# Output 1: Grass and legume genotypes with high forage quality are developed

## 1.1 Selection of *Brachiaria* hybrids for high crude protein and digestibility

### Highlight

- Developed a NIRS calibration equation to measure crude protein in *Brachiaria* hybrids

### 1.1.1 Calibration of NIRS for N in *Brachiaria*

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### 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 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 traditional 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.

We had developed a narrow – based NIRS equation to predict IVDMD in *Brachiaria* hybrids, which had high precision as indicated by low SE of the calibration (0.98). In addition, estimates of IVDMD using NIRS had a high correlation (r = 0.73 to 0.80) with IVDMD values obtained with the two-stage Tilley and Terry in vitro procedure. This year we were interested in developing NIRS equations to estimate crude protein (CP) in *Brachiaria* hybrids.

### Results and Discussion

We applied the NIRS calibration equations for CP with leaves of 50 *Brachiaria* hybrids that form part of a population (*B. ruziziensis* x *B. brizantha* cv. Marandu) used to develop molecular markers for spittlebug. For this we harvested only leaves from replicated (3) pots in the greenhouse. Results showed a high correlation between values of CP estimated using two NIRS equations (Table 1). The CP values ranged from 8.7 to 18.6 % in the first sampling and from 5.4 to 13.9 % in the second sampling. The higher CP content in the first sampling was related to the younger leaf tissue in the genotypes used in the analysis.

These results indicate that the NIRS equation we developed to screen *Brachiaria* hybrids for CP are adequate given the high correlation with protein values measured in the laboratory in tissue of different regrowth.

**Table 1.** Correlation between values of crude protein (CP) in leaves of *Brachiaria* hybrids measured in the laboratory and with NIRS using two equations (NIRS 1 and NIRS 2).

| Sampling Period | No of Samples | CP Lab vs CP NIRS 1 | CP Lab vs CP NIRS 2 |
|---|---|---|---|
| 1 * | 150 | 0.96 | 0.97 |
| 2 ** | 150 | 0.92 | 0.91 |

* 90 days regrowth
** 168 days regrowth

### 1.1.2 Variation in quality attributes in *Brachiaria* hybrids

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## Rationale

We had reported previously very low or no correlation between IVDMD values obtained in samples of the same *Brachiaria* population cut at different times. We had postulated that this lack of correlation had to do with the sampling procedure (whole plant instead of leaves) and to a lesser extent with the processing of the harvested material. Thus we modified the sampling procedure in such a way that only leaves from individual plants were harvested.

Results from three successive samplings in unreplicated plots of the same *Brachiaria* population using the modified sampling scheme indicated a higher correlation (r = 0.5) in IVDMD between sampling periods than had been previously found (r = 0.1).

This year we sampled leaves from replicated (3) plants of 50 *Brachiaria* hybrids derived from a population *B. ruziziensis* x *B. brizantha* cv. Marandu and planted in pots in the greenhouse

### Results and Discussion

Results showed a significant genotype x sampling interaction for CP, which was not the case for IVDMD (Table 2). It was also interesting to observe that for CP the variance associated with genotype was 3 times greater than the variance associated with the interaction genotype x sampling period, which was not the case for IVDMD. This is contrary to what would be expected from previous results.

The only difference from one sampling period to another was the age of the leaves harvested for quality analysis, but still results indicated that the ranking of genotypes of *Brachiaria* was significantly affected by sampling period and a result their were low correlations between sampling periods (CP: r = 0.30; IVDMD: r = 0.01) The reasons for these discrepancies could be related to large differences among genotypes in physiological maturity, known to affect forage quality.

Thus in order to select *Brachiaria* hybrids for quality parameters such as CP and IVDMD we still need to develop a standard sampling procedure, which includes fertilizer management and a uniform chronological or physiological age.

For this reason this year we transplanted 50 *Brachiaria* genotypes in the field in Quilichao in replicated (3) plots (spaced plants). Initially we will cut the plants at a uniform height and after 4 weeks of growth will harvest leaves in two different sampling periods without the application of fertilizer.

All samples (leaves) will be analyzed for CP and IVDMD using NIRS. Subsequently we will repeat the measurements of CP and IVDMD on plants with and without N fertilization.

**Table 2**. Variation in crude protein (CP) and in vitro digestibility (IVDMD) in *Brachiaria* hybrids

| Sampling Period | CP (%) | | IVDMD (%) | |
|---|---|---|---|---|
| | Mean | Range | Mean | Range |
| 1* | 13.1 | 8.7 – 18.6 | 70.0 | 59.5- 77.6 |
| 2** | 10.6 | 5.4- 13.9 | 70.6 | 64.8- 73.7 |
| Significance (P) | | | | |
| Genotype | 0.0001 (5.9552)*** | | 0.0434 (9.8191)*** | |
| Sampling Period | 0.0001 (485.1408) | | 0.0308 (32.2752) | |
| Genotype x Sampling Period | 0.0028 (2.9564) | | 0.0948 (9.0229) | |

* 90 days of regrowth
** 168 days of regrowth
*** Values in parenthesis are the Error Mean Square

## 1.2 Animal Production potential with selected grasses and legumes

### Highlight

- Milk yield with an advanced *Brachiaria* hybrid selection with resistance to some species of spittlebug was as high as in pastures of Mulato and higher than in pastures of other commercial *Brachiaria* cultivars

### 1.2.1 Milk yield with new *Brachiaria* hybrids

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#### Rationale

We had reported that milk yield of cows grazing the commercial *Brachiaria* hybrid cv Mulato and the experimental *Brachiaria* hybrid FM 9503-S046-024 was higher than in the recently released *B. brizantha* cv Toledo. As observed in previous experiments, MUN (Milk Urea Nitrogen) was greater in cows grazing Mulato and the new hybrid FM 9503-5046-024 as compared with *B. brizantha* cv Toledo. Thus we concluded quality of the *Brachiaria* hybrid FM 9503-5046-024 was similar to the quality to the first released hybrid cv Mulato as reflected by milk yields.

This year we wanted to reconfirm the milk yield potential of the experimental hybrid FM 9503-S046-024 as compared to commercial *Brachiaria* cultivars.

Two grazing trials were carried out in a rainy period (September 24 to November 4, 2002) and in a dry period (November 5 to December 16, 2002) using 2 cows/ha. In each experiment a total of 6 cows (3 Holstein and 3 Zebu Crossbreds in 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.

In the two experiment we had the following treatments: T1: *B. brizantha* cv Toledo (control), T2: *B. decumbens* cv Basilisk and T3: *Brachiaria* hybrid FM 9503-S046-024.

#### 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 3. In the first experiment in the dry season we observed that milk yield was higher (10%) in the new *Brachiaria* hybrid FM 9503-S046-024 as compared to *B. decumbens* cv Basilisk and *B. brizantha* cv Toledo. In the second experiment during the rainy season milk yield was 18 to 27% higher in cows grazing the new *Brachiaria* hybrid FM 9503-S046-024 as compared to the two commercial cultivars.

In the two experiment MUN (Milk Urea Nitrogen) values were greater in cows grazing the new hybrid FM 9503-S046-024 as compared with *B. decumbens* cv Basilisk and *B. brizantha* cv Toledo. These higher MUN values have been consistently associated with higher CP in the leaf tissue of the new hybrid.

Our results from this year confirm that the *Brachiaria* hybrid FM 9503-S046-024 with resistance to some species of spittlebug has a higher quality than commercial cultivars and a similar quality to the commercial *Brachiaria* hybrid cv Mulato as reflected by milk yield. This new hybrid is currently under regional evaluation and particular attention is being placed on its seed production potential.

**Table 3.** Milk yield of cows grazing *Brachiaria* pastures in a dry and wet period (Quilichao Research Station).

| Pastures | Dry Period | | Wet Period | |
|---|---|---|---|---|
| | Milk Yield (kg/cow/d) | MUN (mg/dL) | Milk Yield (kg/cow/d) | MUN (mg/dL) |
| *B. decumbens* cv Basilisk | 5.4 b | 6.8 a,b | 5.1 b | 5.2 b |
| *B. brizantha* cv Toledo | 5.5 b | 5.8 b | 5.5 b | 4.5 b |
| *Brachiaria* hybrid FM 9503-S046-024 | 6.0 a | 7.6 a | 6.5 a | 6.8 a |

a, b means with different letters are different ($P<0.05$)

## 1.3 Assessment of the potential of legumes with tannins to reduce methane in ruminants

### Highlights

- The supplementation with *S. saponaria* reduced daily methane release from sheep by over 10%.
- The addition of legumes with high levels of tannins to forage-based supplements reduced methane release per unit of organic matter fermented
- Supplementation with molasses reduced the negative nutritional effects of high concentrations of condensed tannins in legume by enhancing N turnover.

We have made considerable progress in defining the potential of saponin-rich fruits to reduce methane emission from rumen fermentation and enhance N utilization by sheep. The results of experiments carried out during the last two years showed that the fruits of *Sapindus saponaria* are valuable in supplementing tropical forage-based diets since they are effective in improving duodenal microbial protein flow and efficiency of rumen fermentation, and in suppressing ruminal methane release.

This year we confirmed that the inclusion of tannin-rich legumes such as *Calliandra calothyrsus* and *Flemingia macrophylla* in forage-based diets significantly reduces methane release but also inhibits nutrient degradation and N turnover. It was hypothesized that to take advantage of the methane suppressing effect of tannin-rich legumes without affecting nutrient degradation and N turnover, it was necessary to combine them with legumes free of tannin. This hypothesis was partially confirmed this year. However, the effects of legume mixtures were dependent on the type and proportion of tannin rich legumes included in the diet. Although *C. calothyrsus* and *F. macrophylla* presented similar tannin contents, the inclusion of these legumes in mixtures resulted in considerably different effects on rumen fermentation, and *F. macrophylla* was less effective in suppressing methanogenesis than *C. calothyrsus*. Thus, we still need to define the optimal type and proportion of tannin rich legumes in mixtures to take advantage of their methane suppressing potential without affecting nutrient degradation.

### 1.3.1 Rumen fermentation parameters and methane production with legumes of contrasting quality

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**Rationale**

Previous *in vitro*-experiments showed that the inclusion of fruits of *Sapindus saponaria* into tropical diets may suppress methane release by over 10% and that supplementation with leaves from the shrub legume *Cratylia argentea* increased methane release by 300%. However, no information was available on the effect of these tropical forage resources on methane release *in vivo*. Thus a respiratory chamber experiment was carried out to study the influence of a supplementation with fruits of *S. saponaria* and leaves of *C. argentea* on energy utilization and methane release of sheep fed a basal diet of *Brachiaria dictyoneura*.

**Materials and Methods**

Three basal diets with contrasting forage quality were included. The traditional diet consisted of a low quality hay (*Brachiaria dictyoneura* cv. Llanero), in the other two diets 33.3%, respectively sun-dried leaves of the shrub legume Cratylia argentea replaced 66.7% of the grass hay. All three diets were fed either with a concentrate containing 25% *S. saponaria* or with a control concentrate without *S. saponaria*. Animals were offered 60 g/kg $BW^{0.75}$ of forage and 20 g/kg $BW^{0.75}$ of concentrate per day. Six castrated growing lambs of the Swiss White Hill breed with a initial body weight of 30.1±2.8 kg were allotted to one of six treatments in a complete Latin Square design with 3 × 2 factorial arrangement of treatments (3 basal diets × 2 concentrates) and the 6 experimental periods were of 21 days each. The first 12 days of each experimental period were used for adaptation, days 13 to 20 for measurement of forage intake and total collection of feces and urine and day 21 for blood and rumen liquid sampling. Respiratory measurements were carried out during two 22.5 h-periods on days 19 and 20.

The forage required for the trial was harvested and dried at CIAT's research station in Quilichao (Cauca valley, Colombia) and fruits of *S. saponaria* were collected in the rural area near Cali (Cauca valley, Colombia). Forage and fruits were then shipped to Switzerland and stored at the ETH. The two concentrates were formulated to contain similar amounts of protein, fiber and energy. Approximately 180 kg of each concentrate were mixed and pelleted at the Institute of Animal Sciences at the ETH.

**Results and Discussion**

The two concentrates offered were consumed completely by all animals throughout the experiment and total DM and OM intakes per kg of metabolic body weight ($BW^{0.75}$) were not affected ($P>0.05$) by type of concentrate. Total DM and OM intakes responded linearly ($P<0.001$) as legume proportion increased, with the highest DM and OM intakes occurring in the treatments with 2/3 of legume in the basal diet. Intake of CP did not vary with type of concentrate (P>0.05) but responded linearly (P<0.001) to dietary legume proportion and increased from 5.98 g/kg $BW^{0.75}$ in the grass-alone treatments to 13.91 g/kg $BW^{0.75}$ in the 2/3-legume treatments.

Apparent total tract digestibilities of OM, CP, NDF and ADF were reduced (P<0.01) by supplementation with *S. saponaria* and digestibilities of OM, NDF, ADF were linearly reduced with increasing legume proportion. Apparent digestibility of CP, however, increased linearly and quadratically (P<0.001) as legume proportion increased.

Rumen fluid pH did not vary (P>0.05) with dietary treatment and averaged 6.76. Rumen fluid ammonia concentration was not affected (P>0.05) by type of concentrate but responded linearly (P<0.001) as dietary legume proportion increased. In the grass-alone treatments rumen ammonia concentration was as low as 2.45 mmol/l and increased to a level of 13.61in the 2/3-legume treatments.

Total VFA concentration was higher (P< 0.01) when the concentrate containing *S. saponaria* was supplied than with the control concentrate but did not vary (P>0.05) with dietary legume proportion. Supplementation with *S. saponaria* reduced (P<0.001) the molar proportions of iso-Butyrate and iso-Valerate but had no effect (P>0.05) on the other VFA. The molar proportion of acetate responded linearly to increasing legume proportion and decreased from 0.73 in the grass-alone treatments to 0.69 in the 2/3-legume treatments. The molar proportions of propionate, iso-Butyrate, n-Valerate and iso-Valerate increased linearly (P<0.001) as dietary legume proportion was raised. The acetate-to-propionate ratio tended to be lower (P=0.078) in the treatments with *S. saponaria* supplementation and decreased linearly (P<0.001) as legume proportion increased.

Total bacteria count was increased (P<0.01) by *S. saponaria* supplementation but showed no clear trend due to dietary legume proportion (P>0.05). Total ciliate protozoa count was reduced by over 50% in the treatments with *S. saponaria* but did not vary (P>0.05) with legume proportion. No significant interactions of dietary legume proportion and *S. saponaria* supplementation on rumen fluid characteristics were found (P>0.05).

Energy intake did not vary (P>0.05) with type of concentrate but responded to dietary legume proportion. Gross energy (GE), digestible energy (DE) and metabolizable energy (ME) intakes increased linearly (P<0.001) as legume proportion increased. Feeding the *S. saponaria* containing concentrate increased (P<0.001) energy losses through faeces, reduced energy losses through urine (P<0.05) and methane (P<0.01), had no effect (P>0.05) on energy expenditure (heat energy) and tended to increase (P=0.080) total energy losses. Energy losses through faeces, urine and heat, as well as total energy losses increased linearly (P<0.001) with increasing legume proportion, whereas energy losses through methane tended to decrease (P=0.075) with increasing legume proportion. Energy retention did not vary (P>0.05) with dietary treatments. No significant interactions of dietary legume proportion and *S. saponaria* supplementation on energy balance were found (P>0.05).

Daily methane release per kg of body weight was reduced (P<0.001) by 9% on average by *S. saponaria* supplementation and was not affected (P>0.05) by dietary legume proportion. Compared to the control concentrate, the *S. saponaria* containing concentrate reduced (P<0.05) methane release relative to $CO_2$ production and tended to reduce (P=0.063) methane release relative to OM digested but had no effect (P>0.05) on methane release relative to NDF digested. With increasing legume proportion, methane release relative to $CO_2$ produced and OM digested decreased (P<0.01), and methane release relative to NDF digested increased linearly (P<0.001). Methane release relative to energy retained was reduced by 30% (P<0.05) when *S. saponaria* was supplemented bud did not vary (P>0.05) with legume proportion. In contrast, methane release relative to N retained was not affected (P>0.05) by *S. saponaria* and decreased linearly (P<0.05) with increasing legume proportion. On average, methane release per gram of N retained was reduced by 60% due to legume supplementation. Haematocrite values and activity of liver enzymes (ASAT and GLDH) were not affected by dietary treatments and averaged 34.9%, 80.2 U/l and 10.3 U/l, respectively. This indicates that the dietary proportion of *S. saponaria* tested in this experiment did not affect the health status of sheep. The fact that interactions were mostly insignificant indicates that supplementation of *S. saponaria* fruits is a useful means to reduce methane emission from sheep fed tropical grass-alone and legume-supplemented diets. Legume supplementation represents an environmentally friendly way to increase animal performance of tropical livestock, since it was shown to improve N retention and to reduce methane release relative to body protein retention.

### 1.3.2 Effect of mixture of legumes with and without tannins on in vitro rumen fermentation parameters and methane production

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#### Rationale

A previous in vitro experiment had shown that the supplementation of a low-quality grass diet (*Brachiaria dictyoneura*) with *Cratylia argentea* drastically increased organic matter degradation, N turnover and methane release per unit of organic matter degraded, whereas the partial replacement of *B. dictyoneura* by *Calliandra calothyrsus* significantly reduced organic matter degradation, N turnover and methane release. Thus it was hypothesised that, to take advantage of the methane suppressing effect of *C. calothyrsus* without affecting nutrient degradation and N turnover, it was necessary to combine tannin-rich legumes with legumes free of or low in tannins. To test this hypothesis three Rusitec-experiments were performed (see also Activity 1.3.1 in this section) to evaluate the effect of supplementing legumes with contrasting contents of condensed tannins on methane production and N turnover in vitro.

#### Materials and Methods

In each experiment four basal diets were evaluated in four replicates. In experiment 1 the effect of the inclusion of *C. argentea* (0, 25, 50 and 100% of diet DM) in a diet of *C. calothyrsus* was investigated. In experiment 2 the effect of a partial replacement (50% of diet DM) of *F. macrophylla* by *C. argentea*, *C. calothyrsus* or by a mixture of both legumes was evaluated (Table 4). The daily dry matter supply was maintained constant at 15 g DM.

**Table 4.** Composition (% of DM) of the experimental diets

| | Experiment 1 | | | |
|---|---|---|---|---|
| Diet | 1 | 2 | 3 | 4 |
| *Calliandra calothyrsus* | 100 | 75 | 50 | - |
| *Cratylia argentea* | - | 25 | 50 | 100 |
| | Experiment 2 | | | |
| Diet | 1 | 2 | 3 | 4 |
| *Flemingia macrophylla* | 100 | 50 | 50 | 50 |
| *Calliandra calothyrsus* | - | 50 | 25 | - |
| *Cratylia argentea* | - | - | 25 | 50 |
| | Experiment 3 (Activity 3.3) | | | |
| Diet | 1 | 2 | 3 | 4 |
| *Brachiaria dictyoneura* | 100 | 50 | 50 | 50 |
| *Calliandra calothyrsus* | - | 50 | 25 | - |
| *Cratylia argentea* | - | - | 25 | 50 |

#### Results and discussion

In experiment 1, ammonia concentration in the fermenter fluid increased (P<0.001) from 0.81 mmol/l to 13.02 mmol/l when the proportion of *C. argentea* in the diet was increased from 0 to 100%. Counts of ciliate protozoa and bacteria also varied with the composition of the diet and were highest (P<0.05) with 50 and 100% *C. argentea*, intermediate with 25% *C. argentea* and lowest with 100% *C. calothyrsus*. Similarly, total bacteria counts were lowest with 100% *C. calothyrsus* and increased linearly (P<0.01) with increasing dietary proportions of *C. argentea*.

Apparent degradation of nutrients was also related to the level of *C. argentea* in the diet and was lowest in the diet with *C. calothyrsus* alone and increased linearly (P<0.001) with the proportion of *C. argentea*. Apparent organic matter degradation was twice as high in the diet of *C. argentea* alone (35.5%) as in the diet with *C. calothyrsus* alone (17.4%).

Daily methane release increased linearly (P<0.001) from 0.16 to 3.53 mmol/d when the proportion of *C. argentea* increased from 0 to 100%. When 25 or 50% of *C. calothyrsus* were replaced by *C. argentea* no changes occurred (P>0.05) in apparent crude protein degradation and only minor changes were observed in ruminal N turnover. Only the complete replacement of *C. calothyrsus* clearly increased apparent crude protein degradation and enhanced ruminal N turnover. These results suggest that the use of mixtures of *C. calothyrsus* and *C. argentea* is no means to improve ruminal N turnover without increasing methane release. This contrasts with the observations made in the third experiment (Activity 3.3) where the supplementation of low-quality *B. dictyoneura* with a mixture of *C. calothyrsus* and *C. argentea* (50:50) improved the nutritional quality of the diet and increase the ammonia concentration in the fermenter fluid without enhancing methane emission relative to organic matter degraded.

Therefore we suggest that the effects of legume mixtures on rumen fermentation not only depend on the quality and proportion of the different legumes, but also on the remaining components of the diet. Consequently, the evaluation of legume mixtures has to be done in combination with grasses, which represent the most important diet ingredient for ruminants in tropical smallholder livestock systems.

In experiment 2, ammonia concentration in the fermenter fluid was not affected (P>0.05) when 1/2 of *F. macrophylla* was replaced by *C. calothyrsus*, but increased (P<0.05) by 50% when 1/4 of the tannin rich legumes was replaced by *C. argentea*. With 50% of *C. argentea* in the diet, the ammonia concentration was 280% higher (4.6 mmol/l) than with *C. calothyrsus* alone (1.2 mmol/l).

Count of rumen ciliate protozoa was not affected (P>0.05) by the composition of the diet, but total bacteria count was increased (P<0.05) by 45% due to the inclusion of *C. argentea*. A small (-7%) but significant reduction in apparent organic matter degradation was recorded when 1/2 of *F. macrophylla* was replaced by *C. calothyrsus*.

The inclusion of 1/4 of *C. argentea* increased (P<0.05) apparent organic matter degradation by 13%, and with 1/2 of *C. argentea* in the diet, organic matter degradation increased (P<0.05) by 38% relative to the control (*F. macrophylla* alone). Methane release per gram of organic matter apparently degraded, decreased by 24% when 1/2 of *F. macrophylla* was replaced by *C. calothyrsus* and increased (P<0.05) by 27% and 57%, respectively, when 1/4 and 1/2 of *C. argentea* were included in the diet.

When 1/2 of *F. macrophylla* was replaced by *C. calothyrsus* no changes occurred (P>0.05) in apparent crude protein degradation and ruminal N turnover. In contrast, the inclusion of *C. argentea* in the diet resulted in a clear improvement of crude protein degradation and N turnover.

Overall, the replacement of 1/2 of *F. macrophylla* by *C. calothyrsus* resulted in only minor changes in rumen fermentation. Apparent organic matter degradation and methane emission were slightly reduced due to *C. calothyrsus* but the other major fermentation parameters remained unchanged. This suggests that both legumes had similar (negative) effects on rumen fermentation.

In contrast, the inclusion of *C. argentea* in the mixtures with the tannin-rich legumes enhanced rumen fermentation, as indicated by higher ammonia concentration and total bacteria count in the fermenter fluid and by improved apparent nutrient degradation and increased methane emission, both in absolute terms (mmol/d) and relative to organic matter degraded. It's worth mentioning, that apparent crude protein degradation in the mixture of *F. macrophylla* with *C. argentea* (50:50) was twice as high as in the mixture of *C. calothyrsus* with *C. argentea* in experiment 1 (27% vs. 14%). This indicates that *C. calothyrsus* and *F. macrophylla* have similar effects on rumen fermentation when used alone or in combination with each other, but contrasting effects are observed, when these tannin-rich species are mixed with a legume free of or low in tannins.

In general our results suggest that even though *C. calothyrsus* and *F. macrophylla* have similar chemical compositions and tannin contents, the nutritional value of *F. macrophylla* is higher than that of *C. calothyrsus* when these species are used in combination with a good-quality legume, but that is less effective in suppressing methane emission than *C. calothyrsus*.

### 1.3.3 Effect of supplementing low quality grasses with legumes and soluble carbohydrates on in vitro rumen fermentation parameters and methane production

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**Material and Methods**

In this in vitro experiment a grass-alone and three legume supplemented (50% of DM) diets were evaluated. The legume supplements consisted of *C. calothyrsus* (100%), *C. argentea* (100%) or a mixture of both legumes (1:1). All four basal diets were evaluated with and without the addition of sugarcane molasses (10% of DM). The eight treatments were tested during 4 × 10 day periods (n=4).

**Results and Discussion**

The pH of fermenter fluid averaged 6.97 and showed only minor variations between basal diets and due to the addition of molasses. The replacement of 50% of the basal diet by *C. calothyrsus* reduced ($P<0.05$) apparent degradability of N from 350 mg/g (grass-alone diet) to 80 mg/g without affecting fermenter fluid ammonia ($P>0.05$) (Figure 1). Compared to the grass-alone diet, the inclusion of *C. argentea* did not affect ($P>0.05$) apparent degradability of N but drastically increased fermenter fluid ammonia ($P<0.05$) from 1.0 mmol/l (grass-alone diet) to 4.2 mmol/l. When the legume mixture was included, apparent degradability of N was reduced ($P<0.05$) but fermenter fluid ammonia was increased ($P<0.05$).

As expected, the addition of molasses reduced ($P<0.001$) ammonia concentration with all diets. The effect of molasses on apparent N degradability was dependent on the kind of legume supplementation. While no effect was observed ($P>0.05$) when molasses was added to the grass-alone diet and the diets containing *C. argentea*, the addition of molasses to the diet supplemented with *C. calothyrsus* alone, drastically increased apparent N degradability from -45 to 203 mg/g (interaction between diet and molasses, $P<0.001$).

The reasons for this unexpected increase are not well understood but could be related to the higher availability of fermentable energy and hence increased microbial activity when molasses was added, or to the inactivation of condensed tannins due to the formation of complexes between tannins and soluble carbohydrates. Even though we do not completely understand this phenomenon, it is highly interesting, because it indicates that supplementation with molasses could be an alternative to partially reduce the negative

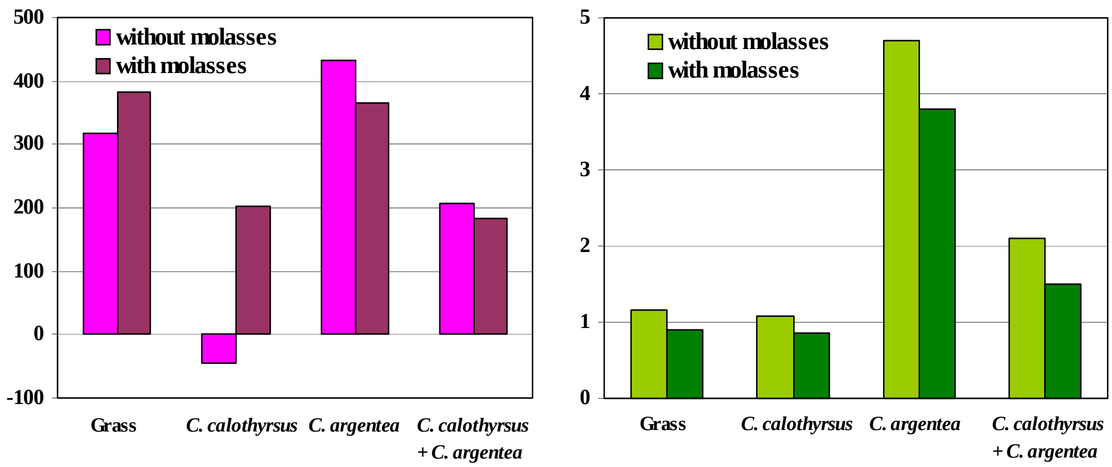


**Figure 1.** Apparent rumen degradability of nitrogen (mg/g) (on the left) and ammonia concentration (mmol/l) (on the right) observed in Rusitec-fermenters supplied with a grass-alone and with legume supplemented (50% of DM) diets. The legume supplements consisted of C. calothyrsus (100%), C. argentea (100%) or a mixture of both legumes (50:50). All diets were evaluated with and without the addition of sugarcane molasses (10% of DM).

nutritional effects of feeding legumes with high concentrations of condensed tannins.

The supplementation with *C. calothyrsus* alone reduced the apparent degradability of organic matter and neutral detergent fiber (P<0.05) (Figure 2, left). The supplementation with *C. argentea*, in contrast, increased organic matter and fiber degradation (P<0.05). Supplementation with the legume mixture increased organic matter degradation (P<0.05) and had no effect on fiber degradation (P>0.05), indicating that the negative effects of supplementing *C. calothyrsus* on fiber degradation can be avoided when this legume is supplied in combination with a legume low in tannins.

Daily methane release (-50%) and methane release relative to organic matter degraded (-40%) were reduced (P<0.05) by supplementation with *C. calothyrsus* alone (Figure 2 ), which agrees with the results from a previous experiment. Supplementation with *C. argentea* alone increased (P<0.05) daily methane release (+100%) and methane release relative to organic matter degraded (+40%), which is also in agreement with the results of the previous experiment.

When the low-quality grass diet was supplemented with the mixture of *C. calothyrsus* and *C. argentea*, daily methane release was increased (P<0.05) by 30% but methane release relative to organic matter degraded remained unaffected (P>0.05). Interactions between basal diet and molasses addition were mostly insignificant, except for apparent N degradation (see above). On average among all diets, molasses addition increased (P<0.05) organic matter degradation and methane release and reduced (P<0.05) fiber degradation.

Results of this experiment confirmed the methane suppressing potential of the tannin-rich *Calliandra calothyrsus* and suggest that supplementing mixtures of legumes with high and low contents of condensed tannins could be a useful alternative to improve nutrient supply and ruminal organic matter degradation avoiding the dramatic increase in methane release typically observed when low-tannin legumes are supplemented alone. Additionally, results indicate that supplementation with molasses could be an alternative to partially reduce the negative nutritional effects of feeding legumes with high concentrations of condensed tannins as it enhances N turnover.

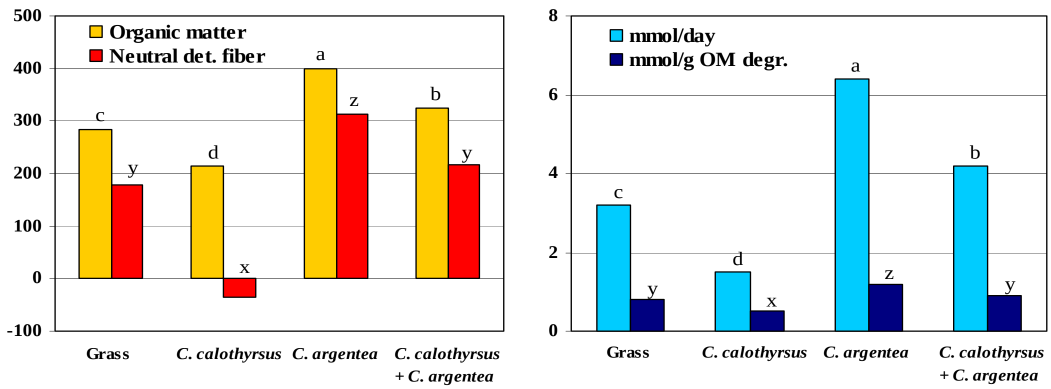


**Figure 2.** Apparent degradability of organic matter and neutral detergent fiber (mg/g) (on the left) and methane release (on the right) observed in Rusitec-fermenters supplied with a grass-alone and with legume supplemented (50% of dry matter) diets. The legume supplements consisted of *C. calothyrsus* (100%), *C. argentea* (100%) or a mixture of both legumes (50:50).

## 1.4 Adjustment of methods for the simultaneous evaluation of tropical legumes for feed and soil improvement

### Highlights

- Mixing tannin free legumes with tannin containing legumes is a possibility to reduce the adverse effects of tannins

- PEG application to bind tannins had positive effects on anaerobic protein degradation but not in the aerobic degradation experiments

### 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 dry matter loss and N release in the rumen or in the soil. Consequently, Animal Nutritionists and Soil Scientists have been interested in defining plant quality parameters that are correlated with the loss of dry matter and the release of nutrients from tropical legumes. However, studies on quality of legumes often have been carried out in an independent manner and there has been little information sharing on methodological aspects between soil and animal nutrition research.

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 for usually several weeks or months. This method provides valuable data for comparing plant species in terms of their relative decomposition and nutrient release patterns but is resource -and time- consuming.

Ruminant animals 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 dry matter loss from plant material used as a feed resource, samples are incubated with rumen microbes using *in-vitro* systems or *in situ* techniques. Principles are similar to the nylon litterbag method used for soil studies. However, soil and rumen processes involved in plant degradation have fundamental differences namely an anaerobic aqueous environment and much faster degradation rates in the rumen compared with soil. Despite these differences, the extent and rate of dry matter loss and nutrient release from plants in the two processes is affected by plant chemical composition, e.g. N, lignin and condensed tannins content.

To test the applicability to aerobic soil- related process of anaerobic methods used to assess quality of forages for ruminants the following studies were carried out:

a) The effect of mixtures of legumes with contrasting quality on anaerobic N degradation and aerobic N release
b) Effect of binding tannins in legumes with PEG on anaerobic degradation in the rumen and aerobic decomposition on the soil
c) Effect of binding tannins in legumes with PEG on anaerobic N degradation and aerobic N release

### 1.4.1 The effect of mixtures of legumes with contrasting quality on anaerobic N degradation and aerobic N release

**Contributors:** K. Tscherning (University of Hohenheim), E. Barrios (CIAT), M. Peters (CIAT), C. Lascano (CIAT), R. Schultze-Kraft (University of Hohenheim)

**Materials and Methods**

Two tropical shrub legumes with contrasting quality *Indigofera constricta* Rydb., (Indigofera, no tannin content), and *Calliandra* sp. nov. (Calliandra, high soluble tannin content), were selected. Freeze-dried plant material was ground to 1 mm and mixed in the following proportions (based on air dry matter basis): 100:0, 75:25, 50:50, 25:75, 0:100 (Calliandra : Indigofera).

**Chemical characterization of plant material:** All samples were subjected to the following chemical analysis: N, C, P, NDF, ADF, IADF, lignin, soluble and bound condensed tannins (sCT and bCT) utilizing standard protocols.

For measuring N release during aerobic degradation, a leaching tube experiment was carried out. Glass leaching tubes (length 15 cm; diameter 5 cm) were filled sequentially with a piece of glass wool, followed by a thin layer of acid-washed sand and a mixture of air-dried soil (45 g)/ acid-washed sand (1:1). A sample of 200 mg of each species and treatment, ground to pass a 1 mm screen, was added on the surface of the soil-sand mixture. A further layer of 10 g sand covered the plant material to avoid soil disturbance during the addition of the leaching solution. The soil-sand mixture was brought to approximately 80% water holding capacity (WHC). Leaching was carried out applying 100 ml leaching solution per tube (1 *mM* $CaCl_2$, 1 *mM* $KH_2PO_4$, 1 *mM* $MgSO_4$) after 7, 14, 28, 42, 70, 84, 112 and 140 days. Leachates were analyzed for mineral N content ($NO_3$-N and $NH_4$-N).

For measuring anaerobic degradation the *in-vitro* dry matter digestibility method (IVDMD) and the gas production method were used.

**Results**

The measured quality parameters of the legume mixtures were compared with estimated values. Estimated values of legumes mixtures were calculated from measured values obtained in analysis of sole samples of Calliandra and Indigofera. The results of sole legume samples were then fitted to the different proportions of Calliandra-Indigofera mixtures. Example: To estimate the value for the 50% Calliandra/50% Indigofera mixture the following formula was

applied:
Value $_{estimated}$ = (Value$_{Calliandra}$/2 + Value$_{Indigofera}$/2).
The estimated values indicate the theoretical or additive value, which should be obtained in mixing two legume materials. If measured and estimated values show no significant differences, it is assumed that no interaction occurred during mixing. If measured and estimated values are significantly different, it is assumed that interaction occurred. Interaction in this case means, that plant components of one species reacted with plant components of the other species.

**Chemical characterization of mixed plant material:** The NDF, ADF, Lignin and IADF content of the tissue decreased linearly with increasing proportion of Indigofera. Soluble CT content decreased with increasing proportion of Indigofera, while N content and IVDMD increased with increasing proportion of Indigofera in the mixture.

In the mixture of 25% Calliandra and 75% Indigofera (Call25/Ind75) measured values of IVDMD were significantly higher (P<0.001) than estimated values. Interactions were also observed for the sCT content, which was lower (P<0.01) than the estimated sCT content and for the bCT content, which has higher (P<0.05) than estimated (Figure 3). In the mixtures (Call25/ Ind75) and (Call50/Ind50) the measured IADF content was lower (P<0.05) than estimated. For ADF, NDF, Lignin and N measured and estimated values were similar indicating that no interactions occurred.

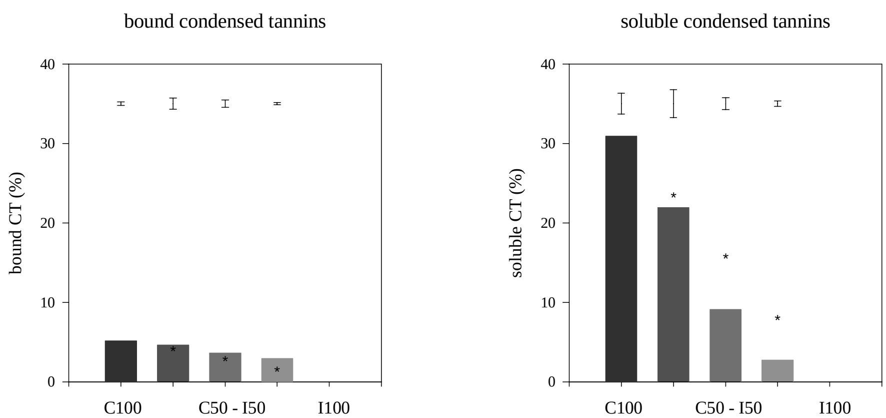


**Figure 3.** Condensed tannin content of Calliandra (C) and Indigofera (I) mixed in the following proportions: 100:0, 75:25, 50:50, 25:75, 0:100 from Calliandra (left side) to Indigofera (right side) * = estimated value, bars = measured value, I = SE of measured values

**Leaching tube experiment** In the aerobic leaching tube, net N mineralization rate( k) showed no differences (P>0.01) between Ind100, Call25/Ind75 and Call50/Ind50. Highest N mineralization for these treatments was observed during week 2 to 6 of the experiment. Maximum N mineralization was higher (P<0.01) for pure Indigofera than for the Call25/Ind75 and the Call50/Ind50 treatment. Extent of N mineralization showed similar values for the Call100 treatment and the Call75/Ind 25 treatment. In both cases N immobilisation was observed. The measured extent of N mineralization was lower (P<0.01) than the estimated extent of N mineralization in treatment Call50/Ind50 (Figure 4) indicating that this mixture did not resulted in additive effects on extent of mineralization.

**Anaerobic gas production and N degradation:** Gas production rate (k) and extent of gas production increased significantly (P<0.01) with increasing proportions of Indigofera in the mixture. The measured gas production in the mixture of Call50/Ind50, was significantly (P<0.01) higher than the estimated value. Measured *in-vitro* dry matter degradation was also higher than the estimated value in the Call25/Ind75 treatment. In contrast, in the Call75/Ind 25 treatment, measured degraded N

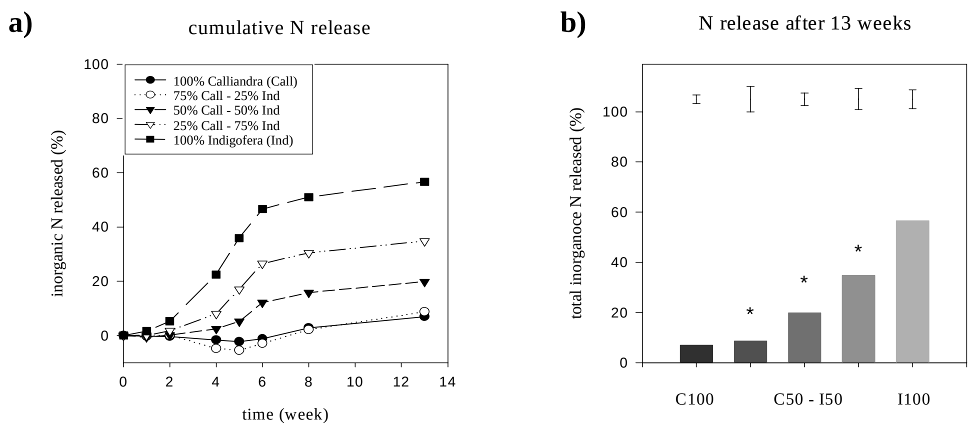


**Figure 4.** a) Cumulative N release during 13 weeks and b) extent of total released N in the aerobic leaching tube experiment. Calliandra and Indigofera mixed in the following proportions: 100:0, 75:25, 50:50, 25:75, 0:100 from Calliandra (C) to Indigofera (I)

* = estimated value, bars = measured value, I = SE of measured values

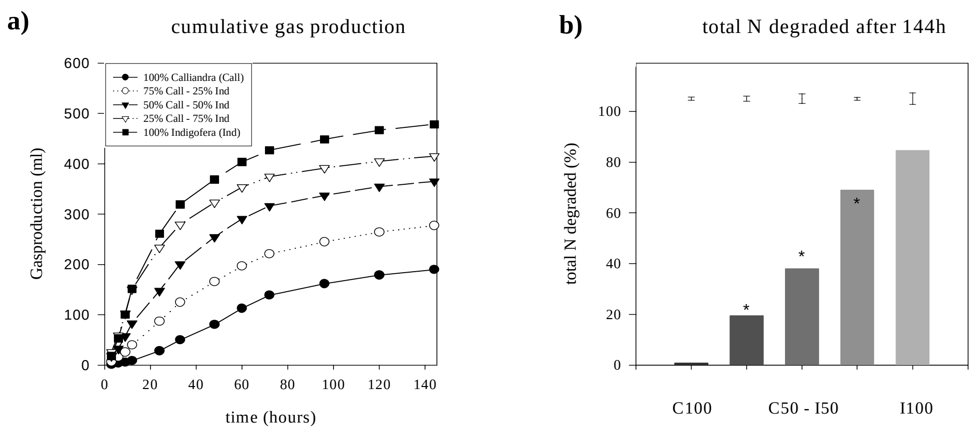


**Figure 5.** a) Gas production during 144 h and b) total degraded N anaerobic gas production experiment Calliandra and Indigofera mixed in the following proportions: 100:0, 75:25, 50:50, 25:75, 0:100 from Calliandra (C) to Indigofera (I)

* = estimated value, bars = measured value, I = SE of measured values

was significantly lower than estimated degraded N (Figure 5).

## Discussion

In the aerobic system the extent of measured mineralized N was lower than estimated for the mixture Call50/Ind50. Stronger N immobilization was observed in the Call75/Ind25 than in Call100. Thus it seem, that mixing prunings of the two legumes altered N mineralization, at the expense of a reduced total amount of mineralized N. In the long run, the remaining N as result of mixing legumes might contribute to the formation of humic and fulvic substances. The strong immobilization of N found in Call75/Ind 25 relative to Call100 might be due to a rapid increase of microorganisms nourished by soluble and easily available C components provided from the 25% of Indigofera.

This may have lead to the incorporation of N by microorganisms, an effect known as short term N-lack. When the mixture consisted of more than 50% of the high-tannin legume Calliandra, the amount of sCT increased sharply in the mixture and reached the additive value. However, no short -term positive effects in N release were observed with any of the legume mixtures during the duration of this study.

Mixing the low quality legume *Calliandra* sp. with the high quality legume *Indigofera constricta* improved the extent of gas production (Call50/Ind50), *in-vitro* dry matter degradation and IVDMD (Call25/Ind75). On the other hand, measured degraded N was not different (P>0.01) from estimated N for Call50/Ind50 and Call25/Ind75. Thus no N loss occurred due to the CT complexation suggesting that a wide range of other tannin-binding compounds such as carbohydrates present in Indigofera may have spared proteins from binding with tannins. In the Call75/Ind25 mixture the sCT content were very high and as a consequences inhibited N degradation.

Soluble and bound condensed tannins did not show an additive behaviour in the 25Call/75Ind and 50Call/50Ind mixtures, which could explain some of the positive and negative effects on the aerobic and the anaerobic systems. For example in the 50Call/50Ind mixture, there was a positive interaction in the anaerobic system as indicated by higher amount of gas production than was estimated for the mixture. Thus it seems that the anaerobic system copes more effectively with the adverse effects on nutrient release or degradation of high sCT content. The high diversity of microorganisms in the rumen liquid may allow a quick adaptation to changing substrates due to the short residence time of the nutrients in the rumen. Degradation processes in the soil are more long term and composition of microorganisms may not change as quick as in the rumen.

### 1.4.2 Effect of binding tannins in legumes with PEG on anaerobic degradation in the rumen and aerobic decomposition on the soil

**Contributors:** K. Tscherning (University of Hohenheim), C. Lascano (CIAT), E. Barrios (CIAT), M. Peters (CIAT), R. Schultze-Kraft (University of Hohenheim)

**Materials and Methods**

Plant samples were collected from four shrub legume species with contrasting qualities (nil, medium and high tannin content) namely: *Indigofera constricta* Rydb., (Indigofera), *Leucaena leucocephala* (Lam.) de Wit. CIAT 21245 (Leucaena) and 2 provenances of *Calliandra calothyrsus* Meisn: San Ramon CIAT 22310 (San Ramon) and Patulul CIAT 22316 (Patulul). Two provenances of Calliandra, San Ramon and Patulul were taken because of differences in the chemical structure of the extractable tannins. While San Ramon's condensed tannins comprise mainly of gallocatechin/epigallocatechin (producing prodelphinidin with butanol/HCL), Patulul contains mainly catechin/epicatechin subunits producing procyanidin with butanol-HCl Leucaena is known to have condensed tannins composition similar to Patulul. Indigofera is a tannin free species.

**Use of Polyethylenglycol (PEG)** (application by soaking the entire plant material): Whole freeze-dried leaves were placed in plastic containers and were soaked with PEG solution to obtain a 5 % PEG treatment (+ PEG). For the control treatment (- PEG) the plant material was soaked with de-ionized water.

**Chemical characterization of plant material**: All samples were subjected to the following chemical analysis: N, C, P, NDF, ADF, IADF, lignin, soluble and bound condensed tannins (sCT and bCT) and ash utilizing standard protocols.

For measuring aerobic degradation a greenhouse litterbag experiment was carried out. Litterbags (nylon mesh bags, 10x10 cm, mesh size 1,5 mm) were filled with 4.5 g of whole plant material and closed with a nylon thread. Litterbags were placed on the surface of soil in plastic pots filled with 2 kg of soil. During the experiment, water-holding capacity (WHC) in the pots was maintained at 60% by weighting the pots weekly and adding water accordingly. Bags were collected at 7, 14, 28, 56, and 112 and were dried for 3 days at 40 °C.

The weight of plant material in each litterbag was recorded and ground in a Wiley mill to pass a 1 mm screen.

For measuring anaerobic degradation the *in-vitro* dry matter digestibility method (IVDMD) and the gas production method were used.

## Results

**Litterbag experiment:** Decomposition rate, averaged over all treatments, was highest for Indigofera, followed by Leucaena, Patulul and San Ramon (Table 5). The extent of decomposition showed significant differences between species following the same order as the decomposition rate (Table 6). The order of decomposition rate and extent was as follows: Indigofera (high N, no CT, low lignin) > Leucaena (intermediate N, intermediate CT, high lignin) > Calliandra Patulul and San Ramon (intermediate N, high CT, high lignin).

Averaged over all species, differences in the extent of decomposition between the +PEG and –PEG treatments were observed. Surprisingly most remarkable differences in dry matter loss between +PEG and –PEG were observed in the species Indigofera and Leucaena (Table 6). In contrast, PEG treatment had no influence on dry matter loss of San Ramon and Patulul.

**Gas production experiment:** Gas production rate and extent was significantly different between species, being highest for Indigofera, followed by Leucaena, San Ramon and Patulul (Tables 5 and 6). In addition, *in-vitro* dry matter degradation was different between species. Further, gas production rate and extent were different between forage tissue treated and not treated with PEG. Likewise, *in-vitro* dry matter degradation showed significant differences due to PEG. Unexpectedly, *in-vitro* dry matter degradation was higher (P<0.001) for +PEG than for the -PEG treatment in the tannin free Indigofera.

**Digestibility:** IVDMD were significantly different among species and in between PEG treatments. Interaction between species and treatments was observed. Likewise, dry matter degradation, and IVDMD were difference (P<0.001) between +PEG and -PEG treatment for the tannin free species Indigofera (Table 6).

**Table 5**. Decomposition rate ($k_D$) and gas production rate ($k_G$) of Indigofera, Leucaena and Calliandra San Ramon and Patulul with and without PEG treatment

| Plant species | Treatment | $k_D$ | $k_G$ | |
|---|---|---|---|---|
| *Indigofera constricta* | + PEG | -0.00725 | 0.065 | |
| | - PEG | -0.00512 | 0.060 | |
| *Leucaena leucocephala* | + PEG | -0.00389 | 0.047 | |
| | - PEG | -0.00222 | 0.040 | |
| Call – San Ramon | + PEG | -0.00112 | 0.038 | |
| | - PEG | -0.00093 | 0.033 | |
| Call – Patulul | + PEG | -0.00192 | 0.038 | |
| | - PEG | -0.00077 | 0.027 | |
| Significance (p) of effects of | | SED | | SED |
| Species | | | *** | 0.0005 |
| Sample pre-treatment | | | *** | 0.0004 |
| Interaction | | | *** | 0.0007 |

SED = Standard error of the difference between means
*** = (P<0.001)

## Discussion

Soluble condensed tannins (sCT) were rapidly reduced in whole leaves during aerobic decomposition in litterbags as shown in Figure 6. As also observed in other decomposition studies, leaching is likely the most important form of sCT loss during decomposition.

Contrary to expectations, losses of sCT in Patulul or Leucaena were not affected by PEG. It is possible, that PEG-tannin complexes were not formed by the application of PEG to the whole leaves. In other words, during the decomposition period in the soil with whole leaf material, PEG only covered the leaves and did not enter into leaf cells vacuoles where tannins are located.

In the case of the two Calliandra provenances there was clear effect of PEG on sCT as indicated by slower loss of sCT in San Ramon as

**Table 6.** Extent of decomposition ($E_D$), gas production ($E_{Gas144}$) and *in-vitro* dry matter degradation ($IVDMD_{Theod}$) and IVDMD of Indigofera, Leucaena and Calliandra San Ramon and Patulul with and without PEG treatment.

| | | aerobic | | anaerobic | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Plant species** | **Treatment** | **$E_D$** | | **$E_{Gas144}$** | | **DM degradation** | | **IVDMD** | |
| *Indigofera constricta* | + PEG | 47.72 | | 495.32 | | 94.73 | | 98.15 | |
| | - PEG | 57.81 | | 478.46 | | 89.12 | | 92.59 | |
| *Leucaena leucocephala* | + PEG | 62.28 | | 375.02 | | 68.66 | | 87.82 | |
| | - PEG | 75.38 | | 348.52 | | 58.46 | | 77.24 | |
| Call – San Ramon | + PEG | 85.83 | | 268.20 | | 49.93 | | 56.88 | |
| | - PEG | 95.89 | | 184.18 | | 37.23 | | 37.80 | |
| Call – Patulul | + PEG | 88.61 | | 266.19 | | 46.75 | | 63.84 | |
| | - PEG | 90.81 | | 173.30 | | 43.36 | | 44.92 | |
| Significance (p) of effects of | | | SED | | SED | | SED | | SED |
| Species | | *** | 3,39 | *** | 8,58 | *** | 0,610 | *** | 0,520 |
| Treatments | | ** | 2,40 | *** | 6,07 | *** | 0,431 | *** | 0,367 |
| Interaction | | n.s. | 4,80 | *** | 12,14 | *** | 0,862 | *** | 0,735 |

SED = Standard error of the difference between means
** = (P<0.01), *** = (P<0.001), n.s. = (P>0.05)

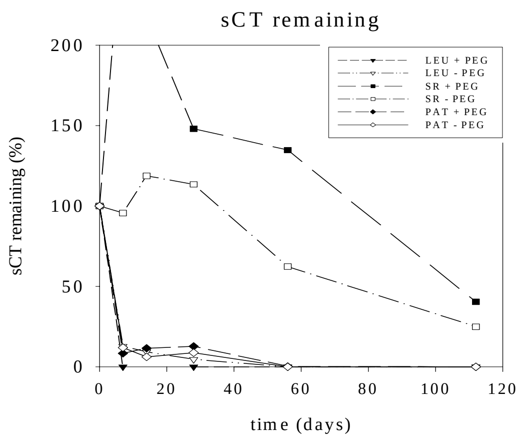


**Figure 6.** Remaining sCT content in litterbags filled with leave material of Leucaena (LEU), San Ramon (SR) and Patulul (PAT), + PEG and –PEG during decomposition of 113 days

compared to Patalul. This might be due to the different composition of condensed tannins in the two provenances. Gallocatechin and Epigalloctechin, which are only present in San Ramon, usually leach more slowly than Catechins, due to their higher solubility. The low solubility of Gallocatechin and Epigalloctechin allowed to complex with PEG.

Both, Indigofera and Leucaena have very soft leaves in comparison to Calliandra (San Ramon and Patulul). The highly hydroscopic property of PEG may have had a stronger impact on the larger and thinner leaves than on small and tough leaves. This could have made leaves more accessible to microorganisms. It could be also hypothized, that the hydroscopic property of PEG influenced cell vacuoles of Leucaena cells and produced the rapid decline of sCT, which was not the case in San Ramon. It is possible that the hygroscopic nature of PEG had an influence on digestibility of the tannin-free species Indigofera.

### 1.4.3 Effect of binding tannins in legumes with PEG on anaerobic N degradation and aerobic N release

**Contributors:** K. Tscherning (University of Hohenheim), M. Peters (CIAT), C. Lascano (CIAT), E. Barrios (CIAT), R. Schultze-Kraft (University of Hohenheim)

#### Material and Methods

Plant samples were collected from four shrub legume species with contrasting qualities (nil, medium and high tannin content) namely: *Indigofera constricta* Rydb., (Indigofera), *Leucaena leucocephala* (Lam.) de Wit. CIAT 21245 (Leucaena) and 2 provenances of *Calliandra calothyrsus* Meisn.: San Ramon CIAT 22310 (San Ramon) and Patulul CIAT 22316 (Patulul). For chemical characteristics of tannins in legumes see 1.4.2.

**Use of Polyethylenglycol (PEG)** (application in ground material): Freeze-dried leave material was ground to pass a 1 mm screen and mixed carefully with PEG solution to receive a PEG application at 7%. Control treatments were mixed with the same amount of de-ionized water.

**Chemical characterization of plant material:** All samples were subjected to the following chemical analysis: N, C, P, NDF, ADF, IADF, lignin, soluble and bound condensed tannins (sCT and bCT) and ash following standard protocols. For measuring N release during aerobic degradation the leaching tube experiment was carried out as as described in 4.1. For the measuring of anaerobic degradation, the *in-vitro* dry matter digestibility method (IVDMD) and the gas production experiment were carried out.

## Results

**Leaching tube experiment** Significant differences between species were observed for the rate (data not shown) and extent (Table 7) of N release. No differences between + PEG and – PEG treatments were observed either for rate or for extent of N release. For the N release rate an interaction between species and treatments was observed.

**Table 7.** Extent of released N ($E_N$ ) and degraded N ($E_{Ndeg}$) of Indigofera, Leucaena and Calliandra San Ramon and Patulul with and without PEG treatment.

| | | Released N (aerob) | | Degraded N (anaerob) | |
|---|---|---|---|---|---|
| **Legumes** | **Treatment** | $E_N$ | | $E_{N\ deg}$ | |
| *Indigofera constricta* | + PEG | 68.13 | | 85.77 | |
| | - PEG | 64.87 | | 84.81 | |
| *Leucaena leucocephala* | + PEG | 41.95 | | 66.12 | |
| | - PEG | 34.75 | | 41.39 | |
| *Calliandra*– San Ramon | + PEG | 34.44 | | 27.68 | |
| | - PEG | 35.96 | | 15.11 | |
| *Calliandra*– Patulul | + PEG | 30.07 | | 25.82 | |
| | - PEG | 27.85 | | 7.32 | |
| Significance (p) of effects of | | | SED | | SED |
| Specie | | *** | 4,34 | *** | 0,937 |
| Treatments | | n.s. | 3,07 | *** | 0,662 |
| Interaction | | n.s. | 6,14 | *** | 1,320 |

SED =Standard error of the difference between means
*** = ($P<0.001$), n.s. = ($P>0.05$)

**Gas production experiment** Significant differences between species were observed for the extent of degraded N (Table 7). In addition, + PEG and -PEG treatment were significantly different. The tannin free control plant showed no differences due to PEG application.

## Discussion

The application of PEG affected N degradation in the anaerobic gas production experiment as expected, but did not show the expected effect in the aerobic leaching tube experiment. In the leaching tube experiment results showed that the application of PEG lowered the sCT in legumes to nill. It is evident, however that these differences in the sCT content due to PEG at time 0 had no influence on N release over time. It was expected that in the +PEG treatment, mineralization would have been higher than in the –PEG treatment, due to the tannin binding capacity of PEG.

It is postulated, that PEG-tannin complexes were not formed in the ground material placed in the leaching tubes given that PEG and sCT need a liquid medium to complex, which was absent during the application of PEG to the ground material. The formation of tannin-protein complexes may have only taken place in the test tubes during the extraction phase in the tannin assay.

In conclusion, both experiments showed that PEG is not suitable for aerobic decomposition and mineralization experiments in which the objective is to assess the effect of tannins from legumes on degradation and N release. This study confirmed however, that PEG is useful to study the effect of tannins in feed quality of tropical legumes.