Tropical and Subtropical Agroecosystems

RUMINAL FERMENTATION AND TANNINS BIOACTIVITY OF SOME BROWSES USING A SEMI-AUTOMATED GAS PRODUCTION TECHNIQUE

[FERMENTACION RUMINAL Y BIOACTIVIDAD DE TANINOS DE ALGUNAS ARBUSTIVAS USANDO UNA TÉCNICA DE PRODUCCION DE GAS SEMI-AUTOMATIZADA]

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SUMMARY

The nutritive value, tannins bioactivity and methane production of some tropical plants e.g. acacia (*Acacia saligna)*, leucaena (*Leucaena leucocephala*), atriplex (*Atriplex caseneace)*, eucalyptus (*Eucalytus globules*), and alfalafa (*Medicago sativa*) hay were evaluated *in vitro* by chemical composition and gas production (GP) technique. Crude protein content was ranged from 95.4 to 204.0 g kg^{-1}DM for eucalyptus and leucaena, respectively. Leucaena and alfalfa had the highest neutral detergent fibre content (543.1 and 546.7 g kg^{-1}DM), respectively. Atriplex had the lowest content (173.8 g kg^{-1}DM) of acid detergent fibre and acid detergent lignin (85.89 g kg^{-1}DM). Alfalfa, atriplex and eucalyptus had negligible condensed tannins (CT) content (0.2, 0.3, 4.9 eq-g leucocyanidin kg^{-1}DM), while acacia and leucaena had the highest CT content (61.4 and 32.5 eq-g leucocyanidin kg^{-1}DM). Acacia and leucaena produced less gas in absence of PEG. Inclusion of PEG in fermentation of tropical browses resulted in an increase of GP (from 28.1 to 87.4 1%) particularly the plants rich in CT. The methane production decreased (P<0.05) by 88, 89 and 90.9 % in leucaena, acacia and eucalyptus samples, respectively in comparison to alfalfa. The ranking order of the browse species on the basis of their potential *in vitro* dry and organic matter degradability was: atriplex > alfalfa > acacia > eucalyptus > leucaena. Partitioning factor values differed (P<0.05) among plants. It is concluded that the four tropical plants represent an important fodder reserve for livestock in harsh conditions according to their chemical composition and fermentation pattern.

Key words: Tropical browses; phenolic compounds; degradability; methane; *in vitro* gas production.

RESUMEN

El valor nutritivo, la bioactividad de taninos y la producción de metano de las plantas tropicales: Acacia (*Acacia saligna*), Leucaena (*Leucaena leucocephala*), Atriplex (*Atriplex caseneace*), Eucalyptus (*Eucalytus globules*), y heno de Alfalfa (*Medicago sativa*) fueron evaluadas mediante la composición química y la técnica de producción de gas (PG) in vitro. El contenido de proteína cruda fluctuó desde 95.4 a 204.0 g Kg^{-1} MS para eucalyptus y leucaena, respectivamente. La leucaena y la alfalfa tuvieron los mayores contenidos de fibra detergente neutra (543.1 y 546.7 g Kg^{-1} MS), respectivamente. Atriplex tuvo el menor contenido (173.8 g Kg^{-1} MS) de fibra detergente ácido y lignina detergente ácido (85.89 g Kg^{-1}MS). Alfalfa, atriplex y eucalyptus tuvieron un contenido de taninos condensados (TC) insignificante (0.2, 0.3, 4.9 eq – g leucocianidina Kg^{-1} MS), mientras acacia y leucaena tuvieron los mayores contenidos de TC (61.4 y 32.5 eq-g leucocianidina Kg^{-1}MS). La acacia y la leucaena produjeron menos gas en ausencia de PEG. La inclusión de PEG en la fermentación de pastos tropicales resultó en un incremento de PG (28.1 y 87.4 %) particularmente las plantas ricas en TC. La producción de metano disminuyó (P<0.05) por 88, 89 y 90.9 % en muestras de leucaena, acacia y eucalyptus, respectivamente en comparación a la alfalfa. El orden del rango de las especies de pastos sobre la base de su potencial de degradabilidad de la materia seca y orgánica *in vitro* fue: atriplex > alfalfa > acacia > eucalyptus > leucaena. Los valores del factor de partición difirieron (P<0.05) entre las plantas. Se concluye que las cuatro plantas evaluadas representan una reserva importante de forraje para la ganadería en condiciones críticas teniendo en cuenta la composición química, la PG y el patrón de fermentación.

Palabras claves: Arbustos tropicales; compuestos fenólicos; degradación; metano; *in vitro*.

INTRODUCTION

The major constraints to increase ruminant productivity in developing countries are the scarcity, fluctuating quantity and quality of the year-round supply of conventional feeds and the inadequate nitrogen supply from low quality forages such as straw and stovers (Leng, 1990), which often contain as low as 20–50 g/kg of crude protein which do not meet the minimum crude protein requirement (80 g/kg DM) for optimal rumen microbial function (Annison & Bryden, 1998). Browse foliages are important components in the diets of cattle, sheep, goats and wild ungulates in arid and semi-arid regions of tropical Africa, including Egypt. The recognition of the potential of tree foliage to produce considerable amounts of high protein biomass and energy especially in harsh and arid conditions has led to the development of animal farming systems that integrate the use of tree foliages with local bulky feed resources (Devendra, 1990).

Despite their potential as feed resources for ruminants, most tropical shrubs contain high levels of anti-nutritional factors such as tannins. At high levels, tannins may have detrimental effects on the nutritive value of forages by reducing their palatability, intake and digestibility (Kumar & D'Mello, 1995). Polyethylene glycol (PEG), a tannin complexing agent, has potential to assess phenolic related anti-nutritive effects in browse plants by forming tannin-PEG complexes (Makkar et al., 1995). Thus, PEG can prevent their formation or liberate protein from tannin-protein complexes (Barry & Manley, 1986), and it has been used to mitigate adverse effects of secondary compounds on rumen fermentation, as well as improve ruminants performance fed diets high in secondary compounds. Increased interest in use of non-conventional feed resources has led to an increase in use of *in vitro* GP technique to provide useful data on digestion and fermentation kinetics of both the soluble and insoluble fractions of browses and tannin bioassay. Therefore, nutritional evaluation of tree forages in terms of phenolics, *in vitro* gas and methane production, substrate fermentability, the partitioning factor (PF) and fermentation activity in the presence of PEG was undertaken to quantify adverse effects of tannins, and the usefulness of these forages, in feeding ruminants in comparison with alfalfa hay as high quality roughage.

MATERIAL AND METHODS

Browses sampling

Browse species were selected: Acacia (*Acacia saligna)*, Leucaena (*Leucaena leucocephala*), Atriplex (*Atriplex caseneace)*, Eucalyptus (*Eucalyptus globules*), Alfalfa (*Medicago sativa*) hay and Tifton-85 (*Cynodon sp*) hay (TIF). *Medicago sativa* hay has been used as reference forage in evaluating the native shrubs. Samples (branches with 5 mm or less of length) were collected at February 2006 from North West coast region of Egypt. In general, the climate of this region is arid Mediterranean where the hard conditions of drought were caused by scarcity of rain and high radiation. Temperature is relatively mild throughout the growing season across the region and frost never occurs along the coast. The lowest temperature is observed in winter around 9^{o}C, whilst in summer it fluctuates between 25- 30^{o}C. The atmospheric relative humidity does not vary greatly through the year; it fluctuates between 50 to 60 percent at noon and 60 to 75 percent in the mornings and evenings. Average annual rainfall is about 100-150 mm, distributed over a period of 15-25 rainy days during autumn and winter, respectively and no rain in summer. North-West and South-West winds are prevalent, and wind blows strongly during winter and early spring at an average speed of 20-23 km/hr., with occasional very strong south-west wind loaded with dust (Khamasin) mostly between February and April. Soils are largely alluvial and tend to afine textured. Samples were dried at 40^{o}C in air-forced oven for 72 h and ground through a 1 mm screen before chemical analysis and *in vitro* trails.

Chemical analysis

Browses were analyzed according to AOAC (1995) (DM: dry matter - ID number 930.15; OM: organic matter - ID number 942.05; CP: crude protein – as 6.25 x N- ID number 954.01; and ADF: acid-detergent fibre – ID number 973.18), (2002) (NDF: neutral-detergent fibre) were determined according to Mertens, 2000), sodium sulphite and alpha amylase were omitted from the NDF procedure

Samples were also analyzed for extractable total phenols (TP), tannins (TT) and condensed tannins (CT). Dried plant material (200 mg) was extracted with acetone: water (10 ml, 70:30 v/v) in an ultrasonic bath for 20 minutes. The contents were centrifuged (4°C, 10 min, 3000 *g*) and the supernatant was kept on ice until analysis. Total phenols were determined with the Folin-Ciocalteau reagent (Makkar et al., 1993, Makkar, 2003). Extractable tannins were determined as the difference in total phenols (measured by Folin-Ciocalteau reagent) before and after treatment with insoluble polyvinyl polypyrrolidone (PVPP), as this polymer binds strongly to tannins (Makkar et al., 1995). TP and TT were expressed as tannic acid equivalents. Condensed tannins were measured by the HCl-butanol method and the results were expressed as leucocyanidin equivalent (Makkar, 2003).

In vitro GP and tannin bioassay

The *in vitro* gas production (GP) assay was carried out using a pressure transducer and data logger (LANA/CENA-USP, Piracicaba/SP, Brazil) for measuring the gas produced in 160 mL serum bottles incubated at 39ºC (Mauricio et al., 1998) with the substrates and the buffered rumen fluid. Five adult rumen cannulated sheep grazing tropical grass pasture and a supplement based on maize and soybean meal (0.7 kg/100 kg of live weight, 20% CP) plus a mineral mixture were used as inoculum donor. Both solid and liquid rumen fractions (50:50, solid : liquid) were collected before the morning feeding through the cannula using a stainless steel probe (2.5 mm screen) attached to a large capacity syringe. Fluids and solids were placed separately in pre-warmed (39ºC) flasks and transported under anaerobic conditions to the laboratory. Equal volumes of solid and liquid phases of rumen digesta were mixed in a blender and then squeezed through a 35 µm nylon filter and kept in a water bath at 39ºC with CO_2 saturation until inoculation (Bueno et al., 2005).

Ground samples (0.5 g) were incubated in 75 ml of diluted rumen fluid (25 ml mixed rumen fluid + 50 ml of Menke's buffered medium) in 160 mL serum bottles. Once filled, all the bottles were closed with rubber stoppers, crimped with aluminium seals, shaken and placed in the incubator at 39 °C. The bottles were shaken manually after the recording of the gas headspace pressure at 3, 6, 9, 14 and 24h of incubation using a pressure transducer (Theodorou et al., 1994). Six replicates each sample were used: two bottles with 0.5 g of PEG (PEG 6000, Merck Schuchardt OHG, Hohenbrunn, Germany) has been used to evaluate tannin activity in browse samples, two for measuring the partition factor (PF) and another two for measuring the dry and organic matter degradability. The PEG tannin bioassay was conducted according to Makkar et al. (1995) as revised by Makkar (2000) with adaptation for semi automated system (Cabral Filho et al., 2005) as measurement of the tannins bioactivity. Four serum bottles containing only rumen fluid were incubated as blanks and used to compensate for GP in the absence of substrate. Two serum bottles with internal standard were used for adjustments and variation between the runs. Three runs were done for the same samples. The amount of GP (GP) at each measuring time was calculated according to the regression equation obtained in our system and conditions from unpublished data on 500 samples between gas volume versus pressure: GP (ml) = 0.0112 psi^2 + 7.3358 psi (r^2=0.98).

Methane production

Ten ml gas from each bottle (two ml each time) was collected at 3, 6, 9, 14 and 24 hr of incubation using syringe and pooled at the vacutainer tubes for methane analyses. Methane determination was done in a Shimadzu gas chromatography 2014 using a standard methane gas (50%) at 240 °C for the detector and at 60°C for the shincarbon ST micro packed column. The test of linearity and calibration were accomplished using the standard gas curve in the range of probable concentration of the samples. Methane production at the end of incubation period was estimated from the volume of gas.

Rumen fermentation

After termination of the incubation at 24h, prior to the filtration about 3 ml of the bottle fluid were collected and stored at -20ºC until NH_3-N analyses. The NH_3-N concentration was measured in the presence or absence of PEG according to Preston (1995).

Dry and organic matter degradability and partitioning factor (PF)

The bottles content were filtered into previously weighed sintered crucibles (100- 160 µm pore size, Schott Duran, Germany), washed with hot distilled water, and the extent of sample disappearance (g kg^{-1}), expressed as in vitro dry matter apparently digested (DMD) and organic matter apparently digested (OMD), were determined by the weight difference of undegraded filtered residue following oven-drying (100ºC) and ashing (500ºC). GP, DMD and OMD were corrected for gas yield and particulate contamination, respectively by the inclusion of blank fermentation bottles containing inoculum only. Two bottles content were quantitatively transferred into a 600 ml spout-less beaker with a total of 70 ml of ND solution (double strength, Blummel & Becker, 1997) and refluxed for 3 h at 105ºC. Residual DM and ash were determined. The ratio of organic matter truly degraded (mg) to gas volume (ml) at 24 h incubation was used as an index of microbial synthesis efficiency (Blummel et al., 1997).

Statistical analysis

Data were subjected to analysis of variance (ANOVA), using the General Linear Model procedure (GLM) of the SAS software package (2000). The used model was: $Y= \mu + Fi + e$, where µ is overall mean, Fi is the plant effect, e is error term. Experimental units were runs and replicates in the same run considered as repetitions. The significant differences between individual means were identified using Tukey test.

RESULTS AND DISCUSSION

Chemical analysis

The chemical composition of the tropical browses is presented in Table 1. The result revealed that there are wide variations in the chemical composition of the investigated browses. In particular CP content was ranged from 95.4 to 204.0 g kg^{-1}DM for eucalyptus and leucaena, respectively. The CP contents of the browses studied had almost similar range as those stated previously (Hove et al., 2001, Vitti et al., 2005). All of the investigated browses had a CP content of above 100 g/kg DM except eucalyptus. The results of the current study and those of Makkar & Becker (1998) indicate that most tropical browse species are high in CP and can be used to supplement poor quality roughages to increase productivity of ruminant livestock in tropical regions. The neutral detergent fibre (NDF) was 352.4, 463.1, 479.5, 543.06 and 546.7g kg^{-1}DM for atriplex, eucalyptus, acacia, leucaena and alfalfa, respectively. Atriplex had the lowest content of NDF (352.4 g kg^{-1}DM), ADF (173.8 g kg^{-1}DM) and ADL (85.9 g kg^{-1}DM) but revealed the highest ash content (248.8 g kg^{-1}DM). The differences in NDF and ADF could be due to the species genotypic differences and to the browses collection period in wet season in Egypt that control fibre accumulation in the plant. As reported by Vitti et al. (2005) in multipurpose trees and shrubs in dry and wet season that the fiber fractions were higher in wet season. Alfalfa, atriplex and eucalyptus have negligible CT content (0.2, 0.3, 4.9 eq-g leucocyanidin kg^{-1}DM), while acacia and leucaena had the highest CT content (61.4 and 32.5 eq-g leucocyanidin kg^{-1}DM). However, acacia and eucalyptus samples were rich in TP (95.1 and 75.2 eq-g tannic acid kg^{-1} DM, respectively) and TT (73.0 and 68.2 eq-g tannic acid kg^{-1} DM, respectively). Acacia and leucaena had condensed tannin values above the level (30–40 g kg^{-1} DM) might have both adverse and beneficial effects (Barry et al., 1986).

In vitro GP and tannin bioactivity

The *in vitro* GP results for the incubated browses without (-) or with (+) PEG at 24h and the methane production (ml g^{-1} DM) are given in Table 2. The cumulative GP profiles of the five substrates with or without PEG incubated in buffered rumen fluid for 24 h are shown in Figure 1. The results showed that, in the absence of PEG, there were significant differences ($P< 0.05$) among browses in cumulative GP. The alfalfa and atriplex produced the highest GP (136.4 and 144.2 ml g^{-1}DM), while acacia sample produced the lowest GP (49.2 ml g^{-1}DM). The gas technique has been widely used for evaluation of nutritive value particularly to various types of tropical plants (Krishnamoorthy et al., 1995, Sallam, 2005, Vitti et al., 2005) and different class's of feeds (Getachew et al., 1998). The acacia and leucaena have high levels of NDF, ADF and phenolics compounds particularly CT indicating that components are limiting fermentation *in vitro*. This is consistent with Haddi et al. (2003) reported that there were significant negative correlation between NDF and ADF, and the rate and extent of GP. The negative effect of cell wall content on GP could be due to reduce the microbial activity through increasing the adverse environmental conditions as incubation time progress. However, GP can be regarded as an indicator of carbohydrate degradation and the low GP in acacia and leucaena are explained by condensed tannin's binding to the carbohydrate and then by the inhibition of enzymes or microorganisms (Griffiths, 1986), complexing with lignocellulose, and preventing the microbial digestion.

Table 1. Chemical composition and tannins content of the investigated plants.

Plants	CP	Ash	NDF	ADF	ADL	TP	TT	CT
Alfalfa	181.9	70.9	546.7	346.0	90.3	10.2	6.6	0.2
Acácia	113.5	99.3	479.5	388.5	159.0	95.1	73.0	61.4
Atriplex	132.4	248.8	352.4	173.8	85.9	6.0	3.2	0.3
Leucaena	204.0	80.0	543.1	351.0	135.2	57.2	49.0	32.5
Eucalyptus	95.4	62.7	463.1	308.1	136.7	75.2	68.2	4.9

† CP: crude protein (g kg^{-1} DM), NDF: neutral-detergent fibre (g kg^{-1} DM), ADF: acid-detergent fibre (g kg^{-1} DM), TP: total phenols (eq-g tannic acid kg^{-1} DM), TT: total tannins (eq-g tannic acid kg^{-1} DM), CT: condensed tannins (eq-g leucocyanidin kg^{-1} DM).

Table 2. The effect of polyethylene glycol (PEG) on *in vitro* gas production (GP, ml g^{-1} DM) for the incubated browses without (-) or with (+) PEG and methane production at 24h of incubation.

Plants	GP 24h (ml/g DM)		Increment	Methane	Inhibition
	(-)PEG	(+)PEG	(%)	(ml/g DM)	(%)
Alfalfa	136.4 [a]	132.8[ab]	-2.6	8.2[a]	-
Acácia	49.2 [d]	92.2[c]	87.4	0.9[c]	89
Atriplex	144.2 [a]	146.6[a]	-1.7	7.3[b]	11
Leucaena	72.7 [c]	93.1[c]	28.1	1.0 [b]	88
Eucalyptus	87.9 [b]	106.2[bc]	20.9	0.8 [b]	90
s.e.d.[‡]	7.0	14.8	-	0.15	-

[a,b,c,d] means with different superscripts, within column, are different (Tueky test, P<0.05)

[†] s.e.d.: standard error of difference between mean.

Inclusion of PEG in fermentation of tropical browses resulted in a significant increase of GP profile particularly in the plants rich in condensed tannins (Fig.1). The percentage increase in GP in the presence of PEG was 20.9, 28.1 and 87.4 1% with eucalyptus, luecaena and acacia, respectively. There was no effect of PEG on GP profile in alfalfa and atriplex (Fig.1) due to less inhibitory compounds content in such plants. Addition of PEG could overcome adverse effects of tannins on nutrient availability as indicated by cumulative GP because PEG has a high affinity and formation of PEG-tannin complexes which inactivates tannins. As observed in the current study with acacia and leucaena, inclusion of PEG during the incubation of tannin-rich plant led to an increase in GP up to 100% and have the potential to influence rumen fermentation (Makkar et al., 1997). However, the little effect for PEG on GP in alfalfa, and atriplex could be due to the absence of inhibitory compounds in such feeds.

The cumulated methane production at 24h of *in vitro* incubation was decreased by 11, 88, 89 and 90 % in atriplex, lucaena, acacia and eucalyptus samples, respectively in comparison to alfalfa (Table 2). The reduction in methane production in lucaena, acacia and eucalyptus samples may be attributed to CTs which inhibit methanogenesis and the fermentation of organic matter or to the inhibitory effects on ciliate protozoa. This is in agreement with Hess et al. (2003) suggesting that extract from CT-containing legumes have shown methanogensis toxicity. There was no inhibitory effect for atriplex substrate on methane production. The CT action on methanogenesis can, therefore, be attributed to indirect effects, by reduced H_2 production and digestibility, and by direct inhibitory effects on methanogens (Monforte-Briceno et al., 2005, Tavendale et al., 2005).

Dry and organic matter degradability, partitioning factor and NH_3-N concentration

In vitro dry and organic matter degradability (DMD, OMD, g kg^{-1} DM), NH_3-N concentration and partition factor (PF) were given in Table 3. The results showed that there were variable responses of *in vitro* digestibility among browses may be due to variable levels of phenolics, tannins activity and cell wall content among plants. The ranking order of the browse species on the basis of their potential *in vitro* degradability of DMD and OMD was atriplex > alfalfa > acacia > eucalyptus > leucaena. The atriplex had the highest values for DMD and OMD (762.75 and 734.24 g kg^{-1} DM, respectively), while the eucalyptus revealed the lowest DMD and OMD (382.57 and 317.98 g kg^{-1} DM, respectively). However, differences in degradability among browses could be due to the extent of lignification of NDF (Van Soest, 1994), and GP was negatively correlated with both NDF and lignin (Table 3). The low *in vitro* degradability of dry and organic matter with eucalyptus, lucaena and acacia could be due to negative relationships between NDF, lignin and phenolics with digestibility (Ammar et al., 2005). Condensed tannins interfere with microbial attachment to feed particles and show significant detrimental effects on the microbial population inhibiting ruminal fermentation to some extent.

The Partitioning factor (PF) values ranged from 3.57 in alfalfa to 6.38 in acacia. PF varied (P<0.05) among investigated browses, with acacia and lucaena having the highest (P<0.05) value (6.38 and 5.33, respectively), with the lowest values being in alfalfa, eucalyptus and atriplex (3.57, 4.11 and 4.30, respectively). Dijkstra et al. (2005) reported that the microbial efficiency using GP technique, in combination with substrate degradation may be useful to rank feeds of interest but, for evaluation in a complete diet, rumen models are more appropriate.

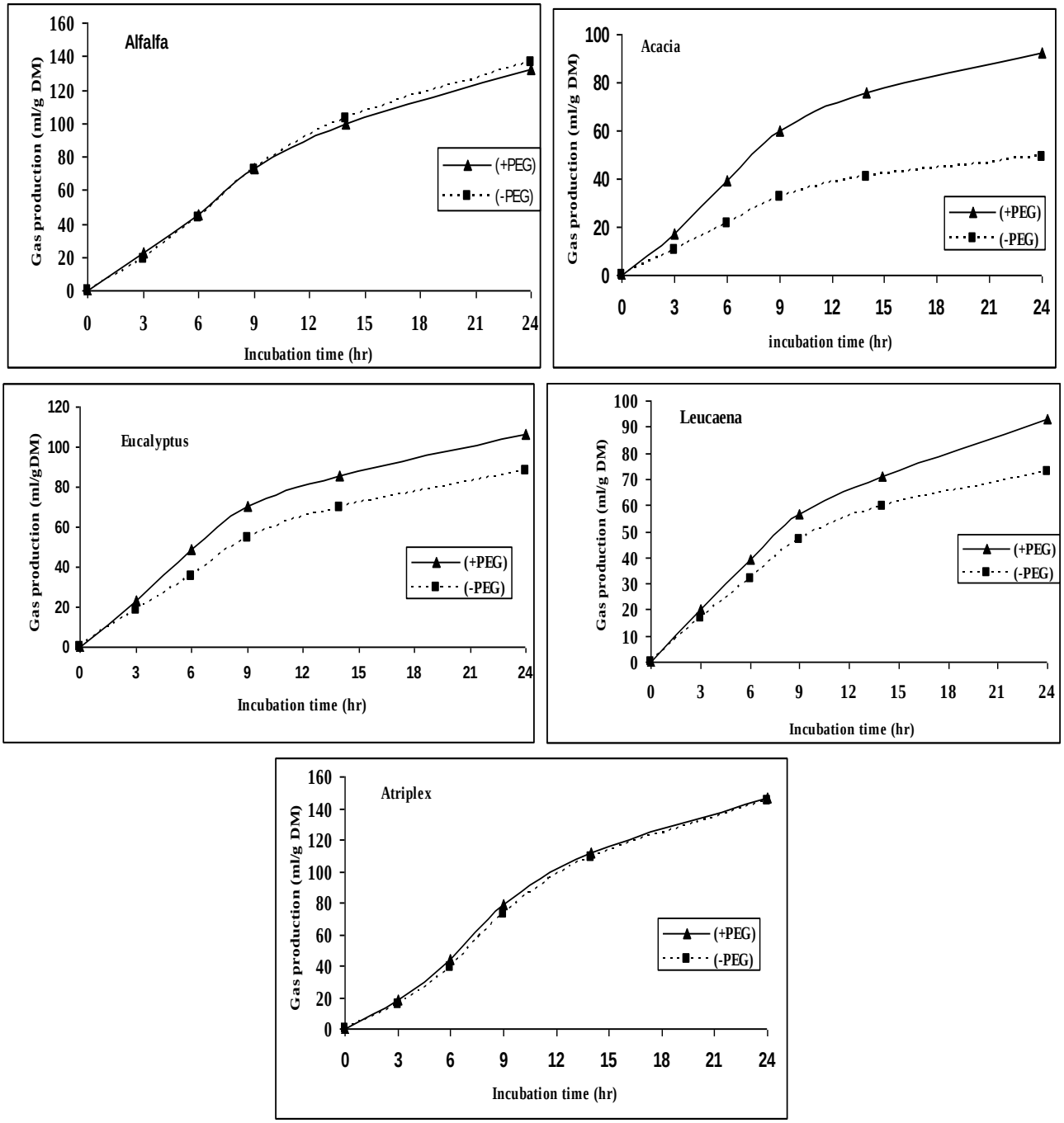

Figure1. *In vitro* effect of PEG on cumulative gas production profiles of the investigated plants.

Table 3. The *in vitro* dry and organic matter degradability (DMD, OMD, g/kg OM) at 24 h of incubation, partition factor (PF) and NH_3-N concentration (mg/l) for the investigated plants.

Plants	PF*	DMD	OMD	NH_3-N		Increment
		24 h	24 h	(-)PEG	(+)PEG	(%)
Alfalfa	3.57 ^{d}	447.6 ^{b}	448.2 ^{b}	194.9 ^{b}	212.8 ^{b}	9.2
Acácia	6.38 ^{a}	438.9 ^{b}	424.5 ^{b}	64.4 ^{d}	222.6 ^{b}	245.7
Atriplex	3.97 ^{cd}	765.1 ^{a}	721.2 ^{a}	247.0 ^{a}	308.0 ^{a}	24.7
Leucaena	5.23 ^{b}	369.6 ^{b}	357.5 ^{b}	129.4 ^{c}	210.0 ^{b}	62.3
Eucalyptus	4.93 ^{bc}	391.7 ^{b}	383.4 ^{b}	76.8 ^{d}	89.6 ^{c}	16.7
† s.e.d.	0.57	56.5	51.3	8.9	9.4	-

^{a,b,c,d} means with different superscripts, within column, are different (Tukey test, $P<0.05$)

† s.e.d.: standard error of difference between means.

* PF: mg truly digested organic matter / ml gas at 24 h.

The PF of feedstuffs can theoretically vary from 2.75 to 4.41 reflecting YATP of 10 to 40 (Blummel et al., 1997). Alfalfa, eucalyptus and atriplex which had a PF value within this range, had very low tannin constituents, supporting this argument. The increase in GP could simply result in lower partitioning of nutrients to microbial protein synthesis (Makkar et al., 1998), and reduced PF value. As found in the current study, PF values for some plants (lucaena and acacia) were higher than the theoretically possible maximum value of 4.41 based on stoichiometric calculation. This was because tannins form complexes with proteins, which were largely insoluble in ND and may contribute to the undegradable fraction (Makkar et al., 1995, 1997).

There are significant variations among species in NH_3-N concentration. The mean values of NH_3-N were 64.4**,** 76.8, 129.4, 194.9 and 247.0 mg/l for acacia, eucaltptus, atriplex, lucaena, and alfalfa in the absence of PEG, respectively. Adding of PEG increased the NH_3-N concentration by 9.2, 16.7, 24.7, 62.3 and 245.7 % in alfalfa, eucalyptus, atriplex, leucaena and acacia indicating that PEG bounded with tannins and released nitrogen for degradation. 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). Increased ammonia N concentrations with addition of PEG could be due to increased CP degradability (Getachew et al., 2000) and/or poor synchronization between N and carbohydrate release in the rumen. The 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. 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). This experiment support the fact that PEG can be added to tanninfirous plants *in vitro* fermentation systems to demonstrate the nutritional importance of tannins on organic matter digestibility and to measure nutritive value of the forage after neutralization (Makkar et al., 1995).

CONCLUSION

A significant variation in *in vitro* degradability was observed among different browse species used in the current study collected from northern Egypt through the wet season. These variations were associated with the cell wall content and/or presence of tannins. Inclusion of PEG improved the fermentation kinetics particularly browses rich in tannins, while the browses poorly in phenolic compounds with high levels of cell wall required another approach to improve their nutritive value. The *in vitro* GP technique could be used in initial screening studies to rank the shrub plants according to their nutritive quality. It is concluded that these browse plants represent an important fodder reserve for grazing ruminants in northern of Egypt by use some detanninfication agents or processing approaches. Also, eucalyptus leaves results suggesting that it is a promising plant for methane mitigation in ruminants feeding.

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