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Effect of polyethylene glycol addition on methane production from some Algerian browse plant species in an *in vitro* gas system

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Abstract. Biological activity of tannins of different browse plants was measured as the change in methane production when plant material was incubated with and without polyethylene glycol (PEG) using the *in vitro* gas production technique. Four dicotyledon browse plants (*Atriplex halimus*, *Artemisia campestris*, *Artemisia herba-alba*, *Calobota saharae*) and three monocotyledon browse plants (*Stipagrostis pungens*, *Lygeum spartum* and *Stipa tenacissima*), collected from an arid zone in Bousâada were evaluated. The increase in gas production upon the addition of PEG, compared with that without PEG, for the browse species varied widely (P<0.05), being particularly high in *S. tenacissima* (+35.0%) and low in *L. spartum* (+1.5%). The methane concentration in fermentation gas ranged from 7.9% with *A. halimus* to 18.6% with *L. spartum*. The higher increase in methane percentage was noted for *S. tenacissima* (+47.4%) and the lower percent value was observed for *L. spartum* (+1.5%). In presence of PEG, the methane production had positive correlation with crude protein (r = +0.78) while in absence of PEG, the methane production was correlated negatively with total condensed tannins (r = -0.88). The strongest correlation (r = 0.89; P<0.01) was between total condensed tannins and methane increase response to the addition of polyethylene glycol, suggesting that tannin compounds appeared to be useful to identify plants possessing antimethanogenic activity.

Keywords. Chemical composition – Browse – Methane – Rumen – Tannin.

Effet de l'addition de polyéthylène glycol sur la production* in vitro *de gaz et de méthane pour des plantes d'Algérie

Résumé. *L'activité biologique des tanins de diverses plantes par le microbiote ruminal d'ovins est mesurée* in vitro*, en présence et en absence de polyéthylène glycol (PEG). La fermentescibilité des substrats est évaluée par la production de gaz, retenus comme marqueurs métaboliques. L'étude est menée sur 4 plantes dicotylédones (*Atriplex halimus, Artemisia campestris, Artemisia herba-alba, Calobota saharae*) et 3 plantes monocotylédones (*Stipagrostis pungens, Lygeum spartum *et* Stipa tenacissima*), collectées de zones arides d'Algérie (Bousâada). Une augmentation nette de la production de gaz est constatée en présence de PEG (P<0,05). Elle est particulièrement élevée pour* S. tenacissima *(+35,0%) et faible pour* L. spartum *(+1,5%). Dans le pool gazeux fermentaire, la production de méthane varie de 7,9 % pour* A. halimus *à 18,6% pour* L. spartum*. Son augmentation la plus élevée est notée pour* S. tenacissima *(+47,4%) et la plus faible pour* L. spartum *(+1,5%). En présence de PEG, la production de méthane est corrélée positivement avec la matière azotée totale (r = +0,78), en son absence la production est corrélée négativement avec les tanins condensés totaux (r = -0,88). La forte corrélation, (r = 0,89 ; P<0,01), observée entre les tanins condensés totaux et l'augmentation de la production de méthane, en réponse à l'addition du PEG, suggère que le test des tanins couplé à la production de gaz peut être un moyen efficace pour identifier les plantes possédant des activités anti-méthanogènes.*

Mots-clés. *Composition chimique – Plantes – Méthane – Rumen –Tanins.*

I – Introduction

Tannins are water-soluble polyphenolic substances that bind and precipitate proteins and can have both negative and positive effects to livestock consuming fodder and shrubs containing a

certain concentration of condensed tannins (Nelson *et al.*, 1995). At high levels, tannin cause over protection of protein resulting in a low utilization of nitrogen (Silanikove *et al.*, 1994), while at concentrations below 50 g kg^{-1} DM, condensed tannins may increase supply of protein to the small intestine (Miller *et al.*, 1995). Due to its property to bind with condensed tannins (CT), polyethylene glycol (PEG) has been used to measure and reduce the adverse effects of CT in ruminant diets (Silanikove *et al.*, 1994; Barry and McNabb, 1999; Jones and Palmer, 2000; McSweeney *et al.*, 2001).

Methane production from ruminants contributes to total global methane emissions, which is an important contributor to global warming (Lassey, 2007). In the recent years, scientists have intensified efforts to exploit plants, plant extracts and natural plant compounds as potential natural alternatives to antibiotic growth promoters for enhancement of livestock productivity while minimizing its environmental impact (Anuraga, 2009; Makkar *et al.*, 2007), including decreased methane production (Soliva *et al.*, 2008). There is a need to develop or identify *in vitro* methods to screen large numbers of plants in a short time and with limited resources.

The objective of the present work was to study the biological activity of tannins of different browse plants collected from an arid zone in Algeria on methane production when plant material was incubated with and without polyethylene glycol (PEG) using the *in vitro* gas production technique.

II – Materials and methods

Plant material was collected in Bousâada district, north central Algeria (N 35° 15.768', E 04° 13.885', 496–981 m altitude), in the Saharan Atlas region, at the northern edge of the Sahara Desert between the Atlas Mountains and the el-Hodna depression and salt lake. The area has a dry desert climate characterized by high temperatures (24 to 41℃) and scarce and erratic annual precipitations (350-700 mm). Selection of the species was based on the available information on their consumption by grazing small ruminants, and on their relative abundance in the area of study. Seven browse plant species were used in this study: four dicotyledon plants, namely *Atriplex halimus* L., *Artemisia campestris* L., *Artemisia herba-alba* Asso and *Calobota saharae* (Coss. & Durieu) Boatwr. & B.-E. van Wyk (formerly *Genista saharae* or *Spartidium saharae*), and three monocotyledon plants, namely *Stipagrostis pungens* (Desf.) De Winter (formerly *Aristida pungens*), *Lygeum spartum* Loefl. ex L. and *Stipa tenacissima* L. Samples were collected in June 2009 (dry season), when plants were at a flowering (*A. halimus* and *L. spartum*) or at a mature stage (the rest of species) and they may be more important for grazing animals. Between six and ten specimens of each plant species were sampled to obtain a representative aliquot of the edible biomass, taken to the laboratory, pooled, oven-dried at 50 ºC (Makkar, 2003), and ground to pass a 1 mm screen.

Chemical composition of the plant material and the corresponding chemical analysis, especially those regarding tannins content (Makkar *et al.*, 1993; Makkar, 2003) are thoroughly described in Boufennara *et al.* (2012).

Six mature Merino sheep (body weight 49.4 ± 4.23 kg) fitted with a permanent ruminal cannula were used as donors of rumen fluid. Animals were fed lucerne hay ad libitum (167 g CP, 502 g NDF, 355 g ADF and 71 g ADL /kg DM) and had free access to water and mineral/vitamin block. Samples of rumen contents were withdrawn prior to morning feeding, transferred into thermos flasks and taken immediately to the laboratory, where rumen fluid was strained through four layers of cheesecloth and kept at 39 ºC under a constant flow of CO_2.

Gas production was measured using an adaptation of the technique described by Theodorou *et al.* (1994). Ground samples (500 mg) with and without the addition of polyethylene glycol (500 mg) were incubated in 50 ml of diluted rumen fluid (10 mL mixed rumen fluid + 40 mL medium (Theodorou *et al.*, 1994) prepared under a CO_2 atmosphere) in 120 mL serum bottles. Incubations were performed using two different inocula (rumen fluid from three sheep) giving

two replicates per treatment. Blanks (bottles containing only diluted rumen fluid) were used to compensate for gas production in the absence of substrate. All the bottles were closed with rubber stoppers, crimped with aluminium seals, shaken and placed in an incubator at 39ºC. The volume of gas produced in each bottle was recorded after 24 h of incubation, using a pressure transducer (Delta Ohm DTP704-2BGI, Herter Instruments SL, Barcelona) and a gas sample (10 ml) was taken into vacuum tubes and stored until analyzed for methane (CH_4) concentration. Methane was determined by gas chromatography (García-González *et al.*, 2008), each gas sample (0.5 ml) being manually injected (Pressure-Lok® syringes A-2 Series, Supelco, USA).

One way analysis of variance (Steel and Torrie, 1980) was performed on methane and gas production data, with browse species as the only source of variation (fixed effect) and source of inoculum (random effect) as a blocking factor. Multiple comparisons were done using LSD test. Pearson linear correlation coefficients were determined pair-wise between the variables studied. Analysis of variance and correlation were performed using the GLM and CORR procedures of the SAS software package (SAS Institute, 2008), respectively.

III – Results and discussion

Chemical composition and tannin composition of the plant material collected from the different species is shown in Table 1. Protein content in dicotyledon species ranged widely from 110 to 154 g/kg DM and was always greater than in monocotyledon grasses. The highest contents of total extractable tannins were observed in the Asteraceae *Artemisia* spp., whereas grasses, *A. halimus* and leguminous plants showed lower concentrations. Total condensed tannins varied widely among species, being highest in *S. tenacissima* and lowest for *A. halimus*.

Table 1. Chemical composition (g/kg DM) and phenolic compounds (g/kg DM, standard equivalent) of Algerian forages

Plant family	Plant species	NDF	ADF	CP	TET	TFC	TCT
Dicotyledons							
Chenopodiaceae	*A. halimus*	360	181	153.6	8.4	42.1	69.1
Asteraceae	*A. campestris*	330	212	115.0	57.1	62.7	114.3
	A. herba-alba	378	258	123.9	36.4	80.6	118.8
Fabaceae - Leguminosae	*C. saharae*	574	427	109.8	9.6	76.4	109.7
Monocotyledons							
Poaceae - Gramineae	*S. pungens*	771	425	95.2	4.8	46.5	78.7
	L. spartum	801	535	72.7	11.1	77.2	102.4
	S. tenacissima	793	476	74.6	3.8	165.5	213.9

NDF, neutral detergent fibre expressed with residual ash; ADF, acid detergent fibre expressed with residual ash; CP, crude protein; TET, total extractable tannins; FCT, free condensed tannins; TCT, total condensed tannins.

Data of gas and methane production of the studied plants without and with the addition of PEG, as well as the percentage of increment in response to PEG addition are shown in Table 2. The increase in gas production upon the addition of PEG, compared with that without PEG, for the browse species varied widely ($P<0.05$), being particularly high in *S. tenacissima* (+35.0%) and low in *L. spartum* (+1.5%). The percentage increase in gas production as result of the blocking effect of PEG on tannins would be an indicator of the biological activity of tannins on rumen microbial fermentation. Improved gas production due to addition of PEG to in vitro fermentation systems indicates a depressing effect of tannins on degradation of feed nutrients in the rumen

and, probably, their poor ruminal digestibility. In fact, PEG binds tannins, inactivates tannin anti-nutritive activity, and recovers feed nutrients bound by tannins (Canbolat *et al.*, 2005; Rubanza *et al.*, 2005). Based on this analysis, the species with a highest tannin anti-nutritional activity would be *S. tenacissima*.

Table 2. Gas and methane production *in vitro* (mmol/g DM) of Algerian forages with (+PEG) and without (-PEG) addition of PEG and percentage of increase (% incr., +PEG/-PEG)

Family	Susbtrate	Gas production			Methane production		
		-PEG	+PEG	% incr.	-PEG	+PEG	% incr.
Dicotyledons							
Chenopodiaceae	*A. halimus*	4.23^{b}	4.65^{c}	10.13^{bc}	0.86^{a}	0.89^{a}	3.96^{c}
Asteraceae	*A. campestris*	5.39^{a}	5.73^{a}	6.25^{cd}	0.76^{b}	0.80^{b}	5.43^{c}
	A. herba-alba	3.64^{c}	4.41^{c}	21.52^{ab}	0.62^{d}	0.75^{c}	20.23^{b}
Fabaceae- Leguminosae	*C. saharae*	5.18^{a}	5.43^{b}	4.74^{cd}	0.80^{b}	0.83^{ab}	4.42^{c}
Monocotyledons							
Poaceae - Gramineae	*S. pungens*	2.76^{d}	3.26^{d}	18.18^{bc}	0.62^{d}	0.69^{d}	12.30^{c}
	L. spartum	4.37^{b}	4.47^{c}	1.49^{d}	0.68^{c}	0.69^{d}	1.49^{c}
	S. tenacissima	2.32^{e}	3.13^{d}	35.00^{a}	0.30^{e}	0.44^{e}	47.41^{a}
R.S.D.†		0.140	0.117	6.071	0.019	0.017	4.652

†Residual standard deviation.
$^{a, b, c, d, e}$ Different superscripts in the same column indicate significant differences (P<0.05).

The higher increase in methane production in response to PEG addition was noted for *S. tenacissima* (+47.4%) and the lower increase value was observed for *L. spartum* (+1.5%) (P<0.05). The variations in methane concentration support the view that tannins decrease methane production. Other dietary components such as NDF also contributed to explaining total variation in methane production. Higher NDF increases methane production by shifting short chain fatty acid proportion towards acetate which produces more hydrogen.

Contrary to the work of Anuraga *et al*. (2009), in the present study we observed a strong positive and significant correlation between total CT and methane increase in response to the addition of PEG (r=0.88; P<0.05). This result suggests a role of total CT in methane reduction and that they seem to be useful to identify plants possessing antimethanogenic activity However, it must be taken into account that although amongst the tannin assays, determination of total CT is relatively simple and can be done using routine laboratory equipment, all chemical tannin assays measure tannins under conditions (i.e., temperature, pH, ionic strength) that differ from those of the rumen. Therefore, the results have limited applicability for predicting the activity of tannins. Nevertheless, PEG is considered to specifically bind tannins, and its use in the *in vitro* rumen assay is a better representation of tannin activity under rumen conditions.

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