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Effects of tannins in *Calliandra calothyrsus* and supplemental molasses on ruminal fermentation *in vitro*[*]

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ABSTRACT

Two experiments were carried out, using rumen simulation technique, to assess the effects of tannins in *Calliandra calothyrsus* and supplemental molasses on ruminal fermentation of tropical grass-based diets. The results demonstrated that tannins were mainly responsible for the suppression of methanogenesis noted when *C. calothyrsus* was included in the diet. Furthermore, results indicated that supplementation of degradable protein or sugars enables rumen microbes to better cope with the limitations given by the high content of condensed tannins in *C. calothyrsus*.

KEY WORDS: methane, rumen fermentation, tannins, tropical legumes

INTRODUCTION

Tropical grass- or straw-based diets are often limited in feeding value because of their low protein content. Forage legumes, which are generally higher in protein than grasses and straw, represent an adequate supplement and may improve protein intake of ruminants. But the number of available legume species adapted to low-P acidic soils is limited. One promising species is the shrub *Calliandra calothyrsus* which is, however, characterized by very high tannin contents. A previous study showed that supplementation with *Cratylia argentea*, another tropical shrub legume, or *Arachis*

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pintoi, a herbaceous legume, clearly improved rumen fermentation which was not the case with *C. calothyrsus* (Hess et al., 2003). On the other hand, supplementing *C. calothyrsus* suppressed *in vitro* methanogenesis per unit of fermented organic matter (OM). However, it remained unclear whether this was the effect of the tannins predominant in this legume or due to a lack of degradable nutrients. The objective of the two experiments carried out in the present study was to separate these two factors in order to be able to develop recommendations for the use of *C. calothyrsus* as a potential component of mixed diets contributing to limit the increase in methane release which is observed when grass diets were supplemented with tannin-free legumes alone (Hess et al., 2003).

MATERIAL AND METHODS

In experiment 1, the test diets consisted of low-quality hay of the tropical grass *Brachiaria dictyoneura* and dried foliage of *C. calothyrsus* in ratio of 1:0, 2:1 and 0:1. The three basal diets were supplemented with 0 and 35 g kg^{-1} (DM basis) of polyethylene glycol (PEG; molecular weight: 8000), in order to inhibit the effects of soluble condensed tannins. Forage samples were sprayed with an aqueous PEG solution and dried prior to incubation. Experiment 2 consisted of four basal diets composed of hay of *B. dictyoneura* and dried foliage of *C. calothyrsus* and *C. argentea* in ratio of 1:0:0, 1:1:0, 2:1:1 and 1:0:1. The four basal diets were tested with and without the addition of 100 mg g^{-1} of sugarcane molasses (DM basis) put homogenously onto the coarsely milled forages prior to incubation.

Both experiments were carried out with a rumen simulation technique (Rusitec) supplying daily portions of 15 g DM of the respective basal diets plus the respective supplements (PEG or molasses). The experimental procedure is described in detail in Hess et al. (2003). Briefly, fermenters were filled with 890 mL strained rumen fluid obtained from two fistulated *Bos indicus* steers and 110 mL McDougall buffer. Buffer infusion rate was 500 mL per day to every fermenter. Feed was supplied in nylon bags of 70 × 130 mm and samples were incubated for 48 h each. Experimental periods lasted for 10 days with the final 6 days serving for the measurement of various fermentation variables inclusive of fermenter fluid ammonia concentration and daily methane release. Data were evaluated by analysis of variance designed for two-factorial arrangements of treatments. Basal diets, supplements and basal diet × supplement interaction were considered as main effects.

RESULTS AND DISCUSSION

In experiment 1, the inclusion of *C. calothyrsus* increased dietary crude protein (CP) content from 35 to 150 mg g^{-1} DM and decreased fibre but increased lignin contents. The apparent degradation of OM and CP was significantly decreased ($P<0.05$) by

increasing *C. calothyrsus* proportions although there was an increase ($P<0.001$) in fermenter fluid ammonia concentration from 0.45 to 1.31 mmol L^{-1} when the grass was completely replaced by the legume. Effects on protozoa and bacteria counts were minor ($P>0.05$). Methane release relative to OM apparently degraded was not affected ($P>0.05$) when 1/3 of the grass was replaced by *C. calothyrsus* but was clearly suppressed ($P<0.05$) when the grass was completely replaced. The respective values for the grass-alone, grass-legume and legume-alone diets were 0.48, 0.47 and 0.16 mmol g^{-1}. Interactions between basal diet and PEG addition were mostly significant ($P<0.05$) and PEG remained without any significant effect in the grass-alone diet, but increased fermentative activity in the treatments with *C. calothyrsus*. Accordingly, the addition of PEG largely prevented adverse effects of tannins on apparent nutrient degradability and led to increased ($P<0.05$) fermenter fluid ammonia concentration and methanogenesis. This was particularly pronounced in the case of methane release relative to OM degraded, which was not affected by PEG in the grass-alone diet but increased by more than threefold when PEG was added to the legume-alone diet. These results clearly indicate that tannins in *C. calothyrsus* are involved in the methane suppressing effect of this legume, and that this suppression is not only a result of a reduced fermentation of OM caused by the tannins.

In experiment 2, both legumes increased dietary CP content to about twice of that of the grass-alone diet. No condensed tannins were detected in the grass-alone and in the grass-*C. argentea* diets, but including *C. calothyrsus* at 1/4 and 1/2 of the diet increased tannin content to 14 and 57 mg g^{-1} dietary DM, respectively. While *C. argentea* increased ($P<0.05$) apparent nutrient degradation, *C. calothyrsus* again was found to be adverse ($P<0.05$) in this respect. Methane release was enhanced ($P<0.05$) from 3.2 to 6.4 mmol d^{-1} and from 0.84 to 1.19 mmol g^{-1} OM degraded when replacing half of the grass by *C. argentea.* The corresponding values found with *C. calothyrsus* were 1.5 mmol d^{-1} and 0.51 mmol g^{-1}, respectively. Ammonia concentration as well as protozoa and bacteria counts in fermenter fluid followed the changes found in apparent nutrient degradation. When including the mixture of both legumes, fermentation characteristics were mostly similar ($P>0.05$) to those in the grass-alone treatment, indicating that positive and negative effects of the two legumes compensated each other. There were, however, two important exceptions: both fermenter fluid ammonia concentration and apparent OM degradation were higher ($P<0.05$) with the legume mixture than with the grass-alone diet. Supplementation of molasses significantly decreased fermenter fluid ammonia concentration and apparent degradation of fibre, but increased ($P<0.05$) degradation of OM and methanogenesis (from 0.81 to. 0.93 mmol g^{-1} OM degraded) across all diets. Protozoa counts were doubled while bacteria counts increased by 28% ($P<0.05$) with molasses supplementation. There were significant interactions between basal diet and molasses supplementation in nitrogen turnover. Molasses supplementation was effective ($P<0.05$) in enhancing dietary CP degradation when

added to the grass-*C. calothyrsus* diet but had no effect (P>0.05) when added to the remaining diets. The same holds true for degraded CP which was not recovered in ammonia N. This fraction is likely to be equivalent to the microbial N leaving the rumen and would suggest that molasses enabled rumen microbes to cope with the tannins in a way that *C. calothyrsus* proteins got accessible to be degraded. Similar effects were observed when half of *C. calothyrsus* was replaced by *C. argentea*.

CONCLUSIONS

The present experiments demonstrated that the tannins present in *C. calothyrsus*, although causing massive depressions in feeding value when provided at too high dietary levels, were favourable to a certain degree in combination with a tannin-free legume such as *C. argentea*. Especially the high increase in methane formation per unit of organic matter degraded, which was observed with supplementation of *C. argentea* alone, could be avoided. Furthermore, it seems that both, supplementary degradable protein and sugars, enabled rumen microbes to cope with the limitations given by *C. calothyrsus* tannins.

REFERENCES

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