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Methane production from the rumen fermentation of Algerian Acacia tree foliage

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Abstract. The present study was carried out to determine the *in vitro* methane production from the rumen fermentation of Acacia tree leaves (*Acacia nilotica, A. cyanophylla, A. albida, A. horrida* and *Albizia julibrissin*) and its reduction by the addition of a tannin-blocking agent (polyethylene glycol, PEG). Gas production was determined when foliage from the five plant species was incubated in diluted rumen fluid for 6, 12, 24 and 48 h, and methane was measured after the incubation at 24 h. The incubations were conducted either without or with the addition of the tannin binder polyethylene glycol (PEG-6000). This has been considered a bioassay of tannin activity, represented by the increase in gas (methane) production upon the addition of PEG (i.e., when tanning are neutralized). The ratio +PEG/-PEG (in total gas production) was highest for *A. nilotica* (2.23 and 1.75 at 12 h and 24h), followed by *A. cyanophylla* (1.73 at 48 h), reflecting the high amount and biological activity of tannins in these plants. PEG addition increased methane production for all the *Acacia* species, thus confirming that tannins in these samples affected methanogenesis. An increase in total volatile fatty acid concentration in samples with addition of PEG was observed only with *A. nilotica* and *A. cyanophylla*. Tannins contained in these plants could be of interest to reduce methane production, providing that other parameters of ruminal fermentation were not inhibited.

Keywords: Rumen – Acacia – Tannin – Polyethylene Glycol – Methane.

Production de méthane issue de la fermentation de feuilles d'acacias collectées en Algérie

Résumé. *La présente étude a été réalisée pour déterminer la production de méthane* in vitro *à partir de la fermentation de feuilles d'acacias* (Acacia nilotica, A. cyanophylla, A. albida, A. horrida et Albizia julibrissin*) dans le jus rumen d'ovins et l'effet inhibiteur des tannins sur cette production par l'ajout d'un agent de blocage des tanins (Polyéthylène Glycol - PEG). Les plantes contenant des tannins ont la propriété de réduire la production de méthane. La production de gaz et de méthane a été déterminée en incubant le feuillage de ces cinq espèces végétales dans du jus de rumen dilué pendant 6, 12, 24 et 48 h. Pour l'estimation de l'activité biologique des tannins, les essais d'incubation ont été réalisés soit en additionnant du polyéthylène glycol (PEG) - 6000 (+PEG) soit sans (- PEG). La neutralisation des tannins, après addition de PEG, a eu pour conséquence l'augmentation de la production des gaz (méthane). Le ratio +PEG / -PEG (dans la production de gaz total) le plus élevé a été enregistré chez* A. nilotica *(2,23 et 1,75 à 12 h et 24 h), suivi par* A. cyanophylla *(1,73 à 48 h), ce qui indique que le feuillage de ces deux espèces végétales est riches en tannins biologiquement plus actifs. L'ajout de PEG a augmenté la production de méthane pour tous les Acacia spp., confirmant que les tannins contenus dans ces échantillons affectent la méthanisation. L'augmentation de la concentration en acides gras volatils totaux dans les échantillons après addition de PEG est observée uniquement avec* A. nilotica *et* A. cyanophylla*. Les tannins contenus dans ces plantes pourraient être d'un intérêt pour réduire la production de méthane, dans la mesure où la fermentation ruminale de ces aliments n'est pas inhibée.*

Mots-clés. *Rumen – Acacia – Tannins – Polyéthylène Glycol – Méthane.*

I – Introduction

Methane emission from ruminants has received special attention because of its contribution to the greenhouse effect and global warming. Methane emitted from ruminant livestock, beyond being a contributor to global warming, entails a loss of feed energy of about 2 to 12% (Johnson and Johnson, 1995) that would otherwise be converted into meat and/or milk.

Nevertheless, methane is a key rumen fermentation end product, and its production is necessary to maintain anaerobiosis in the rumen environment. Understanding why and how methane is produced in the rumen, and its implications to ruminal fermentation, is required before proposing any measure to control its production (Bodas *et al.,* 2012). In this context, plants and plant extracts with high concentrations of secondary compounds appear to be potential candidates for reducing ruminal methanogenesis, without impairing or even enhancing rumen fermentation. Thus, some authors have suggested that tannins, present in the foliage of some Acacia species, may reduce protein degradation in the rumen when used at moderate doses of 20-45 g/kg diet (Min *et al.,* 2003).

The objective of this study was to evaluate the effect of foliage from five *Acacia* spp., rich in condensed tannins, collected from Algerian arid and semi-arid areas, on ruminal gas production and methanogenesis. An *in vitro* gas production bioassay (tannin bioassay) was used to assess the potential biological effect of tannins by incubating tree foliage with and without tannin-binding PEG.

II – Materials and methods

Foliage from five *Acacia* species (*Acacia nilotica*, *Acacia horrida*, *Acacia cyanophylla*, *Acacia albida*, *Albizia julibrissin*) was collected from Algerian arid and semi-arid areas and analysed as described by Bouazza *et al*. (2012). Phenolic compounds were extracted following the procedures described by Makkar (2003). Analytical methods for chemical composition, phenolics and tannins were those described in detail by Bouazza *et al*. (2012).

Three mature Merino sheep fed Lucerne hay (body weight 49.4 ± 4.23 kg) fitted with a permanent ruminal cannula (60 mm diameter) were used for the extraction of rumen fluid. The experiment was designed as a tannin bioassay based on *in vitro* incubations of tannin-containing plant materials with and without polyethylene glycol (PEG) during 48h in buffered rumen fluid (Makkar *et al.,* 1995, Ammar *et al*., 2004). The volume of gas produced in each bottle was recorded at 6, 12, 24 or 48 h after inoculation time, using a pressure transducer. Volatile fatty acids (VFA) were determined by gas chromatography using crotonic acid as the internal standard (Ottenstein and Bartley, 1971). Methane content in fermentation gas was determined according to López and Newbold (2007). Two ways analysis of variance was performed with *Acacia* species and the presence of PEG as the two sources of variation (fixed effects) and source of inoculum (random effect) as a blocking factor.

III – Results and discussion

The chemical composition, phenolic and tannin content of the browse material used in the study have been reported elsewhere (Bouazza *et al*., 2012). The high levels of condensed tannins observed in Acacia leaves are in agreement with values reported by other authors (Rubanza *et al.,* 2005).The concentration of phenolic compounds in the foliage material showed considerable variation among species. These differences may be due not only to plant species, but also to harvest season and plant maturity stage (Makkar, 2003). Leaf samples of *A. albida* were harvested in the late rainy season of May, whereas *A. nilotica* was harvested in autumn. A high proportion of TCT was recovered as FCT, which may be responsible for the possible adverse effects of condensed tannins on microbial fermentation in the rumen (Barry and McNabb, 1999).

Table 1 shows the effect of including PEG during the *in vitro* incubation of *Acacia* species. Polyethylene glycol inclusion increased significantly ($p<0.05$) cumulative gas production of two sub-

strates, *A. nilotica* and *A. cyanophylla*. The greatest and significant (P<0.05) response to PEG was recorded after 12 h incubation with *A. nilotica* (a 2.2 fold increase). The PEG-gas production bioassay is not intended to quantify accurately or to characterize the tannins in animal feedstuffs. It does provide, however, an interesting assessment of the biological effects of tannins on depressing ruminal fermentative activity, and allows identification of plant species containing tannins with greater amounts of active anti-nutritional compounds.

Table 1. Polyphenolics content (g/kg DM, standard equivalent) and effects of PEG on cumulative gas production (ml/g) ay 6, 12, 24 and 48 h of incubation and on volatile fatty acids (VFA) and methane production (CH_4) after 24 h incubation

	Species	*A. nilotica*	*A. horrida*	*A. cyanophylla*	*A. albida*	*A. julibrissin*	S.E.M
TEP		213	99	205	31	105	
FCT		609	386	451	26	502	
TCT		726	476	631	60	587	
G6	+ PEG[1]	47.3^{a}	33.3^{a}	44.5^{a}	16.9	43.1	4.62
	- PEG[2]	27.4^{a}	54.6^{a}	39.7	19.9	50.9	4.70
G12	+ PEG	120.6^{ax}	116.8^{a}	103.1^{ax}	51.9^{b}	112.0^{a}	7.65
	- PEG	54.0^{by}	101.9^{a}	59.9^{by}	35.7^{b}	92.2^{a}	7.08
G24	+ PEG	160.5^{ax}	160.1^{a}	140.3^{ax}	73.0^{b}	159.2^{a}	9.95
	- PEG	91.6^{by}	147.2^{a}	$84.0b^{cy}$	53.7^{c}	135.0^{a}	9.63
G48	+ PEG	182.5^{ax}	191.9^{a}	164.7^{ax}	83.6^{b}	189.7^{a}	11.56
	- PEG	118.6^{by}	169.1^{a}	95.4^{bcy}	58.3^{c}	163.0^{a}	11.57
VFA	+ PEG	0.426^{ay}	0.388^{a}	0.385^{ay}	0.195^{b}	0.452^{a}	0.030
	- PEG	0.240^{bx}	0.387^{a}	0.173^{cx}	0.164^{c}	0.410^{a}	0.035
CH_4	+ PEG	0.748^{ay}	0.771^{ay}	0.733^{ay}	0.307^{by}	0.794^{ay}	0.061
	- PEG	0.458^{bx}	0.560^{ax}	0.283^{cx}	0.173^{dx}	0.451^{bx}	0.046
CH_4 Increase		0.290	0.211	0.450	0.134	0.343	

TEP: Total extractable phenols; FCT: Free condensed tannins; TCT: Total condensed tannins. [1] Incubated with polyethylene glycol. [2] Incubated without polyethylene glycol. $^{a, b, c, d,}$ means in a the same row with different superscripts are significantly different (P<0.05); $^{x, y}$ means in the same column with different superscripts are significantly different (P<0.05). SEM: Standard error of the mean.

The effect of *Acacia* species on total volatile fatty acid (VFA) and methane production is presented in Table 1. Molar concentrations of ruminal VFA were affected by the addition of PEG treatment only when *A. nilotica* and *A. cyanophylla* were incubated (P<0.05). However, PEG affected methane production for all substrates (P<0.05), which may indicate that reduced methane production is a consequence of reduced gas production and not of reduced methane proportion in the gas produced. The greatest CH_4 increment was observed with *A. cyanophylla* (0.450 mmol/g MS) and the lowest was noted in *A. albida*. These results probably reflect the different levels and biological activity of CT in the species studied (Min *et al.,* 2003). Methane production from ruminal fermentation has been decreased up to 50% in response to tannin (Patra and Saxena, 2010; Goel and Makkar, 2012; Bodas *et al.,* 2012). Condensed tannins have a direct toxic effect on methanogens (Bodas *et al.,* 2012). As it can be seen, high amounts of tannin in substrates are able to reduce methane production in the rumen, but this effect is linked to a depressed rumen fermentation and reduced gas and volatile fatty acid production. It is known that high dietary concentrations of condensed tannins can reduce voluntary DM intake and digestibility and thus negatively affect animal performance and may even possibly be toxic (Barry and McNabb, 1999; Min *et al.,* 2003). However, these secondary compounds offer the possibility of being used as additive to decrease CH_4 productions (Patra and Saxena, 2009).

IV – Conclusions

The results from these experiments highlight the potential of tannins from *Acacia* species to reduce methane emissions from ruminants. However, besides their positive effects, tannins exert a powerful inhibition effect on rumen fermentation (i.e. depressing gas and volatile fatty acid production) that must be considered before making practical recommendations, especially on the level of inclusion of this roughage in the diets of ruminants.

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