Chemical structures of condensed tannins affect *in vitro* ruminal methane production and fermentation characteristics

N. T. Huyen[1], C. Fryganas[2], G. Uittenbogaard[1], I. Mueller-Harvey[2], W. H. Hendriks[1,3], W. F. Pellikaan[1]

[1]Animal Nutrition Group, Wageningen University, Wageningen, the Netherlands, [2]Chemistry & Biochemistry Laboratory, School of Agriculture, Policy and Development, University of Reading, Reading, United Kingdom, [3]Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

An in vitro study was conducted to investigate the effects of CT structural properties, i.e. polymer size (or mean degree of polymerization, mDP), percentage of cis flavan-3-ols (% cis) and percentage of prodelphinidins (% PD) in condensed tannin (CT) extracts on methane production (CH4) and fermentation characteristics. Condensed tannins were extracted from eight plants in order to obtain different CT types: black currant leaves, goat willow leaves, goat willow twigs, pine tree bark, red currant leaves, sainfoin whole plants, weeping willow catkins and white clover flowers. They were analysed for CT content and composition by thiolytic degradation, followed by HPLC analysis. Grass silage was used as a control substrate. Condensed tannins were added to the substrate at a concentration of 40 g/kg, either with or without polyethylene glycol (PEG 6000) to inactivate tannins, and then incubated during 72 h in mixed buffered rumen fluid from three lactating dairy cows. Total cumulative gas production (GP) was measured by using an automated gas production system. During the incubation, 12 gas samples (10 μL) were collected from each bottle headspace at 0, 2, 4, 6, 8, 12, 24, 30, 36, 48, 56 and 72 h of incubation and analysed for CH4. A modified Michaelis–Menten model was fitted to the CH4 concentration patterns and model estimates were used to calculate total cumulative CH4 production (GPCH4). Total cumulative gas production and GPCH4 curves were fitted using biphasic and monophasic modified Michaelis-Menten models, respectively. Addition of PEG increased ($P<0.001$) GP, GPCH4, and CH4 concentration compared to without PEG. This means that CT either inhibited or tended to inhibit fermentation. All CTs reduced ($P<0.001$) GPCH4 and CH4 concentration. All CTs increased ($P<0.001$) halftime of GP and GPCH4. Moreover, all CTs decreased ($P=0.0002$) the maximum rate of fermentation for GPCH4. The correlation between CT structure and GPCH4 and fermentation characteristics showed that prodelphinidin content (% PD) had the largest effect on GPCH4 and fermentation characteristics, followed by CT polymer size (or mDP) and cis-flavan-3-ols (% cis).