animal

Effect of the tropical tannin-rich shrub legumes *Calliandra calothyrsus* and *Flemingia macrophylla* on methane emission and nitrogen and energy balance in growing lambs

T. T. Tiemann[1,2], C. E. Lascano[2], H.-R. Wettstein[1], A. C. Mayer[1], M. Kreuzer[1] and H. D. Hess[3†]

[1]ETH Zurich, Institute of Animal Science, Universitätstrasse 2, CH-8092 Zurich, Switzerland; [2]Tropical Grass and Legume Project, CIAT, Cali, Colombia; [3]Agroscope Liebefeld-Posieux Research Station (ALP), Tioleyre 4, CH-1725 Posieux, Switzerland

*The objective of this study was to test whether the use of tannin-rich shrub legume forage is advantageous for methane mitigation and metabolic protein supply at unchanged energy supply when supplemented in combination with tannin-free legumes to sheep. In a 6 × 6 Latin-square design, foliage of two tannin-rich shrub legume species (*Calliandra calothyrsus *and* Flemingia macrophylla*) were used to replace either 1/3 or 2/3, respectively, of a herbaceous high-quality legume (*Vigna unguiculata*) in a diet composed of the tropical grass* Brachiaria brizantha *and* Vigna *in a ratio of 0.55 : 0.45. A* Brachiaria*-only diet served as the negative control. Each experimental period lasted for 28 days, with week 3 serving for balance measurement and data collection inclusive of a 2-day stay of the sheep in open-circuit respiration chambers for measurement of gaseous exchange. While* Vigna *supplementation improved protein and energy utilisation, the response to the partial replacement with tannin-rich legumes was less clear. The apparent total tract digestibilities of organic matter, NDF and ADF were reduced when the tannin-rich plants partially replaced* Vigna*, and the dose–response relationships were mainly linear. The tannin-rich plants caused the expected redistribution of more faecal N in relation to urinary N. While* Flemingia *addition still led to a net body N retention, even when fed at the higher proportion, adding higher amounts of* Calliandra *resulted in body protein mobilisation in the growing lambs. With respect to energy, supplementation of* Vigna *alone improved utilisation, while this effect was absent when a tannin-rich plant was added. The inclusion of the tannin-rich plants reduced methane emission per day and per unit of feed and energy intake by up to 24% relative to the* Vigna*-only-supplemented diet, but this seems to have been mostly the result of a reduced organic matter and fibre digestion. In conclusion,* Calliandra *seems less apt as protein supplement for ruminants while* Flemingia *could partially replace a high-quality legume in tropical livestock systems. However, methane mitigation would be small due to associated reductions in N and energy retention.*

Keywords: Brachiaria, methane, ruminants, tannins, Vigna

Introduction

Due to their abundance, domesticated ruminants affect life on our planet in many ways. Two of their most important impacts are their relevance for income generation in developing countries, mostly located in the tropics and subtropics, and their involvement in global warming due to methane emissions (e.g. Steinfeld *et al.*, 2006). Results of studies carried out over the past 30 years indicated the possibility to increase animal productivity and to mitigate methane emission at the same time by the use of shrub legumes. It was shown for many parts of the tropics that shrub legumes, due to their deep root system and their ability to fix nitrogen, associated with a relatively high crude protein (CP) content, might present an alternative for smallholders to improve protein supply in forage-based diets for ruminants (e.g. Kaitho *et al.*, 1996). At the same time, the high content of condensed tannins (CT) of many species adapted to acidic soils with low fertility is a major constraint for their use in ruminant diets. On the other hand, some CT-containing plants have been shown to reduce methane formation in the rumen with only limited negative side-effects on animal performance (Woodward *et al.*, 2001; Waghorn *et al.*, 2002; Tavendale *et al.*, 2005).

These effects, however, have only been demonstrated for plants from temperate zones. Identifying tropical shrub legumes that perform well in unfavourable growing conditions and contain CT that reduce methane emission at dietary levels not adversely affecting animal productivity would constitute a breakthrough for ruminant livestock systems in the tropics.

The International Centre for Tropical Agriculture (CIAT) in Colombia has identified promising shrub legume species with good agronomic performance under unfavourable conditions. Previous *in vitro* experiments showed a satisfactory nutritional value of these species for ruminant nutrition when used in mixtures with non-tanniniferous legumes to supplement low-quality tropical grasses (Stürm *et al.*, 2007). This strategy, particularly suited for feeding in the dry season, would allow small-scale farmers to cultivate only a small plot of care-intensive, high-quality, annual legumes and combine these with perennial tannin-rich shrub legumes, which are easier to grow and more productive. At the same time, such strategies could contribute to methane mitigation. The annual herbaceous *Vigna unguiculata* (cowpea) chosen as a model for a high-quality annual legume for the present study is cultivated in many parts of the world for human nutrition (grain) and also as animal feed (whole plant or crop residues after harvesting the grains) due to its high protein content. However, the plant requires favourable well-drained soils (Peters *et al.*, 2003). *Calliandra calothyrsus* and *Flemingia macrophylla* are shrub or tree legumes of the subhumid or humid tropics, characterised by having high CT contents ($>$50 g/kg). To our knowledge, the effects on metabolic energy and protein utilisation as well as methane formation of partial replacement of a high-quality legume by CT-rich tropical shrub legumes have not yet been examined in live ruminants.

To define the utility of mixtures of legumes with and without tannins, the following hypotheses were tested in a metabolism experiment with lambs employing the respiration calorimeter technique: (1) supplementation of a tropical grass-hay diet with the tannin-free Vigna increases protein and energy supply; (2) foliage of CT-rich shrub legumes reduces methane emission in ruminants and can partially replace Vigna in a tropical grass-hay based diet without depressing protein and energy utilisation; and (3) the effects of CT-rich plants differ among species due to different CT concentrations and properties.

Material and methods

Experimental forages

Diets consisted of different proportions of dried, leafy biomass of the following tropical forage species: the grass *Brachiaria brizantha* cv. Toledo (CIAT accession no. 26110), the herbaceous legume *V. unguiculata* (CIAT accession no. 391) and the foliages of the tannin-rich shrub legumes *C. calothyrsus* San Ramón (CIAT accession no. 22310) and *F. macrophylla* (CIAT accession no. 17403). All species were

Table 1 *Botanical composition (g/kg dry matter) of the experimental diets*

Diets[1]	Grass	Vigna	Call$_{150}$	Flem$_{150}$	Call$_{300}$	Flem$_{300}$
Brachiaria	1000	550	550	550	550	550
Vigna	0	450	300	300	150	150
Calliandra	0	0	150	0	300	0
Flemingia	0	0	0	150	0	300

[1]Grass = Brachiaria only; Vigna = in the text referred to as Vigna alone.

harvested from February to March 2006 from existing pure, non-fertilised stands established on an acidic Ultisol (pH $\approx$ 5.3) with 76% Al saturation, medium P content (6 mg/kg Bray II) and high (74 g/kg) organic matter content at CIAT's Research Station in Santander de Quilichao, located in the Cauca valley of Colombia (3°60′ N, 76°31′ W, 990-m elevation, 23°C annual mean temperature, 1772 mm annual rainfall). The grass Brachiaria was cut after 12 weeks of regrowth while Vigna was cut before flowering, and the shrub legume foliages were harvested by hand 9 weeks after a standardisation cut. The grass and cowpea were sun dried, while the shrub legume foliage was air dried in a shaded area. All dried feeds were shipped to Switzerland to be fed to growing lambs about 3 months after harvesting.

The forages were used to form six diets: one pure Brachiaria diet and five diets consisting of 550 g/kg Brachiaria and 450 g/kg legumes (on dry matter (DM) basis; Table 1). The legume part of the diets consisted of Vigna alone or Vigna combined with either Calliandra or Flemingia in ratios of 2 : 1 or 1 : 2. To facilitate intake and to limit selection, Brachiaria was chopped to particles of about 10 cm length.

Experimental design and procedures

Six castrated, male lambs of the Swiss White Hill breed with an initial body weight (BW) of 21.6 $\pm$ 4.2 kg were allocated to the six dietary treatments in a 6 $\times$ 6 Latin-square design. Each experimental period lasted for 28 days, consisting of 14 days of adaptation to the respective diet, 7 days of measurement and collection, including 2 days of quantitative measurement of gaseous exchange (two 22.5 h runs) in dual open-circuit respiratory chambers (5.44 m^3 each), and 7 days of resting period. During the adaptation and resting periods, animals were kept in individual boxes (150 $\times$ 180 cm) allowing visual and partial body contact. During the collection periods, animals were kept in individual metabolic crates (70 $\times$ 110 cm) arranged side-by-side. Each animal received the six diets in a different sequence with a daily forage DM allowance of 60 g per kg of metabolic BW (BW$^{0.75}$). This level was derived from previous experiences on intake of Brachiaria hay (Abreu *et al.*, 2004; Hess *et al.*, 2004) and ensured that fluctuations in daily intake and diet selection were limited. Based on estimates using regressions for temperate grasses and legumes (ALP, 2006), as no equations for tropical species were available, the concentrations of net energy for fattening (NEV) of

lambs in the different diets were calculated. Accordingly, the daily amount of NEV offered varied between 0.270 (Brachiaria-only) and 0.286 MJ/kg $BW^{0.75}$ (legume-supplemented diets). This was sufficient to cover maintenance requirements (0.228 MJ/kg $BW^{0.75}$; ALP, 2006) and provided extra energy for 40 to 50 g of average daily gain. However, considering all uncertainties, these values can only be seen as rough approximation. During the resting period, 80 g/kg $BW^{0.75}$ of the Brachiaria/Vigna diet were offered to all animals.

Individual forages were offered in separate troughs as one meal per day at 0900 h. Animals had free access to fresh water, and water consumption was measured daily. All experimental animals received 10 g/day of a commercial vitaminised mineral mixture providing 1.4 g Ca, 0.7 g P, 0.8 g Na, 0.3 g Mg, 12 mg Zn, 10 mg Mn, 0.4 mg I, 0.1 mg Co, 0.15 mg Se, and vitamin A, 200 000 IU; vitamin D_3, 20 000 IU; vitamin E, 2500 IU; vitamin B_1, 300 IU; and biotine, 100 mg.

Animals were weighed directly before feeding in the morning, at the beginning of each experimental period, twice a week during the adaptation periods for adjustment of the daily DM supply, and before (day 14) and after (day 21) the collection periods. During the collection periods no weighing took place and feed allowance was kept constant. The average of days 14 and 21 was used as BW in data evaluation.

From days 13 through 21, feed refusals were recorded daily before the next feed portions were offered. Refusals were collected separately by component and animal, and stored at −20°C. Samples of diet components were taken three times a week. Weight of complete faeces was determined once daily during the collection periods and, after mixing, an aliquot of 0.1 was taken and stored at −20°C. Urine was collected separately in two vessels, one to collect untreated urine, the second containing 1 M sulphuric acid in the amount estimated to be needed to achieve a pH ranging between 2 and 4, to avoid N volatilisation. Both samples were weighed and stored separately at −20°C. At the end of the collection periods, samples of diet components, refusals, faeces and urine were pooled across days within period for each animal. Samples of diet components and refusal were ground in a laboratory mill to pass a 1-mm screen, while faeces were ground in frozen state with a Moulinette kitchen blender. All samples, including urine, were stored at −20°C until being chemically analysed. Sub-samples of faeces were lyophilised.

Before and after the collection periods (days 14 and 21), 4 h after feeding, rumen fluid and blood samples were taken. Approximately 50 ml of rumen fluid was collected through the oesophagus by means of a flexible stomach tube. Special care was taken to avoid contaminations of rumen fluid with saliva. Determinations of pH, ammonia concentration and microbial counts were made immediately after collection, while sub-samples of 1.8 ml were conserved at −80°C for analysis of volatile fatty acids (VFA). Blood samples were drawn from the jugular vein,

heparinised (EDTA-Vacutainer), cooled on ice, centrifuged at $2000 \times g$ for 20 min at 4°C, and the plasma obtained was stored at −20°C until being analysed.

The experiment was carried out in accordance with the Swiss guidelines for animal welfare and the experimental protocol was approved by the Veterinary Department of Zurich, Switzerland.

Laboratory analyses

Feeds and refusals as well as fresh and lyophilised faeces were analysed with a TGA-500, Leco gravimetric furnace (Leco Corporation, St Joseph, MI, USA) for DM and total ash in order to calculate organic matter (OM) content. Feeds, refusals and lyophilised faeces were further analysed for NDF and ADF, feeds also for ADL, all by standard methods (AOAC, 1990). NDF was analysed without the addition of sodium sulphite, and values of NDF and ADF were corrected for ash content. Extractable and fibre-bound CT were determined according to the procedure suggested by Terrill et al. (1992) as modified by Barahona et al. (2003). Nitrogen (CP = N × 6.25) was determined in feeds, refusals, fresh faeces and acidified urine with an automatic C/N analyser (CN-2000, version 2.2; Leco Instrumente GmbH, Kirchheim Germany). With the same device, the C content of feeds, refusals, fresh faeces and non-acidified urine was analysed. Gross energy (GE) contents of feeds, refusals and lyophilised faeces were assessed through an anisothermic calorimeter (C 700 T System; IKA-Analysentechnik GmbH, Heitersheim, Germany).

Ruminal fluid pH and ammonia concentration were determined by a pH meter (model 632; Metrohm, Herisau, Switzerland) equipped with the respective electrodes. Ciliate protozoa and bacteria were microscopically enumerated using 0.1 and 0.02 mm deep Bürker counting chambers (Blau Brand, Wertheim, Germany), respectively. Determination of VFA was performed by HPLC (HP-5890 series II; Hewlett Packard, Waldbronn, Germany) with sample preparation and HPLC protocols following Doane et al. (1998) and Ehrlich et al. (1981), respectively. Blood plasma concentration of urea was analysed using a commercial kit (Roche Diagnostics, Basle, Switzerland) and a COBAS INTEGRA automatic analyser (Roche Diagnostics). The data on the two blood and rumen fluid samples taken per animal and period were combined for further data analysis. The respiratory chambers were equipped with an Oxymat 3 detector for quantification of oxygen (Siemens-Albis AG, Dietikon-Fahrweid, Switzerland) and a Binos 1001 for quantification of carbon dioxide and methane (Fisher-Rosemount, Baar-Walterswil, Switzerland).

Calculations and statistical analysis

Urinary energy was calculated from C and N concentrations (Hoffmann and Klein, 1980). Gaseous exchange data were used to calculate methane energy loss and energy expenditure (heat production) by the equations of Brouwer (1965), but corrected for the carbon dioxide emitted from

fermentation Chwalibog *et al*. (1996). Total body energy retention (RE) was calculated as metabolisable energy (ME) minus energy expenditure. Body fat energy was calculated as the difference between RE and the energy retained in protein.

Data were subjected to analysis of variance using the GLM procedure of SAS (version 9.1.3; 2006; SAS, Cary, NC, USA) with diet, animal and experimental periods as sources of variation. All multiple comparisons among means were performed by the Ryan–Einot–Gabriel–Welsh method (REGWQ; SAS, 1999). Linear and quadratic contrasts were established to identify effects related to the dietary proportion of the CT-rich legumes, while orthogonal contrasts were applied to compare the effects of the CT-rich legumes independent of dietary proportion. The standard errors of the mean (s.e.) are shown for all variables.

Results

The average BW did not differ ($P > 0.1$) among diets and there was also no difference in voluntary water intake. In all diets there were some refusals, but the extent differed among diets. As refusals involved mainly the CT-rich legumes, these differences also affected the composition of the diets consumed, but the intended compositional differences were still achieved (Table 2). As expected, the CP (N) content of the diets increased with supplemented legumes by more than 1.5-fold relative to the grass-alone diet, while at the same time, NDF contents declined. Due to the higher ADF and lignin (ADL) contents of the CT-rich legumes (especially of Flemingia) compared with Vigna, the diets supplemented with these legumes were also higher in

these two diet constituents. Increasing the dietary proportions of the CT-rich legumes increased tannin contents of the diets consumed by up to some 30 g/kg DM. The ratio of extractable to bound CT was lower in Calliandra than in Flemingia. DM and OM intakes (Table 3) were highest for the diet supplemented with Vigna alone and lowest for the diet with high proportion of Calliandra in the legume supplement. This resulted in a linear decrease of intake with increasing proportion of Calliandra (Table 3; $P < 0.01$, linear contrast). The effects on intake of adding Flemingia in the legume supplement tended to be less than those with Calliandra (orthogonal contrast).

The apparent total tract digestibility of OM, CP and ADF clearly differed ($P < 0.001$) among treatments, and the effect was significant ($P < 0.05$) also for NDF (Table 3). Supplementing only Vigna to the grass had small effects on digestibility (trend for an increase in OM and CP digestibility) while replacing part of Vigna by any of the CT-rich legumes decreased ($P < 0.01$, linear contrasts) digestibility of OM, CP, NDF and ADF. Differences in OM and CP digestibility between the grass-alone and the legume-supplemented diets were significant only with high Calliandra supplementation and the high level of Flemingia in case of OM digestibility. However, between the two CT-rich legumes, only apparent CP digestibility differed ($P < 0.05$) at the high level of supplementation where CP digestibility was particularly low with Calliandra (273 g/kg). Both CT-rich legumes resulted in a decreased fibre digestibility ($P < 0.01$, linear contrast). Hemicellulose digestibility was not affected ($P > 0.1$) by treatments.

Ruminal fluid pH did not differ ($P > 0.1$) among treatments and averaged at 7.09 (data not shown in table).

Table 2 *Composition (g/kg dry matter) of the experimental forages and the consumed diets as analysed (*n = 6*)*

	Experimental forages				Experimental diets[1]				
	Brachiaria	Vigna	Calliandra	Flemingia	Vigna	Call$_{150}$	Flem$_{150}$	Call$_{300}$	Flem$_{300}$
Dry matter (DM)[2]	924	899	900	906	913 (15.0)[3]	914 (14.4)	914 (13.9)	913 (12.1)	916 (13.7)
Organic matter	921	908	946	947	915 (4.6)	920 (3.9)	920 (6.0)	922 (3.2)	926 (4.4)
Crude protein	61	166	140	174	108 (10.4)	103 (7.78)	107 (10.0)	93 (3.8)	107 (10.6)
NDF	638	426	407	507	544 (22.2)	542 (26.6)	558 (28.4)	567 (14.3)	571 (33.0)
ADF	376	290	301	381	338 (13.1)	340 (15.9)	351 (14.6)	349 (11.8)	364 (16.7)
ADL	45.9	62	123	185	53.1 (7.00)	60.8 (5.68)	68.6 (11.94)	64.3 (11.45)	85.1 (10.79)
Hemicellulose[4]	262	135	107	127	206 (21.2)	202 (20.7)	207 (25.5)	218 (16.5)	207 (24.1)
Cellulose[5]	330	228	178	196	285 (8.9)	279 (12.1)	282 (12.8)	285 (3.5)	279 (15.4)
Gross energy (MJ/kg DM)	16.6	16.2	18.5	18.8	16.4 (0.20)	16.7 (0.23)	16.8 (0.24)	16.9 (0.29)	17.1 (0.15)
Metabolisable energy (MJ/kg DM)	7.0				7.3 (0.93)	6.5 (0.58)	6.2 (0.48)	6.3 (0.64)	6.0 (0.63)
Condensed tannins[5]									
Total	nd[6]	nd	175	111	nd	22.3 (4.82)	14.2 (6.03)	32.8 (13.27)	28.8 (9.91)
Extractable	nd	nd	98	33	nd	12.5 (2.70)	4.2 (1.79)	18.4 (7.43)	8.6 (2.95)
Bound	nd	nd	77	78	nd	9.8 (2.12)	10.0 (4.24)	14.4 (5.84)	20.2 (6.96)

[1]Chemical composition of diets as actually consumed. Brachiaria was consumed completely. Indices 150 and 300 indicate proportions of the respective legume (Call = Calliandra, Flem = Flemingia) in the diet offered (150 or 300 g/kg DM).
[2]g/kg original substance.
[3]Values in brackets are standard deviations of the diet consumed by individual animals, i.e. accounting for occasional refusals.
[4]Calculated as difference between NDF and ADF.
[5]Calculated as difference between ADF and ADL.
[6]nd = not detected.

Table 3 *Intake and apparent digestibility of nutrients in lambs fed grass alone or grass-legume mixtures with varying proportions of tannin-rich plants (diets n = 6)[1]*

			Experimental diets[3]						+Calliandra		+Flemingia		
	Grass	Vigna	Call$_{150}$	Flem$_{150}$	Call$_{300}$	Flem$_{300}$	s.e.	Diet effect	L	Q	L	Q	Call v. Flem
BW (kg)	26.2	25.3	25.8	25.8	25.5	25.7	0.32	ns	ns	ns	ns	ns	ns
			Intake (g/kg BW$^{0.75}$ and day)										
Dry matter	54.2^{ab}	59.0^{a}	57.6^{ab}	57.7^{a}	51.7^{b}	56.6^{ab}	1.44	*	**	ns	ns	ns	+
Organic matter	49.9^{ab}	54.0^{a}	53.0^{ab}	53.2^{a}	47.7^{b}	52.5^{ab}	1.34	*	**	ns	ns	ns	*
Condensed tannins													
Total	0^{c}	0^{c}	1.29^{ab}	0.84^{b}	1.75^{a}	1.67^{a}	0.176	***	***	+	***	ns	*
Extractable	0^{d}	0^{d}	0.72^{ab}	0.25^{cd}	0.98^{a}	0.50^{bc}	0.083	***	***	*	***	ns	***
Bound	0^{c}	0^{c}	0.57^{b}	0.59^{b}	0.77^{b}	1.18^{a}	0.098	***	***	ns	***	ns	ns
Water	120	145	140	134	141	129	5.5	ns	ns	ns	ns	ns	ns
			Apparent total tract digestibility (g/kg intake)										
Organic matter	614^{ab}	649^{a}	595^{bc}	590^{bc}	563^{cd}	543^{d}	10.7	***	***	ns	***	ns	ns
Crude protein	438^{ab}	524^{a}	391^{b}	437^{ab}	273^{c}	374^{b}	16.7	***	***	ns	***	ns	**
NDF	588^{a}	589^{a}	517^{ab}	534^{ab}	486^{b}	487^{b}	20.6	*	**	ns	**	ns	**
ADF	599^{a}	591^{a}	436^{bc}	454^{b}	372^{bc}	330^{c}	27.9	***	***	ns	***	ns	ns
Hemicellulose	326	305	310	324	298	340	13.9	ns	ns	ns	+	ns	+

[1]Mean values in the same row without common superscript are significantly different at $P < 0.05$.
[2]*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; ns = non-significant; L = linear contrast; Q = quadratic contrast; Call v. Flem = contrast between Calliandra and Flemingia independent of dietary level.
[3]Indices 150 and 300 indicate proportions of the respective legume (Call = Calliandra, Flem = Flemingia) in the diet offered (150 or 300 g/kg DM).

Table 4 *Concentration of ruminal ammonia and blood urea nitrogen as well as nitrogen balance in lambs fed grass alone or grass–legume mixtures with varying proportions of tannin-rich plants (diets n = 6)[1]*

			Experimental diets[3]						+Calliandra		+Flemingia		
	Grass	Vigna	Call$_{150}$	Flem$_{150}$	Call$_{300}$	Flem$_{300}$	s.e.	Diet effect	L	Q	L	Q	Call v. Flem
Rumen fluid ammonia (mmol/l)	3.89^{b}	6.54^{a}	6.05^{ab}	5.62^{ab}	3.83^{b}	5.32^{ab}	0.582	*	**	ns	ns	ns	ns
Blood urea N (mmol/l)	2.44^{e}	6.28^{a}	4.77^{bc}	5.41^{b}	3.38^{d}	4.57^{c}	0.195	***	***	ns	***	ns	***
			Nitrogen balance (mg/kg BW$^{0.75}$ and day)										
Intake	542^{c}	1019^{a}	946^{a}	994^{a}	747^{b}	973^{a}	41.1	***	**	ns	ns	ns	**
N retention	13	92	25	29	−72	55	28.0	ns	**	ns	ns	ns	ns
			Nitrogen balance (g/kg N intake)										
Faeces	562^{bc}	476^{c}	609^{b}	563^{bc}	727^{a}	626^{b}	23.8	***	***	ns	***	ns	*
Urine	423	442	366	420	370	323	31.8	ns	*	ns	*	ns	ns
Retention	16	82	25	17	−97	51	3.3	ns	*	ns	ns	ns	ns
Urinary N (g/kg N excreted)	427^{ab}	484^{a}	374^{ab}	421^{ab}	337^{b}	331^{b}	26.5	**	**	ns	***	ns	ns

[1]Mean values in the same row without common superscript are significantly different at $P < 0.05$.
[2]*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, +$P < 0.1$; ns = non-significant; L = linear contrast; Q = quadratic contrast; Call v. Flem = contrast between Calliandra and Flemingia independent of dietary level.
[3]Indices 150 and 300 indicate proportions of the respective legume (Call = Calliandra, Flem = Flemingia) in the diet offered (150 or 300 g/kg DM).

Ruminal ammonia concentration was low with Brachiaria alone and was numerically increased by all legume supplements, except by the high proportion of Calliandra in the diet (Table 4). Calliandra addition also resulted in a linear decrease of rumen ammonia concentration ($P < 0.01$, linear contrast) compared with the Vigna-only-supplemented diet, which was not the case when Flemingia was supplemented. A linear decrease ($P < 0.01$) in blood urea nitrogen was observed with the inclusion of CT-rich legumes in the supplement, an effect that was more pronounced with

Calliandra than with Flemingia. As was expected, supplementing only Vigna resulted in higher blood urea nitrogen than the Brachiaria-only diet. The total concentration of VFA varied between 98 and 110 mmol/l and did not differ ($P > 0.1$) among diets (data not shown). Also, the molar proportions of individual VFA did not differ between treatments and were high for acetic acid (77% to 79%) and low for propionic acid (11% to 13%) and butyric acid (6% to 7%) (data not shown). Total bacterial counts were not affected by diet, but supplementation of legumes increased ($P < 0.05$) the number of protozoa from 6×10^6 with the grass-only diet to a level of 16 to 20×10^6 in the legume-supplemented diets (data not shown).

Daily N intake differed ($P < 0.001$) among treatments, being higher with any legume supplement. However, with the diet high in Calliandra, N intake was lower ($P < 0.05$) relative to other legume treatments due to its lower consumption and lower CP content. Faecal N losses increased linearly relative to N intake with increasing proportion of the CT-rich legumes in the diet ($P < 0.001$, linear contrasts) while the proportionate urinary N losses decreased for both CT-rich species ($P < 0.05$, linear contrast). The proportion of N in urine relative to total N excretion differed ($P < 0.01$) among diets and decreased linearly ($P < 0.01$) with both CT-rich legumes. Body nitrogen retention was positive for all diets, except for the high-Calliandra diet. There was a trend for the highest N retention when Vigna was fed alone as a supplement.

Intake of GE was not clearly affected ($P > 0.05$) by legume supplementation (Table 5). Intake of digestible energy (DE) increased ($P < 0.05$) with Vigna addition while the replacement of Vigna with the CT-rich legumes decreased ($P < 0.001$, linear contrast) the DE intake. ME intake was equally changed by the different diets tested. Energy loss through heat (energy expenditure) was markedly lower ($P < 0.05$) when high proportions of the CT-rich legumes were fed compared with Vigna alone, and decreased linearly ($P < 0.01$) with increasing dietary proportion of Calliandra or Flemingia. Total energy losses did not differ among treatments (data not shown). Total body energy retention tended to increase ($P < 0.1$) with Vigna addition and decreased linearly ($P < 0.05$) with increasing proportion of CT-rich legumes. The efficiency of utilisation of GE (DE/GE, ME/GE) decreased linearly ($P < 0.05$) with increasing proportion of Calliandra and Flemingia. ME/DE did not differ ($P > 0.05$) between treatments.

Daily methane release was reduced linearly ($P < 0.001$) with supplementation of either Calliandra or Flemingia (Table 6). Energy loss through methane was markedly lower ($P < 0.05$) when CT-rich legumes were fed compared with Vigna alone, and decreased linearly ($P < 0.001$) with increasing dietary proportion of Calliandra or Flemingia. When adjusted for metabolic BW ($BW^{0.75}$) it was particularly evident that methane emissions increased with Vigna supplementation and decreased by 9 and 24% with replacement of Vigna by low and high proportions of CT-rich legumes, respectively. There were no differences in methane emissions between the two legumes. Similar shifts occurred when methane energy was related to intake of DM and GE. However, when methane emissions were expressed per unit

Table 5 *Balance and utilisation of energy in lambs fed grass alone or grass–legume mixtures with varying proportions of tannin-rich plants (diets* n = 6)[1]

	Grass	Vigna	Call$_{150}$	Flem$_{150}$	Call$_{300}$	Flem$_{300}$	s.e.	Diet effect	+Calliandra L	+Calliandra Q	+Flemingia L	+Flemingia Q	Call v. Flem
									Significance[2] / Contrasts				
Energy intake (kJ/kg BW$^{0.75}$ per day)													
Gross energy (GE)	905	972	966	970	878	978	26.6	+	*	ns	ns	ns	*
Digestible energy (DE)	483^b	549^a	484^b	479^b	413^c	434^{bc}	13.2	***	***	ns	***	ns	ns
Metabolisable energy (ME)	378^b	430^a	374^b	364^b	322^b	338^b	13.5	***	***	ns	***	ns	ns
Energy expenditure													
kJ/kg BW$^{0.75}$ per day	371^{ab}	380^a	368^{ab}	358^{ab}	325^c	347^{bc}	66.1	***	***	ns	**	ns	ns
Energy retention (kJ/kg BW$^{0.75}$ per day)													
Total	7^{ab}	50^a	7^{ab}	11^{ab}	−3^{ab}	−8^b	11.7	+	*	ns	**	ns	ns
Fat	5^{ab}	36^a	3^{ab}	7^{ab}	8^{ab}	−17^b	9.88	+	+	ns	**	ns	ns
Energy utilisation (kJ/MJ)													
DE/GE	534^{ab}	565^a	502^{bc}	496^{bc}	470^c	448^c	12.8	***	***	ns	***	ns	ns
ME/GE	417^{ab}	443^a	388^{bc}	377^{bc}	366^{bc}	348^c	12.3	**	**	ns	***	ns	ns
ME/DE	780	782	772	770	779	779	9.1	ns	ns	ns	ns	ns	ns

[1]Mean values in the same row without common superscript are significantly different at $P < 0.05$.
[2]*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, $+P < 0.1$; ns = non-significant; L = linear contrast; Q = quadratic contrast; Call v. Flem = contrast between Calliandra and Flemingia independent of dietary level.
[3]Indices 150 and 300 indicate proportions of the respective legume (Call = Calliandra, Flem = Flemingia) in the diet offered (150 or 300 g/kg DM).

Table 6 *Methane release of lambs fed grass alone or grass–legume mixtures with varying proportions of tannin-rich plants (diets* n = 6)[1]

	Experimental diets[3]								Significance[2]					
										Contrasts				
										+Calliandra		+Flemingia		
	Grass	Vigna	Call$_{150}$	Flem$_{150}$	Call$_{300}$	Flem$_{300}$	s.e.	Diet effect	L	Q	L	Q	Call *v.* Flem
l/day	19.5^{ab}	20.5^a	18.9^b	18.9^b	15.4^c	16.1^c	0.34	*	***	*	***	ns	ns
l/kg BW$^{0.75}$ per day	1.68^b	1.82^a	1.67^b	1.65^b	1.35^c	1.42^c	0.026	***	***	*	***	ns	ns
kJ/kg BW$^{0.75}$ per day	67^b	72^a	66^b	66^b	54^c	56^c	1.0	***	***	*	***	ns	ns
l/kg BW$^{0.75}$ per day	1.68^b	1.82^a	1.67^b	1.65^b	1.35^c	1.42^c	0.026	***	***	*	***	ns	ns
l/kg dry matter intake	31.1^a	30.9^a	29.0^{ab}	28.8^{ab}	26.3^b	25.3^b	0.86	***	**	ns	***	ns	ns
l/kg organic matter digested	55.0	52.2	53.0	52.9	50.7	50.3	1.31	ns	ns	ns	ns	ns	ns
l/kg NDF digested	86.4	97.0	104.0	97.6	95.9	91.0	4.41	ns	ns	ns	ns	ns	ns
kJ/MJ gross energy intake	73.7^a	74.2^a	68.5^{ab}	67.8^{ab}	61.4^{bc}	58.2^c	0.22	***	**	ns	***	ns	ns
kJ/MJ energy digested	138	132	137	137	131	130	3.7	ns	ns	ns	ns	ns	ns

[1]Mean values in the same row without common superscript are significantly different at $P < 0.05$.
[2]*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, +$P < 0.1$; ns = non-significant; L = linear contrast; Q = quadratic contrast; Call *v.* Flem = contrast between Calliandra and Flemingia independent of dietary level.
[3]Indices 150 and 300 indicate proportions of the respective legume (Call = Calliandra, Flem = Flemingia) in the diet offered (150 or 300 g/kg DM).

of digested organic matter, NDF or energy, differences due to diet were no longer evident. The methane conversion rate (energy lost via methane as proportion of GE intake) decreased linearly ($P < 0.01$) with increasing dietary proportion of the CT-rich legumes and was clearly lower at the high dietary level than with Vigna as the only legume supplement.

Discussion

The forages used in the present experiment were typical for tropical livestock systems. The cultivar of *B. brizantha* was, although low in CP (61 g/kg) compared with standards of temperate climate grasslands, a medium-quality grass compared with *Brachiaria dictyoneura* cv. Llanero (now called *B. humidicola*) (37 to 39 g CP/kg), which we previously used for *in vivo* legume supplementation studies (Abreu *et al.*, 2004; Hess *et al.*, 2004). Previous studies with different accessions of *C. calothyrsus* and *F. macrophylla* had indicated considerable intra-specific variation in their chemical composition, feeding value and CT content (Lascano *et al.*, 2003; Andersson *et al.*, 2006). However, both CT-rich species generally have CP values > 150 g/kg DM, which is also the case with the tannin-free *V. unguiculata* (e.g. Peters *et al.*, 2003; FAO, 2007). The legume accessions used were characterised by being well adapted to drought and acidic soils and were promising when used as supplements of low-quality grasses in previous *in vitro* trials (e.g. Stürm *et al.*, 2007).

Effects of the CT-rich shrub legumes on intake

CTs are known to have a wide range of effects on ruminant nutrition, digestion and health. It is known that small amounts of tannins in temperate legumes may improve milk and wool production as well as reproduction in ruminants

(Barry and McNabb, 1999). In the present study, CT content in the diets consumed was increased up to levels of 30 g/kg DM by the supplementation with Calliandra or Flemingia. This intake of CT is similar to the intake reported in other studies (e.g. Carulla *et al.*, 2005) and it is considered a moderate intake level, which may have nutritional advantages (e.g. increased bypass protein and bloat suppression in cattle; Min *et al.*, 2003). However, one often-cited negative effect is the reduced palatability associated with tannin-rich plant species (Woodward and Reed, 1989), particularly when tannin concentrations exceed 60 g/kg DM (Bhatta *et al.*, 2002). Also in the present study, intake of plants with CT concentrations of 175 g/kg (Calliandra) and 111 g/kg (Flemingia) was lower than the daily offer, especially with the high proportion of Calliandra, resulting in a reduced total DM intake. As a consequence, CT intake did not differ between Flemingia and Calliandra at the high level of dietary inclusion. Acceptance of CT-rich species improved in the course of the trial when seen over all experimental periods. This is in line with the observation of Maasdorp *et al.* (1999) that dairy cows feeding on Calliandra may adapt relatively quickly to tannin-rich diets. However, this may also depend on provenance and site of cultivation (i.e. soil fertility). For example, Kaitho *et al.* (1996) stated that *F. macrophylla* has a low palatability and feeding value, as compared to Calliandra, which was found to have a medium palatability. In the present study, the intake of Flemingia was slightly higher than that of Calliandra.

Effects of legume supplementation on protein utilisation

Both ruminal ammonia concentration and blood urea nitrogen are indicators of ruminal degradation of dietary protein. In the present study, the inclusion of Vigna in the diet increased the supply of degradable protein, while this

effect was partially reversed by the inclusion of CT-rich legumes, particularly with the higher proportion of Calliandra in the diet. The effect of Vigna supplementation on ruminal ammonia concentration was lower than in other studies where hay of *B. dictyoneura* cv. Llanero with < 40 g CP/kg DM was supplemented with CT-free legumes (Hess *et al.*, 2003 and 2004; Abreu *et al.*, 2004), but the ammonia values recorded with legume supplementation were in the same range. This, together with the observation that VFA amount and composition, total bacteria counts and fibre digestibility were not affected by the inclusion of Vigna, suggests that the ruminally available nitrogen was not limiting microbial fermentation in the rumen of lambs fed the Brachiaria-only diet.

Apart from balancing potential limitations in ruminal fermentation due to N deficiency, N-rich legumes could also enhance metabolic protein supply more directly. The changes in ruminal ammonia and blood urea nitrogen, as affected by the CT-rich legumes, were closely reflected in urinary N losses and consistent with the findings of other studies (e.g. Carulla *et al.*, 2005). This was expected as it is well documented that CT reduces ruminal protein degradation by complexing proteins and making them less degradable by ruminal microbes (Reed, 1995; Mueller-Harvey, 2006). The crucial question was whether or not the plant protein protected from ruminal degradation was digested and absorbed in the small intestine. Changes in faecal N losses were compensatory to that in urinary N losses resulting in similar total N excretion in all treatments with legume supplementation. This indicates that most, if not all, CT-protein complexes were irreversible and that the rumen-protected protein was not digested and absorbed in high quantities in the lower gut (Barry and McNabb, 1999). Based on allantoin measurements, Carulla *et al.* (2005) concluded that microbial protein synthesis was reduced by a CT extract. However, CT may also increase endogenous N losses (Norton, 2000). Whatever the reason, supplementation of CT-rich legumes seemed to have reduced body N retention compared to Vigna alone. Feeding the diet with the high Calliandra level resulted in an even lower N retention than the grass-only diet. Although not significant, only Vigna without CT-rich legumes appears to have improved metabolic protein supply of the grass-hay fed lambs, as was found earlier for the non-tanniniferous shrub legume *Cratylia argentea* in terms of duodenal protein flow (Abreu *et al.*, 2004) and body protein retention (Hess *et al.*, 2004).

The present findings suggest that CT-rich legumes are useful only when there is a shortage of medium-quality grass or when they have other favourable effects. Among the two CT-rich species used in this study as supplements, Flemingia appeared to be slightly less adverse than Calliandra, in terms of protein supply. Therefore, Calliandra CT seem to be more effective in complexing protein than CT of Flemingia. In Calliandra about 450 g and in Flemingia approximately 700 g/kg of total CT analysed were bound. Assuming that bound tannins are no artefacts as discussed by Makkar (2003), this would mean that more than twice the amount of free CT was present in Calliandra than in Flemingia. Accordingly, more protein of Vigna and of Brachiaria could have been complexed by CT in diets with Calliandra than with Flemingia.

The shift in the excretion of N from urine to faeces as a result of feeding CT-rich legumes is still of practical relevance because the ratio of urinary N to faecal N determines the level of gaseous ammonia losses (Külling *et al.*, 2001). The potential of CT to significantly decrease ammonia N emission from the animal's excreta was demonstrated by Śliwiński *et al.* (2004).

Effects of legume supplementation on energy utilisation
A first indication on the effects on energy supply is given by protozoal counts. The low number of protozoa in the rumen of animals fed grass-only diets could be related to a lack of easily degradable carbohydrates, which was corrected when legumes were supplemented. Supply with digestible (and metabolisable) energy was increased with Vigna supplementation. Although in this context the lack of effect on total VFA concentrations seems surprising, it is in agreement with observations made by Abreu *et al.* (2004), who did not find any effect of legume supplementation on total VFA concentration. Also the proportions of individual VFAs were similar to those reported by Abreu *et al.* (2004) for sheep fed diets based on low-quality Brachiaria hay. The lack of effect in VFA levels and the unchanged respiratory quotients indicate that no major shifts in the type of nutrients utilised took place. Any partial replacement by the CT-rich legumes completely reversed the improvement in energy supply caused by supplementing Vigna. It appears that this was mainly the result of a reduced digestibility of the ADF (cellulose and lignin) fraction of the fibre, while hemicellulose digestion was not affected by the inclusion of CT-rich legumes in the diet. This agrees well with the results of a previous study (Fässler and Lascano, 1995) where ADF digestion was much more affected by increasing the proportion of CT-rich foliage in the diet of sheep than either DM or NDF digestion. Adverse CT effects on ruminal fibre degradation were described earlier (Reed, 1995; Theodorou *et al.*, 2000), most likely without selective suppression of cellulolytic bacteria (McSweeney *et al.*, 2001). However, it was also claimed that fibre digestibility may be affected by analytical problems in fibre determination caused by CT (Makkar *et al.*, 1995). Since similar shifts were also evident in energy digestibility, this seems to have been a minor issue in the present study. As digestion of the grass-only diet appeared not to have been limited by low levels of ruminal ammonia (see above), the reduction in fibre digestion with addition of the CT-rich legumes seems to have been the result of a low digestibility of the fibre present in these plants rather than a side-effect of the CT on ruminal ammonia concentration. With the high dietary proportions of both CT-rich legumes, energy expenditure was similarly reduced, but this was most likely the result of the reduced supply of ME.

Utility of CT-rich shrub legumes for methane mitigation
Daily methane production averaged 13.0 (s.d. ±2.01) g per animal and was hence only between 0.6–0.5 of the mean reported in the literature for growing lambs (Pelchen and Peters, 1998; Hess *et al.* 2004). However, the animals used in the present study had slightly lower BW (18 to 32 kg compared with 25 to 45 kg of BW) and were fed diets with comparably limited digestibility. Methane conversion rate (energy lost via methane as a proportion of GE intake) averaged 67.3 (s.d. ±6.45) kJ/MJ. This value was close to the mean value of 72.2 kJ/MJ reported by Pelchen and Peters (1998), and was very close to the value of 65 kJ/MJ suggested by the IPCC (2006) for cattle fed with less than 90% concentrate.

Some studies (cf. McCrabb and Hunter, 1999) suggested that methane emission per unit of digested DM from the fermentation of C_4 plants (most tropical grasses) could be higher than that from C_3 plants (most shrubs and trees). This was not confirmed in the present study. When expressed as per kg of metabolic BW, methane emission was higher by 8% with the Vigna-supplemented diet than with the grass-only diet. However, Vigna supplementation increased nutrient digestion, thus providing additional substrate for methanogenesis, and when expressed as per unit of digested OM, methane emission did not differ between the Vigna-supplemented diet and the grass-only diet. The decline of methane emission recorded for diets including Calliandra and Flemingia is consistent with the results of a previous *in vitro* study with Calliandra, *C. argentea* and *Arachis pintoi* (Hess *et al.*, 2003), where supplementation of Calliandra to a grass-based diet suppressed methane production, while the two non-tanniniferous legumes increased methane emissions.

The discovery that CT from intact legumes or from extracts can help mitigate methane in ruminants was a recent one (Woodward *et al.*, 2001; Carulla *et al.*, 2005). Carulla *et al.* (2005) demonstrated that a CT-rich *Acacia mearnsii* extract may reduce methane per unit of digested OM but not per digested NDF. In the present study, methane produced per unit of digested NDF was not affected by supplementation with CT-rich legumes. This indicates that the methane-suppressing effect of CT might rather result from reduced fibre digestion and not from specific inhibition of methanogens, although the latter has also been observed in pure cultures (Tavendale *et al.*, 2005). In addition, the fact that the reduction in methane production caused by CT-rich legumes was not associated with a shift in VFA production or a reduction in the number of rumen protozoa suggests that the methane-suppressing effect of CT was mainly the result of a reduced fermentation activity and not of a specific shift in the nutrients fermented or in microbial populations. As long as overall nutrient and energy utilisation are not simultaneously reduced with methane production (i.e. if the decline in methane is not associated with lower feeding value and therefore productivity of the animal), lower fibre digestion *per se* would not be a problem. While this was not the case in the study of Carulla *et al.*

(2005), in the present experiment methane suppression was associated with reduced N (Calliandra) and energy (Flemingia) retention. Therefore it is questionable whether the methane-suppressing effect of Calliandra and Flemingia is of any practical value because it would probably be accompanied with a simultaneous reduction in animal performance (i.e. BW gain or milk yield).

Conclusions

Through an *in vivo* energy and protein balance study it was now demonstrated that *V. unguiculata* is a valuable supplement to tropical grass diets, since it improves not only protein but also energy supply to growing ruminants. The favourable effects of Vigna might be even higher in cases where only very low-quality grasses or crop residues are available to feed ruminants. On the other hand, *F. macrophylla* (CIAT 17403) seems to be a suitable alternative for partial replacement of Vigna in terms of protein supply in diets where energy is not limiting. *C. calothyrsus* (CIAT 22310) seems to be less apt as ruminant feed and cannot be recommended. Although Calliandra and Flemingia were effective in reducing methane production of sheep, the true methane mitigation to be achieved appears to be small due to associated reductions in N and energy retention.

References

Abreu A, Carulla JE, Lascano CE, Dıaz TE, Kreuzer M and Hess HD 2004. Effects of *Sapindus saponaria* fruits on ruminal fermentation and duodenal nitrogen flow of sheep fed a tropical grass diet with and without legume. Journal of Animal Science 82, 1392–1400.

ALP 2006. Fütterungsempfehlungen und Nährwerttabellen für Wiederkäuer (Feeding Recommendations and Nutrient Tables). Online version. Ed Forschungsanstalt Agroscope Liebefeld-Posieux ALP, Posieux. Retrieved July 13, 2007 from http://www.alp.admin.ch/dokumentation/00611/00631/index.html?lang=de

Andersson MS, Lascano CE, Schultze-Kraft R and Peters M 2006. Forage quality and tannin concentration and composition of a collection of the tropical shrub legume *Flemingia macrophylla*. Journal of the Science of Food and Agriculture 86, 1023–1031.

AOAC 1990. Official methods of analysis, 15th edition. Association of Official Analytical Chemists, Washington, DC, USA.

Barahona R, Lascano CE, Narvaez N, Owen E, Morris P and Theodorou MK 2003. In vitro degradability of mature and immature leaves of tropical forage legumes differing in condensed tannin and nonstarch polysaccharide content and composition. Journal of the Science of Food and Agriculture 83, 1256–1266.

Barry TN and McNabb WC 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. The British Journal of Nutrition 81, 263–272.

Bhatta R, Shinde AK, Vaithiyanathan S, Sankhyan SK and Verma DL 2002. Effect of polyethylene glycol-6000 on nutrient intake, digestion and growth of kids browsing Prosopis cineraria. Animal Feed Science and Technology 101, 45–54.

Brouwer E 1965. Report of subcommittee on constants and factors. In Energy Metabolism of Farm Animals. Third Symposium on Energy Metabolism (ed. KL Blaxter), EAAP Publ. 11, pp 441–443. Academic Press, London, UK.

Carulla JE, Kreuzer M, Machmüller A and Hess HD 2005. Supplementation of Acacia mearnsii tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Australian Journal of Agricultural Research 56, 951–969.

Chwalibog A, Jensen K and Thorbek G 1996. Oxidation of nutrients in bull calves treated with adrenergic agonists. Archives of Animal Nutrition 49, 255–261.

Doane PH, Pell AN and Schofield P 1998. Ensiling effects on the ethanol fraction of forages using gas production. Journal of Animal Science 76, 888–895.

Ehrlich GG, Goerlitz DF, Bourell JH, Eisen GV and Godsy EM 1981. Liquid chromatographic procedure for fermentation product analysis in the identification of anaerobic bacteria. Applied and Environmental Microbiology 42, 878–886.

FAO 2007. Grassland Index. For Flemingia: Retrieved July 18, 2007 from http://www.fao.org/AG/AGP/agpc/doc/gbase/data/Pf000154.HTM, for Calliandra: Retrieved July 18, 2007 from http://www.fao.org/AG/AGP/agpc/doc/gbase/data/pf000470.htm

Fässler OM and Lascano CE 1995. The effect of mixtures of sun-dried tropical shrub legumes on intake and nitrogen balance by sheep. Tropical Grasslands 29, 92–96.

Hess HD, Monsalve LM, Lascano CE, Carulla JE, Díaz TE and Kreuzer M 2003. Supplementation of a tropical grass diet with forage legumes and Sapindus saponaria fruits: effects on in vitro ruminal nitrogen turnover and methanogenesis. Australian Journal of Agricultural Research 54, 703–713.

Hess HD, Beuret RA, Loetscher M, Hindrichsen IK, Machmüller A, Carulla JE, Lascano CE and Kreuzer M 2004. Ruminal fermentation, methanogenesis and nitrogen utilization of sheep receiving tropical grass hay-concentrate diets offered with Sapindus saponaria fruits and Cratylia argentea foliage. Animal Science 79, 177–189.

Hoffmann L and Klein M 1980. Die Abhängigkeit der Harnenergie vom Kohlenstoff- und Stickstoffgehalt im Harn bei Rindern, Schafen, Schweinen und Ratten. Archiv für Tierernährung 30, 743–750.

IPCC 2006. Guidelines for National Greenhouse Gas Inventories. Volume 4: Agriculture, forestry and other land use. Retrieved November 1, 2007 from http://www.ipcc-nggip.iges.or.jp/public/2006gl/pdf/4_Volume4/V4_10_Ch10_Livestock.pdf

Kaitho RJ, Umunna NN, Nsahlai IV, Tamminga S, van Bruchem J, Hanson J and van de Wouw M 1996. Palatability of multipurpose tree species: effect of species and length of study on intake and relative palatability by sheep. Agroforestry Systems 33, 249–261.

Külling DR, Menzi H, Kröber TF, Neftel A, Sutter F, Lischer P and Kreuzer M 2001. Emissions of ammonia, nitrous oxide and methane from different types of dairy manure during storage as affected by dietary protein content. Journal of Agricultural Science 137, 235–250.

Lascano C, Avila P and Stewart J 2003. Intake, digestibility and nitrogen utilization by sheep fed with provenances of Calliandra calothyrsus Meissner with different tannin structure. Archivos Latinoamericanos de Producción Animal 11, 21–28.

Maasdorp BV, Muchenje V and Titterton M 1999. Palatability and effect on dairy cow milk yield of dried fodder from the forage trees Acacia boliviana, Calliandra calothyrsus and Leucaena leucocephala. Animal Feed Science and Technology 77, 49–59.

Makkar HPS 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Ruminant Research 49, 241–256.

Makkar HPS, Borowy NK, Becker K and Degen A 1995. Some problems in fibre determination of a tannin-rich forage (Acacia saligna leaves) and their implications in in vivo studies. Animal Feed Science and Technology 55, 67–76.

McCrabb GJ and Hunter RA 1999. Prediction of methane emission from beef cattle in tropical production systems. Australian Journal of Agricultural Research 50, 1335–1339.

McSweeney CS, Palmer B, Bunch R and Krause DO 2001. Effect of the tropical forage Calliandra on microbial protein synthesis and ecology in the rumen. Journal of Applied Microbiology 90, 78–88.

Min BR, Barry TN, Attwood GT and McNabb WC 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Animal Feed Science and Technology 106, 3–19.

Mueller-Harvey I 2006. Unravelling the conundrum of tannins in animal nutrition and health. Journal of the Science of Food and Agriculture 86, 2010–2037.

Norton BW 2000. The significance of tannins in tropical animal production. In Tannins in livestock and human nutrition. Proceedings of an International Workshop (ed. JD Brooker), pp. 14–23. Australian Centre for International Agricultural Research, Canberra, Australia. .

Pelchen A and Peters KJ 1998. Methane emissions from sheep. Small Ruminant Research 27, 137–150.

Peters M, Franco LH, Schmidt A and Hincapié B 2003. Especies forrajeras multipropósito: opciones para productores de Centroamérica. Publicación CIAT No 333. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. 113 pp.

Reed JD 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. Journal of Animal Science 73, 1516–1528.

SAS (SAS Institute, Inc.) 1999. SAS Online Doc version 8. SAS Institute Inc. Cary, NC [http://v8doc.sas.com/sashtml/].

Śliwiński BJ, Kreuzer M, Sutter F, Machmüller A and Wettstein H-R 2004. Performance, body nitrogen conversion and nitrogen emission from manure of dairy cows fed diets supplemented with different plant extracts. Journal of Animal and Feed Sciences 13, 73–91.

Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales M and de Haan C 2006. Livestock's Long Shadow. Environmental Issues and Options. FAO, Rome, Italy.

Stürm CD, Tiemann TT, Lascano CE, Kreuzer M and Hess HD 2007. Nutrient composition and in vitro ruminal fermentation of tropical legume mixtures with contrasting tannin contents. Animal Feed Science and Technology 138, 29–46.

Tavendale MH, Meagher LP, Pacheco D, Walker N, Attwood GT and Sivakumaran S 2005. Methane production from in vitro rumen incubations with Lotus pedunculatus and Medicago sativa, and effects of extractable condensed tannin fractions on methanogenesis. Animal Feed Science and Technology 123–124, 403–419.

Terrill TH, Rowan AM, Douglas GB and Barry TN 1992. Determination of extractable and bound condensed tannin concentrations in forage plants, protein concentrate meals and cereal grains. Journal of the Science of Food and Agriculture 58, 321–329.

Theodorou MK, Barahona R, Kingston-Smith A, Sanchez S, Lascano C, Owen E and Morris P 2000. New perspectives on the degradation of plant biomass in the rumen in the absence and presence of condensed tannins. In Tannins in livestock and human nutrition. Proceedings of an International Workshop (ed. JD Brooker), pp. 44–51. Australian Centre for International Agricultural Research, Canberra, Australia.

Waghorn GC, Tavendale MH and Woodfield DR 2002. Methanogenesis from forages fed to sheep. Proceedings of the New Zealand Grassland Association 64, 167–171.

Woodward A and Reed JD 1989. The influence of polyphenolics on the nutritive value of browse: a summary of research conducted at ILCA. ILCA Bulletin 35, 2–11.

Woodward SI, Waghorn GC, Ulyatt MJ and Lassey KR 2001. Early indications that feeding Lotus will reduce methane emissions from ruminants. Proceedings of the New Zealand Society of Animal Production 61, 23–26.