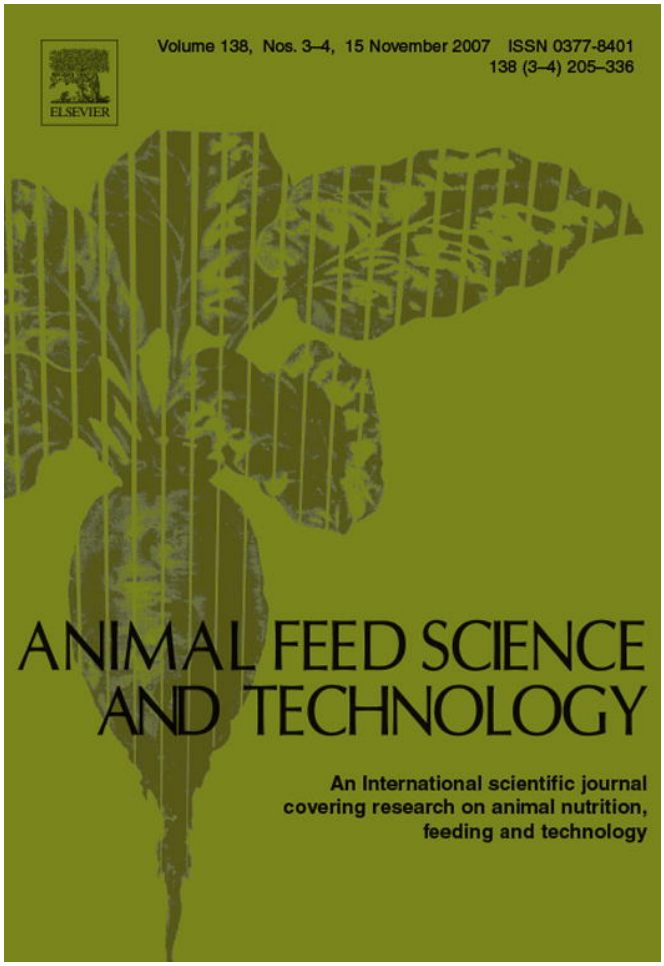

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138 (2007) 318–330
ANIMAL FEED SCIENCE AND TECHNOLOGY
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In vitro fermentation and microbial protein synthesis of some browse tree leaves with or without addition of polyethylene glycol

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Received 10 January 2006; received in revised form 15 November 2006; accepted 30 November 2006

Abstract

To assess the nutritional value of the leaf of four browse trees (*Chorisia speciosa*, *Cassia fistula*, *Schinus molle*, *Eucalyptus camaldulensis*), that are grown in semi-arid regions of northern Egypt, this study evaluated effects of incorporation of polyethylene glycol (PEG) on their nutritional value. *In vitro* gas production after 24 h of fermentation ($IVGP_{24}$), volatile fatty acids (VFA), ammonia concentrations and microbial protein synthesis were determined, and *in vitro* organic matter digestibility (IVOMD) and metabolizable energy (ME) were estimated. The $IVGP_{24}$, VFA and ammonia N concentrations varied ($P<0.001$) among browse species, with the $IVGP_{24}$ and VFA highest ($P<0.001$) for *C. speciosa*, lowest ($P<0.001$) for *E. camaldulensis,* and intermediate for *C. fistula* and *S. molle*. *C. speciosa* had the highest ($P<0.05$) IVOMD, microbial protein synthesis, IVOMD and ME, while these measurements were lowest in *E. camaldulensis* and intermediate in the other browse leaves. In general, *C. speciosa* has

Abbreviations: ADFom, acid detergent fiber; AF, aqueous fraction; ALKA, alkaloids; CP, crude protein; CT, condensed tannins; DM, dry matter; EO, essential oils; IVGP, *in vitro* gas production; IVOMD, *in vitro* OM digestibility; lignin(sa), acid detergent lignin; ME, metabolizable energy; NDFom, neutral detergent fiber; OM, organic matter; PEG, polyethylene glycol; SAP, saponins; TEP, total extractable phenolics; VFA, volatile fatty acids

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0377-8401/$ – see front matter
doi:10.1016/j.anifeedsci.2006.11.026

the highest potential as a ruminant feed, the lowest being *E. camaldulensis* and *S. molle*, with *C. fistula* intermediate. Addition of PEG increased (P<0.001) IVGP24, VFA and ammonia N concentrations, as well as gas production (P<0.05). The highest overall improvement was for *C. speciosa*, intermediate for *E. camaldulensis*, and lowest for *C. fistula* and *S. molle*. Addition of PEG reduced (P<0.01) the amount and efficiency of microbial protein synthesis, and increased (P<0.001) IVOMD and ME in all leaves. The extent of the benefit of PEG on overall nutritive value varied somewhat by browse, with *E. camaldulensis* judged to have the largest overall improvement, *C. fistula* the lowest with *C. speciosa* and *S. molle* intermediate. However PEG addition did not change overall nutritive ranking of these browse leaves.

Keywords: Secondary compounds; Gas production; Microbial efficiency; Digestion; Metabolizable energy

1. Introduction

Tree and shrub leaves are important sources of forage for small ruminants in the semi-arid conditions of northern Egypt, especially during the dry season when the quality and quantity of green herbages is limited. As such, they have potential to alleviate some feed shortages and nutritional deficiencies experienced during the dry season on smallholder farms. Tree and shrub leaves can be an important component of goat and sheep diets (Holecheck, 1984; Papachristou and Nastis, 1996; Salem et al., 2006), and play an important role in nutrition of grazing animals in areas where few, or no, alternative feedstuffs are available (Meuret et al., 1990). However use of tree and shrub leaves by herbivores may be restricted by negative effects on digestion of their generally high levels of secondary compounds (Provenza, 1995; Salem, 2005; Salem et al., 2006).

Although tree and shrub leaves can be rich in N, results from *in vivo* (Ørskov and Grubb, 1978; Salem et al., 2006), in sacco (Nagi et al., 1988) and *in vitro* (Getachew et al., 2000a and 2001; Salem, 2005) studies suggest that the rumen degradable N supply from tree and shrub leaves is often insufficient to meet the N needs of rumen microbes and support acceptable animal performance. This is primarily because phenolic compounds in tree and shrub leaves, particularly tannins, can bind to dietary proteins rendering them undegradable by rumen microbes (Osuga et al., 2005), although other secondary metabolites, such saponins, alkaloids, essential oils and the aqueous fraction of lectins, polypeptids and starch, may also have negative impacts on digestion.

Polyethylene glycol (PEG), a non-nutritive synthetic polymer, has a high affinity for phenolic compounds, especially tannins, and thereby deactivates them by forming tannin-PEG complexes (Makkar et al., 1995b). Thus, PEG can prevent their formation or liberate protein from tannin-protein complexes (Barry and Manley, 1986), and it has been used to mitigate adverse effects of secondary compounds on rumen fermentation, as well as improve performance (*i.e.*, growth and milk yield) of ruminants fed diets high in secondary compounds. For example, addition of PEG to browse and herbaceous legumes high in secondary compounds increased *in vitro* gas production and short chain fatty acid production, although microbial N production and efficiency of microbial protein synthesis was decreased (Norton and Ahn, 1997; Getachew et al., 2001).

This study determined the nutritive value of some tree leaves grown in semi-arid regions of northern Egypt using an *in vitro* ruminal fermentation technique that allowed the quantitative impact of addition of PEG to be assessed. A prior report on other aspects of this study has been published (Salem et al., 2006).

2. Materials and methods

2.1. Tree foliage species and there chemical and secondary compounds contents

Samples of leaves of the tree species *Cassia fistula*, *Schinus molle*, *Chorisia speciosa* and *Eucalyptus camaldulensis* were randomly and manually harvested from different parts of the trees to obtain both young and mature leaves from each tree species. Leaf samples were dried at 40 °C for 72 h in a forced air oven in triplicate to constant weight, ground in a hammer mill to pass a 1 mm sieve and stored in plastic bags for subsequent determination of chemical components, secondary compounds and *in vitro* fermentation. Methods of chemical composition (*i.e.*, ash, N, neutral detergent fiber (NDFom), acid detergent fiber (ADFom) and acid detergent lignin (lignin(sa))), as well as the secondary compounds (*i.e.*, total extractable phenolics (TEP), condensed tannins (CT), saponins (SAP), alkaloids (ALKA), essential oils (EO) and aqueous fraction (AF)) determinations were as previously described (Salem et al., 2006).

2.2. In vitro rumen fermentation parameters

In vitro gas production (IVGP) was determined according to Menke et al. (1979). Approximately 500 mg of dry matter (DM) of each sample was placed in a triplicate 50 ml syringes with or without 1 g of PEG (MW, 4000, analytical grade, Sigma®–Aldrich). Rumen fluid was collected, before feeding, from the rumens of three sheep with permanent rumen cannulae fed a commercial concentrate mixture and rice straw. Rumen digesta was squeezed through four layers of cheesecloth, and the rumen fluids collected from the sheep were mixed to be used in the *in vitro* fermentation. This mixed rumen fluid was homogenized and kept at 39 °C in a water bath, flushed with CO_2 before use, and diluted (1:4, v:v) with a culture medium (Makkar et al., 1995a; FAO/IAEA, 2000), containing bicarbonate buffer, macro-mineral, micro-mineral, resazurine and a reducing solution. Buffered rumen fluid (30 ml) was pipetted into each syringe and syringes were immediately placed in a water bath at 39 °C. Gas volumes were recorded at 2, 4, 6, 10, 12, 24 and 48 h of incubation and corrected for blank syringes incubated in each run.

At the end of the 48 h incubation, contents of each syringe were transferred to tubes and centrifuged at 12,000 × *g* for 20 min at 4 °C (Heraeus Christ GmbH, Model Osrterode/Harz-Cryofuge 20-3, MSE Scientific Instruments, Manor, Crawley, Sussex, UK). Supernatants were pipetted and stored at −20 °C until analysis for ammonia N (McDonald et al., 1960) and volatile fatty acids (VFA; Ottenstein and Bartley, 1971). The residual pellet, which consisted of undigested substrate and microbial mass, was washed with distilled water followed by centrifugation at 15,000 × *g*. Purine, as RNA equivalents (analytical

grade, Sigma®–Aldrich), was spectrophotometrically determined in apparently undegraded residues at 260 nm using the method of Makkar and Becker (1999). All incubations were completed at the laboratory of animal nutrition, Department of Animal Production, Faculty of Agriculture, University of Alexandria, Egypt.

2.3. Calculations

In vitro organic matter (OM) digestibility (IVOMD, g/kg DM) and metabolizable energy (ME, MJ/kg DM) were estimated according to Menke and Steingass (1988) as:

$$\text{IVOMD (g/kg DM)} = [14.88 + 0.889\,\text{IVGP}_{24}\,\text{(ml/0.5 g DM)} + 0.45\,\text{CP (\% DM)}] \times 10$$

$$\text{ME (MJ/kg DM)} = 2.20 + 0.136\,\text{IVGP}_{24}\,\text{(ml/0.5 g DM)} + 0.057\,\text{CP(\% DM)}$$

where IVGP_{24} was 24 h gas volume and CP (% DM) was that of the tree leaves.

Gas production results (ml/0.5 g DM) were fitted using the NLIN option of SAS (1999) to the model of France et al. (2000) as:

$$G = b \times (1 - e^{-k(t-L)})$$

where G is the volume of gas production at time t; b the asymptotic gas production (ml/0.5 g DM); k is the rate of gas production (/h) from the slowly fermentable feed fraction b, and L is the discrete lag time prior to gas production.

2.4. Statistical analysis

Tree foliage nutrient and secondary compound contents were statistically analyzed using the 'GLM' option of SAS (1999), with methods of Steel and Torrie (1980), and differences among foliage species were determined using Duncan's multiple-range test (Duncan, 1955). Data on *in vitro* ruminal fermentation parameters, *in vitro* digestibility and microbial protein syntheses were analyzed as a 4 × 2 factorial experiment (4 tree foliages species × 2 treatments (*i.e.*, with or without PEG)) using the 'GLM' option of SAS (1999) with methods of Steel and Torrie (1980), to determine differences due to tree species and PEG. In the case of significant (*i.e.*, $P<0.05$) interactions, Duncan's multiple-range test (Duncan, 1955) was used to separate means within tree species.

Correlation coefficients between tree foliage chemical and secondary compound contents with the rumen fermentation parameters and microbial protein syntheses were estimated using the CORR option of SAS (1999).

3. Results

3.1. Chemical and secondary compounds contents of the tree leaves

The chemical and secondary compound contents of the tree leaves previously reported by Salem et al. (2006) are in Table 1. In brief, the CP content of the foliages ranged from

Table 1
Nutrient and secondary compound levels (g/kg DM) of foliages (previously reported in Salem et al. (2006))

	Tree species				S.E.M.
	C. speciosa	*C. fistula*	*S. molle*	*E. camaldulensis*	
Chemical composition[a]					
OM	916 c	923 b	909 d	945 a	0.88
CP	128 c	185 a	124 d	154 b	0.95
EE	47 b	39 c	97 a	41 bc	2.64
NDFom	435 c	368 d	515 b	615 a	4.47
ADFom	356 b	200 d	327 c	542 a	5.56
Lignin(sa)	102 c	101 c	160 b	192 a	2.82
Secondary compounds[b]					
TP	29.0 d	44.3 c	67.7 b	102.3 a	2.76
CT	20.8 d	31.6 c	49.2 b	68.1 a	0.96
SAP	3.0 c	8.3 b	10.3 b	14.6 a	0.73
ALKA	0.0 c	1.3 b	1.9 b	5.0 a	0.20
AF	3.9 c	8.6 a	6.6 b	2.4 d	0.28
EO	0.4 c	0.8 c	5.3 b	15.5 a	0.27

Means in the same row with different letters (a,b,c,d) differ (P<0.05).

[a] OM, organic matter; CP, crude protein; EE, ether extract; NDFom, neutral detergent fiber; ADFom, acid detergent fiber; Lignin(sa), acid detergent lignin.

[b] TP, total extractable phenolic components; CT, condensed tannins (as quebracho equivalent); SAP, saponins; ALKA, alkaloids; AF, aqueous fraction (lectins, polypeptides, starch; Cowan, 1999); EO, essential oils (ml/kg DM).

124 to 185 g/kg DM. *C. fistula* had the lowest NDFom, ADFom and lignin(sa) values, *E. camaldulensis* had the highest, and *S. molle* and *C. speciosa* were intermediate. Secondary compounds (*i.e.*, TP, CT, SAP, ALKA, AF, and EO) were lowest in *C. speciosa* and highest in *E. camaldulensis*. Tannins (*i.e.*, TEP and CT) were higher than 50 g/kg of DM in *S. molle* (70 and 50) and *E. camaldulensis* (110 and 70).

3.2. In vitro fermentation and gas production parameters

All fermentation parameters (*i.e.*, IVGP, VFA, NH_3-N) varied (P<0.01) among tree leaves (Table 2). The IVGP24 and VFA were highest (P<0.001) in *C. speciosa*, lowest (P<0.001) in *E. camaldulensis,* and intermediate in *C. fistula* and *S. molle*. Addition of PEG increased IVGP24 and VFA concentrations (P<0.01), as well as NH_3-N concentrations (P=0.05).

C. speciosa had the highest (P<0.05) potential gas production (*i.e.*, fraction *b*) and rate of gas production (*i.e.*, *k*), but the discrete time lag (*i.e.*, *L*) did not differ among leaves. PEG increased (P<0.05) fraction *b* in all leaves, except *C. speciosa*, as well as increasing *k*, and decreasing *L*, overall.

3.3. In vitro microbial protein synthesis and IVOMD

Microbial protein synthesis varied (P<0.01) among tree leaves (Table 2). Purine (μmol) concentrations in undegradable residues after 48 h of incubation were highest (P<0.05) in *C. speciosa*, lowest in *E. camaldulensis* and intermediate in the other leaves. Addition of

Table 2
In vitro ruminal fermentation parameters [$IVGP_{24}$ (gas production volume after 24 h of incubation, ml/500 mg DM), VFA (volatile fatty acids, μmol/ml) and NH_3-N concentrations (μg/ml)], gas production parameters, microbial protein synthesis (purines, μmol) and efficiency of microbial protein synthesis in apparently undegraded residue after 48 h of incubation with rumen fluid of sheep and *in vitro* OM digestibility (IVOMD, g/kg DM) and metabolizable energy (ME, MJ/kg DM) of tree foliage species[a] in the absence (−) or presence (+) of PEG (P)

	Foliage (F)								S.E.M.	Probability			Ranking of foliages (P<0.05)[b]
	C. speciosa (CS)		*C. fistula* (CF)		*S. molle* (SM)		*E. camaldulensis* (EC)			Foliage	PEG	Foliage × PEG	
	−	+	−	+	−	+	−	+					
Ruminal fermentation parameters													
$IVGP_{24}$	44.8	58.8	30. 7	41.1	24.5	42.9	21.0	35.7	1.97	<0.001	0.001	0.278	CS > CF = SM > EC
VFA	25.3	32.5	16.8	22.7	13.1	23.7	11.0	19.7	1.14	<0.001	<0.001	0.236	CS > CF = SM > EC
NH_3-N	137.6	157.3	138.4	158.5	136.2	156.2	142.2	161.8	0.17	<0.001	0.050	0.547	EC > CF > CS > SM
Gas production parameters[c]													
B	60. 9	59.4	43.8 b	51.5 a	32.4 b	55.9 a	27. 6 b	42.7 a	2.4	<0.001	<0.001	0.007	
k	0.05	0.08	0.04	0.05	0.05	0.05	0.05	0.06	0.001	0.033	0.007	0.069	CS ≥ EC ≥ SM ≥ CF
L	3.8	3.8	3.7	2.6	5.9	2.7	3.5	3.4	0.60	0.306	0.019	0.062	SM > CS = EC>CF
Microbial protein synthesis													
Purines	8.2	7.7	7.6	7.3	7.5	7.4	7.3	6.9	0.14	<0.001	0.007	0.618	CS > SM = CF > EC
EMPS[d]	10.80 a	7.90 b	15.08 a	10.72 b	19.08 a	10.41 b	22.12 a	11.68 b	0.50	<0.001	<0.001	0.003	
EMPS[e]	4.32 a	3.16 b	6.03 a	4.29 b	7.63 a	4.16 b	8.85 a	4.67 b	0.50	<0.001	<0.001	0.003	
IVOMD	604	728	504	597	404	534	422	587	17.8	<0.001	<0.001	0.293	CS > CF > SM = EC
ME	9.01	10.92	7.42	8.84	5.92	7.94	6.23	8.75	0.27	<0.001	<0.001	0.288	CS > CF > SM = EC

[a] Different superscripts following means within a row and foliage indicate differences at P<0.05.
[b] Foliages are only ranked overall in the absence of an interaction P<0.05.
[c] *b* is the asymptotic gas production (ml/0.5 g DM); *k* is the rate of gas production (/h); *L* is the initial delay before gas production begins (h).
[d] Efficiency of microbial protein synthesis (EMPS, mol purines/mol VFA).
[e] Efficiency of microbial protein synthesis (EMPS, mol purines/mol VFA-carbon).

PEG reduced (P<0.01) microbial protein yield and its efficiency (although its extent varied by leaf material; P=0.003) in all foliages.

In vitro OM digestibility and metabolizable energy (ME) varied (P<0.001) among leaves, with *C. speciosa* having the highest (P<0.05) values of IVOMD and ME with the lowest values being in *E. camaldulensis* and *S. molle*. Addition of PEG increased (P<0.05) IVOMD and ME in all leaves.

4. Discussion

Browse tree leaves are potential high CP feeds for ruminants fed low quality forages and crop by products, especially during the dry season when availability of higher quality forages is low. Relatively high CP values in our browse leaves (Salem et al., 2006) suggest that they are potential ruminally degradable N supplements during the dry season in semi-arid regions, while the relatively low fibre levels suggest potentially high DM digestibility (Reed, 1986; Salem et al., 2006). However their high levels of secondary compounds will likely negate at least some of these positive effects by, for example, depressing feed intake, impairing digestibility and/or having a toxic effect on rumen microorganisms (Mangan, 1988; Salem et al., 2006).

4.1. Effect of foliage leaves

Gas production parameters suggested differences in nutritional value that were generally closely related to chemical composition (Cerrillo and Juarez, 2004; Kamalak et al., 2005; Salem, 2005). The high $IVGP_{24}$ in *C. speciosa* suggests a higher extent of fermentation in the first 24 h of fermentation *versus* the other leaves, especially *E. camaldulensis* (Fig. 1). Differences in gas production among the leaves could be due to the proportion, and nature, of their fibre (Rubanza et al., 2003). Indeed the higher fibre levels, as well as high (P<0.05) levels of secondary compounds in *E. camaldulensis* (Salem et al., 2006), are almost certainly responsible for its reduced gas production *versus* the other foliages. However, differences in degradability among browses could also be due to the extent of lignification of NDF (Van Soest, 1994; Fonesca et al., 1998), and $IVGP_{24}$ was negatively correlated with both NDFom and lignin(sa) (Table 3). Salem (2005) reported a similar relationship between $IVGP_{24}$ and NDF using rumen fluid of sheep, cattle and buffalo. The low nutritive value of *E. camaldulensis* could also be due to its NDF being bound by polyphenolics (Ndlovu and Nherera, 1997).

Secondary compounds affect ruminal fermentation and forage degradability. Higher *in vitro* fermentation of *C. speciosa* could be due to its lower secondary compound levels (Salem et al., 2006), although some variation among leaves could be due to genotypic characteristics relative to the type of secondary compound activity on digestibility (Muetzel and Becker, 2006; Salem et al., 2006). The reduced fermentation, as well as degradability, of the high secondary compound containing *E. camaldulensis* probably reflects adverse effects of secondary compounds on ruminal (McSweeney et al., 2005) and intestinal bacterial activity (Salem et al., 2004). This suppressing effect probably resulted from a reduction in microbial attachment to feed particles (McAllister et

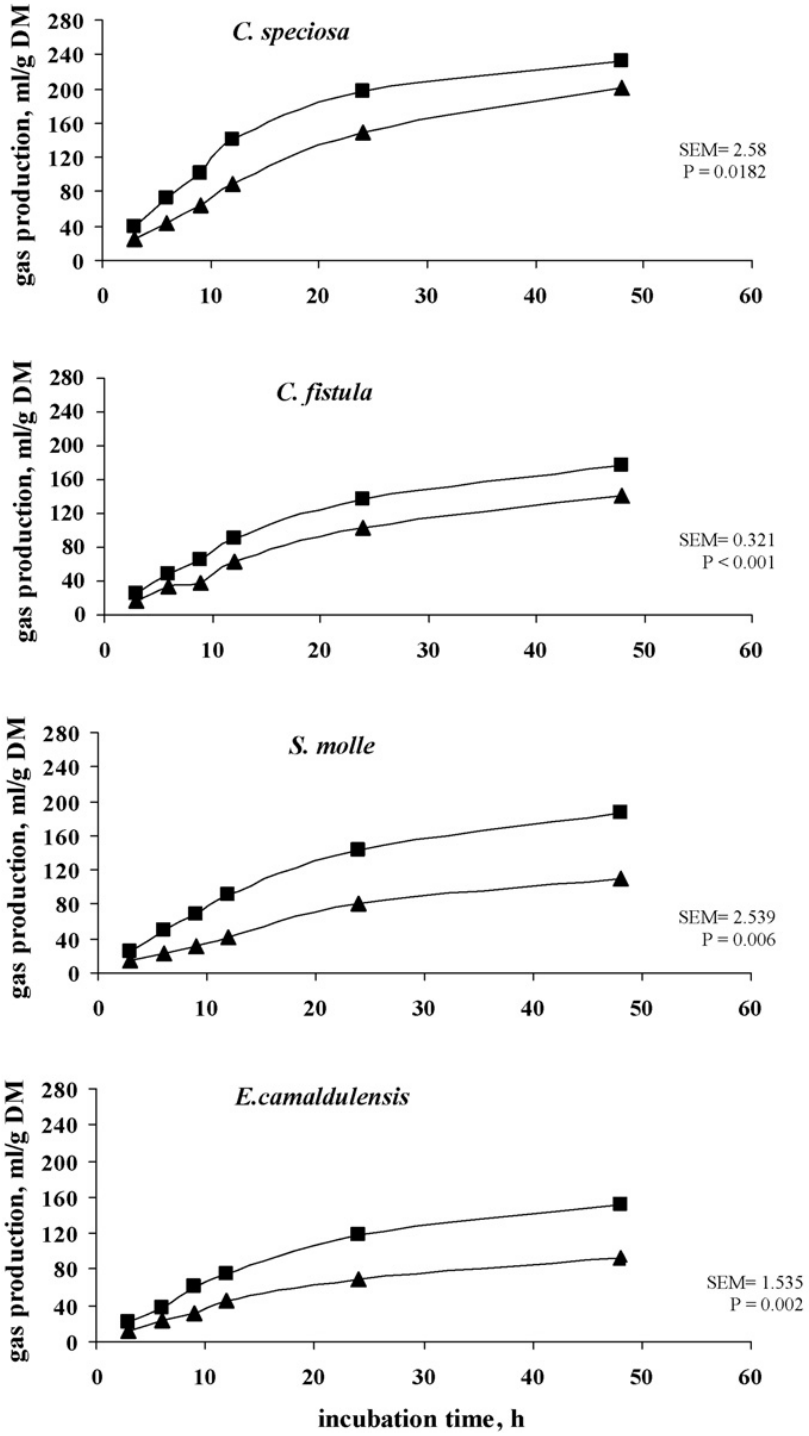

Fig. 1. Cumulative gas production profiles (ml gas/g DM) from *in vitro* fermentation of different foliage species in rumen liquor of sheep. (▲, without PEG; ■, with PEG; S.E.M. is for the overall fit and P' is for the effect of PEG).

al., 1994) and inhibition of microbial growth and enzyme activity (McSweeney et al., 2001).

Increased IVOMD and ME of *C. speciosa* reflect its higher fermentation, and lower secondary compound levels. In contrast, lower values in *E. camaldulensis* and *S. molle*

Table 3
Correlation coefficients (r) between *in vitro* fermentation parameters[a] with chemical and secondary component contents[b] of the four foliage species

	$IVGP^{24}$	NH_3-N	VFA	Purines	EMPS	IVOMD	ME
Chemical components							
CP	−0.23	0.450	−0.22	−0.25	0.06	−0.12	−0.13
EE	0.01	−0.681	−0.01	0.19	0.08	−0.08	−0.06
NDFom	−0.39	0.536*	−0.39	−0.44	0.42	−0.45*	−0.44*
ADFom	−0.25	0.696	−0.25	−0.43	0.29	−0.29	−0.280
Lignin(sa)	−0.53*	0.51*	−0.53*	−0.58**	0.48*	−0.57**	−0.56**
Secondary components							
TEP	−0.58**	0.69***	−0.58**	−0.61**	0.52*	−0.59**	−0.59**
CT	−0.60**	0.63**	−0.60**	−0.59**	0.54*	−0.62**	−0.61**
SAP	−0.68**	0.63**	−0.68***	−0.62**	0.58**	−0.67**	−0.67**
ALKA	0.40	0.36	0.41	0.23	−0.27	0.40	0.40
EO	−0.49*	0.78***	−0.50*	−0.58**	0.46*	−0.51*	−0.50*
AF	−0.22	−0.37	−0.23	−0.03	0.08	−0.17	−0.18

* P<0.05; ** P<0.01; ***P<0.001.

[a] $IVGP_{24}$, gas production volume (ml/0.5 g DM) after 24 h of incubation; VFA, volatile fatty acids; Purines (μmol); EMPS, efficiency of microbial protein synthesis (μmol purines/mmol VFA); IVOMD, *in vitro* OM digestibility (g/kg DM); ME, metabolizable energy (MJ/kg DM).

[b] CP, crude protein; EE, ether extract; NDFom, neutral detergent fiber; ADFom, neutral detergent fiber; lignin(sa), sulfuric acid lignin; TEP, total extractable phenolic components; CT, condensed tannins; SAP, saponins; ALKA, alkaloids; EO, essential oils (ml/kg DM); AF, aqueous fraction.

represent less fermentation and higher secondary compound levels (Salem et al., 2006). Similar results were reported by Peng et al. (2005) who showed reduced IVOMD of lucerne chaff incubated with secondary compounds extracted from *L. sativus*, and suggested that oxalyl-diaminobutyric acid, rather than phenolics, were responsible for inhibition of cellulolytic bacteria. However, digestibility of tree leaves was adversely affected by secondary compounds *in vitro* (Peng et al., 2005; Rakhmani et al., 2005) and *in vivo* (Salem et al., 2006).

The lowest purine concentration, and highest efficiency of microbial protein synthesis (EMPS), occurred in leaves with higher levels of secondary compounds (*i.e.*, *E. camaldulensis*) where lower gas production suggests a role of phenolics as an anti-methanogen that stimulates growth of propionate producing bacteria. Lower methane production, with higher propionate, are consistent with higher EMPS (McCrabb et al., 1997). Higher EMPS in *E. camaldulensis* could be due to the secondary compounds that may act as anti-bacteriophages to reduce efficiency of ruminal fermentation through non-specific bacterial lysis (Klieve et al., 1996).

All fermentation parameters were correlated with chemical composition (Table 3), but fermentation parameters and microbial protein synthesis were not correlated with alkaloid levels, possibly due to the ability of rumen microorganisms to degrade alkaloids (Lanigan, 1970; Wachenheim et al., 1992a,b). Ammonia N concentrations were positively correlated with TEP, CT, SAP, EO and AF as a probable consequence of poor ruminal synchronization between N and energy availability. Negative correlations between TEP, CT, SAP EO and

AF with *in vitro* gas production, IVOMD, VFA and microbial protein synthesis support the hypotheses that secondary compounds have a negative role on digestibility. Other secondary compounds, such as saponins and essential oils, may have another mode of action on ruminal microorganisms, as saponins change cell membrane properties (Moss et al., 2000) and essential oils could change N metabolism of rumen micro-organisms and inhibit growth of bacteria (Mclntosh et al., 2003). Salem (2005) also reported a negative correlation between secondary compounds and *in vitro* gas production and DM degradability of *Acacia saligna* leaves incubated with inoculum of sheep, cattle and buffalo. Similar negative relationships occurred between NDF, lignin(sa) and phenolics with *in vitro* digestibility in some perennial grasses (Casler and Jung, 2006), and Tolera et al. (1997) found similar impacts of proanthocyanidins in some browse legumes. Our results are similar to Rubanza et al. (2003), who reported a negative relationship between chemical composition, and phenolic compounds, with *in vitro* degradability of legumes at 24 h of *in vitro* incubation.

4.2. Effects of PEG

The general improvement in fermentation in each species by adding PEG almost certainly reflects its deactivation of secondary compounds (Makkar et al., 1995b; Salem et al., 2006). The extent of the improvement in fermentation of these tree leaves by addition of PEG probably depended on the level, as well as the nature, of the secondary compounds, especially tannins (Ebong, 1995). In our study, the biggest improvement in fermentation (*i.e.*, IVGP24 and VFA) occurred in *E. camaldulensis,* even though it had the highest levels of secondary compounds, probably due to higher levels of free tannins (Salem et al., 2006). While bound condensed tannins seem to be inert, and do not affect microbial fermentation (Makkar and Becker, 1998), once released into the rumen fluid as a result of microbial activity, they can affect bacterial growth. Normally, PEG has a higher capacity to deactivate free extractable tannins, *versus* bound tannins, from fiber and so reduce their negative effects, which may reflect the negative relationship between fermentation parameters and phenolics (*e.g.*, Table 3). Addition of PEG during incubation of tannin-rich NDF increased gas production (Getachew et al., 2000a), suggesting that tannins released as a result of NDF degradation by rumen microbes are biologically active and can impact rumen fermentation. Secondary compounds in leaves of different tree species, even at the same concentration, produced effects of different magnitudes in gas production rates and digestibility (Makkar et al., 1995b), thereby supporting the importance of the relationship between secondary compound structure and activity (Barahona et al., 2003). However, improvement of foliage nutritive value by PEG supplementation may be due to the nature and activity of the secondary compounds.

Increased ammonia N concentrations with addition of PEG could be due to increased CP degradability (Getachew et al., 2000b) and/or poor synchronization between N and carbohydrate release in the rumen. Rapid release of N that is not matched to availability of carbohydrate can lead to accumulation of ammonia N *in vitro*, or to high absorption of ammonia N from the rumen *in vivo*. Thus, higher levels of ammonia N suggest that utilization of these tree leaves can be improved by inclusion of PEG.

5. Conclusions

Among the browse tree leaves examined, *C. speciosa* had the highest ruminal fermentation, highest microbial protein synthesis and efficiency, as well as a higher digestibility and metabolizable energy, indicating that, of the four browse leaves examined, it had the best potential as a ruminant feed. In contrast, the nutritive value of *E. camaldulensis* and *S. molle* were, in general, lowest with *C. fistula* intermediate.

Addition of PEG sharply increased *in vitro* fermentation, digestibility and metabolizable energy, while reducing efficiency of microbial protein synthesis, almost certainly by reversing effects of their secondary compounds. While the extent of benefits of PEG addition varied somewhat by browse leaf, with *E. camaldulensis* and *S. molle* having the largest overall improvement, *C. fistula* the lowest and *C. speciaosa* intermediate, it did not impact their overall nutritive ranking.

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