Output 1: Grasses and legumes genotypes with high quality attributes are developed

Activity 1.1 Selection of *Brachiaria* genotypes for high digestibility and other quality attributes

Highlights

- Confirmed that the NIRS equation developed in the Forage Quality Laboratory gives reliable predictions of in vitro digestibility in large *Brachiaria* hybrids population
- Found high correlation between saponin activity in *Brachiaria* samples harvested in two successive years.
- Found *Brachiaria* hybrids with high and low saponin activity indicating that there is scope for selecting for this attribute.

Progress towards achieving milestones

- **Efficient and reliable protocol for screening *Brachiaria* hybrids for digestibility**
  We calibrated the NIRS to measure digestibility in large number of hybrids generated in the Brachiaria Improvement Program and the resulting equation predicts in vitro digestibility with high precision. However, we have not been able to get consistent digestibility results between successive samplings of the same *Brachiaria* hybrid population and as a result selection for digestibility is still not part of the improvement program. Thus, we still need to define a sampling procedure in *Brachiaria* hybrids to improve the correlation between samplings.

1.1.1 Calibration and utilization of NIRS to screen *Brachiaria* hybrids for digestibility

Contributors: P. Avila, C. Lascano, J. W. Miles and G. Ramírez (CIAT)

Rationale

Selection for improved forage quality is justified if genetic variance for digestibility or crude protein is greater than the variance resulting from the interaction of genotype with environment (G x E). Previous work at CIAT with accessions of *B. brizantha* and *B. decumbens* had shown that the variance in vitro dry matter digestibility (IVDMD) caused by genotype was four times greater than the variance from G x E.

In the on going *Brachiaria* improvement the main objective has been to breed for spittlebug resistance and for adaptation to acid-low fertility soils. In terms of quality attributes, such as IVDMD and crude protein, our approach has been to maintain the quality of *Brachiaria* bred lines at least as equal to that of *B. decumbens* cv Basilisk, which is the most widely planted cultivar in tropical America.

A justification for this strategy had been that with the current in vitro system in the Forage Quality Laboratory it is not possible to handle the large number of genotypes (over 3,000) generated annually by the breeding program. However, with the acquisition of a Near-Infrared Spectroscopy (NIRS) it is now possible to analyze large number of samples in the Forage Quality Laboratory provided good calibration curves are available.

In 1999 we developed a narrow – based NIRS equation and when applied found that the resulting parameters had high precision as indicated by low SE of the calibration (0.98). In addition, estimates of IVDMD of few samples using NIRS had a high correlation (r = 0.89) with IVDMD values obtained with the two-stage Tilley and Terry in vitro procedure.

Validation of NIRS to predict in vitro digestibility

In 2000, we tested the NIRS calibration curve with leaves of 176 *Brachiaria* hybrids that form part of a population (tetraploid *B. ruziziensis* x *B. brizantha* cv. Marandu) used to develop molecular markers for digestibility. Results showed a high correlation (r= 0.84) between observed and values of IVDMD estimated using NIRS.

This year we were interested in determining the effect of age of plant material on the precision of the NIRS equation to estimate IVDMD in *Brachiaria* and in confirming the precision of the NIRS equation we developed. Thus we sampled the same population (144 entries) following a 7 and 10 week regrowth period and run the samples through a two- stage Tilley and Terry in vitro system. Samples we are also read with the NIRS and results correlated with the in vitro values obtained in the laboratory.

The in vitro digestibility values ranged from 68 to 80 % and from 71 to 83 % in samples of 51 and 71 days of regrowth, respectively, which indicate little effect of maturity on IVDMD. Similar correlations were observed between IVDMD values from the laboratory and values predicted with NIRS in the two sets of samples (Table 1).

Table 1. Correlation between IVDMD values of *Brachiaria* hybrids measured in the laboratory and values estimated using NIRS.

Sampling	No of Samples	Days of regrowth	r	SEP*
1	144	51	0.73	1.5
2	144	71	0.80	1.2

*Standard error of prediction

These results confirm that the NIRS equation we have developed to screen Brachiaria hybrids for IVDMD is adequate given the high correlation with IVDMD values measured in the laboratory and relatively low SE of predicted values.

Last year we reported a very low correlation between IVDMD values obtained in samplings of the same Brachiaria population in different times. These disappointing results affected our ability to relate microsatallites markers to digestibility, which is one of our main objectives.

We had postulated that the main problem we were facing had to do with sampling of the material in the greenhouse and with processing of the harvested material. Individual plants had been harvested after 5 or 10 months regrowth and then freeze dried. Leaves were then separated from stem before grinding in Willey Mill fitted with a 1 mm screen. Thus we thought that separation of leaf from stem was not resulting in uniform material across samplings and that the grinding process was not producing samples with uniform particle sizes.

The modified sampling procedure used was as follows:

1. Take samples (leaf and stem) from individual plants growing in large pots and fertilized with N following a 5 to 7 regrowth
2. Dry samples in a oven at 60°C for 24 hours
3. Grind samples in small laboratory mill

This year, three successive samplings of the same mapping population used before were carried out using the new sampling scheme. All samples were analyzed for IVDMD using the Tilley and Terry method. Results showed higher correlations between samplings than reported previously, but still low (r= 0.4 to 0.5).

It is possible that a small change between samplings in leaf: stem ratio of individual plants is affecting the IVDMD values we are recording. Thus to obtain consist results between samplings we are going to probably need to sample the last expanded leaves in each plant, which is what we will do next year.

1.1.2 Screening of selected *Brachiaria* accessions and hybrids for saponins

Contributors: P. Avila, C. Lascano, J. W. Miles and G. Ramírez (CIAT)

Rationale

A wide spread, but sporadic, toxicity syndrome associated with *Brachiaria decumbens* is hepatogenous photosensitization, which can cause severe losses in LWG and in some cases death, particularly young with young animals. This syndrome has been related to infestation of the grass by the saprophytic fungus *P. chartarum*, which produces spores thought to contain toxic sporidesmin. However, the cause – effect relationship between the fungus and photosensitization in *B. decumbens* has been challenged by some researchers with the argument that: a) strains of P. chartarum isolated in *Brachiaria* pastures where animals have show toxicity do not produce sporidesmin and b) steroidal saponins were isolated for the rumen contents of poisoned animals fed *Brachiaria* and that steroidal saponins have been identified in plants know to cause photosensitization.

Based on the hypothesis that saponins are responsible for photosensitization in *Brachiaria decumbens* last year we determined the presence or absence of these compounds in accessions and hybrids of *Brachiaria*. Results (See AR 2000) indicated differences in saponin activity among the few *Brachiaria* accessions included in the assay. Saponin activity was very high in the commercial cultivar *B. decumbens* CIAT 606, which in fact was as high as that recorded in the positive control. In contrast saponin activity seemed to be low in *B. humidicola* and in *B. brizantha* cv Marandu, which is the source of spittlebug resistance in the Brachiaria breeding program. Saponin activity in the Brachiaria hybrids included in the assay was also variable, ranging from very high (absorbance of 786 to 893) to low (absorbance of 230 to 344). However, among the hybrids included in the assay we did not find any with very low saponin activity.

Thus this year were interested in verifying last years results and in determining saponin- like activity in *Brachiaria* hybrids chosen at random from a *B. brizantha* cv Marandu (apomictic) x *B. ruziziensis* (sexual) population used for developing molecular markers for spittlebug, apomixes and digestibility.

Materials and Methods

A large (>230 sibs), bi-parental (tetraploidized, sexual *B. ruziziensis*-x-*B. brizantha* CIAT 6294 [cv. Marandu]) F_1 hybrid population (full-sib family) was produced and propagated for determinations of saponin content. Unintentional maternal selfs were identified by isozyme analysis, and eliminated.

The laboratory procedure used to estimate saponin activity in the test forage samples is based on the hemolisis of red blood cells obtained from rabbits, with a solution extracted (80% aqueous methanol) from fat free samples (0.1 g) of test forages. A dilution of 1:20 was used to determine absorbance in a spectrophotometer in the Forage Quality Laboratory. Leaves of wheat and of the tree *Entorolubium ciclocarpum* (Orejero) were used as negative (low levels of saponins) and positive (high levels of saponins) controls in the assay, respectively.

Results and Discussion

A high and significant correlation (r = 0.96; P < 0.001) was found between Absorbance value recorded in 2000 and in 2001. However, results shown in Table 2 indicate that the absorbance values recorded in the same accessions and hybrids of *Brachiaria* harvested in 2000 and 2001 were similar in magnitude in some groups but not in others.

Table 2. Saponin like activity in *Brachiaria* accessions and hybrids recorded in samples harvested in two consecutive years.

Classification	Forage Sample	Absorbance (2000)	Absorbance (2001)	Significance
	Entorolubium ciclocarpum (+ Control)	936	762	NS
Very High	*B. decumbens* 606			
	Hybrids:	843	695	**
	1084-3			
	1084-10	794	631	**
		890	808	**
	B. ruziziensis 26164	614	630	NS
High	Hybrids			
	1092-5	696	624	NS
	1092-15	708	520	**
	Hybrids			
Intermediate	1092-14	459	451	NS
	1092-2	478	491	NS
	1092-3	460	589	**
	1092-11	540	462	**
	1094-15		332	
	1094-19		478	
	Hybrids			
Low	1092-1	356	251	**
	1092-4	232	195	**
	1092-13	245	196	**
	1083-7		149	
	Wheat Straw (– Control)	40	28	**
Very Low	*B. humidicola* 16871	54	49	NS
	B. brizantha 6780	64	56	**
	Hybrids:			
	1078-31		28	
	1079-18		30	
	1080- 8		29	
	1081-25		31	
	1093- 31		31	
	1095- 4		29	
	1097- 6		33	

**P<0.01

Absorbance values were greater in samples harvested in 2000 and subjectively classified as very high, high and low, which was not the case for samples classified as intermediate and very low. The reason for this discrepancy is not known, but could be related to differences in maturity of the leaf tissues used for the assay since no attempt was made to harvest samples of the same maturity.

As reported last year, saponin activity was high in the commercial cultivar *B. decumbens* CIAT 606 but low in *B. humidicola* and in *B. brizantha* cv Marandu (CIAT 6780), which is the source of spittlebug resistance in the Brachiaria breeding program. Results from this year also showed that saponin activity in new *Brachiaria* hybrids was more variable than what was found last year. Out of 10 new hybrids included in the test, 7 had very low saponin activity and similar to what was recorded in one of the parents (*B. brizantha* CIAT 6789) used in the cross.

It would seem from the results of this and last year, that two of the parents (*B. decumbens* and *B. brizantha*) used in the *Brachiaria* breeding program have very contrasting levels of saponins and this is reflected in the variability of saponin activity measured in the hybrids included in the test. The high concentration of saponins in *B. decumbens* is consistent with the observations of photosensitization in cattle and sheep fed with this grass.

We conclude from these results that there is justification to screen *Brachiaria* hybrids for saponins. However, before we make this commitment we need to adapt a laboratory screening procedure that is fast, reliable and that allow us to quantify the exact concentration of saponins present. Thus a future priority is to establish collaboration with an advanced laboratory investigate the chemical nature of saponins in *Brachiaria* and to investigate alternative laboratory procedure for quantifying saponins that is more accurate and less time consuming than the qualitative method presently used. Meanwhile we will screen for saponins the elite hybrids selected on the basis of spittlebug resistance and adaptation to high soil Al.

Activity 1.2 Assessment of quality and animal production potential of selected legumes

Highlights

- Using Rubisco as a protein source in an vitro fermentation test we can estimate how the concentration of tannins in tropical legume species could affect the rate and extent of plant - protein degradation in the rumen.
- Differences in tannin structure in *Calliandra* provenances were associated with different proportion of N escaping degradation in the rumen and reaching the duodenum of sheep.
- Supplementing sun-dried or fresh *Calliandra* did not result in milk yield increments of cows grazing a well-managed pasture of *B. decumbens.*

Progress towards achieving milestones

- **Known effect of tannin with different chemical structure on N utilization by ruminants**
  Differences in monomer composition in two *Calliandra* provenances was shown to be associated with the proportion of N escaping degradation in the rumen and reaching the duodenum of sheep. A higher proportion of delphinidin relative to cyanidin in one *Calliandra* provenance resulted in a higher proportion of N consumed escaping the rumen and reaching the lower GI tract, which is consistent with what had been predicted using laboratory astringency assays. These results should now be validated in practical feeding systems where *Calliandra* forage is offered alone to ruminants as a protein supplement.

- **Known value of *Calliandra* as a protein supplement for milking cows**
  We completed a series of experiments with sheep supplemented with *Calliandra* and results indicate differences in quality between provenances, and large effect of soil fertility and drying on intake and digestibility of *Calliandra*. However, we were not able to demonstrate that supplementing fresh or sun-dried *Calliandra* to cows grazing a Brachiaria pasture resulted in more milk yield.

1.2.1 Effect of tannins with different structure on degradation of Rubisco

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Rationale

Previous results had shown that the chemical structure of condensed tannins in tropical legumes could vary among species and that this in turn affected the biological activity of tannins. Purified tannins with high delphinidin: cyanidin ratio extracted form *Calliandra* provenances were more reactive (astringent) with protein than tannins with a high cyanidin: delphinidin ratio.

This year we were interested in defining the significance of different chemical structures of tannins in tropical legumes on N utilization by sheep. Results from a feeding trial are reported in the activity (1.2.3) that follows. In addition, we were interested in defining how in vitro fermentation parameters changed with tannins with different chemical structures and consequently be useful to screen tropical legume for quality.

Materials and Methods

Four woody legume species (*Leucaena leucocephala, Calliandra calothyrsus, Clitoria fairchildiana* and *Bauhinia* sp.) were selected to carry out the study given that they had tannins with different monomer composition (Table 3).

Condensed tannins were extracted from leaf tissues of four legume species using a 70% aqueous acetone solution with ascorbic acid (0.1% w/v). Tannins in the liquid phase following centrifugation were extracted using diethyl ether and ethyl acetate. After evaporation of the organic solvent, samples were freeze-dried and the resulting solid phase was dissolved with 50% aqueous methanol. Tannins were then purified using a column (25 x 5 cm) with 60 ml of LH-20 Sephadex in suspension. A 50% aqueous methanol solution was used to wash out the column and purified tannins were recovered using a 70% aqueous acetone solution.

The ratio of pro-anthocyanidins was measured by hydrolyzing tannins of each legume with Butanol-Hcl (5%). An aliquot (1 ml) of Butane extracted tannins was evaporated and the resulting pro-anthocyanidins were put back in solution with pure methanol + 1% Hcl. The ratio of delphinidin: cyanidin: pelargonidin was then determined using an HPLC fitted with an 8 x 10 cm Nova Pack C 18 Column.

Table 3. Monomer composition of condensed tannins extracted from different tropical woody legumes

Legume	Monomer Composition (%)		
	Pro-Delphinidin	Pro-Cyanidin	Pro-Pelargonadin
Leucaena leucocephala	51.0	45.1	3.9
Calliandra calothyrsus	13.1	50.5	36.4
Clitoria fairchildiana	12.4	87.6	0
Bauhinia sp.	3.4	65.4	31.2

Rubisco was the protein used to form tannin complexes with tannins from extracted from the test legumes. To form the tannin – protein complex 0.1 g of Rubisco was placed in 100 ml centrifuge tubes with 2 ml of the tannins solution (McDougal Buffer) that corresponded to 5 or 10 % concentration. The Rubisco-Tannin mixture was left over night at 39°C before adding 50 ml of the rumen liquor- artificial saliva solution used in the in vitro fermentation system. The rate of degradation by rumen microorganisms of

Rubisco-Tannin complexes was measured stopping fermentation at 6,9, 12, 24, 36 and 48 h. The extent of degradation of Rubisco-Tannin complex was measured weighing the residues after 48 h of incubation with rumen microorganisms.

Results and Discussion

The chemical composition of tannins extracted from the test legumes is shown in Table 3. With the exception of *L. leucocephala*, all species had tannins with more cyanidin relative to delphinidin. It was also interesting to observe that tannins in both *Calliandra* and *Bauhinia* had relatively large proportions of pelargonadin.

Differences in monomer composition of tannins should be associated with their biological properties, such as capacity to bind protein. Results on the effects of tannin monomer composition and tannin level on ammonia production and in vitro DM differences are shown in Table 4.

The addition of tannins from different legumes to Rubisco resulted in a significant reduction of dry matter disappearance and to a lesser extent ammonia N production relative to Rubisco alone (positive control), regardless of legume species or tannin level. A similar trend was observed with rate of DM disappearance and rate of ammonia production.

As expected, both the extent and rates of DM disappearance and ammonia N production were lower when the concentration of tannins was increased. However, it was interesting to observe that with *L. leucocephala* the effect of doubling the concentration of tannins was small in terms of DM disappearance at 48 h as compared to the other three legume species tested. This in turn was associated with a higher delphinidin: cyanidin ratio in L. *leucocephala* as compared to the other species.

Table 4. Extent and rate of in vitro ammonia N production and DM degradation of different Rubisco – Tannin complexes.

Legume	Level of CT (%)	Extent of Degradation of Rubisco-Tannin Complex (48 h)		Rate of Degradation of Rubisco-Tannin Complex	
		NH3-N Production (mg/l)	In vitro DM Disappearance (%)	NH3-N Production (mg/h)	In vitro DM Disappearance (%/h)
Rubisco (Positive Control)	0	24.8	83.5	0.35	1.16
Leucaena leucocephala	5	21.7	68.2	0.35	0.91
	10	20.9	66.5	0.30	0.72
	5	22.7	71.0	0.34	0.87
Calliandra calothyrsus	10	21.6	64.1	0.29	0.66
	5	23.5	80.1	0.36	0.90
Clitoria fairchildania	10	22.7	68.8	0.32	0.74
	5	22.6	73.3	0.35	0.77
Bauihnia sp	10	21.7	63.1	0.31	0.64
Significance					
Legume (Tannin Composition)		0.01	0.01	0.01	0.01
Tannin Level		0.01	0.01	0.01	0.01
Specie x Tannin Level		NS	NS	NS	NS

In *Calliandra calothyrsus* we found that the astringency of tannins was more related to monomer composition than to concentration. Tannins from provenances with higher delphinidin: cyanidin ratio were more astringent than tannins with higher cyanidin: delphinidin ratio. Thus our results suggest that astringency of tannins from L. *leucocephala* is more related to its high delphinidin: cyanidin ratio than to

concentration, which is not the case with the other legume species tested that had higher cyanidin: delphinidin ratios.

In general, our results indicate that using Rubisco as a protein source in an vitro fermentation test we can define how the concentration of tannins in tropical legume species can potentially affect the rate and extent plant - protein degradation in the rumen.

1.2.2 Effect of feeding *Calliandra* with different tannin structure on N utilization by sheep

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Rationale

The evaluation and selection of shrub legumes as a feed resource is an area of interest in the tropics and one that is receiving high priority in CIAT's Tropical Forages Project. Over the last three years we have been evaluating the nutritional characteristics and feed value of two contrasting provenances *of Calliandra calothyrsus* (*Calliandra*) as a part of a collaborative OFI, UK – CIAT, Colombia project funded by DFID, UK.

It is well documented that *Calliandra*, which is native to Central America, is adapted to acid-low fertility soils with high levels of Al saturation and produces high edible biomass rich in protein. However, one commonly cited limitation of *Calliandra,* as source of fodder is the high concentration of condensed tannins (CT) in the edible forage, which have been associated with low palatability, and digestibility.

To address some of the questions related to the feed value of *Calliandra*, we carried out together with OFI a detailed characterization of the chemical composition of the edible forage of two contrasting provenances (CIAT 22310 and CIAT 22316) harvested in two field locations (CIAT) (Quilichao and Palmira) with contrasting soil fertility. An interesting finding was that the chemical composition or structure (the ratio of procyanidin: prodelphinidin) of the soluble CT fraction varied between provenances regardless of location. Soluble CT in CIAT 22316 were comprised mainly by procyanidin subunits, whereas the soluble CT fraction in CIAT 22310 was composed largely of prodelphinidin subunits. Similar results were observed in forage samples harvested in same the *Calliandr*a provenances grown in a greenhouse in the University of Reading, UK

The different tannin composition found in the two *Calliandra* provenances was associated with differences in astringency or ability of tannins to bind protein. In the studies carried out at CIAT using BSA (Bovine Serum Albumin) as a source of protein, we found that astringency was higher with soluble CT of CIAT 22310 (0.90 g protein/g of CT) than with tannins of CIAT 22316 (0.57 g protein/g of tannins), regardless of the location where the plants grew. Similar results were recorded with samples of *Calliandra* harvested from plants grown in a greenhouse in the U. of Reading, UK but the differences between provenances in astringency were smaller (CIAT 22310-0.66 g protein/g of tannins; 22136 – 0.57 g protein/g of tannins), for reasons not understood. In temperate legumes the reactivity of CT with Rubisco was found to be higher with increased proportion of delphinidin relative to cyanidin, which is consistent with what we have found in the laboratory with the two *Calliandra* provenances evaluated.

In order to assess the biological significance of the different monomer composition found in the tannin fraction of two provenances of *Calliandra* grown in two sites with contrasting soil fertility (Quilichao with acid infertile soils and Palmira with Fertile soils), we carried out a feeding trial with sheep housed in metabolic crates.

Materials and Methods

African type sheep (6) fitted with rumen and duodenal cannulas were assigned to one of four treatments in an Unbalanced Simple Crossover Design, where:

T1: *Calliandra* CIAT 22310 grown in Quilichao,
T2: *Calliandra* 22316 grown in Quilichao,
T3: *Calliandra* CIAT 22310 grown in Palmira, and
T4: *Calliandra* CIAT 22316 grown in Palmira.

Animals housed in individual metabolism crates were offered daily 50 g DM / kg BW $^{.0.75}$ (1.7 % of body weight) of sun- dried edible (leaves + fine stems) *Calliandra* forage in two meals (8:00 and 15:00). The 6 animals used in the trial were distributed in the four treatments (T) and across experimental Periods (P) as follows:

Animals	Periods			
	P1	P2	P3	P4
3	T4	T2	T1	T3
5	T2	T4	T3	T1
2	T2	T3	T1	T4
6	T4	T3	T2	T1
4	T2	T1	T4	T3
1	T1	T3	T4	T2

Animals were supplemented via rumen cannula with 4 g/ kg of BW/ d of a high-energy source (extracted cassava meal) with negligible protein concentration and offered a mineral mix ad-lib and water 4 times a day. Sheep used in the trial were allowed to graze for one week between experimental periods, which had duration of 17 days, of which 7 were for adjustment and 10 for measurements.

Forage refused and feces were collected daily and one sub sample was frozen for subsequent freeze drying and chemical analysis and another oven-dried to determine DM content. On days 8 and 9 of the measurement period, samples of duodenal digesta were taken every 6 hours and on the last day rumen samples were collected every 3 hours to determine purines in rumen bacteria. Rumen and duodenal digesta samples were frozen for subsequent analysis.

Samples of legume offered were analyzed for soluble condensed tannins (CT) using the Butanol Hcl method. In addition, forage (offered and refused) and fecal samples were analyzed for N, and fiber (NDF and ADF). Concentration of indigestible acid detergent fiber (IADF) in the forage (offered and refused) and feces was used as an internal marker to estimate flows of the solid phase of digesta to the duodenum. Purines were determined in duodenal samples to estimate microbial N and RNA from torula yeast type II-C from SIGMA (R-6875) was used as a standard. The ratio of N: RNA of bacteria flowing to the duodenum was estimated for each treatment from bacteria isolated from the rumen fluid by differential centrifugation. The ratio of rumen bacteria N: RNA equivalent was used to estimate proportion of bacterial N in duodenal samples.

Data were subject to analysis of variance for an Unbalanced Simple Crossover Design with 4 periods and the Duncan's test was used to compare treatment means.

Results and Discussion

Chemical characterization of *Calliandra*: Results on chemical composition of the forage of *Calliandra* offered are shown in Table 5. The concentration of crude protein (CP) was lower in *Calliandr*a harvested

in Quilichao than in Palmira but on average did not differ between provenances. In contrast, fiber (NDF and ADF) concentration differed between *Calliandra* provenances, being higher in CIAT 22310 than in CIAT 22316, regardless of site.

In other studies carried out in CIAT, fiber content measured as NDF was also higher in CIAT 22310 than in CIAT 22316, and was not affected by drying method or by location, which is in agreement with results found in the U of Reading with the same provenances. The higher fiber concentration in CIAT 22310 was associated with lower in vitro digestibility (IVDMD) as compared with CIAT 22316. It was also observed that IVDMD of *Calliandra* was lower in the edible forage harvested in the site with acid soils (Quilichao) than in the site with fertile soil (Palmira), but these differences due to site were not explained by differences in fiber content.

Table 5. Chemical characterization of *Calliandra calothyrsus* provenances fed to sheep Housed in metabolism crates

Calliandra Provenances (CIAT No. and Site)	Crude Protein	In Vitro Digestibility	Neutral Detergent Fiber (% of DM)	Acid Detergent Fiber	Condensed Tannins Soluble	Condensed Tannins Insoluble
22310- Quilichao	15.5 b	20.1 c	35.0 a	32.1 a	28.3 b	6.3
22310- Palmira	17.0 a	32.2 b	36.7 a	29.6 a	18.0 c	4.4
22316- Quilichao	13.5 c	32.8 b	27.4 b	24.0 b	35.4 a	4.0
22316- Palmira	18.0 a	39.9 a	31.1 b	24.9 b	33.6 a	3.6
SEM	0.4	0.6	0.8	0.9	0.5	0.5
Significance (P) <						
Provenance	NS	0.0001	0.0001	0.0001	0.0001	NS
Site	0.0001	0.0001	0.01	NS	0.0004	NS
Provenance x Site	0.0036	0.004	NS	NS	0.003	NS

a. b, c Values in the same column with the same letters are not different (P < 0.05)

The concentration of soluble condensed tannins (CT) in the forage offered to sheep was higher in CIAT 22316 as compared to CIAT 22310, but the insoluble CT fraction did not change due to provenance (Table 5), which is in agreement with previous findings of CIAT and OFI. However, it was interesting to observe that soluble CT in CIAT 22310 was considerably lower in the edible forage harvested in Palmira as compared to Quilichao. Although the soluble CT fraction was higher in CIAT 22316, IVDMD was also higher with this provenance possibly as a result of having less fiber. Similar results were recorded in other feeding trials where the two provenances were used as supplements to sheep fed a low quality grass diet. These results are interesting since they confirm previous findings that suggest that digestibility of *Calliandra* forage is more related to fiber content than to the concentration of soluble CT.

Intake and digestibility: Results for intake and digestibility are presented in Table 6 for main effects given that the interaction of site x provenance was not significant for the variables measured. Intake of the two *Calliandra* provenances was very low when expressed as proportion of body weight (range 0.5 to 1.2 kg DM/ 100 Kg of BW). The amount of *Calliandra* consumed represented 41% and 63% of the amount offered daily of CIAT 22316 and CIAT 22310, respectively. It was also evident that intake of *Calliandra* was affected by location. Intake of forage harvested in Palmira (fertile soil) was 58 % higher than the intake from forage harvested in Quilichao (infertile soil).

On the other hand, DM intake of *Calliandra* CIAT 22130 was 45% higher than intake of CIAT 22316, which was an unexpected result. In previous feeding trials with sheep we had observed that intake of *Calliandra* CIAT 22316 was higher than *Calliandra* 22130 when fed in combination with a poor quality grass, and this was related to the lower fiber content in CIAT 22136. However, it should be noted that

intake of digestible DM was affected by site and not by provenance as a result of the higher digestibility of *Calliandra* CIAT 22316 as compared with CIAT 22130. The large site effect observed on DM intake of *Calliandra* in this study is consistent with previous results and points out the major influence that soil fertility has on the overall quality of *Calliandra*.

Table 6. Intake and digestibility of *Calliandra calothyrsus* fed to sheep housed in metabolism crates and supplemented with extracted cassava meal[1]

Item	Site Effect		Provenance Effect		SEM
	Quilichao	Palmira	CIAT 22310	CIAT 22316	
Intake of DM (g/kg of BW/d)	6.7 b	10.6 a	10.2 a	7.1 b	0.55
Digestibility of DM (%)	57.8	53.7	51.7 b	59.8 a	2.10
Digestibility of NDF (%)	50.1	50.9	45.5 b	55.6 a	3.60
Intake of Digestible DM (g/kg of BW/d)	3.6 b	5.4 a	5.1	4.0	0.37

a, b, c for each main effect, values in the same row with the same letters are not different (P<0.05)
[1]4 g/ kg of BW/ d of extracted cassava meal was fed via rumen cannula to each sheep

Digestibility (DM and NDF) values were influenced by provenance but not by site (Table 6). In both sites the digestibility of dry matter and fiber were higher with CIAT 22316 than with CIAT 22310, which can be explained by lower fiber content.

Nitrogen utilization: The main objective of running this feeding trial was to test the hypothesis that the higher proportion of delphinidin relative to cyanidin found in the soluble CT fraction of CIAT 22310 would result in greater protection of protein in the rumen (by-pass protein) as compared with CIAT 22316 with soluble CT comprised mainly by procyanidin subunits.

Results shown in Table 7 are again for main effects, given that we did not find a provenance x site interaction for the response variables measured. Total N intake was 44% higher with *Calliandra* CIAT 22310 than with CIAT 22316. In addition, N intake was 85% higher when *Calliandra* harvested in Palmira was fed. These differences are the result of the higher DM intake of *Calliandra* 22310 and of the forage harvested in the fertile soils of Palmira.

Flow of different N fractions to the lower GI tract was affected by provenance fed and by site. A higher amount of total N (solid phase) and non-ammonia non-microbial N (solid phase) reached the duodenum when CIAT 22310 and when *Calliandra* grown in Palmira were fed. On the other hand, estimates of absolute and relative values of escape dietary N were significantly greater when *Calliandra* 22310 was fed, which is consistent with in vitro results that had shown greater astringency of the soluble CT of this provenance relative to CIAT 22316. It could be argued that the greater amount of N reaching the duodenum when *Calliandra* CIAT 22310 was fed is the result of the higher N intake when this provenance was fed.

However, when the N reaching the duodenum was expressed as a proportion of N fed, results also showed more N from *Calliandra* CIAT 22310 by-passing or escaping the rumen as compared with CIAT 22316 (Table 7). The greater level of escape N observed with *Calliandra* 22130 was not only associated with more fecal N but also with more apparently absorbed N, which was associated with the higher N intake recorded when this provenance was fed. It is important to note that the proportion of N absorbed as a proportion of N intake was similar (76 % for CIAT 22136 and 88% for CIAT 22310) for the two provenances.

In previous feeding trials carried out in CIAT we had fed sheep the two *Calliandra* provenances used in this trial in combination with a low quality grass. Results from those feeding trials had shown differences in potential feed value between the two *Calliandra* provenances evaluated and how drying and location can affect their utility as a protein source to ruminants fed a low quality grass. Specifically we found that intake of *Calliandra* CIAT 22316 was higher than intake of CIAT 22310 and that intake of *Calliandra* was improved when the forage was fed dried as opposed to fresh, which is contrary to common belief. The results from this feeding trial contradict previous results, given that DM and N intake of Calliandra CIAT 22310 when fed alone was greater than when CIAT 22316 was fed. However, it should be pointed out that intake of digestible dry matter was similar for the two provenances given the higher digestibility of CIAT 22316 associated with lower fiber content.

Table 7. Nitrogen (N) utilization by sheep housed in metabolic crates and fed two provenances of *Calliandra calothyrsus* grown in contrasting sites[1].

Item	Site Effect		Provenance Effect		SEM
	Quilichao	Palmira	CIAT 22310	CIAT 22316	
N intake, g/d	6.7 b	12.4 a	10.8 a	8.3 b	0.67
Duodenal N, g/d	12.5 b	18.6 a	18.6 b	12.5 a	1.26
NAMNmic- N [2], g/d	5.5 b	9.5 a	11.0 a	4.0 b	1.27
Fecal N, g/d	5.7 b	9.7 a	9.1 a	6.2 b	0.38
Absorbed N [3], g/d	6.9 b	8.9 a	9.5 a	6.3 b	0.10
Escape dietary N, g/d	5.5	9.5	11.0	4.0	1.30
Escape N, % of N intake	77.2	79.0	99.0 a	57.2 b	13.90

[1] 4 g/ kg BW/d of extracted cassava meal was fed via rumen cannula to each sheep
a, b for each main effect, values in the same row with the same letter are not different ($P<0.05$)
[2] NAMNmic- N = Non-ammonia non- microbial nitrogen
[3] Apparently absorbed N = Duodenal N – Fecal N
[4] Escape dietary N = N flow to the duodenum – (Bacterial N flow + Endogenous N)
where: Endogenous N = 2.2 g N/kg of DM intake

On the other hand, results from previous feeding trials did not provide any clues on how the different chemical structures of CT in the two *Calliandra* provenances affect their feed value. It was expected that protein degradation in the rumen of sheep supplemented with *Calliandra* would have been greater with CIAT 22316 than with CIAT 22310, given the higher astringency of the tannins found in the latter. However, we found no evidence of more escape protein when *Calliandra* CIAT 22310 was included as protein supplement as compared to CIAT 22316, possibly as a result of Calliandra forage comprising only a small proportion of the diet fed and consumed by sheep. Thus we postulate that differences in astringency between the two *Calliandra* provenances, which resulted in different levels of by pass - protein when fed to ruminants could have implications in practical feeding systems only when the legume is fed alone, rather than as a protein supplement to a low quality basal diet

An interesting finding of this study is that for the first time it has been shown that the monomer composition of soluble CT in a tropical legume can have an effect on the utilization of N by ruminants. The fact that our in vivo results were in close agreement with results on astringency of soluble CT extracted from *Calliandra* provenances is also a major finding, since they validate the utility of the laboratory astringency tests for screening tropical legumes for quality traits.

The results of this feeding trial indicate that *Calliandra* CIAT 22310 was more consumed than *Calliandra* 22136 when offered to sheep as the only forage source. The higher intake of DM found with CIAT 22310 resulted in more N intake and N apparently absorbed in the small intestine, but not in more intake of

digestible DM given the higher digestibility of CIAT 22316. Thus it is not possible to conclusively infer that the feed value of *Calliandra* 22310 is higher than that of CIAT 22316. However, these results do indicate that differences in tannin structure of the two *Calliandra* provenances had an effect on nitrogen utilization by sheep fed the legumes alone.

1.2.3 Milk production of cows supplemented with sun-dried and fresh *Calliandra*

Contributors: P. Avila, C. Lascano and G. Ramírez (CIAT)

Rationale

Work carried out at CIAT as part of an OFI-CIAT collaborative Project had shown that voluntary intake of *Calliandra* fed to sheep housed in metabolism crates was improved when the forage was fed dried as opposed to fresh. However, the positive effect of feeding sun- dried *Calliandra* on intake of the legume did not translate in higher DM digestibility or N absorption in sheep fed a low quality grass. In fact apparent N absorption was greater when fresh *Calliandra* was fed as a result of increased total N and bacterial flow to the duodenum, which suggest less protein degradation by rumen microbes.

Thus this year we were interested in determining if milking cows grazing a well-managed *Brachiaria decumbens* pasture consumed more of the sun-dried than fresh *Calliandra* when offered as a supplement and if this would result in higher milk yield.

Materials and Methods

A grazing trial was carried out in Quilichao during a wet period (April- May, 2001) to measure milk yield and composition of cows (3 Holstein and 3 crossbred Zebu) grazing a *B. decumbens* pasture stocked with 2 cows/ha. Three treatments (T1: Control; T2: Fresh *Calliandra calothyrsus* CIAT 22310 and T3: Sun Dried *Calliandra calothyrsus* CIAT 22310) were compared using a 3 x 3 Latin Square design. Each experimental period consisted of 7 days adjustment and 7 days measurement phase. At milking (AM and PM) cows were given the legume (leaves, fine stems) supplements at a level of 1.5% DM of BW/day.

Results and Discussion

Results shown in Table 8 indicate that there was no significant effect of supplementing fresh or dry Calliandra on milk yield and composition of cows grazing a well managed *B. decumbens* pasture. These results are interesting given that intake of fresh *Calliandra* was 3.5 times higher (2.4 vs 8.3 g DM/Kg BW/day) than the intake of sun-dried *Calliandra*, which is contrary to what was to be expected based on the results from feeding trials with sheep housed in matabolism cages (See AR 2000). Results with sheep also showed that apparent N absorption was greater when fresh *Calliandra* was fed as a result of increased total N and bacterial flow to the duodenum, which suggest less protein degradation by rumen microbes.

Table 8. Effect of supplementing fresh or dry *Calliandra calothyrsus* on milk yield and composition of grazing a *B. decumbens* pasture during the wet season.

Treatment (Supplement)	Milk Yield (kg/cow)	Fat (%)	Non Fat solids (%)	MUN (mg/dL)
Control	5.4	3.9	8.3	12.6
Calliandra-Fresh[1]	5.6	3.9	8.0	14.7
Calliandra- Sun-Dried[1]	5.3	3.8	8.3	12.9

[1]Cows were supplemented with fresh or sun-dried *Calliandra* (leaf + fine stem) by offering 1.5% DM of BW in two separate meals/day during milking.

It is not known why sheep fed a low quality grass consumed more sun-dried and fresh *Calliandra* as compared to milking cows grazing a *B. decumbens* pasture. However, these conflicting results do point out the danger of extrapolating results from sheep to cattle and from grazing to confinement.

In conclusion, our results indicate that supplementing sun-dried or fresh *Calliandra* did not result in milk yield increments of cows grazing a well-managed pasture of *B. decumbens*. It is possible that cows grazing poorly managed (low forage availability an low forage quality) pastures would respond to supplementation of *Calliandra*, particularly if fed fresh. This hypothesis will be tested next year.

Activity 1.3 Assessment of the potential of saponin-rich tropical fruits to reduce methane in ruminants on grass diets

Highlights

- Out of three saponin-rich fruits evaluated, only *S. saponaria* significantly decreased protozoa count by 54% and daily methane release by 20% relative to the control.
- Defaunation suppressed methane by 43% on average of all diets, but the effect of *S. saponaria* against methane was even higher in defaunated (29%) than in faunated rumen fluid (14%).
- Depression of methane release was related to the proportion of *S. saponaria* in the diet. Methane release was reduced by 10% when the proportion of *S. saponaria* in the diet was increased from 0 to 8%. A further increase of the proportion of S. saponaria, up to 14%, had no further depressing effect on methane release.

Progress towards achieving milestone

- **Known utility of saponin-rich plants and semi-purified saponins to reduce methane by ruminants**
  We made considerable progress in defining the potential of saponin-rich tropical fruits to manipulate rumen fermentation through in vitro experiments carried out with the Rusitec-system. Our findings indicate a high potential for *Sapindus saponaria* to reduce methanogenesis and rumen protozoa populations. We also found a relation between daily methane release and the proportion of *S. saponaria* in the diet. The highest depression occurs in diets with approximately 8% *S. saponaria* in the DM. Future studies, will validate results obtained with the Rusitec-system in feeding trails with confined sheep.

1.3.1 In vitro evaluation of the potential of saponin – rich tropical fruits to manipulate rumen fermentation and to reduce methanogenesis

Contributors: Hess H.D. (ETH, Zurich), Soliva C (ETH, Zurich), Diaz T.E (CORPOICA, Colombia), Kreuzer M, and Machmuller A. (ETH, Zurich)

Rationale

The issue of global warming caused by anthropogenic greenhouse gases is of increasing concern. Although fossil- fuel based industrial development is the major cause of the environmental imbalance, agricultural practices cannot be ignored. Combustion of fossil energy and deforestation are primarily responsible for the increases in of atmospheric CO2. However, ruminant animals have a considerable significance on global warming since they contribute 1/6 of the total atmospheric methane. Thus, efforts to mitigate methane emissions from ruminants and other sources (wet lands, rice paddies, waste management etc) are urgent given that atmospheric methane concentration is increasing at a faster rate than CO2. In

addition, per molecule, methane is 21 times more potent as a greenhouse than CO2, even though it has a relatively shorter half-life.

In tropical countries, where the majority of the world's domestic ruminants are located, production systems are mostly based on forages and crop residues of low nutritive value. Under these conditions, the productivity of the animals is low and the production of methane from microbial fermentation of feed in the rumen represents a loss of 15-16% of the digestible energy consumed. Saponin-rich fodder plants have been shown to reduce protozoa population by 80%, and some methanogens are known to be closely associated with protozoa. There are a variety of tropical plants and fruits differing in contents and types of saponins, and their effect on ruminal fermentation processes and their efficacy against methane is still unexplored.

To address the issue of methane production by ruminants we joined an SDC- ZIL- funded collaborative research effort led by Prof. M. Kreuzer of the Institute of Animal Science in the Swiss Federal Institute of Technology (ETH), Zurich and by Dr. Dieter Hess (ETH) a former Visiting Scientist at CIAT, which includes as partners the U. Nacional in Bogotá and CORPOICA.

The aim of the project is to develop feeding strategies based on locally available forage components and ingredients such as saponins to reduce methane emissions per unit of edible animal product (beef and milk) and to simultaneously improve feed use efficiency in tropical smallholder livestock systems.

The project has three main research components: (a) in vitro screening of different feed and forage sources, (b) in vivo validation of promising feeding components and strategies and (c) assessment of the applicability of the experimental results at local and regional level and definition of diffusion strategies to promote the use of feeding systems leading to reduce methane production.

Two initial experiments were carried out in ETH, Zurich to compare the effects of the dietary inclusion of saponin-containing tropical fruits (*Sapindus saponaria*, *Enterolobium cyclocarpum*, *Pithecellobium saman*) and of increasing proportions of S. *saponaria* on in vitro rumen fermentation parameters including methane production.

Materials and Methods

Experiment 1

The daily control diet consisted of 9.30 g low quality hay, 3.72 g *Arachis pintoi* (a tropical pasture legume), 1.80 g barley straw and 0.18 g urea (on a DM basis). The other diets contained 10% *Sapindus saponaria*, 20% *Enterolobium cyclocarpum* or 20% *Pithecellobium saman*, corresponding to the dietary proportions described to be still applicable *in vivo*. Daily dietary DM supply and crude protein content (13%) were kept constant.

The four diets were evaluated simultaneously with faunated and defaunated (Synperonic) rumen fluid during 4 × 10 day periods in an eight-fermenter Rusitec system (n=4). Feed was incubated for 48 h. Daily rumen fluid samples were taken 4 h before feed introduction and were analyzed for pH, ammonia, VFA and protozoa count. Fermentation gases were collected in gas proof bags and chromatographically analyzed for methane. For statistical analysis mean values from days 5 to 10 were used.

Experiment 2

To follow-up this initial study we determined the effect of increasing proportions of *S. saponaria* fruits in the diet on rumen fermentation parameters. The daily control diet consisted of 14.67 g low quality hay and

0.33 g urea on a DM basis. The other diets contained 2, 4, 6, 8, 10, 12, or 14% of ground *S. saponaria* fruits.

Daily dietary DM supply and crude protein content (13%) were kept constant. The 8 diets were evaluated during 2 x 10 day periods in an eight-fermenter Rusitec system (n=2).

Results and Discussion

Experiment 1

Of the saponin-containing fruits only *S. saponaria* significantly decreased protozoa count by 54% and daily methane release by 20% relative to the control. Defaunation suppressed methane by 43% on average of all diets, but the effect of *S. saponaria* against methane was even higher in defaunated (29%) than in faunated rumen fluid (14%) (Figure 1).

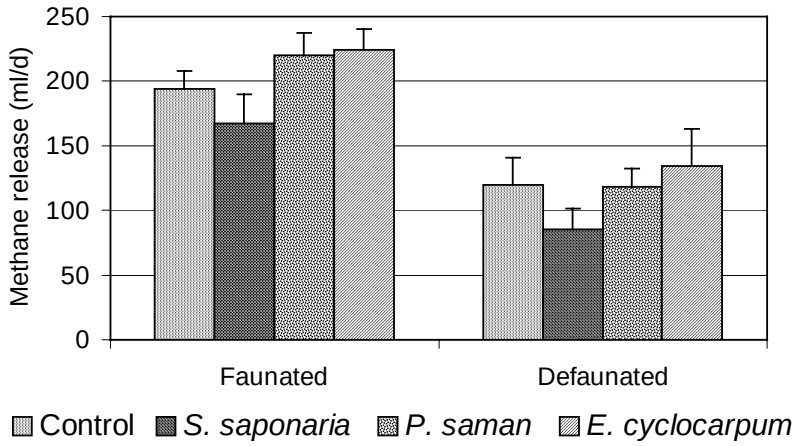

Figure 1. Daily methane release from Rusitec-fermenters supplied with a control diet or with diets containing 10% *Sapindus saponaria*, 20% *Enterolobium cyclocarpum* or 20% *Pithecellobium saman*.

When related to apparently fermented OM, the use of *P. saman* and *E. cyclocarpum* had no effect on methane release, but differences in daily methane release relative to the control remained similar to what was observed with *S. saponaria*. The results form this experiment demonstrated that the fruits of the tropical tree *Sapindus saponaria* (Photo 1) when included at a level of 10% in forage-based diets has the potential to reduce methane release from ruminal fermentation. Since the depression of methanogenesis was also found in defaunated rumen fluid, it can be assumed that saponins or other constituents of *S. saponaria* act directly against methanogens.

Experiment 2

Increasing the proportion of S. *saponaria* in the diet did not affect ammonia (averaged 10.4 mmol/l) content in rumen fluid. Total protozoa count was not affected when lower proportions of *S. saponaria* (2-6%) were included in the diet. However, with higher proportions (12 and 14%) protozoa count was reduced by over 50%. Daily methane release was related to the proportion of *S. saponaria* in the diet.

When the level of *S. saponaria* was increased from 0 to 8%, methane release was reduced by 10%. A further increase of the level of *S. saponaria*, up to 14%, had no depressing effect on methane release. On the contrary, methane release seemed to increase with the highest dose. The reasons for these unexpected

results are not clear, but could be related to other components of the fruit of *S. saponaria*, which, at higher levels, could affect rumen fermentation.

Although reduction of methane release was somewhat lower than in experiment 1, results confirm the potential of *Sapindus saponaria* fruits to reduce methane emissions from ruminal fermentation. The optimal level of *S. saponaria* seems to be around 8% of the daily diet dry matter.

Photo 1. Fruits of *Sapindus saponaria* (Courtesy: CIPAV)

1.3.2 In vitro evaluation of the potential of semi-purified saponins from *Sapindus saponaria* to manipulate rumen fermentation and to reduce methanogenesis

Contributors: H.D. Hess (ETH, Zurich), A. Abreu (U. Nacional, Colombia), A. Cano, (U. Nacional, Colombia), J.E. Carulla (U. Nacional, Colombia), C.E. Lascano (CIAT), and M. Kreuzer (ETH, Zurich)

Rationale

Saponins are a complex group of plant ingredients with highly diverse biological activity, but limited data are available on saponins from typical tropical plants like *Sapindus saponaria*. Therefore we extracted saponins from *S. saponaria* and used the extract to evaluate the effect of semi-purified saponins on rumen fermentation in vitro.

Materials and Methods

The daily basal diet consisted of 8.81 g of *Brachiaria dictyoneura* cv. Llanero hay (a low quality grass; 3% crude protein, 78% neutral detergent fiber, 41% acid detergent fiber) and 5.87 g of sun dried leaves of *Cratylia argentea* (a multipurpose shrub legume with medium forage quality; 18% crude protein, 65% neutral detergent fiber, 38% acid detergent fiber).

Daily dosage of semi-purified saponins (purity approximately 95%) was 0, 0.06, 0.12, 0.18, 0.24, 0.30, 0.36 or 0.42 g per day on a dry matter basis. The eight treatments were evaluated during 4 x 10 day periods in an eight-fermenter Rusitec system (n=4).

Results and Discussion

Due to the moderate forage quality of the basal diet used, ammonia concentration (3.19 mmol/l) was considerably lower than in the first two experiments. However, rumen ammonia decreased linearly from 4.02 to 2.40 mmol/l when the dosage of saponins was increased from 0 to 0.42 g. Lower doses of semi-purified saponins (0.06 to 0.24 g/day) had no effect on total protozoa count, which is in contrast to the 50% reduction in protozoa observed with the highest dose (0.42 g/d).
respectively) (Figure 2).

The effect of saponins on daily methane release was not clear in this experiment. Since none of the saponin levels tested reduced methane release when compared with the control diet. However, methane release from the diet with 0.18 g saponins (1.2% in DM) was approximately 20% lower ($P<0.05$) than release from the diets with 0.24, 0.30 and 0.36 g (1.6%, 2.0% and 2.4% in DM).

Although differences between most of the treatments were not significant ($P>0.05$) it is interesting to note, that the methane release pattern observed in this experiment agrees well with the one from the experiment carried out with complete fruits of *Sapindus saponaria*. In that trial the lowest methane release was measured with proportions between 8 and 10 % of fruit in the diet and when the highest dose (14%) was used, methane release was increased. Taking into account the saponin content in the complete fruits (approximately 12%), 8 to 10% of fruit is equivalent to 1.0 to 1.2% of saponins, and 14% of fruits is equivalent to 1.7% of saponins in the diet.

Finally, results of this experiment suggest that a possible methane depressing effect of saponins from *Sapindus saponaria* (if there is any) would be independent of their potential to suppress rumen protozoa, which agrees with the observations made in the first study.

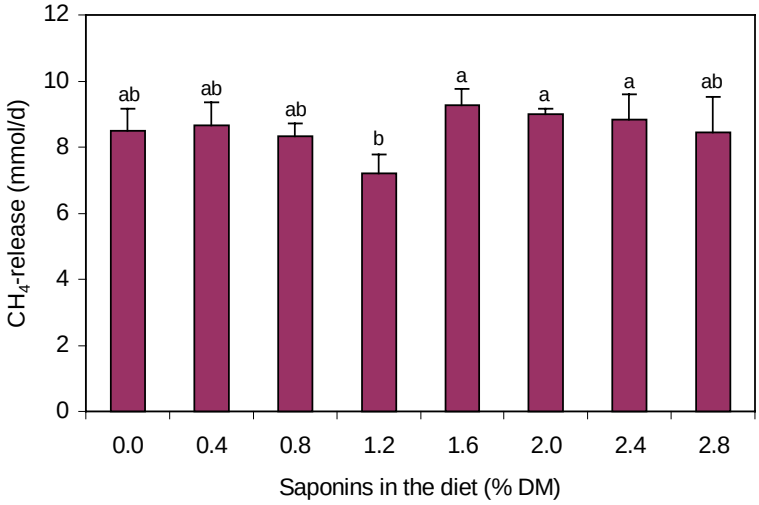

Figure 2. Daily methane release from Rusitec-fermenters supplied with a control diet or with diets containing 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 or 2.8% saponins.

1.3.3 *In vitro* evaluation of fruits of *Sapindus saponaria* in relation to semi-purified saponins and saponin-free diets on their effect on rumen fermentation and methane release

Contributors: H.D. Hess (ETH, Zurich), A. Abreu (U. Nacional, Colombia), A. Cano (U. Nacional, Colombia), J.E. Carulla (U. Nacional, Colombia), C.E. Lascano, (CIAT) and M. Kreuzer (ETH, Zurich)

Rationale

In earlier *in vivo* studies carried out by other groups to evaluate the potential of *S. saponaria* to manipulate rumen fermentation, only the pericarp of the fruit has been fed used. Separation of the pericarp from the residual fruit is quite laborious and time consuming and probably not practical under farm conditions. Thus it was of interest to investigate effects of whole fruits of *S. saponaria* in relation to pericarp and semi-purified saponins on methanogenesis.

Materials and Methods

The control diet in this experiment consisted of 8.8 g of low quality hay (*Brachiaria dictyoneura* cv. Llanero) and 5.9 g sun dried *Cratylia argentea* leaves. The other three diets contained either a) 8% complete and ground *S. saponaria*, b) 5% *S. saponaria*, pericarp or c) 1.2% semi-purified saponins. Daily dry matter supply was kept constant for the evaluation of the 4 diets during 2 x 10 day periods in an eight-fermenter Rusitec system (n= 4).

Results and Discussion

Ammonia concentration across all diets averaged 3.26 mmol/l. Although crude protein content in the diets was constant and the estimates for apparent CP degradability were similar for all diets (45.1%), ammonia concentration was considerably lower (-25%, $P<0.05$) with the fruit and the pericarp than with the control diet. This could indicate a more efficient use of nitrogen by the rumen microbes when the diets contained fruits or pericarp.

Total protozoa counts were not affected when 1.2% of semi-purified saponins were included in the diet, but were reduced by 50% ($P<0.05$) when the diet contained 8% of fruit or 5% of pericarp. The absence of a reduction in protozoa counts when semi-purified saponins were included in the diet could be due to: (a) saponins could have lost their anti-protozoal effect during the extraction process or (b) other constituents (different from saponins) are responsible for the anti-protozoal effect of fruits and pericarp of *S. saponaria*.

Daily methane release averaged 218.0 ml and did no vary with diet ($P>0.05$). These contrasts clearly with the results form the first experiment where 8% of fruits of *S. saponaria* reduced methane release by 14% in faunated rumen fluid. Possible explanation for these contrasting results could be that fiber content in the diets used in the present experiment was considerably higher than in the first one (72.1% vs. 60.8%) and CP content was slightly lower (10.8% vs.13.0%). Since interactions between forage quality and saponin-effects cannot be excluded, this could have contributed to the contrasting results.

Additionally, it is possible that rumen liquid from cattle grazing tropical pastures, as used in the present experiment, contains methanogens resistant to saponins. And finally, it is known that differences in saponin content and composition may be considerable between fruits from provenances or of different age and consequently their effect on rumen fermentation may also be different.

1.3.4 *In vitro* evaluation of the effect of varying proportions of *Arachis pintoi* in a basal diet of a low quality grass on rumen fermentation

Contributors: H.D. Hess (ETH, Zurich), J.E. Carulla (U. Nacional, Colombia), C.E. Lascano (CIAT)

Rationale

Considerable information has been generated on the effect of improved grass and legume species on animal production and soil fertility parameters. However, up to now little information is available on the effect of these improved forage alternatives on rumen methanogenesis. Thus a Rusitec-trail was carried out to evaluate the consequences of the inclusion of varying proportions of *Arachis pintoi* in a low quality diet on rumen fermentation parameters.

Materials and Methods

The daily control diet consisted of 15.0 g of low quality hay (*Brachiaria dictyoneura*; 3.0% CP, 76.1% NDF, 42.6% ADF). The other diets contained 33.3%, 66.7% or 100% *Arachis pintoi* (a high quality pasture legume; 17.0% CP, 50.5% NDF, 38.4% ADF). The four diets were evaluated simultaneously alone and with saponins (1.2% of DM). The 8 treatments were tested in 4 × 10-day periods (n=4).

Results and Discussion

None of the variables evaluated was affected by the addition of saponins (P>0.05), but most of the variables were clearly affected by the proportion of *A. pintoi* in the diet. The pH of rumen fluid decreased from 7.13 with the control diet to 6.86 with the diets containing *A. pintoi*. Ammonia concentration increased linearly from 0.45 to 10.32 mmol/l when the proportion of *A. pintoi* increased from 0 to 100%. Total protozoa as well as bacteria counts were significantly increase when *A. pintoi* was included in the diet and fiber degradation was increase by 100%.

All these results indicate that ruminal fermentation with the pure grass hay diet was strongly limited by the low quality of *B. dictyoneura* (i.e. its low CP content). A proportion of 33.3% of *A. pintoi* in the diet was sufficient to overcome these limitations and to increase microbial activity.

Daily methane release was lowest with the control diet (1.7 mmol/d), intermediate with 33.3% of *A. pintoi* (7.3 mmol/d) and highest with 66.7 and 100% *A. pintoi* in the diet (8.8 and 9.0 mmol/d, respectively). When related to apparently fermented OM the inclusion of *A. pintoi* increased methane release by approximately 150%.

Based on these results, one could argue that, in terms of methane release, pure *B. dictyoneura* hay would be the best diet. However, it has to be taken into consideration that animal production based on such a low quality diet would be very low or even negative (animals would probably lose weight), whereas with diets containing *A. pintoi*, animal production is usually high probably resulting in less methane produced / unit of milk or milk.

Activity 1.4 Assessment of quality and animal production potential of selected grass species

Highlights

- Confirmed that milk yield is higher with the *Brachiaria* hybrid cv Mulato than with the commercial *B. decumbens* cultivar.

- Demonstrated that with proper grazing management milk yield in the recently released *B. brizantha* cv Toledo can be higher than in the commercial *B. decumbens* cultivar

Progress towards achieving milestones

- **Benefits in animal production of new *Brachiaria* hybrids relative to commercial cultivars**
  This year we confirmed that cows grazing *Brachiaria* hybrid cv Mulato (first hybrid released) produce more milk than when grazing the commercial *B. decumbens* cv Basilisk, and that this is associated with a higher protein content in the forage on offer.

1.4.1 Milk yield with new hybrids of *Brachiaria*

Contributors: P. Avila, C. Lascano, J. W. Miles and G. Ramírez (CIAT)

Rationale

Last year we reported that milk yield with the commercial *Brachiaria* Hybrid Mulato was 25% greater than with *B. brizantha* cv Toledo and 7% higher than with *B. decumbens* cv Basilisk. It was interesting to observe that MUN values were two times greater in cv Mulato as compared to the other two *Brachiaria* cultivars, suggesting a higher concentration of CP in the forage on offer.

This year we completed an additional short-term grazing experiments to compare milk yield of the newly released cultivars (Mulato and Toledo) with the commercial *B. decumbens* cv Basilisk in the rainy season.

Materials and Methods

A grazing trial was carried out in October/November 2000 (rainy period) with 2 cows/ha. A total of 6 cows (3 Holstein and 3 Zebu Crossbreds in early-mid lactation) arranged in a 3 x 3 Latin Square were used to measure milk yield in pastures that were mowed 3-4 weeks prior to grazing. Each period was of 14 days of which 7 were for adjustment to the treatment and 7 for measurement of milk yield milk composition parameters and pasture attributes.

Results and Discussion

Our results did not show a significant interaction of cow group and pasture for milk yield, so mean values across cow types are presented in Table 9. Milk yield was higher in cows grazing *B. brizantha* cv Toledo and *Brachiaria* hybrid cv Mulato as compared to what was recorded in the widely used *B. decumbens* cv Basilisk.

As observed last year (See AR 2000), MUN was greater in cows grazing Mulato than the other two cultivars and this was associated with higher CP in the leaf tissue (8.5% in Mulato vs 7.3 % in Basilisk and 7.9 % in Mulato) in the forage on offer. Forage on offer expressed as green DM was also higher in the Mulato pasture (3200 kg/ha) than in the pasture with Basilisk (2000 kg/ha) and Toledo (2300 kg/ha), which is as reflection of the high production capacity under grazing of this Brachiaria hybrid.

Previous results had shown that milk yield in pastures of *B. brizantha* cv Toledo were lower than in *B. decumbens* (See AR 2000) and that this was due to lower protein content in the edible forage. We also suggested that the fast capacity of Toledo to grow following grazing could contribute to a rapid loss of quality if not properly managed. In the experiment being reported, all pastures were grazed after a 3-4 weeks regrowth periods using high stocking rate and as a result the forage on offer in the three pastures had high digestibility and was not limiting in protein.

Table 9. Milk yield of cows grazing contrasting *Brachiaria* pastures (Quilichao Research Station).

Pastures	Milk Yield (kg/d)	MUN (mg/dL)
B. decumbens cv. Basilisk	7.0 b	4.4 b
B. brizantha cv. Toledo	8.5 a	3.8 b
Brachiaria Hybrid cv Mulato	8.1 a	5.7 a

a, b, c, P<0.05

In general, these results confirm that *Brachiaria* hybrid released as cv Mulato has a high quality and milk yield potential when compared to other Brachiaria cultivars. In addition, it would seem that an additional advantage of Mulato is that it has the potential to produce more edible biomass than Basilisk and Toledo in the absence of N fertilizer and when grown in an acid soil with high OM, as is the case of Quilichao.

Activity 1.5 Adjustment of methods for the simultaneous evaluation of tropical legumes for feed and soil improvement

Highlights

- Showed that decomposition of legume plant material in the soil using the litterbag technique is highly correlated with DM disappearance using vitro anaerobic methods. The advantage of this finding is in terms of time and cost savings.
- Initial results indicate that decomposition of legumes in the soil and rumen is not a function of total cell wall in the plant but rather it is a function of indigestible fractions of the cell wall such as lignin alone or corrected for presence of condensed tannins

Progress towards achieving milestone:

- **Established correlation between in vitro anaerobic systems and aerobic soil-based systems in the decomposition of legumes with contrasting quality**
  We confirmed with legumes of contrasting quality that there is a high correlation in the decomposition of plant material using an anaerobic in vitro fermentation method and the aerobic litterbag technique.

1.5.1 Assessment of the effect of species and drying method on aerobic and anaerobic decomposition of legumes

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Rationale

It is recognized that legume species are useful to enhance existing feed resources and to contribute to soil fertility in mixed livestock-cropping systems through their use in associated grass-legume pastures, as green manure or as mulch through prunings.

In mixed crop-livestock production systems legume quality is a key factor for obtaining maximum benefits in terms of rate and extent of N release in the rumen or soil. Consequently, Animal Nutritionist and Soil Scientist have been interested in defining plant quality parameters that are correlated with release of nutrients from topical legumes. However, research in quality of legumes as it relates to ruminants or soil has been carried out in an independent manner and consequently there has been very little information sharing on methodological aspects.

Microbial populations mainly mediate the decomposition of plant material in the soil with lesser effects from soil macrofauna. Decomposition is often studied using the litterbag technique whereby plant material is placed in or on the soil in series of nylon litterbag. Decomposition is determined by sampling the bags over time of usually several weeks or months and relating the results (DM disappearance and N release) to initial compositional factors of the plant material. This method is resource -and time- consuming but provides valuable data for comparing plant species in terms of their relative decomposition and nutrient release patterns.

Ruminants also decompose plant material through microbes that degrade plant protein and cell wall constituents to ammonia, amino acids and energy for the host animal. To assess the extent and rate of nutrient release from plant material used as a feed resource, samples are incubated with rumen microbes using in vitro systems or alternatively using in situ techniques, which follow the same principle of the soil nylon litterbag method.

It is recognized that soil and rumen processes involved in plant degradation have fundamental differences namely an anaerobic aqueous environment in the rumen, higher number of microbes and much faster degradation rates in the rumen compared with soil. Despite these differences, the extent and rate of nutrient release from plants in the two processes is greatly affected by compositional factors of the plant (i.e. N level, lignin, condensed tannins).

Thus we are interested in testing the hypothesis that similar plant chemical entities control decomposition and the release on nutrients in the rumen and soil and that in vitro values on rates and extents of digestion by rumen microbes can be used for predicting decomposition values of legume plant material in the soil.

To test these hypotheses we setup a research program, which involves three phases:

a) Laboratory studies to determine rates and extent of aerobic and anaerobic degradation of plant material from legumes with contrasting quality subject to different drying treatments and using different methods.
b) Laboratory studies to determine relationships between plant chemical entities and aerobic and anaerobic decomposition and release of nutrients in a range of legumes of contrasting quality.
c) Field studies using selected legumes as green manures and indicator crops to validate predictions of equations of nutrient release and in vitro anaerobic and aerobic results.

We hope that through this research we can produce the following outputs:

a) Know applicability of in vitro methods used to assess feed value of forages to define potential decomposition and release of nutrients from legumes used as feed resource or to improve soil fertility.
b) Known chemical entities in plant material that controls the extent and rates of decomposition of tropical legumes in the rumen and soil.
c) Guidelines for quick and reliable assessment of the value of tropical legumes as feed resources and to improve soils.

In this report we summarize results from the first series of laboratory studies in which measured anaerobic and aerobic decomposition of plant material from shrub legumes with contrasting quality and subject to different drying treatments.

Materials and Methods

The following woody tropical legumes were selected for the initial studies: a) *Indigofera constricta* (low tannin content), b) *Cratylia argentea* (medium tannin content) and c) *Calliandra* sp (high tannin content).

Plant material from the three legumes growing in a hillside site (Pescador, Cauca) was harvested after 6 weeks of regrowth and cuttings (leaf + fine stem) where subject to the following drying treatments prior to aerobic and anaerobic incubation: fresh, frozen, freeze-dried, oven-dried (60°C) and air-dried.

All samples were subjected to the following chemical analysis: N, C, P, Fiber (NDF and ADF), lignin, soluble and bound condensed tannins and ash following standard protocols.

For measuring anaerobic degradation of DM we used two procedures:

a) **Tilley and Terry In Vitro method**, which comprises an incubation of the samples with rumen microorganisms followed by pepsin extraction and

b) **In Vitro Gas Production**, which involves the incubation of samples with rumen microbes and measurement of gas produced at regular intervals using a transducer.

For measuring aerobic decomposition and nutrient release two procedures were used:

a. **Litterbag-Technique:** A greenhouse decomposition trial was carried out to observe decomposition and disappearance-rate of the legume prunings. Litterbags (10 cm x 10 cm, mesh size 1 mm) were filled with 5 g dry matter and placed on the soil surface. Soil from the upper layer obtained in Pescador was air-dried and filled in pots of 17 cm diameter. Pots were arranged in a randomized block design with 5 replicates. Moisture content of the soil was maintained at 60 % of water holding capacity. Sampling of litterbags was done after 1, 2, 4, 8 and 20 weeks. Bags were oven-dried (40°C) to constant weight with the plant material inside. Later plant material was manually cleaned from soil particles and weeds to determine dry weight and nutrient concentrations at different sample times.

b. **Leaching Tube Assay:** An aerobic leaching tube incubation method (Photo 2) was used to measure N-release rates from legume pruning. Glass tubes (5 cm diameter and 20 cm length) with a funnel bottom were filled (from the bottom to the top) with a fine layer of glass fiber wool, 10g of acid-washed sand, 90 g of soil/sand mixture (1:1), and 200 mg of the different legume samples. Tubes were arranged in a randomized block design with 5 replicates and kept in a dark room at 26°C +/- 1°C. Leaching will be performed 8 times (1, 2, 4, 6, 8, 12, 16 and 20 weeks) with 100 ml of leaching solution (1mM $CaCl_2$, 1mM $MgSO_4$ and 1mM $KHPO_4$). Leachates will be analyzed for NO_3^-, NH_4^+ and condensed tannin content. Results of this experiment will show amount and period of N-release of the different legumes during degradation.

Results on gas production and DM decomposition over time were fitted to appropriate regression models to estimate rates, which were then subject to an analysis of variance with drying treatment and legume species as sources of variation.

Results and Discussion

The effect of drying method on chemical composition of the three legumes used in the study is shown in Table 10. As expected, there were large differences among legumes in cell wall content, lignin and N, which could results in different decomposition rates when exposed to rumen and soil microorganism.

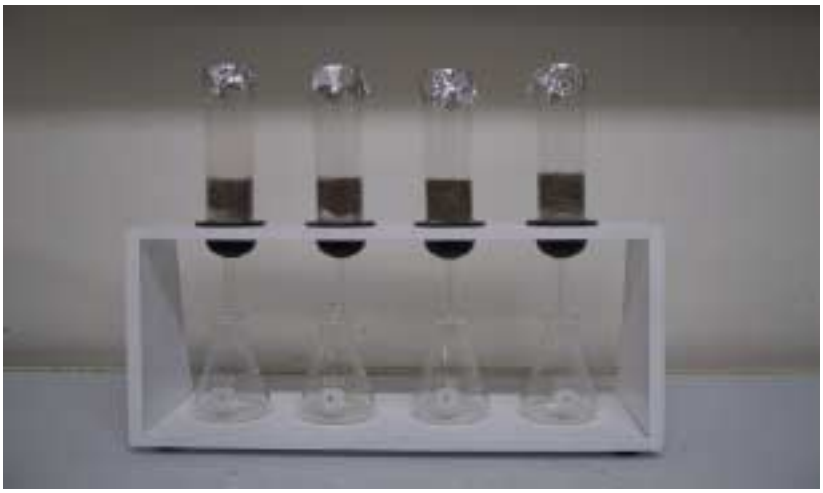

Photo 2. Leaching Tube Setup

The legume with the highest quality was *Indigofera constricta* (no condensed tannins) given its lower fiber and lignin concentration and higher N level when compared to the other two species. In the case of *Cratylia argentea* with low levels of condensed tannins, the main factor affecting its quality would seem to be the high and lignified cell wall fraction. In contrast, degradation of *Calliandra sp* could be more related to its high tannin content than to fiber and lignin.

Also as expected, drying treatment had a significant effect on the chemical composition of the three legumes included in the test. Results shown in Table 10 indicate that in all legume species, oven drying resulted in more fiber and lignin than freeze-drying or air-drying possibly as a result of artifacts formed by heat damage (Maillard reaction). However, this effect did not result in consistent reduction in the soil or rumen of DM degradation of the legumes under test as we had expected based on results in the literature.

The extent of DM decomposition of the three legumes by rumen microbes using two methods was highly correlated ($r = 98$; $P<0.01$) as has been shown in other studies. We also found a high correlation ($r = 0.87$; $P<0.0001$) between anaerobic in vitro DM loss and aerobic decomposition of DM in the soil, which had been shown in previous studies carried out in CIAT.

One important finding was that in vitro DM degradation by rumen microbes was more affected by legume specie than by drying method, regardless of the in vitro method used. The extent of degradation of *I. Constricta* was 1.5 times greater than *C. argentea* and almost 3 times greater than *Calliandra* sp, which is a reflection of the different chemical composition of the plant material used in the experiments.

Another parameter measured in the in vitro fermentation and litterbag trials was the rate of degradation of the three legumes subject to different drying treatments. Results indicated a positive correlation ($r = 0.49$; $P<0.05$) between anaerobic rate of in vitro gas production and aerobic rate of DM disappearance using the litterbag technique.

Table 10. Chemical composition of three tropical legumes subject to different drying treatments prior to aerobic and anaerobic incubation.

Treatment	NDF (%)	Lignin (%)	N (%)
*Infigofera constricta**			
Freeze-dried	27	5.0	4.58
Oven-Dried (60 ^{o}C)	**43**	**5.4**	5.04
Air-dried	30	4.5	5.3
*Cratylia argentea***			
Freeze–dried	57	12.0	3.65
Oven-Dried (60 ^{o}C)	**77**	**13.4**	3.75
Air-dried	67	12.6	3.89
Calliandra sp.***			
Freeze-dried	36	10.3	2.01
Oven-dried (60 ^{o}C)	**43**	**13.3**	2.71
Air-dried	35	8.5	2.27

*No tannins
**Low tannins (1-2 %)
***High tannins (17 to 22 %)

Results also showed that rates of aerobic and anaerobic rates of degradation were significantly influenced by legume species as shown in Table 11. However, the effect of legume species on rates of degradation was greater when samples were incubated under aerobic than under anaerobic conditions.

Table 11. Rates of anaerobic (gas production with rumen microbes) and aerobic (DM disappearance in litter bags) of three legumes (Data presented is as an average across drying treatments).

Legume Species	Anaerobic Conditions-Rumen Microorganisms Rate of in vitro gas production (% / h)	Aerobic Conditions-Soil Microorganisms Rate of DM disappearance (% /d)
Indigofera constricta	8.57 a	1.354 a
Cratylia argentea	6.16 b	0.334 b
Calliandra sp	2.51 c	0.190 c

The rate of DM disappearance of *I. constricta* under aerobic conditions was 4 times greater than *C. argentea* and 7 times greater than with *Calliandra* sp. However, under anaerobic conditions the rate of gas production of *I. constricta* when averaged across drying treatments was only1.4 times greater than with *C. argentea* and 3.5 times greater than with *Calliandra* sp.

One of the objectives of this work is to establish functional relationships between plant chemical components and decomposition and release of nutrients from legumes with contrasting quality in an anaerobic rumen system and in an aerobic soil system. Initial results indicate that cell wall content (ADF) was poorly correlated to DM loss in the anaerobic in rumen vitro system and in the aerobic soil litterbag system, but that negative and significant correlations were observed with ADF (cellulose + lignin) and lignin content (Table 12). By correcting the lignin fraction with condensed tannins and with N the correlations with observed DM decomposition under aerobic and anaerobic conditions significantly improved.

Table 12. Correlation between different plant chemical components and dry matter (DM) loss in an anaerobic in vitro gas production system and an aerobic soil litterbag system.

Plant Chemical Components	Anaerobic Conditions-Rumen Microorganisms DM loss (%) r	Aerobic Conditions-Soil Microorganisms DM loss (%) r
NDF	- 0.13 (NS)	- 0.28 (NS)
ADF	- 0.64 (P<0. 0045)	- 0.66 (P<0.0014)
Lignin	- 0.74 (P<0.0014)	- 0.78 (P<0.0002)
Lignin + Total Condensed Tannins	- 0.95 (P<0.0001)	- 0.91 (P<0.0001)
Lignin: N	- 0.98 (P<0.0001)	- 0.96 (P<0.0001)

In general, these results confirm that decomposition of legume plant material in the soil using the litterbag technique is highly correlated with DM disappearance using vitro anaerobic methods. The advantage of this finding is in terms of time and cost savings. While with the litterbag it takes 20 weeks to determine the extent and rate of decomposition of plant material in the soil with the in vitro anaerobic system it only takes 48 h to determine extent and rate of degradations of DM from plant material.

Finally, our results suggest that differences in plant quality attributes could be more important than sample preparation in determining the extent and rate of decomposition of plant material in the soil and rumen. Initial results indicate that decomposition of legumes in the soil and rumen is not a function of total cell wall in the plant but rather it is a function of indigestible fractions of the cell wall such as lignin alone or corrected for presence of condensed tannins.