Effects of condensed tannins from *Salix* varieties on methanogenesis *in vitro*

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Introduction

Nowadays, global warming is an increasing concern throughout the world. Greenhouse gases, among which methane (CH_4) emissions, are responsible for this global warming (Beauchemin et al., 2007), with CH_4 having a greenhouse warming potential 21 times higher than that of carbon dioxide (CO_2; EPA, 2006). Further, enteric CH_4 production represents a loss to the animal of around 6 % of the ingested dietary gross energy (Johnson and Johnson, 1995). All these aspects have lead research to find methane mitigation strategies without detrimental effects on animal performance. Condensed tannins (CT) have been reported to decrease methane emissions, 1) directly by inhibiting methanogens' growth and 2) indirectly by decreasing rumen fiber degradability and hence, a reduced H_2 availability for CH_4 production (Tavendale et al., 2005). However, the extent of effects of tannins on ruminants depends on factors such as the basal diet (Hess et al., 2006), dose (Oliveira et al., 2007) and the tannin chemical and physical structure (Waghorn and McNabb, 2003). Hence, the objective of this study was to screen 18 condensed tannin (CT) extracts for their effect on rumen fermentation and methanogenesis in relation to the extract composition and tannin structure and dose.

Material and methods

For this screening test, a range of doses was tested: 0, 0.25, 0.50, 1.0, 2.0, 5.0, 10 and 25 mg of CT per incubation flask. The latter range was chosen based on *in vitro* and *in vivo* studies from literature and this study went above and below doses found in literature. This experiment was performed in one run using 18 different tannin crude extracts from *Salix* spp. (willow), with one replicate per dose, and a control (in quadruplicate), where no tannins were added. Extracts were isolated by L. Falchero with methodology according to Gea et al. (2011). Samples were incubated in 125 ml flasks. Flasks were sealed and flushed with CO_2. To each flask 25 ml of a CO_2 saturated bicarbonate/phosphate buffered rumen fluid was added (buffer:rumen fluid 4:1, v:v) before the onset of the 24h *in vitro* incubations at 39°C in a shaking incubator. The substrate used was composed of 100 mg of cellobiose, 100 mg of starch and 20 mg of casein. An external source of unsaturated fatty acids (20 mg) was also added: a 50:50 mix of linseed oil (omega-3 source) and corn oil (omega-6 source). Before the start of the incubation, 1 ml of ethane (C_2H_6) was added to each flask as an internal standard for gas measurements. After 24 h of incubation, a subsample was taken from the headspace and analyzed by a micro-GC equipped with two gas chromatographic modules and with a thermal conductivity detector (3000 micro-GC, Agilent, USA) for CH_4 production (Hassim et al., 2010). Rumen fluid was collected at 0h for volatile fatty acids (VFA) analysis performed on a Shimadzu 2010 (Shimadzu Corporation, 's-Hertogenbosch, the Netherlands) equipped with a Nukol column (30 m × 0.25 mm × 0.25 µm, Supelco).

Results and discussion

In this study, some of the tested extracts were more effective in inhibiting CH_4 production than others (Figure 1) at the same dose. For example, the same inhibition level (44 % CH_4 inhibition) is achieved at a lower dose for extract 13 (2 mg CT/incubation flask) and as compared with extract 14 (25 mg CT/incubation flask). This suggests differences in the chemical structure of the CT to play a role.

At 2 mg CT/incubation flask, extracts 11 and 13 inhibited CH_4 by around 40 % whereas extracts 5 and 10 did not inhibit CH_4 production. This difference in CH_4 inhibition could be related to the *cis*/*trans* ratio, which describes the orientation of functional groups within a molecule. Indeed, the *cis*/*trans* ratio for extracts 11 and 13 is 82.1 and 89, respectively; whereas for extracts 5 and 10 this ratio was lower (43.9 and 53.4 for extracts 5 and 10, respectively).

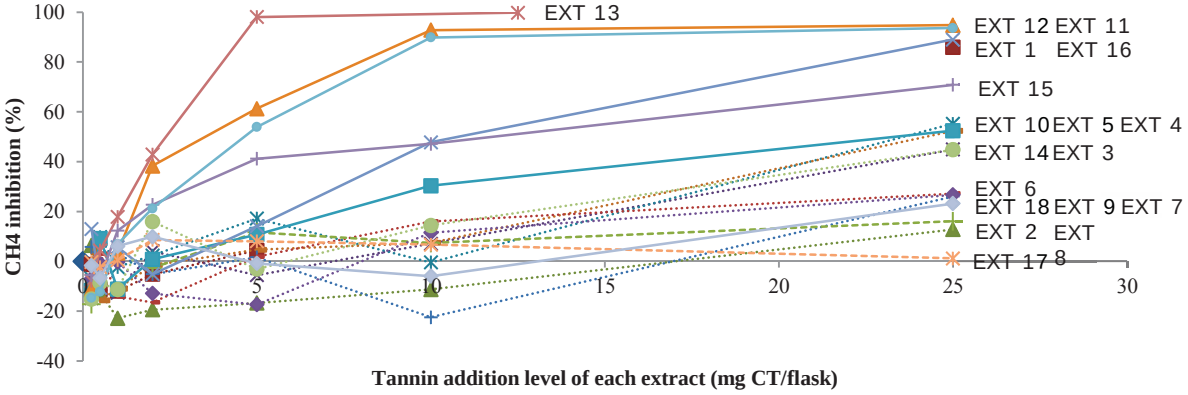

Figure 2. Methane (CH_4) inhibition compared with control (400 µmol CH_4 produced) after 24h incubation in the presence of different CT extracts and supplemented at different doses (extract 1; extract 2; extract 3; extract 4; extract 5; extract 6; extract 7; extract 8; extract 9; extract 10; extract 11; extract 12; extract 13; extract 14; extract 15; extract 16; extract 17; extract 18)

However, at the highest dose tested (25 mg CT/ incubation flask), the *cis*/*trans* ratio seems to be of less importance for the effect of the CT on ruminal methanogenesis. Indeed, extracts 2 and 4, with low *cis*/*trans* ratios (2.1 and 7.4 for extracts 2 and 4, respectively), resulted in different degrees of CH_4 inhibition: with extract 2 CH_4 was inhibited by 12 % while CH_4 production inhibited by extract 4 by 55 %. This suggests other parameters to be important to link the structure of the CT to their biological effects. In fact, looking at the procyanidin/prodelphinidin ratio (PC/PD ratio), that compares similar molecules with one extra hydroxyl-group (prodelphinidin) or not (procyanidin) on the B ring of the tannin, extract 2 is characterized by a low PC/PD ratio (15.1) compared with extract 4 (49.8).
Nevertheless, net total VFA productions and relative proportions of VFA data are currently being analyzed. So it is not possible yet to conclude if the tested extracts are effective to reduce CH_4 production without detrimental effects on ruminal fermentation.

Acknowledgments

L. Falchero received an Intra-European Fellowship from Marie-Curie. P. Faure received a scholarship from Rhône-Alpes French region for student mobility abroad.

References

Beauchemin, K.A., McGinn, S.M., Martinez, T.F., McAllister, T.A., 2007. Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle. *J Anim Sci*, 85(8): 1990-1996.

EPA, 2006. Global anthropogenic Non-CO_2 Greenhouse Gas Emissions: 1990-2010. http://www.epa.gov/climatechange/economics/downloads/GlobalAnthroEmissionsReport.pdf, accessed 26-1-2012.

Gea, A., Stringano, E., Brown, R.H., Mueller-Harvey I., 2011. *In situ* analysis and structural elucidation of sainfoin (Onybrychis viciifolia) tannins for high thoughput germplasm screening. *J Agric Food Chem*, 59: 495-503.

Hassim, H.A., Lourenço, M., Goel, G., Vlaeminck, B., Goh, Y.M., Fievez, V., 2010. Effect of different inclusion levels of oil palm fronds on *in vitro* rumen fermentation pattern, fatty acid metabolism and apparent biohydrogenation of linoleic and linolenic acid. *Anim Feed Sci Technol*, 162: 155-158.

Hess, H.D., Tiemann, T.T., Noto, F., Carulla, J.E., Kreuzer, M., 2006. Strategic use of tannins as means to limit methane emission from ruminant livestock. *International Congress Series*, 1293(0): 164-167.

Johnson, K.A., Johnson, D.E., 1995. Methane Emissions from Cattle. *J Anim Sci*, 73(8): 2483-2492.

Oliveira, S.G. de, Berchielli, T.T., Pedreira, M.D., Primavesi, O., Frighetto, R., Lima, M.A., 2007. Effect of tannin levels in sorghum silage and concentrate supplementation on apparent digestibility and methane emission in beef cattle. *Anim Feed Sci Technol*, 135(3-4): 236-248.

Tavendale, M.H., Meagher, L.P., Pacheco, D., Walker, N., Attwood, G.T., Sivakumaran, S., 2005. Methane production from *in vitro* rumen incubations with *Lotus pedunculatus* and *Medicago sativa*, and effects of extractable condensed tannin fractions on methanogenesis. *Anim Feed Sci Technol*, 123: 403-419.

Waghorn, G.C., McNabb, W.C., 2003. Consequences of plant phenolic compounds for productivity and health of ruminants. *Proc Nutr Soc*, 62(02): 383-392.