiment 1: Duplicate dried samples were obtained from three trees per site for three sites with different soil fertilities and climates for the species *Flemingia congesta* (= F *macrophylla*) and *Leucaena leucocephala*. The trees for each species were from single genetic sources. The gas production technique revealed significant ($P<0.001$) site differences for both species, the sites with the more fertile soils having the more digestible leaves.

6. Experiment 2: Duplicate dried samples were obtained from nineteen different tree species which ranged from being highly palatable to not palatable (as classified by local farmers and forestry experts). There was no apparent relationship between palatability and gas production characteristics as measured by any parameter investigated. This indicated 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 on what trees animals eat is of vital importance, it is not a good indicator of the availability of nutrients. For 4 of the 19 species the residues recovered at the end of the gas production experiment did not correspond to the amount of gas produced. This indicated that either methods based on the recovery of insoluble material or the gas production technique used at NRI are not reliable indicators of the digestibility of all fodder tree species.

7. Fresh and dried samples from twelve species were assayed for tannins by the radial diffusion technique. Losses in the protein precipitation activity (PPA) of extractable tannins were observed for all species due to the drying process (oven drying at 50°C). The falls ranged from 18.7% for Flemingia up to 70.1% for Leucaena. Differences in times between harvesting and drying, and in drying time itself, did not significantly affect gas production characteristics.

8. The variable tannin loss during sample preparation reinforces the need for testing assessment methods on fresh material and comparing the results with those obtained from dried material. Further analyses of the samples are required to explain the gas production data and to identify simple assays which could be used for routine screening purposes. Above all else it must be established that the broad conclusions regarding the ability of the gas

production technique to rank tree fodders correctly predict relative animal performance (and similarly degradation of green manures and mulches). Experimental trials on the degradation of leaves on the ground are ongoing in Bolivia.

Appendix 1

FILE NOTE

Visit to Bolivia to obtain dried tree leaf samples for the assessment of their use as feeds, green manures and mulches: 16 January 1993 - 19 February 1993

Dr C D Wood
Project No Q0010

ABBREVIATIONS

BTAM	British Tropical Agricultural Mission
CIAT	Centro de Investigacion en Agricultura Tropical
LIDIVET	Laboratorio de Investigacion y Diagnostico Veterinario
	Natural Resources Institute
	Protein Precipitation Activity

OBJECTIVES

1. The objective of this project (Q0010) is to assess the value of tree leaves as feeds, green manure and mulch. The particular objectives of this visit were:

   (a) to obtain samples from different trees and sites from 2 species to assess intraspecies variability

   (b) to obtain information and samples of species of good to poor quality to see if NRI's techniques can correctly classify them

   (c) to assess the effect of drying on tree leaf tannin by radial diffusion.

SUMMARY AND CONCLUSIONS

2. Duplicate dried samples were obtained from three trees per site for three sites with different soil fertilities and climates for the species *Flemingia congesta* (= F *macrophylla*) and *Leucaena leucocephala*. The trees for each species were from single genetic sources.

3. Duplicate dried samples were obtained from nineteen different species which ranged from being highly palatable to not palatable. Additionally dried samples of *Erythrina poeppigiana* handled in different ways were prepared to investigate the effects of handling on subsequent assessment.

4. Fresh and dried samples from twelve species were assayed for tannins by the radial diffusion technique. Losses in the protein precipitation activity (PPA) of extractable tannins were observed for all species due to the drying process (oven drying at 50°C). The falls ranged from 18.7% for Flemingia up to 70.1% for Leucaena.

5. The variable tannin loss during sample preparation reinforces the need for testing assessment methods on fresh material and comparing the results with those obtained from dried material.

BACKGROUND

6. NRI is developing techniques for the assessment of the nutritive value of tropical feeds. Many tropical feeds contain anti-nutritive factors such as tannins which can considerably reduce their nutritive value. Conventional proximate analysis can therefore be misleading. Two techniques have been adopted recently and appear to be promising. The radial diffusion assay gives a measure of the activity of acetone extractable tannins in precipitating protein. Assays of this type are widely believed to be better indicators of the biological effects of tannins than any of the chemical assays currently available. The radial diffusion assay requires little sophisticated equipment, making it particularly suitable for use in less developed

countries. Earlier studies conducted jointly by NRI and BTAM on tree leaf samples indicated general relationships between protein precipitation activity, palatability to animals and rate of degradation of fallen leaves.

7. The gas production technique provides information on the rate and extent of fermentation of substrates by rumen microbes. The value of the technique is still being investigated (such as in this project) and it has still to be calibrated against established digestibility assays (eg Tilley and Terry) and animal performance.

ACTIVITIES

Review of information on nutritive value etc of tree leaves:

8. A review of BTCAM/CIAT and other reports indicated that there has been little investigation of the nutritive value of tree leaves in Bolivia, and none on the value of tree leaves for use as green manure and mulch. Claure (1971) reported analytical data on a range of native and introduced forages, and produced lists of what appeared to be good quality forages. The assays used included an enzymic protein digestibility assay, but the methods used must be considered unreliable for forages which contain anti-nutritive factors such as tannins. For example Claure (1971) identified Curupau (*Piptademia macrocarpa*) as a high quality forage due its proximate composition, whereas in fact it has a high tannin content and is said to be completely unpalatable to cattle. Sumur *et al* (1980) reported improved weight gains for cattle with access to *Leucaena leucocephala*, Paterson *et al* (1980) identified *Leucaena leucocephala* and *Glycine wightii* as useful legumes in cattle feeding systems, others were identified as promising.

9. Conversations with farmers in the Montero area, and with CIAT and BTAM experts indicated that there was a high awareness of the palatabilities of different tree leaves (ie species which cattle ate readily and ones which were never eaten, as well as ones which could be eaten but which made the animals ill). The information collected enabled samples of highly palatable and unpalatable species to be collected. While palatability is not synonymous with nutritive value, it is clearly of major importance. There is a need to collect this information in a systematic way and it is understood that work on this will start shortly at CIAT. Some studies are currently being initiated on the degradation of tree leaves on the ground and some co-ordination between these studies and the nutritive value studies is planned. These studies currently include *Leucaena leucocephala, Erythrina poeppigiana, Flemingia congesta* and *Inga marginata* which will also be assessed by gas production, it is suggested that other species are included for comparative purposes such as Jorori, Curupau and Ajunao which are all considered to be unpalatable. For both studies it will be possible to rank the species with respect to their degradation characteristics. It is likely

that there is an optimum range for the degradation characteristics rather than a simple "faster degradation = higher nutritive value/manure value" relationship. Nevertheless the techniques should distinguish poorly degradable material from that which is readily degraded and should provide more information when further work on nutritive value and manure/mulch value allow a fuller interpretation of the data on degradation characteristics.

Sampling procedure:

10. 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.

11. Samples were sent to Santa Cruz for drying. The delay between harvesting was variable depending upon the location of the trees. Typical delay times were 4 hours for samples from Saavedra, 8 hours for Yapacani and 24 hours for San Pedro. In all cases drying was started within 48 hours of collection. Samples were oven dried at 50°C for 16-24 hours and ground to pass through a 1mm sieve.

12. To assess the affect of different delays before drying a single bulked sample of fresh leaves of *Erythrina poeppigiana* was obtained, subdivided into 8 samples and duplicate samples were subjected to 4 different handling conditions as below:

2 hours delay at ambient temperatures, dry 24 hours at 50°C
2 hours delay at ambient temperatures, dry 48 hours at 50°C
24 hours " " " , dry 24 hours at 50°C
96 hours " " " , dry 24 hours at 50°C

Intraspecies variability:

13. Samples of *Leucaena leucocephala* (highly palatable, low tannin) and *Flemingia congesta* (believed to be of low to moderate palatability, high tannin) were obtained in duplicate from 3 sites, 3 different trees per species per site. The 3 sites were Yapacaní (soils not very fertile, slightly acidic), San Pedro (soils alluvial, fertile) and Saavedra (soils generally light to medium, medium fertility). The trees of each species were from a single genetic source. At all three sites the weather had been particularly wet for several months so short term weather differences were probably unimportant. Yapacaní is normally wetter than the other two sites (annual rainfall about 2000mm and 1200mm respectively, mainly falling from October to April).

Trees of different palatability:

14. Samples from trees of the following species were obtained in duplicate.

High palatability

From Mairana (W of Santa Cruz)
Kare, Melendre, Quiñe (*Acacia sp)*, Yareta

From Cañada Larga
Choroquete

From Saavedra/Montero
Chamba (*Leucaena leucocephala*), *Gliricidia sepium*
Erythrina poeppigiana

Medium Palatability (from Saavedra/Montero)

Flemingia congesta (= F *macrophylla*)
Inga marginata
Inga ingoides
Pica pica - leaves sting, but said to be eaten occasionally by cattle.
Erythrina fusca

Not palatable (from Saavedra/Montero)

Leche leche - low palatability thought to be due to white resin in stems

Jorori (*Schwartzia jorori*)
Bibosi (*Ficus sp*)
Curupau (*Piptademia macrocarpa*) - bark used as a source of tannins.
Ajunao (*Pterogyne nitens*)

The duplicate samples originated from the same tree

Effect of drying on tree leaf tannins:

15. Fresh leaf samples from selected species were extracted and analysed for tannins by the radial diffusion method. The results obtained are summarised in Annex 1. Losses in PPA were observed for all species, ranging from 18.7% for *Fleminga congesta* to 70.1% for *Leucaena leucocephala*.

16. With the use of facilities at LIDIVET the assay was successfully performed, but involves working in two buildings (CIAT and LIDIVET) and in some 5 different laboratories. One CIAT staff member (Sra. Margarita Cabrera) and an undergraduate student working with CIAT (Aida Tapia) have been trained to perform the assay. CIAT could be made self sufficient with respect to equipment at modest cost, and this would be advisable if it was to be conducted on a regular basis. Recommendations have been made to upgrade the CIAT laboratory and provide technical

support to develop an animal nutrition programme (Gill, 1992). The assay has been introduced but without some commitment to continued work on nutritive value it is unclear whether its use will be sustained.

FUTURE WORK AND CONCLUSIONS:

17. The dried samples will be assessed by the gas production method at NRI, and their analyses done as appropriate. Gas production work is due to be completed by the end of March and a report prepared by the end of May. It is hoped that data and rankings produced by the gas production method can be compared with those obtained from degradation studies on leaves on the ground. It is hoped that the data will contribute to a scientific publication in due course.

18. In the longer term NRI intends to perform gas production assays on fresh leaves and compare rankings to those indicated by dried samples (as per the initial proposals for the current visit). Further work will then be required to validate the approach. This will require some measurement of animal performance fed particular diets. Also the research must be applied to practical situaticns to provide advice on fodder tree selection, feed management etc. It is hoped contacts between BTAM/CIAT and NRI will be maintained to the benefit of both programmes.

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ANNEX 1

1. Changes in Protein Precipitation Activity (PPA) of extractable tannin when selected Bolivian tree leaf samples were dried at 50°C for 16-24 hours.

Species	Average Changes (%)[a]
Jorori	-30.9
Bibosi	
Leucaena leucocephala	
Fleminga congesta	
Curupau	-47.4
Ajunao	-30.5
Erythrina olei	-39.3
Inga ingoides	-49.6
Kare	-43.5
Melendre	-27.4
Quine	-54.8
Yareta	-55.2

Note [a] - negative changes signify losses on drying

ANNEX 2

Dried tree leaf samples obtained

Two species, different trees, different sites

Leucaena leucocephala

Codes	LL Y	1 or 2 or 3	x 6 samples
	LL SP	1 or 2 or 3	x 6 samples
	LL S	1 or 2 or 3	x 6 samples
			Sub total x 18 samples
Similarly Flemingia congesta			Sub total x 18 samples

Different species of different "quality"

1.	Kare	1 & 2
2.	Melendre	"
3.	Quiñe	"
4.	Yareta	"
5.	Choroquete	"
6.	Leucaena	"
7.	Gliricidia	1, 2, 3, & 4
8.	Erythrina poeppigiana	1 & 2
9.	Flemingia congesta	1 & 2
10.	Inga marginata	"
11.	Inga ingoides	"
12.	Pica pica	"
13.	Erythrina fusca	"
14.	Leche Leche	"
15.	Jorori	"
16.	Bibosi	"
17.	Curupau	"
18.	Ajunao	"
19.	Erythrina ulei	"

	Sub total x 40 samples
Different handling conditions	Sub total x 8 samples
Grand Total	x 84 samples

Appendix 2 Draft paper

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

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Abstract

An *in vitro* gas production technique was used to evaluate the degradability of leaves from different tree species. Variability within two species (*Leucaena leucocephala* and *Flemingia congesta*) grown from single genetic sources at three sites was assessed. 19 species, which were considered by local farmers to range from being highly palatable to completely unpalatable, were ranked according to their gas production characteristics. The degradability of tree leaves varied significantly ($P<0.001$) between sites for both species; these differences could have been due to differences in soil fertility. Between species differences were much greater than site related differences for these two species. Apparent Digestibility as measured by filtration correlated well (R^2 = 92.47%) with the gas production data for 15 of the 19 species, but for 4 species filtration apparently overestimated degradability (by as much as a factor of 3 for 1 specie). This may indicate that methods based on the recovery of insoluble material may not be reliable indicators of the degradability of all fodder tree species.

There was no apparent relationship between palatability and degradability as measured by any parameter investigated. *Gliricidia sepium* and *Leucaena leucocephala*, both regarded as having high nutritive values, were highly ranked by the gas production technique, but other highly palatable species were of low degradability. This indicated that both voluntary intake and degradability must be investigated to assess the nutritive value of fodder trees.

Introduction

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 are 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).

Theodorou *et al* (1991) have developed an *in vitro* gas production method to provide an estimate of nutritive value. This provides information on the kinetics as well as the extent of digestion of the feed. Further, digestibility methods which rely on the physical separation of soluble and insoluble material may overestimate the nutritive value of feeds which contain soluble but indigestible material. The gas production method may provide a more reliable index of nutritive value for certain types of feed. The objective of this work was to investigate the variability in digestibility of leaves from genetically similar trees growing at different sites and to rank tree leaves from different species according to their ease of digestion as judged by *in vitro* gas production. The tree species used for ranking were classified by local farmers on the basis of their palatability to cattle and the rankings by gas production characteristics compared with the palatability 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, = F *macrophylla*) were taken in duplicate from each of three trees at three agronomically 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 soils of medium fertility. All three sites had similar altitudes (300 to 500m above sea level). Yapacani had a wetter climate than the other two sites althouth for several months before the samples were taken all 3 sites had received atypically high rainfalls. For each of these 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 of each classification, the species and palatability classification being given in Table 1.

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, large stalks removed 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 1mm sieve.

To assess the effect of different delays before drying a single bulked sample of fresh leaves of *Erythrina poeppigiana* (EP) was obtained, subdivided into 8 samples and duplicate samples were subjected to 4 different handling conditions as below:
2 hours delay at ambient temperatures, dry 24 hours at 50°C
2 hours delay at ambient temperatures, dry 48 hours at 50°C
24 hours delay at ambient temperatures, dry 24 hours at 50°C
96 hours delay at ambient temperatures, dry 24 hours at 50°C

Gas production procedure

The method outlined by Theodorou *et al* (1991), Merry *et al* (1991) was used. This involves the fermentation of dried ground substrates in a buffer solution inoculated with microbes from fresh rumen fluid. The same inoculum was used for all the treatments in each experiment. The following modifications suggested by Theodorou were used (M Theodorou, personal communication): trypticase peptone was omitted from the basal medium, 5 ml of inoculum was used for each bottle.

Each fermentation was performed in triplicate and gas production monitored at intervals up to a total duration of 166 hours. 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 and oven dried at 100°C.

LL, FC samples from different sites and EP samples prepared in different ways were fermented in one experiment and the samples for comparisons of different species were fermented in a second experiment.

Computation of data and statistical analysis

Cumulative gas production data were corrected to a common basis of 1 g dry substrate. 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), and the correlation equation obtained used to calculate Apparent Digestibility from cumulative gas production.

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 C S, Wood C D and Sampath K T, unpublished data) indicated that *in vitro* digestibilities calculated from gas production data were similar to *in vivo* digestibilities at about 52 hours. Additionally the equation:

$y = a + br^t$ (equation 1

was fitted to gas production data obtained from 12 hours (inclusively) using the Genstat programme. Equation 1 is essentially the same as the expression fitted by Merry *et al* (1991) with the following modification:

$k = -\ln r$ (equation 2)

Constants b (the asymptote of the curve at infinite time called the Gas Pool Size), r and k (the Rate Constant) were used for ranking to see if different fermentation characteristics gave different rankings and if any of these characteristics were indicators of palatability.

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.

Results and Discussion

Different handling treatments

Table 2 presents means for the different handling treatments of the pooled EP sample. Differences between the samples (as judged by CG52, CG166, Gas Pool Size and r) were not statistically significant (P>0.05).

The delay between harvesting and drying did not affect the fermentation characteristics of EP. Similarly there was no evidence that drying for 24 or 48 hours had an effect. Nevertheless tannin levels were reduced by the drying procedure used, the falls ranging from 18.7% for FC up to 70.1% for LL. Mahyuddin *et al* (1988) noted that drying could affect in vitro dry matter digestibility, although similar rankings were obtained with fresh and dried forages. Thus the relative digestibility of the samples were probably reflective of differences in fresh material, but when fresh material is consumed by animals and dried samples are assessed for nutritive quality artefacts may occur.

Parameters to compare sites and species

Equation 1 fitted the experimental data well (R^2 between 99.0% and 99.8%) although there were indications of a systematic lack of fit. CG52, CG166 and Gas Pool Size gave closely similar rankings.

The Rate Constant and constant r often did not show the same trends as the other parameters and correlated poorly against CG52, CG166 and Gas Pool Size (R^2 = 32.60%, 21.66% and 18.98% respectively). Orskov and Ryle (1990) proposed the calculation of an index value for data from dacron bags based on the rate constant, soluble material content and degradable but insoluble material content. A similar index may prove useful for interpreting the gas production data although calibration against animal performance would be required before such an index could be developed.

Correlation of apparent digestibility and gas production

Data for 4 species (K, Y, C and PM) clearly did not lie on the derived line, their apparent digestibility being greater than that expected from the gas production data. Table 3 presents data comparing the measured apparent digestibility and that predicted from CG166 using the correlation equation obtained from the 15 species for which CG166 and apparent digestibility did appear to correlate. The greatest difference was found in species C where filtration gave an apparent digestibility nearly 200% higher than that obtained by the gas production technique, but all four outlying species gave values not less that 50% higher.

Although gas production and apparent digestibility generally correlated well, it was noteworthy that 4 of the 19 species clearly did not lie on the correlation line. Apparent digestibility overestimated the digestibility of these species compared to the gas production method. This 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. Any method which relies on the recovery or retention of material by physical means may be unreliable for samples of this type. Conversely, if for certain substrates the end products include increased levels of soluble materials rather than gas the *in vitro* gas production method would be an inferior indicator of nutritive value.

Site to site differences

Table 4 presents mean data 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. Using CG52, CG166 and Gas Pool Size as parameters for comparison, 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. Significant differences (P<0.001) between species were also found using constant r for comparison, however this parameter (and the Rate Constant, k, derived from it) did not indicate between site differences which were consistent between species. For neither species did this parameter generally indicate that trees from Yapacani were less digestible than those from the other sites, indeed for FC the leaves from Yapacani appeared superior.

There was an apparent relationship between soil fertility and gas production with the infertile soils of Yapacani giving leaves of lower degradability. Yapacani also has a wetter climate than the other two sites which could have been a factor, although at the time when the samples were taken all three sites had had atypically high rainfalls for several months. Soil analysis work was not undertaken during this work 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 that soils with sub-optimal nutrient availability will lead to fodders of reduced nutrient content, also levels of tannins may be increased.(TO ANALYSE).

Differences between species

Mean data together with their standard errors for the various parameters used for comparison are given in Table 5. The palability 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 all the parameters except r which is inversely related to the rate constant k). Rankings are given in Table 6 together with the palatability classes of the species ranked according to CG52 and Rate Constant. The ranking orders given by CG52, CG166 and the Gas Pool Size were very similar. Ranking by Apparent Digestibility (by filtration) showed less agreement particularly for the 4 species which deviated from the general correlation between Apparent Digestibility and Gas Production. These were more highly ranked by the filtration technique considerably more highly for species Y (2nd by filtration, 8th or 9th by gas production).

Ranking by Rate Constant (which is equivalent to ranking by r) gave a different ranking again although there were many similarities. The most marked differences were that PM was ranked 1st, species M 14th according to their Rate Constants while the other parameters indicated rankings of 15th to 18th for PM, 3rd or 4th for M.

Large differences between species were observed with CG166, for example, ranging between 38ml/g to 223ml/g. This was a much wider range than that found for between site differences (158 to 191 ml/g CG166 for LL, 109 to 155 ml/g CG166 for FC). While rankings 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 and Martinez Cairo, 1983), were both 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 and Schultze-Kraft, 1990).

Species rankings with respect to palatability

There was no apparent relationship between any of the degradability rankings and palability. This is illustrated in Table 6 using CG52 and the Rate Constant, a similar picture being obtained using the other parameters. Although the palatability clasifications were obtained from brief, non-systematic interviews it is considered unlikely that the clasifications 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 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 use 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 digestibility although this must be confirmed by comparison with animal performance. In the longer term this data may be calibrated against animal performance to develop an index of nutritive value particularly for diets which contain anti-nutritive factors.

Conclusions

Apparent Digestibility as measured by filtration correlated well (R^2 = 92.47%) with the gas production data for 15 of the 19 species, but for 4 species filtration apparently overestimated degradability (by as much as a factor of 3 for 1 specie). This indicated that methods based on the recovery of insoluble material or the gas production method used for this study may not be reliable indicators of the digestibility of all fodder tree species.

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 nutritive value.

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

Local Name	Palatability Class[a]	Scientific Name	Species Code
Kare	H		K
Melendre	H		M
Quine	H	*Acacia* sp	Q
Yareta	H		Y
Choroquete	H		C
Chamba	H	*Leucaena leucocephala*	LL
	H	*Gliricidia sepium*	
	H	*Erythrina poeppigiana*	EP
	M	*Fleminigia congesta* (= F *macrophylla*)	
	M	*Inga marginata*	
	M	*Inga ingoides*	
Pica pica	M		PP
	M	*Erythrina fusca*	EF
Leche leche	U		LE
Jorori	U	*Schwartzia jorori*	SJ
Bibosi	U	*Ficus* sp	B
Curupau	U	*Piptademia macrocarpa*	PM
Ajunao	U	*Pterogyne nitens*	PN
	U	*Erythrina olei*	EO

Note [a] H = High, M = Medium, U = Unpalatable

Table 2 Effect of handling on various parameters obtained from the *in vitro* gas production of *Erthrina peoppigiana* leaves

Handling Regime		Cumulative gas production after 52 hrs (CG52) (ml)	Cumulative gas production after 166 hrs (CG166) (ml)	Gas Pool Size (ml)	Constant r(hr^{-1})[a]
Delay before drying (hrs)	Drying time (hrs)				
2	24	134.5	164.0	161.9	0.96767
2	48	136.7	166.7	164.8	0.96783
24	24	141.9	169.0	166.7	0.96767
96	24	142.3	168.0	164.8	0.96717
se[b]		10.23	9.63	9.31	0.001848

Note [a] Derived from equation

Note [b] se = standard error

TABLE 3 Apparent digestibility of Bolivian tree leaves measured by filtration compared with the apparent digestibility calculated from the in vitro gas production

Species code[a]	Apparent digestibility (%) calculated from gas production[b]	Apparent digestibility (%) estimated by filtration	difference (%)[c]
K	26	41	61
M	65	66	1
Q	43	42	-3
Y	50	75	50
C	13	40	199
LL-1[d]	53	53	-1
LL-2[d]	61	61	1
GS	75	64	-14
EP	48	50	5
FC	46	48	5
IM	28	30	7
II	22	24	13
PP	57	52	-10
EF	44	44	0
LE	76	75	-1
SJ	33	36	7
B	50	52	2
PM	25	43	73
PN	41	45	10
EO	53	54	1

Note a Species codes given in Table 1
Note b Calculated using the correlation equation
Apparent digestibility (%) = (0.338 x CG166) + 0.227 R = 92.47%
Note c +ve values indicate a relative overestimation of digestibility as determined by filtration
Note d LL-1 and LL-2 were two separately prepared samples

Table 4 **Influence of site on various parameters obtained 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**	**se[a]**
Cumulative gas production after 52 hrs, 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
Gas Pool Size[b] (ml	190.9	154.6	183.0	154.8	109.6	131.8	5.18
Constant $r^{b}(hr^{-1})$	0.958	0.960	0.964	0.984	0.975	0.975	0.00176
Rate constant[c] $(hr^{-1}$	0.0432	0.0412	0.0368	0.01573	0.0254	0.0248	nd[d]

Note [a] se = standard error

Note [b] Derived from equation 1

Note [c] Derived from equation 2

Note [d] nd = not determined

TABLE 5 Parameters obtained using the in vitro gas production technique on leaf samples from 19 Bolivian tree species

Species code[a]	Palatability class[b]	CG52[c] (ml)	Parameters from in vitro gas production CG166[d] (ml)	Gas Pool Size (ml)	Apparent Digestibility (%)	r (per hr)[e]	Rate constant (per hr)[f]
K	H	55	75	76	41.41	0.97883	0.021397
M	H	146	192	193	66.01	0.9735	0.026857
Q	H	94	127	128	42.11	0.97533	0.024979
Y	H	119	147	141	74.70	0.96067	0.040124
C	H	31	38	37	39.50	0.97417	0.026169
LL-1[g]	H	132	157	153	52.78	0.96183	0.038918
LL-2[g]	H	152	179	174	61.13	0.95683	0.04413
GS	H	193	220	217	64.43	0.95383	0.04727
EP	H	118	140	137	50.25	0.96417	0.036488
FC	M	94	134	137	48.16	0.97883	0.021397
IM	M	64	82	81	29.80	0.97467	0.025656
	M	46	63	65	24.36	0.98033	0.019866
PP	M	134	169	165	58.97	0.96517	0.035451
EF	M	109	130	126	44.02	0.959	0.041864
LE	U	196	223	216	75.13	0.9525	0.048665
SJ	U	83	98	96	35.60	0.96383	0.03684
B	U	118	148	143	51.51	0.96533	0.035285
PM	U	64	73	71	43.16	0.94767	0.053749
PN	U	90	119	117	44.63	0.97067	0.029769
EO	U	126	156	154	53.59	0.96917	0.031315
se		5.652	7.372	7.49	1.822	0.00315	nd

se = standard error
nd = not determined
Note a Species codes given in Table 1
Note b Palability class H = high, M = medium, U = unpalatable
Note c CG52 = cummulative gas production after 52 hours fermentation
Note d CG166 = cummulative gas production after 166 hours fermentation
Note e r = constant from equation 1
Note f Rate Constant derived from equation 2
Note g LL-1 and LL-2 were two separately prepared samples

TABLE 6 Ranking of 19 species[a] of Bolivian trees in descending order of digestibility by parameters from in vitro gas production

RANKINGS	CG52[b]	Palatability class[c] (CG52 ranking)	CG166[d]	Gas Pool Size	Apparent Digestibility	Rate Constant	Palatability class[c] (Rate Constant ranking)
1	LE	U	LE	GS	LE	PM	U
2	GS	H	GS	LE	Y	LE	U
3	LL-2[e]	H	M	M	M	GS	H
4	M	H	LL-2[e]	LL-2[e]	GS	LL-2[e]	H
5	PP	M	PP	PP	LL-2[e]	EF	U
6	LL-1[e]	H	LL-1[e]	EO	PP	Y	H
7	EO	U	EO	LL-1[e]	EO	LL-1[e]	H
8	Y	H	B	B	LL-1[e]	SJ	U
9	B	U	Y	Y	B	EP	H
10	EP	H	EP	EP	EP	PP	M
11	EF	M	FC	FC	FC	B	U
12	Q	H	EF	Q	PN	EO	U
13	FC	M	Q	EF	EF	PN	U
14	PN	U	PN	PN	PM	M	H
15	SJ	U	SJ	SJ	Q	C	H
16	PM	U	IM	IM	K	IM	M
17	IM	M	K	K	C	Q	H
18	K	H	PM	PM	SJ	FC	M
19	II	M	II	II	IM	K	H
20	C	H	C	C	II	II	M

Note a Species codes given in Table 1
Note b CG52 = cummulative gas production after 52 hours fermentation
Note c Palability class H = high, M = medium, U = unpalatable
Note d CG166 = cummulative gas production after 166 hours fermentation
Note e LL-1 and LL-2 were two separately prepared samples