

An *in vitro* evaluation of some drought-tolerant native range plants in terms of ruminal microbial nitrogen, microbial biomass and their fermentation characteristics utilising a gas-production technique

M.R. AL-MASRI

Department of Agriculture, Atomic Energy
Commission, Damascus, Syria

Abstract

Native drought-tolerant perennial range shrubs (*Alhagi camelorum*, *Salsola vermiculata*, *Peganum harmala*) and herbaceous range plants (*Poa sinaica*, *Erodium cicutarium*, *Schismus arabicus*) grown naturally on the south-eastern semi-desert of Syria and harvested in the early bloom stage or at seeding were evaluated in terms of microbial nitrogen (MN) and biomass (MBM) production after incubation with or without polyethylene glycol (PEG, 6000) at a ratio of 2:1 PEG:substrate for 96 h. Fermentation kinetics were assessed using an *in vitro* incubation technique with rumen fluid. The relationships between *in vitro* gas production (GP), MN and MBM were studied. MN and MBM production varied between species. The response of the range plants to PEG treatment in terms of increased gas production varied between species and with time of incubation, the greatest response occurring between 6 and 24 h incubation. Plants harvested at early bloom produced more gas per unit weight than those harvested at seeding. Initial gas production (*a*) was highest for *E. cicutarium*, whereas gas production during incubation (*b*) and potential gas production (*a + b*) were high for *P. sinaica* and *E. cicutarium* but low for *A. camelorum*, *S. vermiculata* and *P. harmala*. The amount of MN produced from 100 mg substrate was dependent on species and amounted to 0.02–0.54 mg without PEG and 0.17–0.79 mg with PEG. Corresponding values for MBM were 1.62–6.25 mg without PEG and 0.35–9.10 mg with PEG. Microbial nitrogen and MBM production were negatively correlated with GP.

Introduction

Range shrubs and herbaceous plants form an integral part of livestock feed in many tropical and subtropical regions, where crop residues form a major component of the feed supply. Both *in vivo* (Campling *et al.* 1962; Ørskov and Grubb 1978) and *in vitro* (Getachew *et al.* 1998) studies have indicated that the rumen-degradable nitrogen supply from crop residues would not meet the maintenance requirements of animals. In the tropics, forages from shrubs and trees and agricultural by-products are used as dietary supplements to make up for dietary deficiencies in nitrogen, energy, minerals and vitamins during regular feed shortages and droughts (Siebert and Hunter 1982; Goodchild and McMeniman 1994; Al-Masri 2003). Correction of dietary deficiencies can increase microbial degradation of feed in the rumen and improve the animal's metabolic capacity to use energy, both of which increase the voluntary intake of digestible organic matter and animal production. Some range plants, which are tolerant of the seasonal changes in environmental conditions, can be used as protein supplements to straws for ruminants. *Erodium cicutarium*, *Poa sinaica* and *Schismus arabicus* are classified as herbaceous range plants and *Alhagi camelorum*, *Salsola vermiculata* and *Peganum harmala* as perennial range shrubs (Al-Rabbat and Abouzakhem 1987). The annual *E. cicutarium* belongs to the Geraniaceae family, and grows on drought soils and wastelands up to 1500 m elevation. It is widely spread in Middle East regions and is grazed well by ruminants. *P. sinaica*, a perennial grass which reproduces by bulbils and seeds, is widely spread in arid and semi-arid zones of Syria, where it may constitute 80% of the spring vegetative cover, and is grazed well by small ruminants. *S. arabicus*, an annual grass, reproduces only by seeds, grows on poor stony soil, is widely distributed on wastelands and is also grazed well by small ruminants (Al-Rabbat and Abouzakhem 1987). *A. camelorum*, a leguminous shrub,

invades fallow lands up to 1200 m elevation and is grazed by sheep and camels. It is tolerant of saline soil and is distributed in western Caucasia, western Siberia, Iran and central and eastern Asia. *S. vermiculata* is a shrub that belongs to the Chenopodiaceae. It is grazed well by ruminants, usually spreads on sandy saline soils, seashores, fields and wastelands and is distributed through southern and central Europe, north Africa and Asia. *P. harmala* belongs to the Zygophyllaceae, and is a xerophyte distributed in southern Europe, north Africa, south-west Asia, eastwards to Tibet and North America. It can be poisonous to animals in its early vegetative stages. Seeds and roots contain b-carboline, alkaloids, mostly harmine, as well as peganine, isopeganine, dipegene, harmalol, harmaline, vasicinone and deoxyvasicinone. Leaves are used for medicinal purposes, whereas seeds are used as incense (Bischof 1978; Al-Rabbat and Abouzakhem 1987).

The phenolic compounds (particularly tannins) in some range plants and shrubs may bind to protein, thus rendering the protein undegradable by rumen microbes. The extent to which tannins in drought-tolerant range shrubs and herbaceous plants bind to proteins and make them undegradable by rumen microbes is not known. Polyethylene glycols (PEG) of various molecular masses (2000–35 000) have been used to prevent binding between tannins and proteins (Silanikove *et al.* 1994; Makkar *et al.* 1995; Khazaal *et al.* 1996). PEG can also displace protein from a pre-formed tannin-protein complex (Barry and Manley 1986).

Compared with other laboratory techniques, the gas-production technique has proved accurate in predicting animal performance and voluntary feed intake of roughages (Blümmel and Ørskov 1993; Khazaal *et al.* 1993a, 1995; Blümmel and Becker 1997) and was suggested as being more efficient than the nylon bag technique for determining the nutritive value of feeds containing anti-nutritive factors (Khazaal *et al.* 1993b). In the gas method, kinetics of fermentation can be studied by simply reading the increase in gas production at a series of chosen time intervals and using the exponential equation $P = a + b(1 - e^{-ct})$ (Ørskov and McDonald 1979).

The objectives of the present study were to: evaluate, by the use of *in vitro* incubation techniques with rumen fluid, some drought-tolerant native range plants (*E. cicutarium*, *P. sinaica*, *S. arabicus*, *A. camelorum*, *S. vermiculata* and

P. harmala) in terms of microbial nitrogen and biomass production; study the fermentation kinetics of these plants; determine the relationship between gas production and synthesis of microbial biomass; and assess the impact of polyethylene glycol on these parameters.

Materials and methods

Plant materials tested

Different species of native drought-tolerant range plants, grown naturally on the south-eastern desert of Syrian, were collected from different locations (Sikhne 34°48'N, 38°42' E and Gabajeb 35°16'N, 39°42'E), which receive a total annual precipitation of 100–120 mm. The shrubby range plants, *A. camelorum*, *S. vermiculata* and *P. harmala*, were harvested twice, at the early bloom (EB) stage in May or at seeding (S) in August. The stem percentages in the harvested material were 28.4, 21.7 and 18.8% at the EB stage and 24.3, 23.3 and 24.7% at the S stage for *A. camelorum*, *S. vermiculata* and *P. harmala*, respectively. The herbaceous range plants were harvested in April when *E. cicutarium* and *S. arabicus* were at EB and *P. sinaica* was seeding. Plants were cut at 3 cm from ground level for the plants harvested in April and May and at 25 cm for those harvested in August. Subsamples (8 kg and 4 kg from each shrubby and herbaceous range plant, respectively) were randomly taken, dried at room temperature (25–30°C) for 3 weeks, ground to pass through a 1 mm sieve, well mixed and stored frozen at –20°C in sealed nylon bags for later analysis and evaluation. Table 1 gives some nutritive components of the experimental range plants (Al-Masri 2006).

Fermentation kinetics of feeds and the biological activity of tannins

The experimental samples were incubated in 100 mL calibrated glass syringes at 39°C with the ruminal fluid mixed with the medium, basically by the procedures of Menke *et al.* (1979) to determine the rate of gas production during 96 h incubation. As a modification, the syringes were incubated standing upright in a water-bath instead of being stacked horizontally on a slowly turning rotor housed in an incubator (Blümmel

Table 1. Nutritive components of the experimental range plants (g/kg DM)

Plant	Harvesting Stage ¹	Crude ash	Crude protein	Crude fibre	Crude fat	GE ² (MJ/kg DM)	ME (MJ/kg DM)
<i>P. sinaica</i>	S	52.2	81.0	294.7	29.2	16.9	7.08
<i>E. cicutarium</i>	EB	132.3	101.1	275.0	33.5	15.49	7.60
<i>S. arabicus</i>	EB	118.9	98.9	270.5	36.7	15.18	7.65
<i>A. camelorum</i>	EB	77.9	206.5	315.0	17.1	16.31	7.11
<i>A. camelorum</i>	S	139.4	104.1	238.7	43.2	15.88	5.81
<i>S. vermiculata</i>	EB	364.1	200.5	147.1	21.4	11.55	6.94
<i>S. vermiculata</i>	S	332	159.3	166.7	20.3	12.7	5.91
<i>P. harmala</i>	EB	160.1	190	175.3	35.7	15.3	7.42
<i>P. harmala</i>	S	138.1	143.2	240.0	66.4	16.6	6.23

¹ EB = early bloom; S = seeding.

² GE: gross energy.

and Ørskov 1993). The method of Menke *et al.* (1979) was used to study the digestion kinetics of feed samples over 96 h according to the exponential equation $P = a + b(1 - e^{-ct})$ of Ørskov and McDonald (1979), and the biological activity of the tannins according to Makkar *et al.* (1995) with or without adding polyethylene glycol during *in vitro* incubation of rumen fluid.

The rumen fluid was collected from 3 rumen-fistulated Awassi rams, which were fed twice daily on a predominantly roughage diet (lentil straw and alfalfa) and received 185 g crude protein and 10.6 MJ ME per day (Al-Masri 2003). Rumen fluid samples were taken once every 7 days, 17 h after the last feed. The rumen fluid was homogenised and strained through 100µm nylon cloth into a warm flask (39°C) filled with CO₂. A total of 30 mL of medium, consisting of 10 mL of rumen fluid and 20 mL of bicarbonate-mineral-distilled water mixture (1: 1: 2 by vol.), was pumped with an automatic pipette into the warmed syringes containing the samples (200 mg) and into the blank syringes. The syringes were shaken by hand for a couple of seconds, twice in the first hour and once again after 3, 6 and 8 h of incubation.

Gas production with or without adding polyethylene glycol (PEG, 6000; Fluka Firm No. 81260) at a ratio of 2:1 PEG:substrate was recorded after 3, 6, 8, 11, 24, 30, 48, 72 and 96 h of incubation. If gas production for a syringe exceeded 60 mL, the syringe was removed from the bath and the gas was released until the piston returned to 35 mL position, when it was returned to the incubator. Triplicates of each range sample were used in 3 runs. Gas production from the forage sample was calculated by subtracting the volume of gas produced from

the blank with or without the addition of PEG depending on treatment.

Determination of microbial nitrogen and biomass

¹⁵N-labelled urea (>95% ¹⁵N) was added to 30 mL of the rumen fluid mixture and incubated for 96 h with the samples (200 mg), with or without added PEG, to estimate the microbial nitrogen (MN) and microbial biomass (MBM) production (Hardarson and Danso 1990; Al-Masri 2003). Triplicates of each range sample were used in 3 runs. Total nitrogen, as well as ¹⁵N atom excess in the N pool of the sample and fluid mixture incubated for 96 h or in the fluid mixture alone (blank) were measured with an emission spectrometer (JASCO N-150, Japan Spectroscopic Com. Ltd, Tokyo, Japan). The following equations were used to estimate MN and MBM production:

$$\text{MN (mg/96 h/mg sample)} = [1 - (\% \text{ } ^{15}\text{N atom excess in the N-pool of the sample and fluid mixture incubated for 96 h} / \% \text{ } ^{15}\text{N atom excess in the fluid mixture})] \text{ mg N in the sample.}$$

$$\text{MBM (mg/96 h/ mg sample)} = \text{MN} / 0.0864$$

Czerkawski (1986) indicated that the rumen microbes contain 8.64% nitrogen.

Data and statistical analyses

Data on gas production were fitted to the exponential equation $P = a + b(1 - e^{-ct})$ of Ørskov and McDonald (1979), where P (mL) was defined as gas production at time t , a (mL) was the initial gas production, b (mL) was the gas production during incubation, $a + b$ (mL) was the potential

gas production and c (mL/h) was the fractional gas production. The difference in gas production as a result of treatment with PEG was calculated and expressed as a proportion of that for the untreated sample (*i.e.*, % increase). Predicted daily intake (Y) (g DM / kg $M^{0.75}$) was estimated according to Khazaal *et al.* (1995) using the following equation:

$$Y = 18.9 - 0.23(a + b) + 687(c) + 0.11 \text{ crude protein (g / kg DM).}$$

Means of the studied parameters were subjected to an analysis of variance (ANOVA) test, using a Statview-IV program (Abacus Concepts, Berkeley, CA, USA) on an IBM system, and employing the Fisher's least significant difference (LSD) test at the 95% confidence level. Correlation coefficients (r) between the studied parameters were calculated.

Results

Chemical composition

Both stage of growth and species affected the crude protein (CP) levels in the harvested material, with the shrubs displaying higher CP concentrations than the herbaceous species

(Table 1). In all shrubs, CP and ME declined between early bloom and seed set, despite the fact that cutting height was raised to 25 cm for the seeding cut.

Gas production and fermentation kinetics

Changes in gas production by the studied range plants after incubation with or without PEG and their fermentation kinetics are presented in Table 2. Initial gas production (a), potential gas production ($a + b$) and rate of gas production (c) differed between species. *Erodium cicutarium* produced the most gas in the initial stages, and total gas production over 96 h was significantly higher in *E. cicutarium* and *P. sinaica* than in *P. hamala* and the shrub species (Figure 1). Addition of PEG increased the level of gas production in all species but for most species and incubation times, differences failed to reach significant levels. The increase (%) in gas production by the studied range plants when treated with PEG varied between species and harvesting stage and tended to decline as incubation progressed, with the highest increase during the first 24 h of incubation.

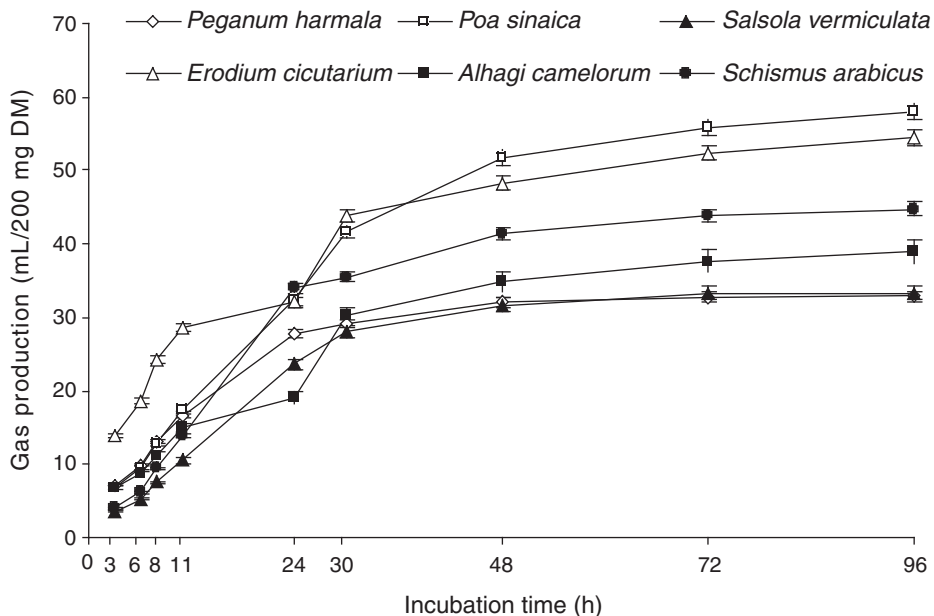


Figure 1. Cumulative gas production (*in vitro*) over 96 h from the experimental range plants harvested in May.

Table 2. Cumulative gas production *in vitro* (mL/100 mg DM) from some range plants after incubation with or without polyethylene glycol (PEG, 6000) for 96 h and the characteristics of fermentation obtained by fitting data for gas production after 3, 6, 8, 11, 24, 30, 48, 72 and 96 h incubation to the equation $P = a + b(1 - e^{-ct})^1$.

Range plant	Harvesting stage ²	PEG	Gas production after				<i>a</i>	<i>b</i>	<i>a + b</i>	<i>c</i>	RSD ³	Y ⁴
			24 h	48 h	72 h	96 h						
<i>Poa sinica</i>	S	No	16.2	25.9	28.0	29.0	0.987	31.465	30.478	0.018	0.946	38.5
		+	19.1	29.5	31.2	33.2	0.818	34.724	33.906	0.020	1.207	
Increase (%) ⁵			18	14	12	14						
<i>Erodium cicutarium</i>	EB	No	16.1	24.2	26.2	27.3	5.212	22.596	27.808	0.020	1.460	44.0
		+	17.3	25.6	26.9	28.2	5.353	23.114	28.467	0.021	1.443	
Increase (%)			8	6	3	3						
<i>Schismus arabicus</i>	EB	No	17.0	20.7	22.0	22.4	3.39	26.197	22.808	0.027	1.141	56.4
		+	18.4	23.2	24.5	25.0	3.074	28.598	25.740	0.026	1.194	
Increase (%)			8	12	12	12						
<i>Alhagi camelorum</i>	EB	No	9.6	17.4	18.9	19.5	0.985	19.789	20.774	0.018	1.122	56.8
		+	17.2	19.5	20.2	20.6	1.368	18.655	20.023	0.043	0.589	
Increase (%)			79	12	7	6						
<i>Alhagi camelorum</i>	S	No	9.7	12.1	12.4	12.4	0.113	12.666	12.553	0.032	0.295	68.7
		+	15.6	17.7	17.8	18.2	1.781	15.856	17.637	0.065	0.638	
Increase (%)			61	46	44	47						
<i>Salsola vermiculata</i>	EB	No	11.8	15.9	16.7	16.7	2.122	19.381	17.259	0.026	0.818	68.1
		+	13.7	17.3	17.9	18.5	2.917	21.633	18.717	0.028	0.896	
Increase (%)			16	9	7	11						
<i>Salsola vermiculata</i>	S	No	8.6	12.4	13	12.9	0.481	14.042	13.561	0.026	0.664	65.2
		+	11.5	14.2	14.7	15.1	0.418	15.655	15.237	0.029	0.418	
Increase (%)			34	15	13	17						
<i>Peganum harmala</i>	EB	No	13.9	16.1	16.4	16.5	0.111	16.843	16.733	0.034	0.464	78.1
		+	14.6	16.9	17.2	17.5	0.188	17.372	17.559	0.034	0.499	
Increase (%)			5	5	5	6						
<i>Peganum harmala</i>	S	No	9.8	12.3	12.9	13.1	2.436	10.862	13.297	0.024	0.194	60.8
		+	10.5	13.5	14.1	14.2	2.000	12.620	14.620	0.023	0.310	
Increase (%)			7	10	10	8						
LSD (0.05)			2.0	3.0	3.5		1.790	3.954	4.356	0.015		
Mean			13.9	18.6	19.5		0.272	20.115	20.387	0.029		
± s.e.			0.5	0.8	0.8		0.356	0.933	0.904	0.002		

¹ *a*, *b* and *c* are constants in the exponential equation $P = a + b(1 - e^{-ct})$. *a*, initial gas production; *b*, gas production during incubation; *a + b*, potential gas production; *c*, rate of gas production. Number of replicates, 3.

² EB = early bloom; S = seeding.

³ RSD, residual standard deviation of fitting the mean of gas production to the exponential equation.

⁴ Y, predicted daily intake (g DM/kg M^{0.75}).

⁵ Increase (%) = $\{[(\text{gas volume with PEG}) - (\text{gas volume without PEG})] / (\text{gas volume without PEG})\} \times 100$.

Table 3. Changes in the microbial nitrogen and biomass synthesis of the experimental range plants after incubation with or without polyethylene glycol (PEG, 6000) for 96 h.

Range plant	Harvesting stage ¹	Microbial nitrogen (mg/100 mg DM)				Microbial biomass (mg/100 mg DM)			
		With PEG	Without PEG	LSD (P<0.05)	² Pooled (n=6) (±s.e.)	With PEG	Without PEG	LSD (P<0.05)	² Pooled (n=6) (±s.e.)
<i>Poa sinica</i>	S	0.030	0.021	0.001	0.026 (0.002)	0.347	0.243	0.013	0.295 (0.025)
<i>Erodium cicutarium</i>	EB	0.167	0.140	0.037	0.154 (0.008)	1.933	1.620	0.370	1.776 (0.091)
<i>Schismus arabicus</i>	EB	0.215	0.193	0.012	0.204 (0.005)	2.488	2.234	0.082	2.361 (0.068)
<i>Alhagi camelorum</i>	EB	0.400	0.330	0.132	0.365 (0.026)	4.630	3.819	1.559	4.223 (0.307)
	S	0.233	0.165	0.057	0.199 (0.018)	2.700	1.910	0.593	2.305 (0.200)
<i>Salsola vermiculata</i>	EB	0.785	0.530	0.119	0.658 (0.060)	9.086	6.134	1.368	7.610 (0.684)
	S	0.483	0.420	0.137	0.452 (0.026)	5.590	4.861	1.559	5.226 (0.298)
<i>Peganum harmala</i>	EB	0.768	0.540	0.126	0.654 (0.055)	8.889	6.250	1.406	7.570 (0.639)
	S	0.517	0.498	0.118	0.508 (0.023)	5.984	5.764	1.374	5.874 (0.262)
LSD (P<0.05)		0.073	0.074		0.050	0.838	0.842		0.571
³ Pooled (n=27) (±s.e.)		0.400 (0.049)	0.315 (0.036)	0.024		4.597 (0.568)	3.648 (0.419)	0.272	

¹ EB = early bloom; S = seeding.

² Total effect of plant species.

³ Total effect of PEG.

Microbial nitrogen and biomass

The levels of microbial nitrogen or microbial biomass produced from 100 mg substrate depended on species, stage of growth and presence or absence of PEG (Table 3). In general, the shrub species produced more microbial nitrogen and microbial biomass than the herbaceous species and maturity reduced production of both in the shrub species where comparisons existed. Overall, while addition of PEG increased production of microbial N and biomass, this was not true for individual species. Overall, *S. vermiculata* and *P. harmala* at early bloom produced the most microbial N and biomass.

There was a negative correlation between gas production at 96 h of incubation and crude fibre concentration ($r = -0.61$), crude protein ($r = -0.49$) and microbial nitrogen or biomass production ($r = -0.69$) for the studied species without addition of PEG. Predicted daily intake was negatively correlated with potential gas production ($a + b$) ($r = -0.80$) and crude fibre concentration ($r = -0.74$) and positively correlated with crude protein ($r = 0.63$) and microbial nitrogen or biomass production ($r = 0.77$). Microbial nitrogen or biomass production was negatively correlated with crude fibre concentration ($r = -0.73$) and positively correlated with crude protein concentration ($r = 0.84$).

Discussion

This study has provided interesting information on the value of a number of plant species adapted to arid environments for use as ruminant feeds. This information may prove useful for herders, farmers and ruminant nutritionists in developing appropriate feeding strategies for ruminants in the dry season.

Composition, gas production and fermentation kinetics

The decline in crude protein concentration with maturity in the shrub species was to be expected as were the negative correlations between crude protein and crude fibre concentrations ($r = -0.50$). Similar correlations between crude protein and crude fibre concentrations ($r = -0.83$) of some shrubs were obtained by

Al-Masri (2003). It is of interest that crude fibre concentration in *A. camelorum* declined with maturity, while the reverse was the case with *S. vermiculata* and *P. harmala*. The leaf proportion in *A. camelorum* was higher at the S stage than at the EB stage, which could explain the apparent anomaly. The range in predicted intake of the studied range plants was wide (39–78 g DM/kg M^{0.75}) and may be attributed to differences in their nutrient concentrations, particularly crude fibre and crude protein. Further work is needed to determine *in vivo* the feed intake and palatability of these species.

The amount of gas produced per unit of fermented material reflects the level of fermentation of the forages. High CH₄ production from ruminants is undesirable from both economic and environmental aspects. While gas production increased as time of digestion increased, the greatest proportion of the production occurred in the first 24 hours. Shrub material harvested at early bloom produced more gas than that harvested at seeding, which is indicative of the relative digestibilities of the forage at the two stages of maturity.

The addition of PEG in the fermentation process increased the level of gas production in all cases with the magnitude of the increase varying with species and stage of maturity, and the greatest increase also occurring during the first 24 h of the fermentation process. This, allied with the higher production of MN and MBM in the presence of PEG, suggests that the PEG might have bound with tannins, releasing proteins for microbial breakdown. Pritchard *et al.* (1988) indicated that the low intake and feed value of mulga (*Acacia aneura*) leaf was related to its content of condensed tannins, which bound with the proteins in the leaves. Further work is needed to study the anti-nutritional factors (tannins, saponins) and deleterious substances (alkaloids, harmine, peganine, etc.) in the range plants at different stages of maturity.

The higher gas production and MN and MBM production following inclusion of PEG in our study are at variance with other findings. Getachew *et al.* (2000) indicated that addition of PEG 6000 to tannin-containing plants (*Acacia cyanophylla*, *Acacia albida*, *Acacia barberi* and *Quercus ilex*) significantly ($P < 0.05$) increased *in vitro* gas and short-chain fatty acid (VFA) production, and *in vitro* degradability of N, but reduced the efficiency of microbial protein synthesis.

However, the overall negative correlation ($r = -0.69$) between gas production and microbial nitrogen production in our study in the absence of PEG is in agreement with results of other studies. Al-Masri (2003) found a similar negative relationship ($r = -0.61$) between gas production and microbial biomass production per unit of substrate degraded for some unconventional feeds incubated for 24 h. In addition, Blümmel (1994) indicated a significant negative correlation ($r = -0.64$) between the amount of substrate converted to microbial cells and the gas produced from a given unit of truly fermented substrate. Hillman *et al.* (1993) and Blümmel *et al.* (1997a; 1997b) also showed that gas production was negatively related to microbial protein synthesis.

Microbial nitrogen and biomass

The amount of microbial nitrogen (MN) produced from 100 mg substrate varied with species and amounted to 0.02–0.54 mg without PEG and 0.17–0.79 mg with PEG. Corresponding values for microbial biomass (MBM) were 1.62–6.25 mg without PEG and 0.35–9.10 mg with PEG. These figures suggest that some proteins may have been bound by complexes (possibly tannins) and the PEG displaced the proteins from the complexes, making proteins available for microbial degradation and formation of MBM and MN. This needs verification. The different responses in different species and at different stages of maturity suggest different levels of tannins or other anti-nutritional factors in the different situations. Whether the protein protected by tannins from microbial degradation in the rumen is available to animals post-ruminally is a matter for further research.

The net microbial nitrogen or biomass production would depend on the balance between decreased degradable dry matter and higher microbial mass production per unit dry matter digested. While *Poa sinaica* is considered highly by farmers, it produced only a small amount of microbial protein compared with other species. Al-Masri (2003) indicated that the amount of MN and MBM reported from 100 mg of truly fermented organic matter of some browses and range plants (*Atriplex leucoclada*, *Prosopis stephaniana*, *Moringa oleifera*, *Jatropha curcas*) amounted to 0.7–2.9 mg and 8–34 mg, respectively. In a further study, Al-Masri (2007)

indicated that MN and MBM values amounted to 1.29 and 14.93 mg/100 mg DM for *Sesbania aculeata* harvested at 60 days after planting and 0.68 and 7.87 mg/100 mg DM when harvested at 120 days after planting, respectively.

The levels and types of tannins in feeds and their biological activity can have negative or positive effects on N utilisation to produce microbial nitrogen. It is interesting that the addition of PEG to *S. vermiculata* and *P. harmala* had much greater effects on production of MN and MBM at the early bloom stage than at the seeding stage. Al-Masri and Mardini (2007) indicated that condensed tannin concentration (17.1 g/kg DM) was higher in leaves of *Sesbania aculeata* than in stalks (3.5 g/kg DM). This could be a contributing factor.

The degradability of protein in tannin-containing feeds is depressed, resulting in low $\text{NH}_3\text{-N}$ concentration. Addition of PEG results in increased levels of $\text{NH}_3\text{-N}$. However, this does not necessarily increase microbial protein synthesis, probably due to poor synchronisation between N release and carbohydrate fermentation. A rapid degradation of N not matched with the availability of energy could lead to accumulation of $\text{NH}_3\text{-N}$ in the *in vitro* system or to a high absorption of $\text{NH}_3\text{-N}$ from the rumen *in vivo*. *In vivo*, the $\text{NH}_3\text{-N}$ not captured in the rumen is absorbed and converted into urea, and the synthesis of urea in the liver requires expenditure of energy, each mole of urea requiring 4 moles of ATP (Van Soest 1994). Synchronisation of the rate of degradation of N and carbohydrate components in the rumen is extremely important for efficient utilisation of rumen $\text{NH}_3\text{-N}$ for synthesis of microbial protein. Therefore, utilisation of browses with high tannin levels would possibly be improved by inclusion of tannin-binding agents such as PEG and an additional energy source to trap the N resulting from the fermentation. Getachew *et al.* (2000) indicated that incubation of tannin-containing feeds in the absence of PEG resulted in higher microbial protein synthesis than in the presence of PEG. However, incubation of tannin-containing feeds in combination with PEG and starch yielded higher microbial protein synthesis than with PEG alone. Norton and Ahn (1997) infused PEG into sheep fed on *Calliandra calothyrsus* and reported higher microbial efficiency in the non-PEG group compared with those infused with PEG.

Addition of PEG can be advantageous if the tannin content of the feed is sufficiently high that it depresses microbial activity and digestibility of feeds drastically. On the other hand, addition of PEG to low-tannin feeds may result in negative effects by reducing the amount of by-pass protein and also by decreasing the efficiency of microbial protein synthesis (Getachew *et al.* 2000). The beneficial effect of low levels of tannins in forages could be due to the tannins protecting protein from microbial degradation in the rumen (Barry *et al.* 1986; Waghorn *et al.* 1994); this increases the amount of by-pass protein to the lower gut as well as causing a higher flow of microbial protein to the intestine as a result of higher efficiency of microbial protein synthesis. Montossi *et al.* (1997) reported that addition of PEG to tannin-containing diets reduced liveweight gain. Inclusion of PEG in the diet resulted in reduced non-NH₃-N digested post-ruminally, increased N excreted in urine, and increased rumen NH₃-N (Barry *et al.* 1986). However, Degen *et al.* (1998) used PEG with *Acacia saligna* diets containing Quebracho tannin, and tannic acid, and found that addition of PEG increased dry matter intake and body weight gain.

These preliminary studies would suggest that all species except *P. harmala* are suitable as supplements for livestock in the dry season in tropical regions. More detailed studies are needed to determine the anti-nutritional factors (tannins) and deleterious substances (alkaloids) in the range species at different stages of maturity. Feeding of PEG with the browse forages should improve microbial activity at some stages and presumably animal performance. However, these hypotheses would require testing with animals and the economics of feeding this compound would need investigation before feeding strategies could be recommended for use commercially.

Acknowledgements

The author thanks the Director General and Head of Agriculture Department, Atomic Energy Commission of Syria, for their encouragement and financial support.

References

AL-MASRI, M.R. (2003) An *in vitro* evaluation of some unconventional ruminant feeds in terms of the organic matter

- digestibility, energy and microbial biomass. *Tropical Animal Health and Production*, **35**, 155–167.
- AL-MASRI, M.R. (2006) Nutritive value of some indigenous range plants and their *in vitro* biochemical fermentable characteristics. *AECS-A/FRSR 361*, 43 p. (Atomic Energy Commission: Syria).
- AL-MASRI, M.R. (2007) Effects of harvest time and cutting regimen on the *in vitro* nutritive value and fermentable characteristics of dhaincha (*Sesbania aculeate*). *AECS-A/FRSR 380*, 46 p. (Atomic Energy Commission: Syria).
- AL-MASRI, M.R. and MARDINI, M. (2007) Effect of harvest time and sampling site on the nutritive value of *Sesbania aculeate* and *Kochia indica*. *AECS-A/RSS 710*, 28 p. (Atomic Energy Commission: Syria).
- AL-RABBAT, M.F. and ABOUZAKHEM, M. (1987) *Some Economically Important Range Plants*. 3rd Edn. 237 p. (Publication Department, University of Damascus: Syria).
- BARRY, T.N. and MANLEY, T.R. (1986) Interrelationships between the concentrations of total condensed tannin, free condensed tannin and lignin in *Lotus* sp. and their possible consequences in ruminant nutrition. *Journal of Food Science and Agriculture*, **37**, 248–254.
- BARRY, T.N., MANLEY, T.R. and DUNCAN, J. (1986) The role of condensed tannins in the nutritional value of *Lotus pendunculatus* for sheep. 4. Sites of carbohydrate and protein digestion as influenced by dietary reactive tannin concentration. *British Journal of Nutrition*, **55**, 123–137.
- BISCHOF, F. (1978) *Common Weeds From Iran, Turkey, The Near East and North Africa*. 212 p. (Deutsche Gesellschaft für Technische Zusammenarbeit, GTZ: GmbH, Germany).
- BLÜMMEL, M. (1994) *Relationship between kinetics of stover fermentation as described by the Hohenheim *in vitro* gas production test and voluntary feed intake of 54 cereal stovers*. Ph.D. Dissertation. University of Hohenheim, Germany.
- BLÜMMEL, M. and ØRSKOV, E.R. (1993) Comparison of *in vitro* gas production and nylon bag degradability of roughages in predicting feed intake in cattle. *Animal Feed Science and Technology*, **40**, 109–119.
- BLÜMMEL, M. and BECKER, K. (1997) The degradability characteristics of fifty-four roughages and roughage neutral-detergent fibres as described by *in vitro* gas production and their relationship to voluntary feed intake. *British Journal of Nutrition*, **77**, 757–768.
- BLÜMMEL, M., MAKKAR, H.P.S. and BECKER, K. (1997a) *In vitro* gas production: a technique revisited. *Journal of Animal Physiology and Animal Nutrition*, **77**, 24–34.
- BLÜMMEL, M., STEINGASS, H. and BECKER, K. (1997b) The relationship between *in vitro* gas production, *in vitro* microbial biomass yield and ¹⁵N incorporation and its implications for the prediction of voluntary feed intake of roughages. *British Journal of Nutrition*, **77**, 911–921.
- CAMPLING, R.C., FREER, M. and BALCH, C.C. (1962) Factors affecting the voluntary intake of food by cows. 3. The effect of urea on the voluntary intake of oat straw. *British Journal of Nutrition*, **15**, 115–124.
- CZERKAWSKI, J.W. (1986) *An Introduction to Rumen Studies*. (Pergamon Press: Oxford).
- DEGEN, A.A., MISHORR, T., MAKKAR, H.P.S., KAM, M., BENJAMIN, R.W., BECKER, K. and SCHWARTZ, H.J. (1998) Effect of *Acacia saligna* with and without administration of polyethylene glycol on dietary intake in desert sheep. *Animal Science*, **67**, 491–498.
- GETACHEW, G., MAKKAR, H.P.S. and BECKER, K. (1998) The *in vitro* gas coupled with ammonia nitrogen measurement for evaluation of nitrogen degradability in low quality roughages using incubation medium of different buffering capacity. *Journal of Food Science and Agriculture*, **77**, 87–95.
- GETACHEW, G., MAKKAR, H.P.S. and BECKER, K. (2000) Effect of polyethylene glycol on *in vitro* degradability of nitrogen and microbial protein synthesis from tannin-rich browse and herbaceous legumes. *British Journal of Nutrition*, **84**, 73–83.

- GOODCHILD, A.V. and McMENIMAN, N.P. (1994) Intake and digestibility of low quality roughages when supplemented with leguminous browse. *Journal of Agricultural Science*, **122**, 151–160.
- HARDARSON, G. and DANSO, S.K.A. (1990) Use of ^{15}N Methodology to Assess Biological Nitrogen Fixation. In: *Use of Nuclear Technique in Studies of Soil-Plant Relationships. Training Course Series No. 2*. pp. 129–160. (International Atomic Energy Agency: Vienna).
- HILLMAN, H.K., NEWBOLD, C.J. and STEWART, C.S. (1993) The contribution of bacteria and protozoa to ruminal forage fermentation *in vitro*, as determined by microbial gas production. *Animal Feed Science and Technology*, **42**, 193–208.
- KHAZAAL, K., DENTINHO, M.T., RIBEIRO, R. and ØRSKOV, E.R. (1993a) A comparison of gas production during incubation with rumen contents *in vitro* and nylon bag degradability as predictors of the apparent digestibility *in vivo* and voluntary intake of hays. *Animal Production*, **57**, 105–112.
- KHAZAAL, K., MARKANTONATOS, X., NASTIS, A. and ØRSKOV, E.R. (1993b) Changes with maturity in fiber composition and levels of extractable polyphenols in Greek browse: effects on *in vitro* gas production and in sacco dry matter degradation. *Journal of Science of Food Agriculture*, **63**, 237–244.
- KHAZAAL, K., DENTINHO, J.M., RIBEIRO, J.M. and ØRSKOV, E.R. (1995) Prediction of apparent digestibility and voluntary intake of hays fed to sheep: comparison between using fibre components, *in vitro* digestibility or characteristics of gas production or nylon bag degradation. *Animal Science*, **61**, 527–538.
- KHAZAAL, K., PARISSI, Z., TSIIOUVARAS, C., NASTIS, A. and ØRSKOV, E.R. (1996) Assessment of phenolics-related anti-nutritive levels using the *in vitro* gas production technique: A comparison between different types of polyvinylpyrrolidone or polyethylene glycol. *Journal of Food Science and Agriculture*, **71**, 405–414.
- MAKKAR, H.P.S., BLÜMMEL, M. and BECKER, K. (1995) Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in *in vitro* techniques. *British Journal of Nutrition*, **73**, 897–913.
- MENKE, K.H., RAAB, L., SALEWSKI, A., STEINGASS, H., FRITZ, D. and SCHNEIDER, W. (1979) The estimation of the digestibility and metabolizable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor *in vitro*. *Journal of Agricultural Science, Cambridge*, **93**, 217–222.
- MONTOSI, F., LIU, F., HODGSON, J., MORRIS, S.T., BARRY, T.N. and RISSO, D.F. (1997) Influence of low-level condensed tannins concentrations in temperate forages on sheep performance. *Proceedings of the XVIII International Grassland Congress, Winnipeg, Manitoba and Saskatoon, Saskatchewan, Canada*, **1**, 8.1–8.2.
- NORTON, B.W. and AHN, J.H. (1997) A comparison of fresh and dried *Calliandra calothyrsus* supplements for sheep given a basal diet of barley straw. *Journal of Agricultural Science*, **129**, 485–494.
- PRITCHARD, D.A., STOCKS, D.C. O'SULLIVAN, B.M., MARTIN, P.R., HURWOOD, I.S. and O'ROURKE, P.K. (1988) The effect of polyethylene glycol (PEG) on wool growth and live weight of sheep consuming mulga (*Acacia aneura*) diet. *Proceedings of the Australian Society of Animal Production*, **17**, 290–293.
- ØRSKOV, E.R. and GRUBB, D.A. (1978) Validation of new systems for protein evaluation in ruminants by testing the effect of urea supplementation on intake and digestibility of straws with or without sodium hydroxide treatment. *Journal of Agricultural Science, Cambridge*, **91**, 483–486.
- ØRSKOV, E.R. and McDONALD, I. (1979) The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Agricultural Science, Cambridge*, **92**, 499–503.
- SIEBERT, B.D. and HUNTER, R.A. (1982) Supplementary feeding of grazing animals. In: Hacker, J.B. (ed.) *Nutritional Limits to Animal Production from Pastures*. pp. 409–426. (Commonwealth Agricultural Bureaux: Slough).
- SILANIKOVE, N., NITSAN, Z. and PEREVOLTSKY, A. (1994) Effect of a daily supplementation of polyethylene glycol on intake and digestion of tannin-containing leaves (*Ceratonia siliqua*) by sheep. *Journal of Agriculture and Food Chemistry*, **42**, 2844–2847.
- VAN SOEST, P.J. (1994) *Nutritional Ecology of the Ruminant*, 2nd Edn. (Cornell University Press: Ithaca, NY).
- WAGHORN, G.C., SHELTON, I.D., McNABB, W.C. and McCUTCHEON, S.N. (1994) Effect of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 2. Nitrogenous aspects. *Journal of Agricultural Science*, **123**, 109–119.

(Received for publication March 23, 2007; accepted October 1, 2007)