Relationship between phenolic contents in tropical plants and *in vitro* ruminal methane concentration

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Introduction

Tropical plants are known to contain considerable amounts of secondary compounds, including the group of phenolics. Adding phenols to ruminant diets is, besides the addition of plant derived oil, a promising nutritive strategy to reduce methanogenesis in cattle and sheep (Sliwinski et al., 2002). In this experiment, several tropical plants from Indonesia were investigated for their effect on methane formation during ruminal fermentation *in vitro*, without or with addition of linseed oil. The hypothesis to be tested was that there is a negative relationship between the content of phenolic compound and *in vitro* methanogenesis across the plants investigated; this intensified with the addition of linseed oil.

Materials and Methods

The leaves from 27 plant species were obtained from the area of Bogor, Indonesia. These leaves, originating from grasses (1 species), herbs (4), shrubs (3) and trees (19), are often fed to ruminants in rural areas or are used as traditional veterinary medicines. Based on information from the literature all of these plant species are assumed to contain relatively high amounts of phenolic compounds (e.g. Lowry et al., 1992). The *in vitro* incubation of the plants was performed using the Hohenheim gas production method as described by Soliva and Hess (2007). Approximately 200 mg dry matter (DM) of each plant was incubated with 30 ml of ruminal fluid/buffer mixture (1:2; v/v) for 24 h at a constant temperature of 39°C. Each plant was incubated either without or with 10 mg (5%) linseed oil addition to plant DM. Each plant was incubated four times (n=4, represented by one incubation unit per experimental run). The ruminal fluid was collected before the morning feeding from a rumen-fistulated Brown Swiss cow receiving hay *ad libitum* and a regular concentrate (0.5 kg/day). Variables measured were total gas production by manual reading of the scale on the syringes, and methane concentration by using gas chromatography (Hewlett Packard model 5890 Series II, Avondale, PA, USA) following the procedure of Soliva and Hess (2007). The plant samples were determined for their phenolic contents using the Folin-Ciocalteu method

(Makkar, 2003). The phenolic contents were expressed as gallic acid equivalent. Crude protein and ether extract were analysed according to standard methods. Net energy for lactation (NEL) of each plant was calculated using an equation from Menke and Steingass (1988). Data was analysed statistically by factorial ANOVA, followed by Tukey's test for comparing different means using the GLM procedure of the SAS software version 9.1.

Results and Discussion

The contents of total phenolics of the plant species varied between 1.4 and 23.6% in plant DM. The lowest content was found in *Canna indica* and the highest in *Acacia villosa*. There were effects of both plant species and linseed oil addition on methane concentration ($P<0.05$), but no interaction took place. The incubation of *Swietenia mahagoni* leaves led to the lowest methane production measured (6.9% of total fermentation gas), while it was highest with *Hibiscus tiliaceous* (19.3%). Other plants species that resulted in methane concentrations of <10% were *Acacia villosa, Eugenia aquea, Myristica fragran,* and *Pithecelobium jiringa*.

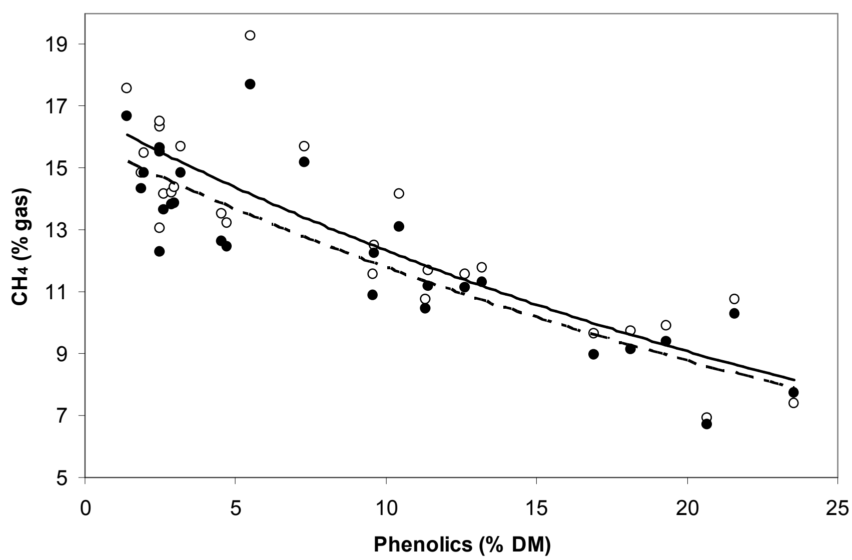

Figure 1: Relationship between total phenolic content and methane concentration in fermentation gas, without (○; full regression line) or with (●; dashed regression line) linseed oil addition.

The relationship between total phenolic content and methane concentration is presented in Figure 1. Methane concentration decreased exponentially as the phenolic content increased, both without ($r^2 = 0.77$) and with ($r^2 = 0.77$) linseed oil addition. Both tannin phenolics and non-tannin phenolics could have been involved in this effect (Jayanegara et al., 2009), either directly through inhibition of the growth of methanogens or indirectly through reduction in fiber digestion, which decreases H_2

production, or both (Tavendale et al., 2005). Addition of linseed oil reduced methane concentration in fermentation gas within the same plant species ($P<0.05$) and, hence, shifted the regression line downward by approximately 0.6 vol % on average. Figure 2 shows that there is not a general correlation between concentrations of phenols and NEL. Rather, the variance of NEL concentration decreased with increasing concentrations phenols, restricting higher NEL concentrations in the respective plants. But within the plants with the lowest phenol levels some contained as low NEL as with the highest phenol concentrations. This indicates that phenols in the analysed sample set were a clear but not the only factor limiting metabolism of energy sources.

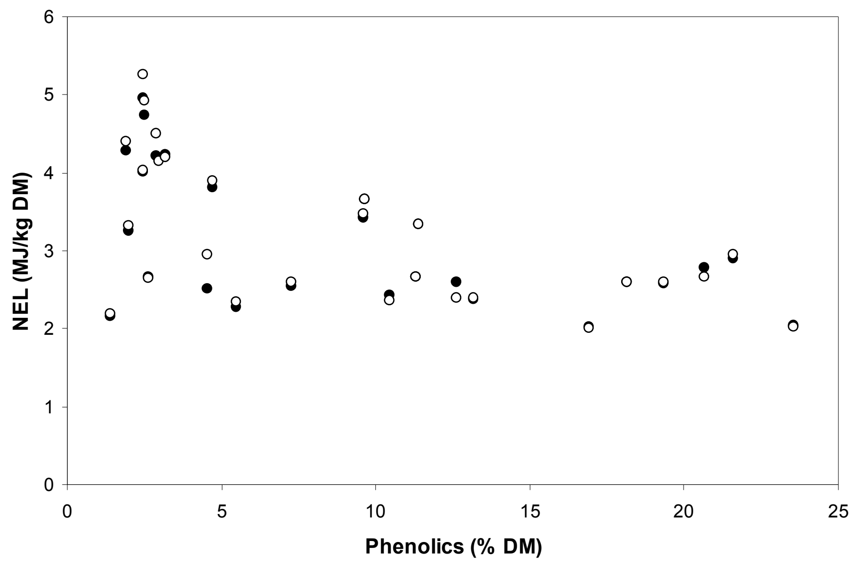

Figure 2: Relationship between total phenolic content and net energy lactation (NEL), without (○) or with (●) linseed oil addition.

Conclusion

The results suggest that phenols clearly decrease ruminal methane production but also limit digestive utilization of energy sources. Also, added linseed oil has a very constant but low inhibiting effect on methane production. Phenol concentration appears as an easily analysable indicator for a plant’s potential to inhibit methanogenesis, but a clear antagonism to productivity of respective forages has to be solved for practical application in animal nutrition.

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