Methane reduction properties of tannin containing plants, simple phenols and purified tannins in *in vitro* rumen fermentation system

A. Jayanegara[a,b], H.P.S. Makkar[a], K. Becker[a]

[a]Institute of Animal Production in the Tropics and Subtropics (480b), University of Hohenheim, Stuttgart, Germany

[b]Departement of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University, Indonesia

E-mail address of main author: anu_jayanegara@yahoo.com

This study was conducted in a series of 3 experiments, and aimed at evaluating (1) polyphenol containing plants, (2) simple phenols in the form of phenolic acids, and (3) purified tannins for their potential to reduce methane production *in vitro*.

In experiment 1, polyphenol containing plants (n=17) were analysed for chemical composition (dry matter, ash, crude protein, ether extract, neutral detergent fibre (NDF) and acid detergent fibre (ADF)), polyphenols content and activity (total phenols (TP), total tannins (TT), condensed tannins (CT) and tannin bioassay) [1], and methane production *in vitro*. Methane was expressed as ml net CH_4 in 100 ml net gas production, decrease of CH_4 compared to methane from hay, and increase of CH_4 after polyethylene glycol (PEG) addition. Regression and correlation analyses were performed between each tannin assay and other chemical constituents for their effect on methane production. Each plant was analysed in duplicate. In experiment 2, six sources of simple phenols (benzoic, cinnamic, phenylacetic, caffeic, p-coumaric and ferulic acids) were evaluated for their potential to reduce methane. Two levels of each phenol (2 and 5 mM) were added to hay diet before *in vitro* incubation. The simple phenols were prepared by solubilizing the phenols in sodium phosphate buffer pH 6.7 to avoid pH>7.5, and adding 130 µl of NaOH (10 M) to completely dissolve the phenols. An appropriate aliquot of solubilized phenolics (≤1 ml) was injected into the syringe from the syringe nozzle before dispensing rumen liquor. The measured variables were gas production, methane, organic matter digestibility (OMD), and short chain fatty acids (SCFA: C_2, C_3, C_4, iso-C_4, C_5, iso-C_5, total SCFA) and ratio of C_2/C_3. In experiment 3, effects of four purified tannins [2] from chesnut, mimosa, quebracho and sumach in hay:concentrate diet (70:30 w/w) were evaluated at three different concentrations (0.5, 0.75 and 1.0 mg/ml). The measured parameters were gas production, methane, OMD and individual SCFA. Chesnut and sumach are hydrolysable tannins, whereas mimosa and quebracho are condensed tannins.

The results from experiment 1 showed that there were negative relationships between TP, TT or tannin activity and methane production. The correlation (r) values ranged from –0.59 to –0.75 with $P<0.05$ for the relationships. Very weak relationship was found between CT and methane production. There were positive and significant relationships between TP, TT or tannin activity and the methane decrease, as well as with methane increase by PEG addition. The highest correlation of 0.79 ($P<0.001$) was obtained between tannin activity and the methane decrease. *Amongst the tannin assays, tannin bioassay (a reflection of tannin activity) was the best predictor of the methane reduction potential of a plant.*

All simple phenols studied in experiment 2 were not effective in decreasing methane production at lower concentration (2 mM). At higher concentration (5 mM), benzoic and phenylacetic acids were not effective. Cinnamic, caffeic, p-coumaric and ferulic acids decreased methane production significantly ($P<0.05$) when added at 5 mM. *Caffeic acid at 5 mM was the most effective out of the simple phenols tested and it decreased methane by 6.3% from the control. The magnitude was higher when expressed as decrease of methane per unit organic matter digested and the decrease was 9.4% from control. After caffeic acid, the order of simple phenols to decrease methane was: p-coumaric > ferulic > cinnamic.*

The observation from experiment 3 showed that addition of purified chesnut and sumach tannins at 1 mg/ml to hay:concentrate (70:30) diet significantly decreased ($P<0.05$) methane production by 6.5 and 7.2% from control, respectively. Lower concentrations (0.5 and 0.75 mg/ml) of these hydrolysable tannins did not significantly ($P>0.05$) decrease methane production. The addition of mimosa and quebracho tannins (condensed tannins) did not significantly decrease methane production, even at the highest concentration. For all tannins, increase in concentration led to increase in methane reduction (*FIG. 1*). Condensed tannins decreased gas production and OMD more than hydrolysable tannins. *The results suggested that hydrolysable tannins are more effective in decreasing methane emissions than condensed tannins, while at the same time hydrolysable tannins did not significantly decrease OMD. The condensed tannins appear to decrease methane more through reduction in fibre digestion (indirect effect), while hydrolysable tannins act more through inhibition of the growth and/or activity of methanogens and/or hydrogen producing microbes (direct effect)*. Work on the study of the microbial ecology using quantitative PCR is in progress and the results from this study will also be presented.

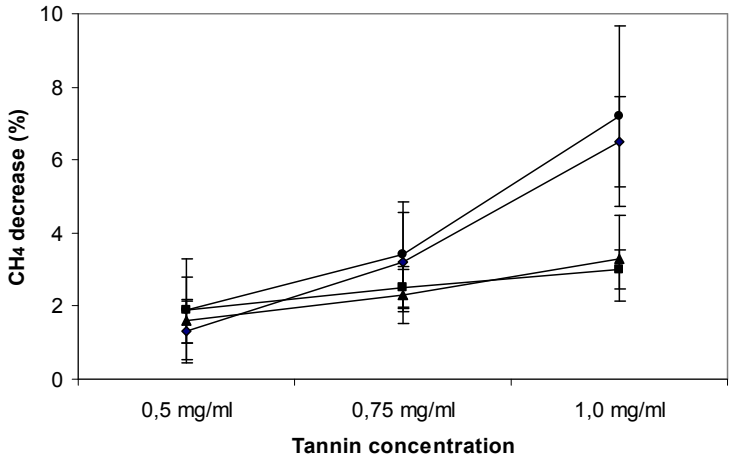

FIG. 1. Effect of purified tannins from chesnut (-♦-), mimosa (-■-), quebracho (-▲-) and sumach (-●-) on CH_4 decrease when added to hay:concentrate diet (70:30 w/w) at 0.5, 0.75 and 1.0 mg/ml concentrations.

REFERENCES

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[2] MAKKAR, H.P.S., BECKER, K., 1994. Isolation of tannins from leaves of some trees and shrubs and their properties. Journal of Agricultural and Food Chemistry 42 (3): 731–734.