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Effects of tannins on ruminal degradation and excretory pattern of N and implications for the potential N emission from the manure

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Abstract. Two experiments were carried out to assess the effects of supplementing tannin-rich legumes or extracted tannins on ruminal degradation of crude protein and the excretory pattern of nitrogen (N). In Experiment 1, using the in vitro gas-transducer technique, various tannin-rich forage legumes were supplemented alone or in combination with the tannin-free legume *Vigna unguiculata* to a tropical grass diet. In Experiment 2, six growing lambs fed temperate grass or grass–legume diets were supplemented with 0 or 25 g of *Acacia mearnsii* tannins/kg dietary dry matter. In Experiment 1, supplementation of the grass with tannin-rich legumes (i.e. *Calliandra calothyrsus* or *Flemingia macrophylla*) alone did not increase the supply with degradable protein compared to the pure grass diet, and suppressed apparent ruminal protein degradability. By contrast, *V. unguiculata* clearly increased amount and proportion of rumen-degradable protein in the diet. When combined with *V. unguiculata*, the tannin-rich legumes could be used in proportions of up to 10% of the complete diet without affecting protein degradation rate. In Experiment 2, tannin supplementation decreased ruminal ammonia concentration and resulted in a shift in N excretion from urine to faeces without affecting body N retention. This shift in N excretory pattern is of practical relevance because urinary N is prone to ammonia emission during manure storage and application. These results confirm that tannin-rich legumes suppress ruminal protein degradability and suggest that dietary tannin supplementation can be useful in mitigating potential gaseous N emissions from animal excreta.

Keywords: Legume; Nitrogen excretion; Protein degradation; Rumen fermentation; Tannin

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1. Introduction

In tropical as well as in temperate climates, there is growing interest in the use of grass–legume associations and in the identification of efficient natural feed additives and forage species with secondary plant compounds. One major goal is to improve the metabolic protein supply of the animals without the need of exogenous mineral N. Ideally, such farming strategies should aim at limiting environmentally harmful emissions. Both forage grasses and legumes from temperate regions are typically rich in rumen-degradable protein which may result in a low efficiency of N utilisation and high metabolic energy costs for N excretion with urine. Reducing ruminal protein degradation in forage-based diets could result in decreased metabolic energy losses and gaseous nitrogen emissions from manure during storage and application. It was hypothesised that a supplementation with tannin-rich legumes or extracted tannins to diets free of or low in tannins would result in a suppression of ruminal protein degradation and modification of the excretory pattern of nitrogen. To test this hypothesis, one in vitro experiment and one in vivo experiment were conducted.

2. Materials and methods

In Experiment 1, the test diets consisted either of the tropical grass *Brachiaria humidicola* alone or the grass supplemented with legumes [1/3 of dietary dry matter (DM)]. Legume supplements were either single legumes [*Vigna unguiculata* (no detectable condensed tannins), *Leucaena leucocephala* (48 mg/g condensed tannins), *Calliandra calothyrsus* (263 mg/g condensed tannins), or *Flemingia macrophylla* (48 mg/g condensed tannins)] or mixtures of *V. unguiculata* with one of the three tannin-rich legumes in ratios of 1 : 2 or 2 : 1. All diets were evaluated with and without the addition of polyethylene glycol (PEG; 35 mg/g of dietary DM) to inactivate the soluble condensed tannins in the legumes. The experiment was performed using the gas transducer technique [1]. Briefly, samples of 1.0 g DM were placed in triplicate in serum bottles and incubated with buffer and mineral solution, reducing agent and strained rumen fluid obtained from a rumen-cannulated Zebu steer. 3, 6, 9, 12, 24, 36, 48, 60, 72, 96, 120 and 144 h after initiating the fermentation, the pressure inside the flask was determined and the volume of the gas produced was measured with a syringe. After 144 h of incubation, the content of the bottles was vacuum filtered, and the residues as well as the feeds were analysed for their contents of DM and total N.

In Experiment 2, six castrated growing lambs with an initial body weight (BW) of 25.4 ($\pm$ 2.1) kg were subjected to a total of six treatments and six experimental periods in a 6×6 Latin square with a 3×2 factorial arrangement ($n = 6$). Basal diets were either ryegrass silage alone (*Lolium perenne*) or 1 : 1 (on a DM basis) silage mixtures of ryegrass and red clover (*Trifolium pratense*) or ryegrass and alfalfa (*Medicago sativa*). All three diets were evaluated with and without the addition of 25 g/kg of diet DM of condensed tannins obtained from the bark of *Acacia mearnsii*. The experimental periods lasted for 21 days each with the last 8 days being reserved for measurements. In that period, lambs were placed into metabolic crates and refusals, faeces and urine were quantitatively collected. Rumen fluid samples were analysed for volatile fatty acids (VFA) and ammonia concentration. Lambs were fed 75 g DM/kg $BW^{0.75}$ of silage per day. Nitrogen determinations in feed, refusals, faeces and urine were performed with an automatic

C/N analyser (CN-2000, version 2.2, Leco Instrumente GmbH, Kirchheim Germany). Data of both experiments were subjected to analysis of variance.

3. Results and discussion

In Experiment 1, the highest gas production and in vitro DM digestibility (IVDMD) were observed with the pure *B. humidicola* diet and the mixtures of *B. humidicola* with *V. unguiculata* ($P<0.05$). The lowest ($P<0.05$) IVDMD was found in the mixture that contained 1/3 of *F. macrophylla*. There was a highly significant interaction ($P<0.001$) between diet and PEG addition which was mainly due to the fact that the addition of PEG increased gas production and IVDMD in the diets with high proportions of tanniniferous legumes, but had no effect in the other diets. The highest amount of crude protein was apparently degraded in the diets containing 1/3 of *V. unguiculata* and the lowest amount in the pure *B. humidicola* diet ($P<0.05$; Fig. 1, left). Supplementation with *C. calothyrsus* or *F. macrophylla* alone did not increase ($P>0.05$) the supply with degradable protein when no PEG was added. With PEG, however, the amount of protein degraded in the diets containing 1/3 of *C. calothyrsus* or *F. macrophylla* was clearly higher than in the pure *B. humidicola* diet ($P<0.05$; Fig. 1, left). The apparent degradability of crude protein was highest ($P<0.05$) in the diets containing 1/3 of *V. unguiculata*. The diets containing *L. leucocephala* or low proportions of *C. calothyrsus* or *F. macrophylla* or only *B. humidicola* showed intermediate values. The lowest ($P<0.05$) apparent crude protein degradability was observed in the diets containing 1/3 of *C. calothyrsus* or *F. macrophylla*. There was a highly significant interaction ($P<0.001$) between diet type and PEG addition. In all diets containing *C. calothyrsus* or *F. macrophylla*, as well as in the diets containing medium or high proportions of *L. leucocephala*, the apparent degradability of crude protein was significantly higher ($P<0.05$) when PEG was added, while PEG remained

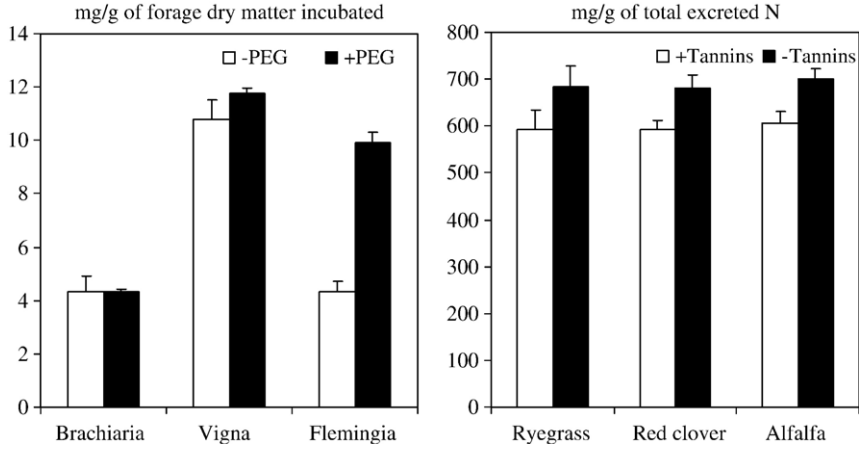

Fig. 1. Effect of the addition of PEG to selected diets (*Brachiaria humidicola* alone or supplemented with *Vigna unguiculata* or *Flemingia macrophylla*) on the amount of apparently degraded N (left) and proportion of urinary N in lambs fed three different diets (ryegrass alone, ryegrass–red clover or ryegrass–alfalfa) either with or without tannins (right).

without any effect ($P>0.05$) in the other diets. Among the three tanniniferous legumes, *L. leucocephala* showed the least negative effects on crude protein degradability.

In Experiment 2, there was no significant interaction between basal diet and tannins. With the addition of tannins, the ruminal fluid ammonia concentration was decreased ($P<0.05$) by 9% on average. Total concentration of VFA was similar ($P>0.05$) in all dietary treatments. The addition of tannins decreased ($P<0.05$) the molar proportion of acetate and the acetate-to-propionate ratio and increased ($P<0.05$) the proportions of propionate, *n*-butyrate and *n*-valerate. Daily N intake was not affected ($P>0.05$) by the addition of tannins. The addition of tannins clearly increased ($P<0.001$) daily N excretion with faeces and decreased ($P<0.001$) excretion with urine, but had no significant effect ($P>0.05$) on the amount of N retained in the body. The proportion of urinary N (mg/g of total N excreted) clearly decreased ($P<0.001$) with the addition of tannins (Fig. 1, right). When expressed as a proportion of total dietary N intake, N excretion with faeces was increased ($P<0.001$) with tannin supplementation while the proportion of N excreted with urine was decreased ($P<0.001$) and had no effect ($P>0.05$) on the proportion of N retained. The shift in N excretory pattern from urine to faeces as a result of feeding tannins is of practical relevance because urinary N is prone to ammonia emission during manure storage. This was clearly demonstrated by comparing excretory pattern and ammonia and total gaseous N emissions from storage of manure of dairy cows receiving different forage-alone diets [2] or diets supplemented with concentrates [3] with contrasting N contents. Furthermore, previous own studies [4], testing very low tannin doses in cows, indicated that this dietary measure might have the potential to decrease ammonia N emission from animal excreta. The favourable effects of tannins in this respect could be twofold: (i) reducing the amount of easily volatile urine N and (ii) continuing their protein-binding activity during manure storage.

The present results confirmed that the condensed tannins, prevalent in many tropical legumes, suppress ruminal protein degradability and decrease rumen fluid ammonia concentration and urinary N excretion. Tannin supplementation, therefore, represents a useful means to mitigate potential gaseous nitrogen emissions from animal excreta when supplemented to diets excessive in nitrogen. As this is mainly the case in intensive feeding systems, additions of tannin extracts rather than unprocessed tannin-rich tropical legumes could be implemented in feeding practice.

References

[1] M.K. Theodorou, et al., A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds, Anim. Feed Sci. Technol. 48 (1994) 185–197.
[2] D.R. Külling, et al., Ammonia, nitrous oxide and methane emissions from differently stored dairy manure derived from grass- and hay-based rations, Nutr. Cycl. Agroecosyst. 65 (2003) 13–22.
[3] D.R. Külling, et al., Emissions of ammonia, nitrous oxide and methane from different types of dairy manure during storage as affected by dietary protein content, J. Agric. Sci. Camb. 137 (2001) 235–250.
[4] B.J. Śliwiński, et al., Performance, body nitrogen conversion and nitrogen emission from manure of dairy cows fed diets supplemented with different plant extracts, J. Anim. Feed Sci. 13 (2004) 73–91.