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## Tropical Plants Containing Condensed Tannins Inhibit Ruminal Methanogenesis In Vitro

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Tropical plants are characterized by a high content of secondary compounds such as condensed tannins (CT). Condensed tannins, depending on their chemical structure and concentration, are able to reduce enteric methane (CH<sub>4</sub>) but they might also have adverse effects on rumen fermentation. An in vitro experiment was performed using 3 tannin-rich plants (TRP) largely available in the tropics to determine CH<sub>4</sub> production and fermentation parameters using a dose-response approach.

Tannin-rich *Glyricidia sepium* (G), *Leucaena leucocephala* (L), and *Manihot esculenta* (M) leaves were used as substrates for in vitro incubations. Their CT content was 75, 39, 92 g/kg dry matter, respectively. These substrates were incubated alone or mixed with tannin-free grassland hay (C), so that proportions of TRP were 0:100, 25:75, 50:50, 75:25 and 100:0. Three series of incubations were carried out for 24 h using ruminal fluid taken from 3 rumen-cannulated Texel wethers before morning feeding. Total gas, CH<sub>4</sub> and volatile fatty acids (VFA) were analysed at the end of the incubation. Fermented organic matter (FOM) was calculated from VFA production according to a stoichiometric equation. Statistical analyses were performed using the mixed procedure of SAS with plant, dose, plant x dose interaction as fixed effects and series as random effect. Linear, quadratic and cubic contrasts were determined for dose effect.

**Table 1. Gas and CH<sub>4</sub> production (mL/24h), VFA concentration (mM) and composition of rumen liquid of sheep fed a control grassland hay (C) or tannin-rich plants *Leucaena leucocephala* (L), *Glyricidia sepium* (G) and *Manihot esculenta* (M)**

	C					G					M						
Dose, %	0	25	50	75	100	25	50	75	100	25	50	75	100	s.e.	Effects		
Gas	66.2	60.0	59.1	56.3	54.5	65.3	64.1	60.7	64.6	65.9	56.9	53.0	48.2	4.68	P D (l) I		
CH <sub>4</sub>	18.1	15.7	15.4	13.2	12.4	16.8	16.7	15.6	17.3	17.2	14.7	13.8	12.5	1.23	P D (l)		
VFA	59.8	59.2	52.4	50.8	48.2	60.9	58.4	57.0	52.8	61.5	54.9	47.4	32.9	3.05	P D (l, q) I		
VFA, mol/100 mol																	
C2	66.4	66.3	61.4	62.2	61.6	65.1	61.7	63.6	62.5	64.3	67.3	66.4	63.8	2.39	-		
C3	20.4	20.3	21.2	20.8	20.1	20.7	21.0	20.0	20.1	20.2	17.9	17.2	16.4	0.81	P		
C4	6.4	6.2	7.3	7.3	7.8	6.7	7.5	6.8	7.0	7.3	7.3	7.4	8.3	0.68	-		
C2:C3	3.3	3.3	2.9	3.0	3.0	3.2	3.0	3.2	3.1	3.2	3.8	4.0	4.0	0.28	P		
FOM,%	50.6	49.2	43.7	42.4	40.7	51.2	49.1	48.3	44.7	51.5	46.2	40.4	28.3	2.74	P D (l, q) I		

C2= acetate, C3= propionate, C4= butyrate

Statistical effects are mentioned when  $P < 0.01$ . P=nature of TRP; D=dose (i.e., proportion of TRP in the fermented substrate); I = interaction between P and D. Analysis of contrasts: l=linear, q=quadratic (cubic contrasts were not significant)

This experiment confirms the potential of TRP for limiting CH<sub>4</sub> production, as shown in vivo with the same plant species (Rira *et al* 2013). Tannin-rich plants also decreased VFA production and thus FOM decreased too, especially for M. However, while the effect on VFA was observed at TRP concentrations of 50% or more, the effect on CH<sub>4</sub> was observed at a lower concentration. The global effect of CT was more pronounced for L and M than for G, because of a lower concentration in CT in this latter TRP, without significant interaction between dose and TRP type. The response to increasing doses of TRP was linear for most variables. This *in vitro* experiment provides new insights on the role of tannin content on CH<sub>4</sub> and VFA production.

Rira M., Marie-Magdeleine C., Archimède H., Morgavi D.P. and Doreau M. (2013). In "Energy and protein metabolism and nutrition in sustainable animal production" (Eds J.W. Oltjen, E. Kebreab and H. Lapierre) p. 501-502 (Wageningen Acad. Publ.: Wageningen, the Netherlands).

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