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

1.1 Screening of *Brachiaria* hybrids for high digestibility and protein

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Highlight

- Found acceptable correlations in quality parameters measured in different sampling periods in *Brachiaria* hybrids planted as spaced plants in the field

Rationale

We had previously reported a low correlation between IVDMD values obtained in samples of the same *Brachiaria* population cut at different times. We had postulated that this lack of correlation between samplings had to do with the sampling procedure 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).

Last year we sequentially 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. 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 periods (CP: r = 0.30; IVDMD: r = 0.01).

Thus we concluded that in order to select *Brachiaria* hybrids for quality parameters such as CP and IVDMD we still needed to develop a standard sampling procedure, which included fertilizer management and a uniform chronological or physiological age for harvesting the forage.

Materials and Methods

In 2003 we transplanted 50 *Brachiaria* hybrids (*B. ruziziensis* x *B. brizantha* cv. Marandu) in the field in Quilichao in replicated (3) plots (spaced plants). Initially the plants were cut at a uniform height and after 6 weeks of growth, leaves were harvested in three successive samplings (September 25, November 18 and December 18, 2003). After each harvest 2 g of urea were applied per plant. All samples (leaves) were analyzed in CIAT's Forage Quality Laboratory for CP and IVDMD using NIRS. Results were subject to an analysis of variance and to correlation analysis.

Results and Discussion

Results showed that mean CP and IVDMD values differed between sampling periods. While CP increased from sampling period 1 to 3, the reverse occurred with IVDMD (Table 1). In the three sampling periods the mean CP and IVDMD observed were high for a tropical grass probably related to both genetic factors as well as management factors (i.e. N fertilization and age of leaf at harvest).

The ANOVA showed that for both CP and IVDMD there was a significant sampling period ($P < 0.0001$) and genotype ($P < 0.002 – 0.006$)

Table 1. Variation in crude protein (CP) and in vitro digestibility (IVDMD) in *Brachiaria* hybrids

Sampling Period (No of Samples)	CP (%)		IVDMD (%)	
	Mean	Range	Mean	Range
1 (143)	14.9	12.1-17.2	70.2	65.4-74.1
2 (141)	16.1	12.6-19.4	68.3	64.2-72.4
3 (150)	17.7	13.5-21.8	66.7	63.5-71.2
Significance (P)				
Sampling Period	0.0001		0.0001	
Rep (sampling period)	NS		0.0025	
Genotype				
Genotype x Sampling Period	0.0026		0.0064	
	NS		NS	

effects (Table 1). However, it was interesting to observe that the sampling x genotype interaction was not significant for both quality variable measured. With the 50 *Brachiaria* hybrids evaluated the variance associated with CP and IVDMD was 5.1 and 3.7 times greater, respectively than the variance associated with the interaction of genotype x sampling period.

The correlations between sampling periods for CP ranged from 0.58 to 0.73 (Figure 1), while in the case of IVDMD the correlation between periods ranged from 0.51 to 0.55 (Figure 2).

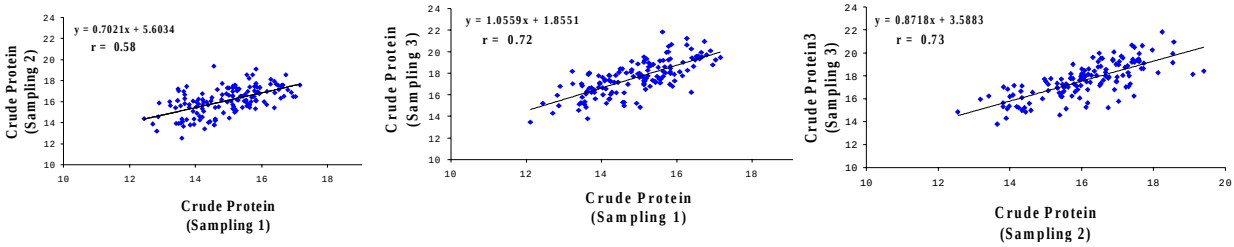

Figure 1. Relationship between crude protein (CP) values measured with NIRS in *Brachiaria* hybrids harvested in three sampling periods

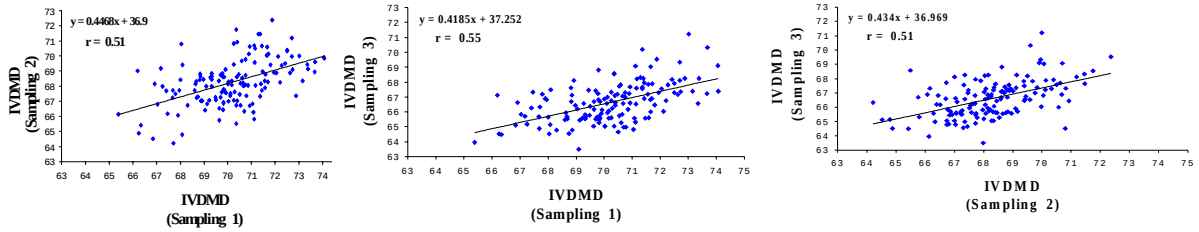

Figure 2. Relationship between in vitro digestibility (IVDMD) values measured with NIRS in *Brachiaria* hybrids harvested in three sampling periods.

From these results we conclude that with the methodology used it is possible to detect with NIRS differences in CP and IVDMD between *Brachiaria* hybrids planted as spaced plants in the field, even though the variability in these quality attributes seemed to be lower than expected. One of the advantages of *Brachiaria* hybrid cv Mulato is its high crude protein (CP) content relative to commercial cultivars when growing in good soils. Thus we also believe that hybrids should be screened for both CP and IVDMD, recognizing that selection for improved forage quality is justified if genetic variance for quality traits 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 IVDMD caused by genotype was four times greater than the variance from G x E. This may not be the case for CP since this quality parameter is significantly affected by soil N content.

To implement screening for IVDMD and for CP in the *Brachiaria* improvement program we still need to adjust a protocol for plants grown in pots in the greenhouse. From the results of this year with plants grown in the field, the greenhouse methodology to be implemented will include:

a) Transplanting vigorous cuttings in replicated pots with water and nutrients not being limiting.
b) Plants well established in the pots will be subject to a uniformity cut and allowed to growth for 6 weeks to harvest leaves for quality analysis

This protocol will be implemented and results will be reported next year.

1.2 Animal production potential with selected grasses and legumes

Highlights

- Sorghum silage with or without molasses had better fermentation (lactic acid process) than Cratylia silage (acetic process).
- Adding molasses improved the intake of Cratylia silage by goats.

1.2.1 Milk production in Brachiaria pastures supplemented with LabLab hay

Collaborators: P. Avila (CIAT), J. Miles (CIAT) and C. Lascano (CIAT)

Rationale:

The forage Project has been evaluating different annual legumes such as cowpea (from IITA) and Lablab (from CSIRO) as green manures in crop –livestock systems. In addition, we have been interested in looking at the feed value in the dry season of cowpea and Lablab hay harvested at pre-flowering and after grain harvest.

There is abundant literature on production and utilization of silage and hay, but in most cases the technology available is not useful to small farmers given that it relies on machinery (i.e. tractors and mechanical forage harvesters) out of the reach of these farmers. Thus we have been investigating alternative technologies such as the “bag silage” and hay packed in bags that could be more appropriate for smallholders livestock systems. Results from last year indicated that intake of cowpea hay was higher than the intake of Cratylia silage, but that this difference did not translate into a significantly higher milk yield. The supplementation of Cowpea hay resulted in18% more milk yield of cows grazing *B. decumbens* pastures when compared with production of cows receiving no supplement.

In this section we report the results of feeding LabLab hay to milking cows grazing different Brachiaria pastures.

Material and Methods

A grazing trial was carried out in a rainy –dry transition period (August 25 to October 7, 2003) in the Quilichao research station using 8 crossbred (Holstein x Zebu) arranged in a 3 x 3 Latin Square design. Four cows received Lablab hay (0.5% of BW) and the other for cows were left as control. All cows grazed pastures of *Brachiaria brizantha* cv Toledo, *Brachiaria* hybrid cv Mulato and *Brachiaria* hybrid CIAT 36087. 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. The hay of Lablab used in the experiment was harvested after 12 weeks of regrowth. The whole plant (leaves, stems) were sun dried (3 days) and packed and stored in a well-ventilated room prior to being fed to the cows.

Results and Discussion

Milk yield was not affected by the supplementation of Lablab, but was affected by pasture type, being higher ($P< 0.05$) in the *Brachiaria* hybrid CIAT 36087 pasture than in Mulato and Toledo (Table 2). In the case of milk composition only the milk non- fat solids were higher in cows receiving the Lablab hay as supplement. The higher milk yield in CIAT 36087 was related to higher CP (14.4 %) in the forage (leaves) on offer relative to the other grasses (9.0 % and 12.9 % for Toledo and Mulato, respectively).

In general, our results indicate that the lack of response to the supplementation of the high quality Lablab hay may have been associated with the low level of supplementation (0.5% of BW) of the hay and/or to the fact that the grasses had relatively high leaf protein contents.

The average consumption of Lablab hay was 4.2 kg DM per day/ cow (79% of the forage offered), which is lower than expected and maybe the reflection of the high quality of the grass in the pastures grazed by the cows.

Table 2. Milk yield and composition of cows grazing *Brachiaria* pastures with and without supplementation of Lablab hay

Treatments	Milk Yield (l/cow/d)	Milk Fat (%)	Milk Non-Fat Solids (%
Supplementation Effect			
- Lablab	8.0	4.3	9.1 a
+ Lablab	7.7	4.0	8.7 b
Pasture Effect			
B. brizantha CIAT 26110	7.9 b	3.9	8.8
Brachiaria Hybrid Mulato	7.5 c	4.2	8.9
Brachiaria Hybrid CIAT 36087	8.3 a	4.3	8.9

a, b means different ($P < 0.05$)

1.2.2 Quality and goat intake of *Cratylia argentea* and *Sorghum* sp. silage mixed in different proportions in plastic bags

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Rationale

The legume *C. argentea* cv. Veraniega (Cratylia) is an important forage legume mainly used by farmers either fresh or as silage to supplement the diet of milking cows during the dry period in Costa Rica and elsewhere. Silage making is almost always associated with large cattle

feeding systems, which implies the use of sophisticated machinery and abundant resources. However, it has been shown that forages can be conserved using simple technology such as plastic bag silage. This forage conservation method is less costly and available to small farmers with no machinery, particularly for those who live along the dry or sub-humid tropics that experience a significant lack of forage during prolonged dry periods, with subsequent losses in animal production.

Finally, little attention has been paid to the benefits of mixing cereals and legumes for the production of high quality silage for livestock feeding.

Materials and Methods

An 111 days old re-growths of Cratylia and 88 days old re-growths of sweet forage *Sorghum* sp. were harvested and mechanically chopped into 2-5 cm pieces, and mixed in different proportions, with or without molasses to form eight silage treatments. An unrestricted randomized block design was used and treatments arranged in a 4 x 2 factorial for a total of 8 treatments. Molasses was used at 5% (on forage fresh weight basis), and three plastic bags (30-kg capacity) were used for each treatment. Plastic bags were hand packed and a vacuum machine was used to extract the air before sealing the bags with plastic bands as shown in Photo 1.

Photo 1. A30-kg plastic bag silage of *Sorghum* sp.

Plastic bags were stored in a protected room and opened 2 months later. Silage pH, color, smell, DM and OM contents, CP and NDF were measured.

Goat intake was measured only with Cratylia silage with and without molasses, and with the mixture of 66% Cratylia 33% sorghum plus molasses, namely treatments 1, 5 and 6. For this test, eight -50 kg additional bags were prepared for each treatment.

The different proportions of forage and molasses components for each treatment used were as follows:

Treatment	Cratylia (10%)	*Sorghum* (%)	Molasses
1	100	0	–
2	66	33	–
3	33	66	–
4	0	100	–
5	100	0	+
6	66	33	+
7	33	66	+
8	0	10	+

Silage coming from treatments 1, 5 and 6 was offered *ad libitum* to 4 goats per treatment in a complete randomize block design. The observation lasted 10 days and the daily silage offered and rejected was measured during the last 3 days to estimate animal intake and in vivo digestibility.

Results and Discussion

With the exception of maize and sorghum species, it is admitted that tropical grasses and legumes are not ideal material to ensile because at harvesting they have low contents of water-soluble carbohydrates that are essential for successful fermentation. However, mixing legumes with cereal crops such as sorghum, is a practice that contributes to improve the levels of fermentable carbohydrates reduces buffering and prevents proteolysis. In this case the silage color and smell averaged a value of 2.7, which indicated a silage of acceptable quality (Table 3).

Silage with pH 4 or less indicates a proper fermentation process since epiphytic lactic acid bacteria ferments the water-soluble carbohydrates. The bag silage made of pure sorghum, either with or without molasses (treatments 4 and 8) had the lowest pH content (3.5 and 3.9 respectively) (Table 3); however pure Cratylia silage had a pH of 5.0 (treatment 1) that was slightly reduced to pH 4.8 by adding molasses (treatment 5), indicating a strong buffering capacity and a more acetic than a lactic acid fermentation process.

In general, the fermentation process was acceptable for the different proportions of forage mixtures ensiled. Adding molasses at 5% (fresh weight basis) did not have a significant effect in this process, and as has been suggested in other experiments a proportion of at least 10% is needed to overcome the strong buffering effect of Cratylia silage.

It is commonly accepted that a crude protein (CP) content over 7% is required for animal production, and in this case only the silage made of pure sorghum (treatment 4) could be considered as limiting in protein. As expected, as the proportion of legume decreased in the mixture so did the CP content, particularly when no molasses was added.

The residues remaining after neutral detergent extraction (NDF) represents the proportion of plant dry matter made up of cellulose, hemicellulose, lignin, lignified N, and insoluble ash. This component is inversely correlated with intake and digestibility. In our study NDF ranged from 60.3-67.2% for silages without molasses and from 51.4-56.7% for silages with molasses, indicating that this additive may increase both digestibility and intake of the silage.

Silage intake with goats ranged from 0.09 kg/day/ animal for the Cratylia pure silage to 0.52 kg/day/ animal for the Cratylia plus molasses silage. The intake of the 66% Cratylia, 33% sorghum silage plus molasses was intermediate (0.20 kg/day/ animal).

An intake value of 0.52 kg/day of silage represented only 1.3 dry matter (DM) intake per 100 kg body weight (BW), which is low and may be associated with the high cell wall contents recorded in the silages mainly due to plant age and shown by both the Cratylia and sorghum silage. Associated to this were the low organic matter digestibility values observed that range from 52.1% for Cratylia silage, to 48.7% when molasses was added, and a slight improvement in the mixed silage with sorghum (53.2%).

This demonstrated that small bag silage is a viable practice for forage conservation and that as expected, sorghum silage with or without molasses had better fermentation (lactic acid process) than Cratylia silage (more acetic

Table 3. Smell, color and some quality characteristics of *C. argentea* cv. Veraniega and *Sorghum* sp. silage mixed in different proportions with and without molasses in plastic bags.

Treatment	Smell*	Color*	pH	CP (%)	NDF (%)	DM (%)
1	2	2	5.0 a **	11.8 ab	66.8 ab	30.2
2	3	3	4.2 b	10.7 abc	67.2 a	27.1
3	2	3	3.8 c	9.3 bc	60.3 bc	27.1
4	2	2	3.5 c	6.8 d	66.3 a	24.1
5	3	3	4.8 a	12.0 a	55.7 dc	29.1
6	3	3	4.1 b	11.1 ab	56.7 c	29.7
7	3	3	4.0 bc	9.7 bc	51.4 d	29.6
8	3	3	3.9 c	7.7 d	56.5 c	22.4

* Related to smell and color: 1, is poor; 2, medium and 3, good (silage with lactic acid smell and greenish color)

**Within columns means followed by the same letter are not statistically significant (P< 0.05)

process). On the other hand, intake of Cratylia silage by goats was low and this may be associated with the type of fermentation produced; however, adding molasses to Cratylia improved the intake of Cratylia silage.

1.3 Assessment of the potential of tanniniferous legumes to improve ruminant nutrition

Highlights

- Selecting appropriate planting sites may improve the feeding value of *Calliandra calothyrsus* cv Patulul
- High quality legumes may be partially replaced by tanniniferous legumes without any negative effect on ruminal fermentation characteristics of the complete diet

Relations between the plant nutritional status and forage quality defined

While the tanniniferous shrub legume *Calliandra calothyrsus* is widely used by smallholders to supplement dairy cattle and goats in Kenya, farmers in Colombia have hardly adopted this supplementation strategy. We hypothesized that this was due to contrasting environmental conditions, which may affect the forage quality of this legume species. Results from in vitro studies carried out this year showed that foliage from *C. calothyrsus* var. Patulul cultivated in Kenya had a higher nutritional value than foliage from the same variety cultivated in Colombia, which was mainly due to a much lower concentration of condensed tannins in the foliage produced in Kenya.

Future work will concentrate in defining the environmental factors (e.g. soil type and fertility) responsible for differences in forage quality and type and concentration of condensed tannins of a range of tanniniferous shrub legumes. For this purpose experimental plots have been established at two contrasting sites in Colombia.

Suitable combinations of tanniniferous legumes with forages free of tannins for improved protein supply and efficiency of rumen fermentation available

Results from in vitro and in vivo experiments carried out during the last two years showed that condensed tannins in tropical legumes contribute to lower methane emission from ruminal fermentation. However, this methane suppressing effect was generally associated with a decrease in the ruminal degradation of nutrients. This year, our work was therefore focused on the identification of types and proportions of tannin rich legumes in mixtures with legumes free of tannins, which improve protein supply without negatively effecting ruminal nutrient degradation.

The results indicate that the tanniniferous legumes *C. calothyrsus* and *Flemingia macrophylla*, when supplemented in a mixture with *Vigna unguiculata* (a legume free of tannins), may be included in the diet at a proportion of up to 13% of total DM without negative effects on rumen fermentation. For *Leucaena leucocephala*, another tanniniferous legume, this maximal proportion seems to be around 22% of the total diet. When higher proportions of tanniniferous legumes were included, the apparent degradation of crude protein was drastically suppressed.

It is likely that this decrease would result in a greater flow of non-ammonia nitrogen to the lower digestive tract of ruminants fed such mixtures which in turn could result in improved animal performance. This hypothesis will be tested in future feeding trials, using sheep fitted with ruminal and duodenal cannulas.

1.3.1 Assessment of the effects of plant nutritional status on forage quality and concentration and chemical properties of condensed tannins of selected legume species

1.3.1.1 Effect of the cultivation site (Kenya or Colombia) on ruminal fermentation characteristics of *Calliandra calothyrsus* var. Patulul

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Rationale

Insufficient quality of ruminant diets, as is prevalent in tropical agriculture, in conjunction with low feed conversion efficiency, leads to low levels of animal production. Protein deficiency is by far the most important cause of low performance of ruminants maintained on low-quality forages, and ensuring adequate ammonia levels in the rumen for microbial growth is the first priority in optimizing fermentative digestion of forage. In this respect, shrub legumes are adequate supplements as small-scale farmers can grow them with additional advantage that they supply both rumen-degradable and undegradable protein in higher amounts than most grasses and crop residues.

Calliandra calothyrsus is a shrub legume with excellent agronomic characteristics and high crude protein content. However its feeding value may be limited by low palatability and high concentrations of condensed tannins. Although *C. calothyrsus* is widely used by smallholders to supplement dairy cattle and goats in Kenya, this supplementation strategy has hardly been adopted by farmers in Colombia. We hypothesized that this was mainly due to differences in the forage quality of this legume in the two countries.

To test this hypothesis, an in vitro experiment was performed to compare the nutritional value and the ruminal fermentation characteristic of *C. calothyrsus* var. Patulul cultivated in Colombia, Quilichao (site with acid soils) and Kenya, Embu (site with fertile soils) when used as supplements to a low-quality grass hay.

Material and Methods

In this experiment a grass-alone diet (negative control), a urea-supplemented diet (positive control) and five legume supplemented diets were evaluated. The legume supplements (33% of dietary dry matter) consisted of *Cratylia argentea* (100%), *Calliandra* Colombia (100%), *Calliandra* Kenya (100%), or mixtures (1:1) of *C. argentea* with *Calliandra* Colombia or *Calliandra* Kenya (Table 4). Foliage from *C. calothyrsus* var. Patulul was harvested from mature plants grown in Kenya and Colombia and oven dried at 50°C. Plant material from *Brachiaria humidicola* CIAT 6133 (grass) and *Cratylia argentea* was harvested in Colombia and sun dried. The different diets were tested using a rumen simulation technique (Rusitec) during 4×10 day periods (n=4) with four days for adaptation and 6 days for data collection. The daily dry matter supply to the fermenter was maintained constant at 15 g (Table 4).

Results and Discussion

Ammonia concentration in the fermenter fluid was increased ($P<0.05$) by supplementation with urea or *C. argentea* alone but was not affected ($P>0.05$) by supplementation with *Calliandra* alone. While supplementation with the legume mixture containing *Calliandra* Kenya increased ($P<0.05$) ammonia concentration, the mixture containing *Calliandra* Colombia had no effect on ammonia concentration. Independent of the growing site, ammonia concentration decreased linearly ($P<0.05$) with increasing *Calliandra* proportion in the diet. Overall, supplementation with *Calliandra* Kenya resulted in higher ($P<0.01$) ammonia concentrations than

Table 4. Composition (% of dry matter) of experimental diets.

	Diet						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Diet ingredients							
B. humidicola CIAT 6133	100	100	67	67	67	67	67
C. argentea			33			16.5	16.5
C. calothyrsus Colombia				33		16.5	
C. calothyrsus Kenya					33		16.5
Urea		1					
Analyzed composition							
OM	88.9	88.9	89.8	90.2	90.5	90.2	89.9
CP	3.5	5.9	9.7	9.1	9.6	9.4	9.7
NDF	72.1	70.4	63.8	55.5	57.6	60.1	60.7
ADF	39.2	38.8	37.7	33.1	34.7	35.5	36.8

supplementation with *Calliandra* Colombia. Counts of ciliate protozoa and bacteria were not affected (P>0.05) by supplementation.

The apparent degradation of organic matter (OM) increased (P<0.05) with any type of supplementation, except when the supplement consisted of pure *Calliandra* Colombia. Apparent crude protein (CP) degradation was increased (P<0.05) by supplementation with urea or *C. argentea*, but was not affected (P>0.05) by supplementation with the legume mixtures, and was decreased (P<0.05) by supplementation with pure *Calliandra* from Colombia or Kenya. Supplementation with urea, *C. argentea* or the legume mixtures increased (P<0.05) apparent degradation of neutral detergent fiber (NDF), but supplementation with pure *Calliandra* had no effect (P>0.05) on NDF degradation. Independent of growing site, the apparent degradation of OM, CP and NDF decreased linearly (P<0.05) with increasing *Calliandra* proportion in the diet. However, apparent nutrient degradation was higher (P<0.05) in diets containing *Calliandra* Kenya than in those containing *Calliandra* Colombia.

Daily methane release as well as methane release relative to OM degraded increased (P<0.05) with any type of supplementation, except when the supplement consisted of pure *Calliandra* Colombia. Diets containing *Calliandra* Colombia resulted in a 10-20% lower methane release (P<0.01) than those containing *Calliandra* Kenya. With increasing proportions of *Calliandra*, methane release decreased linearly (P<0.05), independent of the site where the legumes were harvested.

Nitrogen supply to the fermenters increased with any type of supplementation. However, depending on the type of supplement, there were significant shifts between N fractions. The proportion of N recovered as ammonia was increased (P<0.05) by supplementation with urea or *C. argentea*, but supplementation with *Calliandra* alone or in mixture with *C. argentea* had no effect on this N fraction. The fraction of apparently not degraded N was increased (P<0.05) by supplementation with *Calliandra* alone, but was not affected (P>0.05) by supplementation with the mixtures, and decreased (P<0.05) when urea or *C. argentea* alone were supplemented. Compared to *Calliandra* Colombia, supplementation with *Calliandra* Kenya resulted in higher proportions of N degraded, of N recovered as ammonia and of N apparently incorporated in microbial protein, but in a lower proportion of apparently not degraded N (P<0.001).

Although the two provenances of *C. calothyrsus* tested in this experiment had similar contents of OM, CP and NDF, they differed in nearly all fermentation properties and the material from Kenya showed a higher apparent nutrient degradability. Thus we conclude from these

results that foliage from *C. calothyrsus* var. Patulul cultivated in Kenya had a higher nutritional value than foliage from the same variety cultivated in Colombia, which is mainly due to a much lower concentration of condensed tannins in the foliage produced in Kenya (20 vs. 38% in DM). Both materials had a much lower nutritional value than foliage of *C. argentea*. The effects of urea supplementation on ruminal fermentation were almost identical to the effects caused by supplementation with *C. argentea*. This indicates that the *C. argentea*-related effects on ruminal fermentation were mainly the result of an increased availability of fermentable nitrogen.

1.3.1.2 Establishment of experimental plots in Palmira and Matazul

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Rationale

Type and concentration of secondary metabolites in plants may be affected by environmental factors. Results resported in Activity 1.3.1.1 indicate that the forage quality of tanniniferous shrub legumes could be improved by the selection of appropriate planting sites or by improving the growing conditions (e.g. by fertilization). Therefore we are carrying out research to definine the environmental factors (e.g. soil type and fertility) responsible for differences in forage quality and type and concentration of condensed tannins of a range of tanniniferous shrub legumes. For this purpose, experimental plots were established at two contrasting sites in Colombia. The aim of this activity is to identify growing conditions, which could contribute to an improved forage quality of tanniniferous legumes.

Material and Methods

Based on the current knowledge, five multipurpose legume species with contrasting forage quality and types and contents of tannins were selected from the germplasm collection held at CIAT. Namely these were *Cratylia argentea* (CIAT 18516), *Calliandra calothyrsus* (CIAT 22310), *Flemingia macrophylla* (CIAT 17403), *Leucaena leucocephala* (CIAT 17502) and *Desmodium velutinum* (CIAT 33443). In experiment 1, one legume plot was established on a Mollisol at CIAT's headquarters in Palmira and one on an Oxisol in Matazul in the Eastern Plains of Colombia in order to assess the effect of soil type on forage quality. At both sites two fertilization levels are tested. Experiment 2 was established on an Oxisol in Matazul and three contrasting fertilization treatments awere applied to assess the effect of sulfur and phosphorus fertilization on forage quality of shrub legumes. The five experimental species and the fertilization treatments were arranged in randomized complete block designs with three replicates each. The two plots in experiment 1 consist of 30 subplots each (5 species $\times$ 2 fertilization levels $\times$ 3 replicates) and the plot in experiment 2 consists of 45 subplots (5 species $\times$ 3 fertilization treatments $\times$ 3 replicates). Subplots are of 6×5 m each with 1 m between subplots. In each subplot 30 individual plants have been established.

Seven to nine months after the establishment, the experimental plants will be cut for the first time. This will be done at a height of 75cm for *L. leucocephala* and of 50 cm for the remaining species. Thereafter dry matter production will be evaluated every 8 weeks and twice year edible forage will be harvested to determine forage quality. Plant tissues will be analyzed for C, P, N, K, S, Ca, Mg, condensed tannin, astringency, monomer composition and molecular weight. At the end of the experiment the total dry matter accumulated in the different organs of the plant will be evaluated and the total amount of C, N and P in the soil profile will be measured.

1.3.2 Assessment of in vitro ruminal fermentation dynamics of tanniniferous legumes using the gas transducer technique

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Rationale

Ruminants play an important role as assets and sources of high quality food and income for rural populations in the developing world. Ruminant productivity is usually low due to inadequate and insufficient nutrition, frequently based on roughages, which are deficient in fermentable protein. Promising forage legume species have been identified to overcome these limitations. Many of these legumes contain tannins that could be either advantageous or disadvantageous in terms of feed efficiency and metabolizable protein supply to the animal. Four multipurpose legume species, *Flemingia macrophylla*, *Cratylia argentea*, *Calliandra calothyrsus* and *Leucaena leucocephala*, have shown great agronomic potential and could serve as forage supplements for ruminants fed tropical grass-based diets.

Prior in vitro studies showed that the incorporation of the tannin-rich legumes *C. calothyrsus* and *F. macrophylla* did not improve the feeding value of low-quality grass diets when used alone. However, in combination with legumes free of tannins (e.g. *Vigna unguiculata*), these tanniniferous legumes could contribute to improve animal nutrition. Very little information is available on the fermentation dynamics and the tannin-related effects of legume mixtures when supplemented to tropical low-quality grass diets. Furthermore it is completely unknown which combinations and proportions of individual legumes species would be most efficient in improving animal nutrition. Therefore several in vitro experiments were performed, to evaluate the effects of contrasting mixtures of tanniniferous and tannin-free legumes as supplements to the tropical grass *Brachiaria dictyoneura* on ruminal fermentation. Additionally, the tannin related effects were assessed by incubating different diets with and without the addition of PEG (a tannin-binding compound which inactivates soluble condensed tannins).

Material and Methods

The plant material of the four shrub legumes, *C. calothyrsus*, *C. argentea*, *F. macrophylla* and *L. leucocephala*, was harvested manually eight weeks after the last cut. The youngest three fully developed leaves of actively growing branches were used for these experiments. This material was immediately stored at 20°C. Subsequently plant samples were freeze-dried. The material of *B. humidicola* CIAT 6133 was cut after a growing period of 12 weeks and sun- dried for two days. In the case of *V. unguiculata*, eight-week-old herbage (the whole plant, before flowering) was harvested and sun dried for two days. The dried plant material of all species was ground in a laboratory mill using a 1 mm screen.

In experiment 1, *B. humidicola* CIAT 6133, *V. unguiculata* and *C. calothyrsus* were tested either alone or in combination with each other. The legume supplemented diets contained either 1/3 or 2/3 of legume and the remaining part was *B. humidicola* CIAT 6133. The legume supplements consisted either of *V. unguiculata*, *C. calothyrsus* or mixtures of the two legumes (4:1, 3:2, 2:3 or 1:4).

In experiment 2, *B. humidicola* CIAT 6133 was evaluated either alone or supplemented with legumes (1/3 of dietary DM). The legume supplements consisted either of a single legume (*V. unguiculata*, *C. calothyrsus*, *F. macrophylla*, *C. argentea* or *L. leucocephala*) or of a mixture of the herbaceous legume *V. unguiculata* with one of the four shrub legumes in ratios of 1:2 or 2:1. Overall, a total of 15 diets were evaluated in

experiment 1, and 14 diets in experiment 2. In both experiments all diets were evaluated with and without the addition of PEG (3.5% of DM).

Results and Discussion

Experiment 1: The highest (P<0.05) in vitro DM degradability (IVDMD) was observed in the pure *B. humidicola* CIAT 6133 and *V. unguiculata* diets and in grass-legume mixtures with high proportions of *V. unguiculata*, whereas the mixtures with high *C. calothyrsus* proportions and the pure *C. calothyrsus* diet showed the lowest IVDMD (P<0.05). The IVDMD decreased linearly (P<0.001) with increasing *C. calothyrsus* proportion in the legume mixture. This decrease was much lower when PEG was added to the diets than without PEG (interaction: P<0.05). The maximal gas production after 144 h was highest in the pure *B. humidicola* CIAT 6133 diet and lowest in the pure *C. calothyrsus* diet (P<0.05). The pure *V. unguiculata* and the mixed diets showed intermediate values.

The maximal gas production decreased linearly (P<0.001) with increasing *C. calothyrsus* proportion in the mixture, regardless of the legume proportion This decrease was greater in diets with high legume proportion than in the diets with low legume proportion. The interaction between diet and addition of PEG was highly significant (P<0.001) and the PEG-related increase in total gas production was larger in the diets containing high proportions of *C. calothyrsus* than in the remaining diets. The gas production rate was highest with the pure *V. unguiculata* diet and lowest with the pure *C. calothyrsus* diet and mixtures containing high proportions of this legume (P<0.05). In the remaining grass-legume mixtures and the pure *B. humidicola* CIAT 6133 diet intermediate values were observed. The interaction between diet and PEG addition was highly significant (P<0.001) and the PEG-induced increase in the gas production rate was much larger in the diets containing large proportions of *C. calothyrsus* than in the other diets.

The highest amount of protein was degraded in the pure *V. unguiculata* diet and the lowest amount in the pure *B. humidicola* CIAT 6133 diet (P<0.05). The interaction between diet and PEG addition was highly significant (P<0.001). The addition of PEG increased (P<0.05) the amount of apparently degraded crude protein in the pure *C. calothyrsus* diet and in all diets containing more than 13% of *C. calothyrsus* but had no effect in the remaining diets. Additionally, there was a quadratic effect (P<0.001) of the *C. calothyrsus* proportion on the amount of crude protein degraded when no PEG was added to the diets. With PEG, this effect was not apparent in the diets with low legume proportion, and in the diets with high legume proportion the negative effect of *C. calothyrsus* was much less pronounced than without PEG.

The inclusion of *C. calothyrsus* in the legume mixture had a quadratic effect on crude protein degradation. As a result, crude protein degraded was not affected by *C. calothyrsus* when a small proportion of the legume was used in the diet. In contrast, with higher proportions of legume (>13%) the amount of crude protein degraded decreased very sharply. The effects of diet and PEG on the apparent degradability of crude protein were very similar to the effects on the amount of crude protein apparently degraded described above. Crude protein degradability was high in the pure *V. unguiculata* diet and low in the pure *C. calothyrsus* diet and there was a highly significant interaction (P<0.001) between diet and PEG addition. In all *C. calothyrsus* - containing diets, the addition of PEG significantly increased protein degradability. With increasing *C. calothyrsus* proportion in the legume mixtures, protein degradability decreased quadratically (P<0.001).

Experiment 2. The highest IVDMD was observed the pure *B. humidicola* CIAT 6133 diet and the mixtures of *B. humidicola* CIAT 6133 with *V. unguiculata* and *C. argentea* (P<0.05). The partial or complete replacement of *V. unguiculata* by *C. argentea* did not affect IVDMD, but with other shrub legumes the IVDMD decreased with increasing proportion of

the legume in the diet (P<0.05). The lowest (P<0.05) IVDMD was found in the mixture that contained 1/3 of *F. macrophylla*.

There was a highly significant interaction (P<0.001) between diet and PEG addition which was mainly due to the fact, that the addition of PEG increased IVDMD in the diets with high proportion of tanniniferous legumes, but had no effect in the other diets. The maximal gas production after 144 h was highest (P<0.05) with the pure *B. humidicola* CIAT 6133 diet and lowest with the diets containing the tanniniferous legumes at a proportion of 1/3. The replacement of *V. unguiculata* by *C. argentea* had no effect on the maximal gas production, but the inclusion of the other shrub legumes suppressed the gas production.

The addition of PEG increased the maximal gas production in the diets with high proportion of tanniniferous legumes but not in the remaining diets (interaction: P<0.001). The highest gas production rates were observed in the mixtures of *B. humidicola* CIAT 6133 with *V. unguiculata* and *C. argentea*, whereas the lowest values were found in the pure *B. humidicola* CIAT 6133 diet and the diets which consisted of 1/3 of *F. macrophylla* or *C. calothyrsus* (P<0.05). The partial or complete replacement of *V. unguiculata* by *C. argentea* did not affect gas production rate. With other shrub legumes the gas production rate decreased with increasing proportion of the legume in the diet (P<0.05). The interaction between diet and PEG addition was highly significant (P<0.001). The gas production rate increased when PEG was added to the diets consisting of 1/3 of *C. calothyrsus* or *F. macrophylla*, but not when PEG was added to the other diets.

The highest amount of crude protein was degraded in the diets containing *C. argentea* and the lowest amount in the pure *B. dictyoneura* diet (P<0.05). The diets containing *L. leucocephala* and the diet containing *V. unguiculata* to 1/3 showed intermediate values. In the diets containing *C. calothyrsus* or *F. macrophylla*, the amount of protein degraded was lower than in any other legume supplemented diet, but still higher than in the pure *B. dictyoneura* diet (P<0.05). The amount of crude protein degraded decreased (P<0.05) with increasing proportion of tanniniferous legumes in the diet when no PEG was added, but with PEG this effect was not observed (interaction: P<0.001). The apparent degradability of crude protein was highest (P<0.05) in the diets consisting of 1/3 of *C. argentea* or *V. unguiculata* or mixtures of these two legumes. The diets containing *L. leucocephala* or low proportions of *C. calothyrsus* or *F. macrophylla* as well as the pure *B. dictyoneura* diet showed intermediate values. The lowest (P<0.05) apparent crude protein degradability was observed in the diets consisting of 1/3 of *C. calothyrsus* or *F. macrophylla*.

There was a highly significant interaction (P<0.001) between diet type and PEG addition. In all diets containing *C. calothyrsus* or *F. macrophylla* as well as in the diets containing medium or high proportions of *L. leucocephala*, apparent degradability of crude protein was significantly higher (P<0.05) when PEG was added. With the other legume diets the addition of PEG had no effect (P>0.05). Additionally, the apparent crude protein degradability decreased (P<0.05) with increasing proportion of tanniniferous legumes in the diet when no PEG was added. In the treatments with PEG this effect was not observed or much less pronounced. Among the three tanniniferous legumes, *Leucaena* showed the lowest negative effects on crude protein degradability.

In summary, these results indicate that *B. humidicola* CIAT 6133 has a high potential in vitro dry matter degradability (>65%) but an extremely low crude protein content (<5%). Therefore it may be a good source of fermentable energy, provided that it is adequately supplemented with a source of fermentable protein. The herbaceous legume *V. unguiculata* has a great potential as protein supplement for ruminants fed low quality grasses, because it improves protein supply without negatively affecting IVDMD.

The effects of partial or complete substitution of *V. unguiculata* in the legume supplement for foliage of shrub legumes strongly depends on the tannin content of the shrub species. The supplementation with the tannin-free *C. argentea* results in similar or even slightly better fermentation characteristic of the mixed diet than the supplementation with *V. unguiculata*. Thus, *C. argentea* represents a valuable alternative for smallholders to improve protein supply to ruminant livestock, particularly because, compared to *V. unguiculata,* it has several agronomic advantages like being drough tolerant, and being perenial. In contrast to the tannin-free legumes, supplementation with *C. calothyrsus* or *F. macrophylla* does not increase the amount of degraded protein and also suppresses IVDMD compared to the pure *B. humidicola* CIAT 6133 diet. However, when these high tannin legumes are supplemented in a mixture with *V. unguiculata,* both may be included in the diet at a proportion of up to 13% of total DM without negative effects on rumen fermentation. For *L. leucocephala* this maximal proportion seems to be around 22% of the total diet. When higher proportions of tanniniferous legumes are included in the diet, the apparent degradation of crude protein is drastically decreased. It is likely that such a decrease would result in a greater flow of non-ammonia N to the lower digestive tract of ruminants fed such mixtures. However, as in this study only ruminal fermentation processes were considered, effects on N digestion and utilization in the lower digestive tract have to be assessed in animal feeding trials. If a certain amount of the protein-tannin complexes gets dissolved in the lower digestive tract and is available for digestion and absorption, a high proportion of these tanniniferous legumes in mixed diets could result in an increased animal performance.

1.3.3 Assessment of the effects of legume mixtures on rumen fermentation parameters using the Rumen Simulation Technique (Rusitec)

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Material and Methods

Two experiments were carried out to evaluate the effects of the partial replacement of *Vigna unguiculata* by tanniniferous shrub legumes of contrasting quality in a basal diet of *Brachiaria humidicola* CIAT 6133.

In experiment 1, all experimental diets consisted of 50% *B. humidicola* CIAT 6133 and 50% legume supplement. The legume supplements consisted either of *V. unguiculata* alone or a mixture (3:2) of this legume with *Calliandra calothyrsus*, *Flemingia macrophyllus* or *Leucaena leucocephala*. The four diets were evaluated with and without the addition of PEG (1% of DM).

In experiment 2, a grass alone diet (negative control), a urea supplemented diet (positive control) and six legume -based diets (50% of DM) were evaluated. The legume supplements consisted either of *V. unguiculata* alone or in mixtures (3:2) with *C. calothyrsus*, *F. macrophyllus* or *L. leucocephala*. In the case of *C. calothyrsus* and *F. macrophylla* two accessions were tested per species, one with low in vitro dry matter digestibility (L-IVDMD) and one with high IVDMD (H-IVDMD). The experimental diets were tested in a Ruistec apparatus during 4 × 10 day periods (n=4) and daily dry matter supply to the fermenters was maintained constant at 15 g.

Results and Discussion

Experiment 1. The partial replacement of *V. unguiculata* by any tanniniferous shrub legumes used decreased ($P<0.05$) ammonia concentration in the fermenter fluid, but the decrease was more pronounced with *C. calothyrsus* than with *F. macrophylla* or *L. leucocephala*. On average,

L. leucocephala. On average, PEG addition increased (P<0.05) ammonia concentration from 3.3 to 3.7 mmol/l and no interaction (P>0.05) between PEG addition and diet was observed. Counts of ciliate protozoa and bacteria were not affected (P>0.05) by diet but bacteria counts increased (P<0.05) with PEG addition.

Apparent degradability of organic matter (OM) and crude protein (CP) decreased (P<0.05) when *V. unguiculata* was partially replaced by *C. calothyrsus* or *F. macrophylla*, but not when *L. leucocephala* was included in the diet (P>0.05). The inclusion of any shrub legume decreased (P<0.05) the apparent degradability of fiber (NDF), but the decrease was more pronounced with *C. calothyrsus* and *F. macrophylla* than with *L. leucocephala*. Addition of PEG had no effect (P>0.05) on the apparent degradability of OM and CP but increased (P<0.05) degradability of NDF. No interaction (P>0.05) between PEG addition and diet on apparent nutrient degradability was observed. Daily methane emission as well as methane emission relative to OM degraded were similar with all diets and were not affected by PEG addition (P>0.05). Taken as such, the lack of an interaction between PEG addition and diet would suggest, that tannins present in the shrub legumes had no effect on ruminal fermentation. However, the changes caused by the inclusion of these tanniniferous legumes agreed well with those observed in previous experiments (see also Activity 1.3.2) and indicate that microbial activity was suppressed in the same way, as it would be expected from tannin addition. We therefore assume that the level of PEG added to the diets (1% of DM) was not sufficient to inactivate all tannins and to avoid negative effects on ruminal fermentation.

Experiment 2. The supplementation with urea or legumes clearly increased (P<0.05) ammonia concentration in the fermenter fluid. The largest increase was observed with urea supplementation, the smallest with the legume mixture containing *C. calothyrsus* L-IVDMD. Ciliate protozoa and bacteria counts were not affected (P>0.05) by supplementation. Legume supplementation increased (P<0.05) the apparent OM degradability regardless of species or mixture. The largest increase was achieved with the supplement consisting of pure *V. unguiculata*, the smallest with the legume mixtures containing any of the *C. calothyrsus* accessions or *F. macrophylla* L-IVDMD.

Apparent CP degradability was increased (P<0.05) by supplementation with urea, *V. unguiculata* alone or in mixture with *F. macrophylla* H-IVDMD, but was not affected (P>0.05) by the other legume supplements. Apparent NDF degradability was increased (P>0.05) by supplementation with urea, *V. unguiculata* alone and the mixtures containing *F. macrophylla* H-IVDMD or *L. leucocephala*. Daily methane release was not affected (P>0.05) by supplementation with mixtures containing any *C. calothyrsus* accession, *F. macrophylla* L-IVDMD or *L. leucocephala*, but increased (P<0.05) when urea, *V. unguiculata* alone or the mixture containing *F. macrophylla* H-IVDMD were supplemented. Methane release relative to OM degraded was not affected (P>0.05) by dietary treatments.

In summary, these results confirm the high potential of *V. unguiculata* as a supplement to low-quality diets and suggest that the effects of supplementing this legume on ruminal fermentation are mainly the result of an increased supply of fermentable nitrogen. The partial replacement of *V. unguiculata* by *F. macrophylla* H-IVDMD resulted only in small changes in the fermentation characteristics of the complete diet. Therefore, this shrub legume seems to have a high potential as a supplement for ruminants in combination with high-quality legumes. Based on the present results, the evaluated shrub legumes can be ranked according to their feeding value as follows: *F. macrophylla* H-IVDMD > *L. leucocephala* > *F. macrophylla* L-IVDMD = *C. calothyrsus* H-IVDMD > *C. calothyrsus* L-IVDMD.