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## *In vitro* screening of tropical forages for low methane and high ammonia generating potential in the rumen

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### Introduction

Goat farming is a livelihood activity which helps ensure food security for small and marginal farmers, landless labourers and rural folk in Sri Lanka. Goats are fed on a diverse range of tree leaves which are their primary food source in rural areas, whereas in peri-urban areas they are fed with other feedstuffs due to limited supply of tree leaves (Seresinhe and Marapana 2011). The poor growth performance of local goats is associated with low digestibility of feeds which may be due to the presence of condensed tannins (CT) present in the feed. Therefore, this study evaluated the suitability of several combinations of low tanniniferous non-legume foliage mixed with high tanniniferous legume foliage on *in vitro* gas production and rumen degradability characteristics.

### Methods

Edible forage samples were analysed for proximate, cell wall composition and condensed tannins using the following standard procedures. Eight treatments (Table 1) were tested in a randomized complete block design using four non-legumes with high tannins and two shrub legumes with low tannins at a ratio of 3:1. *In vitro* gas production was determined using Hohenheim gas method. At the end of the fermentation period, *in vitro* dry matter digestibility (IVDMD) and ammonia concentration in the fermentation liquid were determined. Methane (CH<sub>4</sub>) was measured using a Hewlett Packard Gas Chromatograph (Model 5890, Series II, Avondale, PA, USA). Protozoa and bacteria were counted with Bürker counting chambers (0.1 and 0.02 mm depth, respectively; Blau Brandw, Wertheim, Germany). Analysis of variance (ANOVA) to test the effects of the treatments was performed on chemical composition, *in vitro* digestibility and gas production data. The mean differences were tested using the Duncan's Multiple Range Test (DMRT). Correlation coefficients were calculated using MS EXCEL version 2000.

### Results

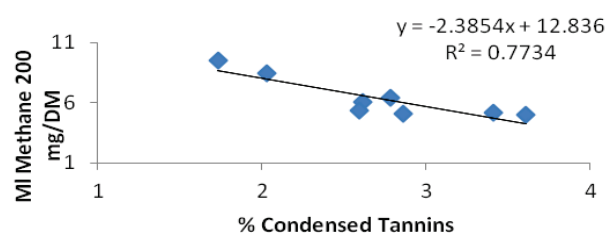
The condensed tannin content of non-legume and legume combinations (Table 1) ranged from 1.73% (*S. spicata* x CC [Trt 4]) to 3.61% (*S. caryophyllatum* x CC [Trt 8]) indicating that mixing of two forages with high and low

tannins can reduce the content of of tannins in the diet by a reasonable amount. There was a steady increase in the gas production during 48 hours of incubation and a significant differences between forage mixtures in net gas volume. The highest net gas production was observed with mixtures containing low levels of tannin (T3 , T4) while the lowest values ( $P<0.05$ ) were observed in mixtures with higher levels of tanins. (T7, T8). The findings (Fig. 1) are in agreement with those of Getachew *et al.* (2002), who reported strong correlations between CT and gas production.

Methane production was affected by treatment and the lowest values ( $P<0.05$ ) were observed for T7 and T8. The significant correlation between methane production and tannin content in forage mixtures suggests that tannins have an effect on mitigating methane production (Fig. 1). Soliva *et al.* (2008) also confirmed that plants known to contain plant secondary metabolites were able to suppress methanogenesis. Significantly lower protozoa populations in T7 and T8 compared with the other treatments provide evidence that tannins did not affect on the population of protozoa. This is in contrary to Hess *et al.* (2003) who reported that tannins may cause significant shifts in rumen microbial populations. On the other hand, tannins did not affect feed protein degradation, with a correlation of 0.73 between crude protein content and ammonia production.

### Conclusion

Supplementing low tannin non-leguminous forages by incremental substitution with high tannin legume forage appears to be found promising to approach the goal of an improved nutrition and reduced energy loss in goats through mitigation of methanogenesis.



**Figure 1. Correlation between condensed tannins and methane production.**

**Table 1. Condensed tannins (CT), total gas production, methane and protozoa counts in experimental treatments (forage mixtures). Values are mean  $\pm$  SE.**

Treatment	CT in mixture (%)	Gas production (ml /200 mg DM)	CH <sub>4</sub> production (ml/ 200 mg/DM)	Protozoa number x 10 <sup>4</sup> /ml
Trt 1; <i>Terminalia catappa</i> + <i>Acacia (A.) auriculiformis</i>	2.86a $\pm$ 0.31	36.8 av $\pm$ 6.95	5.07 C $\pm$ 0.96	1.70 AB $\pm$ 0.1282
Trt 2; <i>T.catappa</i> + <i>Calliandra (C.) calothyrsus</i>	2.61a $\pm$ 0.34	40.5 a $\pm$ 5.45	6.07 B C $\pm$ 0.73	2.15 A $\pm$ 0.4621
Trt 3; <i>Symplocos (S.) spicata</i> + <i>A auriculiformis</i>	2.03b $\pm$ 0.23	45.5 a 8.54	8.42 AB $\pm$ 1.37	4.44 D $\pm$ 0.2220
Trt 4; <i>S. spicata</i> + <i>C. calothyrsus</i>	1.73b $\pm$ 0.14	46.5 a 3.42	9.56 A $\pm$ 1.53	4.66 D $\pm$ 0.2220
Trt 5; <i>Mangifera (M.) indica</i> + <i>A. auriculiformis</i>	2.78a $\pm$ 0.32	40.3 a3.86	6.43 B C $\pm$ 1.57	1.26 BC $\pm$ 0.2563
Trt 6; <i>M. indica</i> + <i>C. calothyrsus</i>	2.59a $\pm$ 0.24	42.3 ah $\pm$ 4.92	5.38 B C $\pm$ 0.64	2.00 A $\pm$ 0.5874
Trt7; <i>Syzygium caryophyllatum</i> + <i>A. auriculiformis</i>	3.61a $\pm$ 0.31	30.5 b $\pm$ 6.76	5.04 C $\pm$ 0.54	1.04 BC $\pm$ 0.3391
Trt 8; <i>S. caryophyllatum</i> + <i>C. calothyrsus</i>	3.41a $\pm$ 0.34	29.0 b $\pm$ 4.36	5.15 c $\pm$ 1.52	1.63 AB $\pm$ 0.5587

Values in the same column with different letters are significantly different ( $P < 0.05$ )

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