Tannins in Tropical Browses: Effects on in Vitro Microbial Fermentation and Microbial Protein Synthesis in Media Containing Different Amounts of Nitrogen

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Four species of browses (*Acacia angustissima*, *Acacia salicina*, *Calliandra calothyrsus*, and *Dichrostachys cinerea*) were used to study the effect of tannins on microbial fermentation and microbial protein synthesis in incubation media containing high nitrogen (HN) and low nitrogen (LN) in the presence and absence of polyethylene glycol (PEG, MW 6000). The additional nitrogen in HN medium was supplied through ammonium bicarbonate. The use of HN medium significantly ($P < 0.05$) increased the in vitro gas and short-chain fatty acid (SCFA) production and microbial protein synthesis compared to the LN medium. Incubation of tannin-containing browses alone produced significantly ($P < 0.05$) lower gas and SCFA compared to in the presence of PEG in both HN and LN media. Inclusion of PEG in tannin-containing browses significantly ($P < 0.05$) reduced the molar proportion of propionate compared to in its absence. Higher N in the media resulted in 10.4 and 9.9% increases in in vitro gas and SCFA production, respectively, whereas inclusion of PEG to tannin-containing feed to remove the effect of tannins increased the in vitro gas and SCFA production by 186 and 195%, respectively, indicating that the low fermentation of tannin-containing browses could be due to the depressive effects of tannins on microbial activity and only partially accounted for by unavailability of N for rumen microbes. Incubation of browses with straw significantly ($P < 0.05$) decreased ammonia nitrogen concentration but increased the in vitro gas and SCFA production and microbial protein synthesis compared to straw alone.

Keywords: *Tannins; browses; microbial protein synthesis; polyethylene glycol (PEG)*

INTRODUCTION

Livestock productivity in tropical regions is limited mainly by inadequate supply and poor quality of feeds. Browse species, because of their resistance to harsh environmental conditions (heat, drought, salinity, alkalinity, drifting sand, grazing, and repeated cutting), are the major available feed resources during the dry season (Fagg and Stewart, 1994). Browses are known to be higher in protein content than grasses (McDonald and Ternouth, 1979; Rittner and Reed, 1992), which tend to remain green longer into the dry season than herbaceous legumes, and their chemical compositions vary very little with season. Browses are used freshly lopped in the dry season or as dry leaves conserved for feed after collection. In addition to their extensive use in arid and semiarid regions, browse trees are becoming particularly important in more humid, agriculturally productive areas where the increasing human population has necessitated the cultivation of grazing land. A number of entry points have been identified for such an integration, for example, live fences, fodder banks, alley farms, hedge crops, wind breaks, improved fallows, and multistrata systems, as sources of home-grown supplements for low-quality crop residues (Larbi et al., 1998). Despite their potential as feed resources for animals, most tropical browse species contain secondary metabolites, particularly tannins. During disintegration of plant material, such as chewing by ruminants, tannins interact with dietary proteins to form insoluble tannin−protein complexes (Jones and Mangan, 1977). Tannins at higher concentration reduced the nutritive value of tannin-containing browses (Kumar and Singh, 1984; Terrill et al., 1992; Silanikove et al., 1996). It has not been well established whether the depression in nutritive value caused by tannins was due to low nitrogen availability (as a result of reduced protein degradability due to tannins) to the microbes in the rumen or reduced microbial activity. The effect of tannins on the nutritive value of tannin-containing browses can be studied with the use of tannin-binding agents such as polyethylene glycol (PEG), which strongly binds to tannins and thus inhibits their biological effects. Comparison can then be made between the biological properties of tannin-containing browses with and without PEG, the difference being a measure of the effect of tannins found in these browses.

The principal objective of the present study was to determine whether the degradation of tannin-containing browses is affected by low nitrogen availability for rumen microbes or reduced microbial activity or a combination of both processes. This was examined by incubating tannin-containing browses in buffered rumen fluid containing high nitrogen (HN) and low nitrogen (LN) media. PEG was used to remove the biological activity of tannins. The supplementary effect of tannin-containing browse leaves to cereal straw was also assessed.

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Table 1. Contents (Grams per Kilogram of DM) of OM, CP, NDF, ADF, ADL, TP, TT, and CT in Browses and Straws Incubated in Medium Containing Different Levels of Nitrogen[a]

species	OM	CP	NDF	ADF	ADL	TP	TT	CT
A. angustissima	902.0	224.5	320.7	471.4	339.5	230.5	211.5	16.5
A. salicina	875.5	135.5	391.9	353.5	215.3	173.0	104.0	262.5
C. calothyrsus	904.0	197.7	333.5	418.0	267.4	202.5	139.0	60.5
D. cinerea	914.5	140.8	378.8	502.0	335.1	214.5	138.0	124.0
teff straw	947.5	26.9	769.0	543.9	123.2	ND	ND	ND
wheat straw	920.5	23.4	768.5	612.7	122.9	ND	ND	ND

[a] CT, as leucoanthocyanidin equivalent; TP and TT, as tannic acid equivalent; ND, not determined.

MATERIALS AND METHODS

Experiment 1. Leaf samples from four browse species (*Acacia angustissima*, *Acacia salicina*, *Calliandra calothyrsus*, and *Dichrostachys cinerea*) were collected from the International Livestock Research Institute (ILRI) seed multiplication site at Zwai in the Rift valley of Ethiopia. Two cereal straws (teff and wheat straws) were obtained from the Institute of Agricultural Research (IAR, Ethiopia). Freeze-dried browse leaves and straws were ground to pass through a 1 mm screen. The incubation was carried out in the presence and absence of PEG (PEG, MW 6000) using the in vitro gas method of Menke et al. (1979). Rumen fluid was taken before morning feeding from a rumen-cannulated dairy cow receiving ~3 kg of grass hay and ad libitum straw. Rumen fluid was collected into a prewarmed Thermos bottle, homogenized in a laboratory blender, strained using nylon cloth of 100 μm, and then filtered through glass wool. All handling was carried out under continuous flushing with CO_2. The amounts of sample and PEG in the incubation medium were 0.5 and 1 g, respectively. The preparation of incubation medium was similar to that described in Makkar et al. (1995) except that for the LN medium, ammonium bicarbonate was omitted. Therefore, in the LN medium the nitrogen source was only that which was contributed by rumen fluid, whereas the HN medium contained nitrogen from the rumen fluid and the added ammonium bicarbonate. The incubation medium (40 mL) at the start of the incubation contained 7.43 ± 0.197 and 0.78 ± 0.157 ($n = 9$) mg of N in the HN and LN systems, respectively. Incubations were carried out in two sets. The first set was terminated at 16 h and the second at 24 h. At the end of the incubation, gas production was recorded and the contents of the syringes were transferred to centrifuge tubes and centrifuged at 23000*g* for 20 min. The supernatant was pipetted into 50 mL plastic bottles and stored in a freezer until it was analyzed for ammonia nitrogen (NH_3-N) and short-chain fatty acids (SCFA). The syringes were washed twice with a total of 50 mL of distilled water. To transfer quantitatively all of the contents of the syringes, each time the washing solution was emptied from the syringes into the same centrifuge tubes containing the pellet from the first centrifugation. The tubes were centrifuged once more at 23000*g* for 20 min. The residual pellet was frozen and lyophilized overnight in the tubes. The residual moisture, if any, was removed by drying the tubes overnight at 60 °C. After the tubes had been weighed, the pellet (the apparently undegraded residue) was used for the determination of purine bases (adenine and guanine) (Makkar and Becker, 1999). Purines were used as markers for the determination of microbial protein synthesis (Broderick and Merchen, 1992). Values for SCFA and NH_3-N were expressed per 40 mL of incubation medium.

Experiment 2. Three species of browse leaves (*C. calothyrsus*, *D. cinerea*, and *A. salicina*) were used to supplement teff straw (*Eragrostis tef*) in vitro. Incubations were carried out using the LN medium as described in experiment 1. Browse leaves (100 mg with or without 200 mg of PEG) were incubated with teff straw (400 mg) in 40 mL of medium. Four sets of incubations (in triplicate) were used for *Calliandra* (incubations were terminated after 8, 12, 16, and 24 h) and three sets for *Dichrostachys* and *Acacia*, for which incubations were terminated after 8, 16, and 24 h. Rumen NH_3-N and SCFA in the supernatant and purine bases in the apparently undigested substrate were determined.

Chemical Analysis. Dry matter and organic matter were determined according to AOAC (1980) methods. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined following the method of Van Soest et al. (1991). Crude protein (N × 6.25) was measured by Kjeldahl digestion followed by distillation (AOAC, 1980). NH_3-N in the supernatant was also determined using the Kjeldahl procedure by distilling 15 mL of the supernatant under alkaline condition (Makkar and Becker, 1996). Total phenol (TP) was determined by using Folin−Ciocalteu reagents, total tannins (TT) as the difference of phenolics before and after tannin removal using the insoluble polyvinylpyrrolidone (Makkar et al., 1993), and condensed tannin (CT) using the butanol−HCl−iron reagent (Porter et al., 1986). TP and TT were expressed as tannic acid equivalents and condensed tannins as leucoanthocyanidin equivalents. Purine determination in residues after different times of incubation was carried out using the method described by Makkar and Becker (1999). SCFA in the supernatant were determined using a Hewlett-Packard gas chromatograph (Aiple, 1993).

Statistical Analysis. In vitro gas, SCFA, and purine data were subjected to analysis of variance (ANOVA) using the General Linear Model of SAS (1988). Differences in mean values between treatment groups were tested using the *t* test, and all pairwise multiple comparisons were carried out using Tukey's test (Montgomery, 1984). Values are expressed as mean ± SD.

RESULTS

Experiment 1. *Chemical Composition and Phenolic Contents.* Organic matter (OM), crude protein (CP), NDF, ADF, and phenolic compound concentrations of browses and straws used in the experiment are presented in Table 1. *A. angustissima* had the lowest CT content, but its total phenolic content was comparable to those of the other three species. *A. salicina* and *D. cinerea* were lower in CP content than *A. angustissima* and *C. calothyrsus*.

In Vitro Gas and SCFA Production. The in vitro gas production on incubation of browses and straw in media containing different amounts of N at different times of incubation is presented in Table 2. The addition of N (HN medium) significantly ($P < 0.05$) increased the in vitro gas production in *C. calothyrsus* at 16 h and in *A. salicina* at 16 and 24 h of incubation. In *A. salicina*, the HN in the medium increased gas production even in the presence of PEG. The response in gas production to the HN medium was higher for the tannin-containing feed alone as compared to the tannin-containing feed plus PEG. The added nitrogen in the medium increased gas production by 0−13.9 and 0−23.6% after 16 and 24 h, respectively, when tannin-containing browses were incubated alone. Addition of PEG to tannin-containing browses significantly increased gas production ($P < 0.05$) in all of the browses after 16 and 24 h incubations in both LN and HN media. The increase in the gas production by the added nitrogen in the presence of PEG ranged from 0 to 4.8%. The HN medium resulted in 18.3 and 12.7% higher gas than LN medium from teff and wheat straw, respectively.

Table 2. In Vitro Gas Production (Milliliters)[a] on Incubation of Tannin-Containing Feeds (with and without PEG) and Straws in LN and HN Media

	16 h		24 h	
feed	LN	HN	LN	HN
A. angustissima	8.5 aA ± 0.8	9.3 aA ± 0.8	13.4 aA ± 1.1	13.8 aA ± 0.8
A. angustissima + PEG	56.9 bA ± 1.0	58.2 bA ± 0.3	60.7 bA ± 0.8	61.9 bA ± 0.8
C. calothyrsus	16.4 aA ± 0.6	18.3 aB ± 0.3	17.3 aA ± 1.5	19.9 aA ± 1.3
C. calothyrsus + PEG	57.7 bA ± 1.2	59.5 bA ± 1.5	65.9 bA ± 0.8	67.1 bA ± 1.0
D. cinerea	24.6 aA ± 0.3	23.1 aA ± 1.0	30.8 aA ± 0.8	30.1 aA ± 0.9
D. cinerea + PEG	66.7 bA ± 0.2	63.5 bB ± 1.3	78.7 bA ± 1.3	77.2 bA ± 1.4
A. salicina	37.5 aA ± 1.0	42.7 aB ± 0.3	44.4 aA ± 2.0	54.8 aB ± 0.2
A. salicina + PEG	78.6 bA ± 0.5	80.2 bB ± 0.4	83.8 bA ± 1.3	87.8 bB ± 0.9
teff straw	ND	ND	57.9 A ± 1.6	68.5 B ± 0.1
wheat straw	ND	ND	58.1 A ± 1.6	65.5 B ± 0.6

[a] Corrected for respective blank incubations. Capital letters in rows within incubation time indicate significant difference at $P < 0.05$. Small letters in columns within species indicate significant difference at $P < 0.05$. ND, not determined. Values are expressed as mean ± SD ($n = 3$).

Table 3. Net Production of SCFA (Millimoles per 40 mL) from Incubation of Tannin-Containing Browses and Straws in LN and HN Media[a]

	16 h		24 h	
feed	LN	HN	LN	HN
A. angustissima	0.112 aA ± 0.003	0.160 aB ± 0.005	0.256 aA ± 0.024	0.340 aB ± 0.024
A. angustissima + PEG	1.370 bA ± 0.066	1.414 bA ± 0.053	1.470 bA ± 0.008	1.598 bB ± 0.015
C. calothyrsus	0.331 aA ± 0.023	0.343 aA ± 0.024	0.465 aA ± 0.014	0.476 aA ± 0.030
C. calothyrsus + PEG	1.310 bA ± 0.081	1.363 bA ± 0.051	1.607 bA ± 0.044	1.554 bA ± 0.027
D. cinerea	0.493 aA ± 0.029	0.530 aA ± 0.017	0.757 aA ± 0.032	0.776 aA ± 0.025
D. cinerea + PEG	1.372 bA ± 0.073	1.455 bA ± 0.030	1.794 bA ± 0.034	1.721 bA ± 0.055
A. salicina	0.731 aA ± 0.026	0.802 aB ± 0.016	0.812 aA ± 0.000	1.069 aB ± 0.024
A. salicina + PEG	1.212 bA ± 0.038	1.546 bB ± 0.029	1.825 bA ± 0.034	1.856 bA ± 0.050
teff straw	ND	ND	1.139 A ± 0.017	1.346 B ± 0.002
wheat straw	ND	ND	1.103 A ± 0.005	1.194 B ± 0.017

[a] Capital letters in rows within incubation time indicate significant difference at $P < 0.05$. Small letters in columns within species indicate significant difference at $P < 0.05$. ND, not determined. Values are expressed as mean ± SD ($n = 3$).

The net production of SCFA on incubation of browses and straws is indicated in Table 3. The HN medium significantly ($P < 0.01$) increased the SCFA production in *A. angustissima* and *A. salicina*, but had no effect ($P > 0.05$) in *C. calothyrsus* and *D. cinerea* after both 16 and 24 h. In *A. salicina*, the HN medium increased the SCFA even in the presence of PEG when measured at 16 h of incubation. Incubation of tannin-containing browses with PEG significantly ($P < 0.01$) increased the SCFA production. HN in the medium significantly ($P < 0.05$) increased SCFA production from incubation of straws.

In the absence of PEG, HN medium significantly ($P < 0.05$) increased the molar proportion of acetate in *A. angustissima* and *C. calothyrsus* and tended to increase in the other two species (Table 4). Addition of PEG to tannin-containing browses to remove the effect of tannins significantly ($P < 0.05$) decreased molar proportion of propionate with the exception of *C. calothyrsus*, which tended to yield higher propionate on the addition of PEG after 24 h of incubation. Addition of PEG increased the production of butyrate in both LN and HN media. HN in the medium increased the proportion of propionate from straws.

Ammonia Nitrogen Concentration and Microbial Protein Synthesis. Ammonia N concentration in the supernatant on incubation of browses and straws is given in Table 5. Incubation of browses with PEG resulted in higher ammonia N concentration in the supernatant as compared to its absence in both LN and HN media. In the absence of PEG, there was a reduction in ammonia N at both 16 and 24 h of incubation of *D. cinerea* and *A. salicina* in LN medium compared to the amount at the beginning of incubation.

Table 6 shows the net production of purines (purines were considered as microbial marker) on incubation of browses and straws in media containing different amounts of N. The HN medium significantly ($P < 0.05$) increased microbial protein synthesis in *A. angustissima* and *D. cinerea*. Addition of PEG to *A. angustissima* and *D. cinerea* tended to increase microbial protein synthesis at 16 h of incubation in both LN and HN media. However, at 24 h of incubation, PEG resulted in significantly ($P < 0.05$) lower microbial protein synthesis compared to its absence.

Experiment 2. *In Vitro Gas and SCFA Production.* The extent of in vitro gas and SCFA production on supplementation of browse leaves to teff straw varied markedly with species (Table 7). Supplementation of straw with browse leaves increased the in vitro gas and SCFA production compared to straw alone. However, the values obtained by supplementation were lower than those predicted from separate incubations of the individual components. Generally, tannins in tannin-containing browses depressed SCFA production compared to that incubated with PEG. Addition of PEG to *C. calothyrsus* increased SCFA production by 82.3, 70.2, 47.6, and 44.3% at 8, 12, 16, and 24 h of incubation, respectively. The corresponding values for *D. cinerea* were 25.9, 52.5, and 46.3% at 8, 16, and 24 h of incubation. In *A. salicina* the increase in SCFA production due to PEG was 36.7, 34.6, and 26.0% at 8, 16, and 24 h of incubation, respectively.

Table 4. Molar Proportions (Percent) of Acetate, Propionate, and Butyrate from Incubation of Tannin-Containing Browses and Straws in LN and HN Media[a]

	16 h			24 h		
feed	C2	C3	C4	C2	C3	C4
A. angustissima LN	45.1d	54.9a	0.0	69.2c	30.8a	0.0
A. angustissima HN	60.8c	39.2b	0.0	73.7b	26.3b	0.0
A. angustissima + PEG LN	75.3a	19.9c	4.9	75.6a	19.4c	4.8
A. angustissima + PEG HN	76.1a	19.2c	4.6	76.1a	19.0c	4.9
C. calothyrsus LN	76.4b	19.9a	3.6	80.8b	17.2a	2.0
C. calothyrsus HN	78.6a	19.1b	2.3	82.9a	15.4b	1.7
C. calothyrsus + PEG LN	75.6c	18.7b	5.7	76.6c	17.7a	5.6
C. calothyrsus + PEG HN	76.1bc	18.4b	5.5	76.9c	17.6a	5.5
D. cinerea LN	66.5d	29.5a	4.0	73.3a	25.2a	1.6
D. cinerea HN	67.7c	29.0a	3.0	73.6a	25.2a	1.2
D. cinerea + PEG LN	72.1ba	22.7b	5.2	73.9a	21.2b	5.0
D. cinerea + PEG HN	72.5a	22.5b	5.0	73.7a	21.1b	5.2
A. salicina LN	72.2a	26.9a	0.9	73.4a	25.8a	0.8
A. salicina HN	73.0a	26.1ab	1.0	74.8b	24.6b	0.6
A. salicina + PEG LN	69.7b	25.7bc	4.6	70.4c	24.2bc	5.4
A. salicina + PEG HN	70.1b	24.9c	5.0	70.8c	23.9c	5.3
teff straw LN	ND	ND	ND	71.5a	22.5a	6.0
teff straw HN	ND	ND	ND	69.0b	24.6b	6.4
wheat straw LN	ND	ND	ND	69.6a	23.8a	6.5
wheat straw HN	ND	ND	ND	68.2b	25.5b	6.4

[a] C2, acetate; C3, propionate; C4, butyrate. Different letters in columns within feeds indicate significant difference at $P < 0.05$. ND, not determined.

Table 5. NH_3-N Concentration in Supernatant (Milligrams per 40 mL) on Incubation of Tannin-Containing Browses (with and without PEG) and Straws in LN and HN Media[a]

	16 h		24 h	
feed	LN	HN	LN	HN
media[b] (0.58 ± 0.05 and 7.19 ± 0.05)				
A. angustissima	0.95B ± 0.04	7.25B ± 0.07	0.87B ± 0.05	7.31B ± 0.14
A. angustissima + PEG	3.97A ± 0.06	9.93A ± 0.00	6.58A ± 1.39	11.72A ± 0.10
C. calothyrsus	0.63B ± 0.05	6.85B ± 0.18	0.64B ± 0.11	6.94B ± 0.15
C. calothyrsus + PEG	4.15A ± 0.25	10.35A ± 0.08	7.23A ± 0.03	12.55A ± 0.21
media[b] (0.85 ± 0.02 and 7.50 ± 0.05)				
D. cinerea	0.62B ± 0.05	7.44B ± 0.39	0.47B ± 0.03	6.58B ± 0.12
D. cinerea + PEG	2.70A ± 0.06	8.86A ± 0.09	4.29A ± 0.18	10.05A ± 0.15
teff straw	ND	ND	0.85 ± 0.02	7.50 ± 0.05
media[b] (0.92 ± 0.01 and 7.61 ± 0.05)				
A. salicina	0.22B ± 0.07	5.77B ± 0.43	0.13B ± 0.01	5.62B ± 0.02
A. salicina + PEG	3.11A ± 0.13	8.90A ± 0.15	4.71A ± 0.01	10.54A ± 0.26
wheat straw	ND	ND	0.61 ± 0.03	6.29 ± 0.07

[a] ND, not determined. Different letters in columns within species indicate significant difference at $P < 0.05$. Values are expressed as mean ± SD ($n = 3$). [b] NH_3-N (mg) in the incubation medium at the beginning of incubation for LN and HN, respectively.

Table 6. Net Productions of Purines (Micromoles) in Apparently Undegraded Residues after Incubation of Tannin-Containing Browses and Straws in LN and HN Media[a]

	16 h		24 h	
feed	LN	HN	LN	HN
A. angustissima	5.7 aA ± 0.153	6.1 aB ± 0.115	6.4 aA ± 0.404	6.5 aA ± 0.100
A. angustissima + PEG	6.4 bA ± 0.100	6.6 bB ± 0.058	5.4 bA ± 0.058	5.5 bA ± 0.306
C. calothyrsus	5.6 aA ± 0.153	5.7 aA ± 0.100	5.5 aA ± 0.289	5.6 aA ± 0.208
C. calothyrsus + PEG	5.0 bA ± 0.252	5.4 aA ± 0.208	4.0 bA ± 0.058	4.4 bB ± 0.000
D. cinerea	3.5 aA ± 0.566	5.0 aB ± 0.361	5.5 aA ± 0.566	6.8 aA ± 0.919
D. cinerea + PEG	4.6 aA ± 0.495	5.6 aA ± 0.529	3.8 bA ± 0.346	4.1 aA ± 0.212
A. salicina	5.5 aA ± 0.424	5.5 aA ± 0.404	4.4 aA ± 0.265	6.8 aB ± 0.141
A. salicina + PEG	5.5 aA ± 0.346	7.1 bB ± 0.636	4.6 aA ± 0.265	4.0 bA ±0.141
teff straw	ND	ND	3.5 A ± 0.240	5.1 B ± 0.503
wheat straw	ND	ND	2.9 A ± 0.321	3.8 B ± 0.361

[a] Capital letters in rows within incubation time indicate significant difference at $P < 0.05$. Small letters in columns within species indicate significant difference at $P < 0.05$. ND, not determined. Values are expressed as mean ± SD ($n = 3$).

Ammonia Nitrogen Concentration and Microbial Protein Synthesis. The response of browse supplementation on NH_3-N concentration varied with species (Table 8). The NH_3-N concentration on incubation of teff straw alone as well as when incubated with browse (in the absence of PEG) was markedly lower than that at 0 h (at the beginning of incubation). The lowest NH_3-N concentration was observed on incubation of *C. calothyrsus* and straw together. Supplementation of straw with *C. calothyrsus* and *A. salicina* reduced the NH_3-N concentration after 8, 16, and 24 h of incubation. *D. cinerea* supplementation tended to increase NH_3-N concentration after 8 and 16 h of incubation but decreased at 24 h of incubation. Incubation of browses with PEG resulted in 75, 126, and 48% higher NH_3-N after 24 h in *C. calothyrsus*, *D. cinerea*, and *A. salicina*,

Table 7. In Vitro Gas and SCFA Production on Incubation of Teff Straw Supplemented with Tannin-Containing Browses in the Presence and Absence of PEG in LN dsMedium[a]

	in vitro gas (mL)				SCFA (mmol/40 mL)			
feed	8 h	12 h	16 h	24 h	8 h	12 h	16 h	24 h
C. calothyrsus	4.0D ± 0.6	5.5D ± 0.2	5.0D ± 0.6	7.0E ± 0.5	0.16D ± 0.010	0.22D ± 0.024	0.27D ± 0.018	0.33E ± 0.008
C. calothyrsus + PEG	9.7C ± 0.0	11.8C ± 1.0	11.7C ± 0.3	14.5D ± 1.0	0.29C ± 0.022	0.37C ± 0.028	0.39C ± 0.033	0.48D ± 0.043
teff straw	23.7B ± 1.0	33.2B ± 1.2	40.0B ± 0.3	56.3C ± 1.3	0.56B ± 0.029	0.73B ± 0.004	0.91B ± 0.014	1.24C ± 0.032
C. calothyrsus + straw	24.8B ± 0.6	34.2B ± 1.2	40.0B ± 0.6	59.2B ± 0.6	0.60B ± 0.004	0.69B ± 0.034	0.91B ± 0.023	1.37B ± 0.026
C. calothyrsus + PEG + straw	31.7A ± 0.0	43.8A ± 1.0	49.3A ± 1.0	71.8A ± 0.3	0.73A ± 0.016	0.99A ± 0.030	1.14A ± 0.053	1.65A ± 0.027
D. cinerea	4.8D ± 0.0	ND	8.2D ± 0.0	10.8D ± 0.6	0.20C ± 0.005	ND	0.26D ± 0.035	0.33E ± 0.022
D. cinerea + PEG	9.8C ± 0.0	ND	15.0C ± 0.3	17.5C ± 0.3	0.25C ± 0.031	ND	0.40C ± 0.024	0.48D ± 0.024
teff straw	20.2B ± 0.6	ND	36.7B ± 0.9	51.0B ± 0.8	0.51B ± 0.017	ND	0.82B ± 0.027	1.01C ± 0.011
D. cinerea + teff straw	19.3B ± 0.5	ND	35.3B ± 1.3	49.0B ± 2.8	0.55B ± 0.012	ND	0.80B ± 0.031	1.15B ± 0.042
D. cinerea + PEG + teff straw	29.8A ± 0.0	ND	51.7A ± 0.5	68.8A ± 0.6	0.67A ± 0.074	ND	1.11A ± 0.033	1.39A ± 0.029
A. salicina	8.8E ± 0.0	ND	12.3E ± 0.6	13.7E ± 0.3	0.30E ± 0.032	ND	0.36E ± 0.074	0.47E ± 0.011
A. salicina + PEG	15.2D ± 0.0	ND	18.0D ± 0.0	18.8D ± 0.0	0.40D ± 0.015	ND	0.48D ± 0.002	0.59D ± 0.024
teff straw	25.7C ± 0.3	ND	43.2C ± 0.3	59.0C ± 0.3	0.55C ± 0.032	ND	0.96C ± 0.029	1.28C ± 0.004
A. salicina + teff straw	31.7B ± 0.6	ND	49.5B ± 0.9	67.3B ± 0.0	0.69B ± 0.021	ND	1.12B ± 0.037	1.57B ± 0.018
A. salicina + PEG + teff straw	39.2A ± 0.3	ND	59.8A ± 1.0	79.3A ± 0.0	0.89A ± 0.055	ND	1.31A ± 0.030	1.71 ± A0.020

[a] ND, not determined. Different letters in columns within species indicate significant difference at $P < 0.05$.

Table 8. Ammonia Nitrogen in the Supernatant and Purines in Apparently Undegraded Residue on Incubation of Teff Straw Supplemented with Tannin-Containing Browses in the Presence and Absence of PEG in LN Medium[a]

	ammonia-N (mg/40 mL)				purines (μmol in apparently undegraded residues)			
feed	8 h	12 h	16 h	24 h	8 h	12 h	16 h	24 h
medium[b] (0.83 ± 0.02 mg of N)								
C. calothyrsus	1.47B ± 0.027	1.68B ± 0.052	1.85B ± 0.083	2.30B ± 0.043	0.82D ± 0.138	1.23D ± 0.085	1.21D ± 0.064	1.21C ± 0.128
C. calothyrsus + PEG	1.99A ± 0.048	2.51A ± 0.004	3.01A ± 0.078	4.03A ± 0.082	0.80D ± 0.063	1.11D ± 0.124	1.19D ± 0.124	0.87D ± 0.018
teff straw	0.40D ± 0.027	0.31D ± 0.053	0.28D ± 0.0	0.47D ± 0.026	3.26C ± 0.121	4.00C ± 1.71	3.63C ± 0.117	3.67B ± 0.097
C. calothyrsus + straw	0.33D ± 0.011	0.21D ± 0.030	0.26D ± 0.050	0.31E ± 0.033	3.82B ± 0.138	4.50B ± 0.009	4.44B ± 0.056	4.26A ± 0.038
C. calothyrsus + PEG + straw	0.56C ± 0.064	0.48C ± 0.007	0.62C ± 0.038	1.30C ± 0.017	4.52A ± 0.224	5.52A ± 0.129	4.99A ± 0.096	4.42A ± 0.071
medium[b] (0.79 ± 0.02 mg of N)								
D. cinerea	1.02B ± 0.022	ND	1.24B ± 0.034	1.33B ± 0.011	1.93D ± 0.012	ND	1.22D ± 0.121	1.33B ± 0.060
D. cinerea + PEG	1.68A ± 0.035	ND	2.29A ± 0.016	3.00A ± 0.132	1.57D ± 0.179	ND	0.80D ± 0.195	0.71C ± 0.058
teff straw	0.48D ± 0.041	ND	0.25D ± 0.020	0.33D ± 0.016	3.03C ± 0.248	ND	2.47C ± 0.054	3.22A ± 0.365
D. cinerea + straw	0.50D ± 0.009	ND	0.31D ± 0.020	0.25D ± 0.007	3.53B ± 0.086	ND	3.07B ± 0.082	3.19A ± 0.024
D. cinerea + PEG + straw	0.60C ± 0.020	ND	0.44C ± 0.017	0.70C ± 0.024	4.11A ± 0.120	ND	4.16A ± 0.252	3.65A ± 0.306
medium[b] (0.76 ± 0.02 mg of N)								
A. salicin	1.27B ± 0.013	ND	1.88B ± 0.035	2.48B ± 0.038	1.52D ± 0.132	ND	1.38C ± 0.122	1.13C ± 0.094
A. salicina + PEG	1.93A ± 0.019	ND	2.88A ± 0.069	3.68A ± 0.028	1.52D ± 0.165	ND	1.03C ± 0.013	1.03C ± 0.130
teff straw	0.39C ± 0.038	ND	0.32D ± 0.016	0.57D ± 0.013	3.00C ± 0.141	ND	3.39B ± 0.234	3.19B ± 0.139
A. salicina + teff straw	0.14D ± 0.034	ND	0.24D ± 0.016	0.47E ± 0.041	3.64B ± 0.149	ND	3.37B ± 0.206	3.95A ± 0.315
A. salicina + PEG + straw	0.39C ± 0.026	ND	0.59C ± 0.016	1.09C ± 0.050	4.35A ± 0.151	ND	3.95A ± 0.306	4.39A ± 0.244

[a] ND, not determined. Different letters in columns within species indicate significant difference at $P < 0.05$. [b] NH_3-N at the beginning of incubation (0 h).

respectively. Supplementation of straw with browse leaves in the presence of PEG increased NH_3-N concentration by >100% compared to samples without PEG. The NH_3-N concentration on incubation of teff straw alone was markedly lower than that at 0 h (at the beginning of incubation).

Supplementation of straw with browse significantly increased microbial protein synthesis compared to straw alone (Table 8). Addition of PEG to *C. calothyrsus* and *D. cinerea* significantly ($P < 0.05$) decreased microbial protein synthesis at 24 h of incubation. Supplementation of *C. calothyrsus* to straw tended to increase the efficiency of microbial protein synthesis (micromoles of purines per millimole of SCFA) at 8 and 12 h of incubation (Table 9). The higher efficiency was observed at 8 h of incubation of *D. cinerea*. In all three species tested, addition of PEG to remove the effect of tannins resulted in significantly lower microbial efficiency compared to incubation without PEG.

DISCUSSION

Chemical Composition. Nitrogen and phenolic compound contents of browses were variable among species but broadly comparable to values obtained in previous studies (Silanikove et al., 1996; Balogun et al., 1998). The presence of phenolic compounds, such as tannins, due to their complex formation with protein and fiber fraction makes the use of the detergent method of fiber analysis difficult (Makker et al., 1997; Aregheore et al., 1998). The higher ADF value than that of NDF indicated the formation of tannin−protein or tannin−fiber complexes, which became insoluble in acid detergent solution. The high ADL values in browses recorded in the present and previous studies (Aregheore et al., 1998) may be due to the presence of cutin, biogenic silica, or pectin, as these substances are insoluble in acid detergent solution (Van Soest, 1994).

Effect of Nitrogen on Rumen Fermentation. The presence of tannins in browses depressed the in vitro gas and SCFA production. This inhibitory effect was

Table 9. Efficiency of Microbial Protein Synthesis (EMPS, Micromoles of Purines per Millomole of SCFA) on Incubation of Browses and Teff Straw with and without PEG[a]

	EMPS			
feed	8 h	12 h	16	24
C. calothyrsus	5.8a ± 0.24	6.2ab ± 0.23	4.9a ± 0.20	3.6a ± 0.38
teff straw	5.8a ± 0.36	5.7b ± 0.05	4.0b ± 0.17	3.0b ± 0.15
C. calothyrsus + PEG	2.8b ± 0.37	3.3c ± 0.11	2.7c ± 0.25	1.8c ± 0.17
C. calothyrsus + teff straw	6.4a ± 0.26	6.5a ± 0.33	4.9a ± 0.06	3.1ab ± 0.05
C. calothyrsus + teff straw + PEG	6.2a ± 0.19	5.6b ± 0.23	4.4ab ± 0.22	2.7b ± 0.08
D. cinerea	9.6a ± 0.30	ND	4.7a ± 1.31	4.1a ± 0.09
teff straw	6.0b ± 0.34	ND	3.0ab ± 0.06	3.0b ± 0.14
D. cinerea + PEG	6.1b ± 0.30	ND	2.0b ± 0.52	1.5c ± 0.12
D. cinerea + teff straw	6.4b ± 0.23	ND	3.8ab ± 0.29	2.8b ± 0.12
D. cinerea + teff straw + PEG	6.2b ± 0.86	ND	3.7ab ± 0.20	2.5b ± 0.01
A. salicina	4.8bc ± 0.24	ND	3.6a ± 0.22	2.4a ± 0.22
teff straw	5.8a ± 0.19	ND	3.6a ± 0.36	2.4a ± 0.03
A. salicina + PEG	4.1c ± 0.30	ND	2.2b ± 0.03	1.7b ± 0.15
A. salicina + teff straw	5.3ab ± 0.25	ND	3.0a ± 0.32	2.5a ± 0.25
A. salicina + teff straw + PEG	4.7bc ± 0.26	ND	3.0a ± 0.21	2.6a ± 0.12

[a] ND, not determined. Different letters in columns within species indicate significant difference at $P < 0.05$.

removed on addition of PEG. This observation is in agreement with previous studies (Makkar et al., 1995; Silanikove et al., 1996). The depression in the fermentation could be a result of either direct interaction between tannins and the bacterial cell wall (Jones et al., 1994) or the effect of tannin on microbial enzymes (Makkar et al., 1988; Bae et al., 1993).

The higher gas production as a result of higher N in the medium was consistent with that obtained with hay (Wood and Manyuchi, 1997). The low response in SCFA production due to increased level on N in the medium could be attributed to the combined effect of LN (reduced protein degradability due to tannins) and reduced microbial activity as a result of tannin binding to microbial cells/enzymes. Rumen microbes that ferment cellulose and hemicellulose utilize ammonia as a nitrogen source for microbial protein synthesis (Russell et al., 1992). Therefore, if binding to dietary protein was the only direct effect of tannins, then there should have been a marked response in microbial fermentation with the increased N in the media. The higher production of SCFA from straws compared to browses indicated the low energetic value of browses. This confirms that tannin-containing browses alone cannot support the maintenance energy requirement of animals.

The net negative NH_3-N released from tannin-containing feed indicates that the amount of NH_3-N utilized by microbes was more than the amount of N released from feed. *D. cinerea* and *A. salicina*, which were relatively low in CP content, resulted in the lowest NH_3-N concentrations at both 16 and 24 h of incubation. In tannin-containing browses, the degradability of protein is markedly depressed by tannins, resulting in a low NH_3-N concentration. Addition of PEG, on the other hand, dramatically increased the NH_3-N level beyond that required for optimum microbial activity. Addition of PEG resulted in an increase in SCFA production by ~2-fold in *A. salicina* and ~12−fold in *A. angustissima*. This significantly higher degradation of tannin-containing browses in the presence of PEG did not increase the microbial protein synthesis, probably due to the poor synchronization of energy availability and protein degradability. Although NH_3-N is a major source of nitrogen for microbial growth, Russell and Hespell (1981) mentioned the importance of peptides and amino acids, particularly for microbes degrading nonstructural carbohydrates. Insufficiency of these compounds in a matched manner may be a major factor causing energetic uncoupling, resulting in the continued production of fermentation products such as SCFA without a concomitant increase in microbial growth (Russell and Hespell, 1981). Therefore, synchronization of the rate of degradation of N and energy components from browses is extremely important for the efficient utilization of rumen NH_3-N for synthesis of microbial protein.

When tannin-containing browses were incubated alone in LN medium, the net production of NH_3-N ranged from 3.2 to 21.8 mg/L. These values were much lower than the amount required for optimum microbial fermentation, which is ~235 mg/L (Ørskov, 1982). However, addition of PEG increased NH_3-N by ~7 and ~35-fold in *A. angustissima* and *A. salicina*, respectively. The NH_3-N concentration after 24 h was lower than after 16 h in the absence of PEG, but in the presence of PEG, the 24 h values were higher than at 16 h. This indicates that in the absence of PEG, the N released from feed was lower than the N uptake, whereas in the presence of PEG, the N release was higher than N uptake, leading to the NH_3-N accumulation in the system.

A depressive effect of tannins in browses on NH_3-N concentration in vivo has been reported by various workers (Waghorn et al., 1987; Terrill et al., 1992; Silanikove et al., 1996; Ben Salem et al., 1997; Osakwe et al., 1998). The NH_3-N concentration in the rumen of sheep decreased as the level of *A. cyanophylla* in the diet increased (Ben Salem et al., 1997). This clearly demonstrates the protective effect of tannins on the degradability of proteins by rumen microbes. HN resulted in a 10.4% increase in in vitro gas production, whereas addition of PEG yielded a 186% increase. HN medium increased SCFA production by 9.9%, whereas inclusion of PEG in tannin-containing feed to remove the effect of tannins resulted in an increase in SCFA production by 195%. The relatively low response in microbial fermentation to high nitrogen in the medium compared to the values obtained by inclusion of PEG shows that the effect of tannins in tannin-containing browses on microbial fermentation is not only through protecting protein but also reducing microbial activity through binding to microbial enzymes and making them less active. The results shown in the present work and previous studies with temperate legumes (Waghorn et al., 1994; Stienezen et al., 1996) confirm that depression of microbial fermentation by tannin-containing browses is not due to only low degradability of protein. The antinutritional effect of tannins is exerted through the

reduction of protein availability and depression of digestive enzyme activity (Robins et al., 1987; Jones et al., 1994).

When the effects of HN in the medium and addition of PEG on in vitro parameters were compared, the effect of PEG was substantially larger than that of HN. In addition, there was a poor response in SCFA production due to HN on incubation of *C. calothyrsus* and *D. cinerea*. These results suggest that the depression in microbial fermentation on incubation of tannin-containing browses could largely be due to antifermentative effects of tannins rather than availability of N to rumen microbes.

Supplementation. The lower gas volume observed on incubation of teff straw with browse leaves compared to gas predicted from individual components may suggest a change in the pattern of microbial fermentation toward higher microbial yield when protein and energy sources were incubated together. Supplementation of nutrients that were deficient in straw might have stimulated microbial fermentation by creating a favorable environment for microbial growth.

Incubation of tannin-containing browses alone resulted in low microbial degradability of protein. Addition of PEG to tannin-containing browses, on the other hand, substantially increased microbial degradability of protein and thereby increased NH_3-N accumulation in the in vitro system with a corresponding decrease in microbial growth probably due to the uncoupled fermentation. Uncoupled fermentation in the presence of PEG occurs because energy is released much more quickly than it can be trapped and utilized for growth by rumen microbes. The incubation of straws with browse leaves in the presence of PEG in this study allowed ruminal bacteria to utilize the energy for growth more efficiently because the energy is released in a more uniform pattern throughout the incubation period. This study showed that supplementation of poor-quality roughages with browse leaves in the presence of PEG can improve microbial fermentation and microbial growth, thereby increasing animal performance from roughage-based diets.

The higher uptake of N compared to N released from browses in the absence of PEG would result in higher nitrogen retention. Although addition of browse leaves to straw increased the SCFA production and microbial protein synthesis, the N level after 24 h of incubation appears to be lower than the critical level for optimum microbial activity. This indicated that the low level of supplementation (20%) used in this study did not alleviate the nitrogen deficiency of the substrate. This was confirmed from positive response in microbial growth on addition of PEG, which released additional N to the system through its binding effect to tannins. The higher N uptake on supplementation of straw with browse leaves was reflected in increased microbial yield both in the presence and in the absence of PEG. The positive N retention was also reported from the in vivo studies by Reed et al. (1990), Bonsi et al. (1994), and Wiegand et al. (1995) using tropical browse supplements in ruminant diets. In in vivo experiments, higher microbial protein production was recorded from the supplementation of maize stover with *C. calothyrsus*, which was higher in condensed tannins compared to the *Leucaena* species with low condensed tannins (Nherera et al., 1998). The high microbial protein produced from *C. calothyrsus* resulted in a higher rate of daily gain compared to the *Leucaena* species (Nherera et al., 1998).

The efficiency of microbial protein synthesis decreased with time of incubation (less purine was recovered per millimole of SCFA at 24 h than at 16 h). The decline in efficiency of microbial protein synthesis with time was much higher in the presence of PEG than without PEG, which could be attributed to microbial lysis. Following the exhaustion of fermentable substrate, SCFA continued to be produced due to microbial turnover, and, as a consequence, microbial protein was decreased. The time at which microbial yield reaches its maximum may depend on the rate of degradation of feeds. Blümmel et al. (1998) mentioned that microbial nitrogen recovery was less after 12 h of incubation of grass hay than earlier hours of incubation. Therefore, knowledge of the rate of degradation of feeds appears to be important for deciding appropriate times of incubation.

Conclusion. The depression of in vitro microbial fermentation of tannin-containing browses could partly be due to the low degradation of feed proteins that limits the availability of ammonia nitrogen for microbial growth, but the larger effect could be attributed to the inhibitory effect of tannins on microbial cells/enzyme activity. The use of PEG in supplementation strategies would be of immense advantage in improving the nutritive value of browses.

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