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Introduction

Enteric methane (CH_4) produced by domestic ruminants represents approximately 15% of the global emissions of this potent greenhouse gas. For reducing rumen CH_4 emission various compounds have been tested as feed additives. Among these compounds, tannins are considered a promising group of natural additives. A meta-analysis by Jayanegara *et al.* (2012) showed that condensed and hydrolysable tannins might reduce CH_4 production. However, it is still unclear (1) whether tannin supplementation reduces rumen CH_4 in every situation and (2) to which extent this is associated with adverse effects on digestibility and their potential toxicity to some rumen micro-organisms (Goel *et al.*, 2005). In this experiment we investigated the effect of tanniniferous tropical plants on enteric CH_4 production and on numbers of methanogens, protozoa, and total and main cellulolytic bacteria.

Material and methods

Two sheep breeds were used, Texel (T, n=4) of temperate origin and Blackbelly (B, n=4) of tropical origin in two 4×4 Latin square designs. Diets, given ad libitum twice daily, consisted in tropical natural grassland based on *Dichanthium spp.* fed alone (C) or in association with 3 different tanniniferous forages given as pellets at 44% of the daily ration on average. The tanniniferous forages were leaves of *Leucaena leucocephala* (L), *Glyricidia sepium* (G) or *Manihot esculenta* (M). Total contents in condensed tannins measured by the vanillin-H_2SO_4 method were 75, 39 and 92 g/kg dry matter (DM) for L, G and M, respectively. Intake, total tract digestibility and CH_4 production using the SF_6 method were determined. For microbial parameters, rumen contents were sampled before the morning feeding. Microbial groups (total bacteria, *Fibrobacter succinogenes, Ruminococcus albus, Ruminococcus flavefaciens* and total methanogens) were enumerated by quantitative PCR (qPCR) using group-specific primers targeting the *rrs* gene for bacteria and the *mcrA* gene for methanogens. Protozoa were counted by microscopy. Statistical analyses were performed using the mixed procedure of SAS with period, diet, breed, and the diet x breed interaction as fixed effects and animal as random effect. Statistical differences were declared significant at $P \leq 0.05$. Orthogonal contrasts between control diet and all tanniniferous forages were also determined.

Results and discussion

The addition of tannin-rich plants given as pellets increased DM intake probably due to the physical presentation (Table 1). Within tannin-rich plants, it was higher for M than for G diet. Intake per kg body weight was higher for Blackbelly than for Texel. Organic matter digestibility did not differ among diets and breeds, although contrast analysis showed a higher digestibility for C than for other diets. Daily CH_4 production did not vary among diets and breeds, but CH_4 production per kg DM intake was higher with C diet compared with tannin-rich diets. Within these latter diets, CH_4 production was higher for G than for M and L diets. Concentration of total bacteria and *R. flavefaciens* was higher for C and L diets than for G and M diets; concentration of *R. albus* was lowest for C diet. The methanogens population was higher for Texel than for Blackbelly. In contrast the addition of condensed tannins did not influence the population of protozoa and *F. succinogenes.*

Table 1. Intake, digestibility, methane emission and ruminal microbial populations in sheep fed tropical grassland hay (C) alone or associated with tannins-containing plants Leucaena leucocephala *(L),* Glyricidia sepium *(G) and* Manihot esculenta *(M).*

Breed	Texel				Blackbelly				SEM	*P* value[1]
Diet	C	L	G	M	C	L	G	M		
DM intake, g/kg body weight/d	16.28	24.45	20.72	27.68	16.62	25.96	24.49	31.54	1.877	B 0.04 D<0.01
Organic matter digestibility, %	69.66	64.94	62.69	62.36	70.12	65.74	62.78	66.97	3.639	NS
CH4, g/kg DM intake	32.06	24.23	28.83	24.28	29.98	15.89	24.98	15.26	2.147	B 0.03 D<0.01
Protozoa, $\log_{10}$ cells/ml	4.97	5.01	5.04	5.03	4.96	5.01	4.97	5.06	0.046	NS
Total bacteria[2]	11.91	11.90	11.80	11.84	11.89	11.96	11.85	11.81	0.041	D 0.04
F. succinogenes[2]	9.71	9.79	9.61	9.72	9.67	9.85	9.64	9.68	0.079	NS
R. Albus[2]	8.00	8.79	8.33	8.49	7.60	8.42	8.34	8.16	0.261	D 0.04
R. flavefaciens[2]	8.96	8.71	8.53	8.65	8.94	9.03	8.67	8.85	0.154	D 0.05
Methanogens[3]	10.00	9.85	9.72	9.84	9.76	9.71	9.70	9.74	0.082	B 0.04

[1] B = breed; D = diet. Breed × diet interaction was never significant.

[2] *rrs* copy number /g DM ($\log_{10}$)

[3] mcrA copy number /g DM ($\log_{10}$)

Our results confirm that tannin-rich plants can limit CH_4 production per kg DM intake. The low effect of *Glyricidia sepium* on reduction of CH_4 production could be explained by a low tannin concentration or by the intrinsic characteristics of *Glyricidia sepium* tannins. The presentation of tannin-rich plants as pellets probably decreased ruminal DM retention time which resulted in an increase in DM intake and can partially explain the reduction in CH_4 production. As methanogens and protozoal numbers were not changed, further research is necessary to elucidate the relations between methane production and microbial activity in tannin-rich diets.

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