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In vitro fermentative traits of Australian woody perennial plant species that may be considered as potential sources of feed for grazing ruminants

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ABSTRACT

A range of Australian woody perennial plant species ($n = 128$ samples) were screened *in vitro* for overall fermentability and the capacity to induce favourable metabolic pathways in the rumen. Plants were tested in a batch rumen culture system, where gas pressure, pH and total volatile fatty acids were used as indicators of overall plant fermentability, and concentrations of methane, ammonia, acetate and propionate indicated changes in fermentation end products. *In vitro* fermentation of 52 of the plants yielded a gas pressure that was similar to, or higher than, the positive control (*i.e.*, oaten chaff). Five plants produced less methane (mL/g dry matter supplied) without reducing total gas production, 54 reduced both methane and gas production, 47 reduced neither, and 22 reduced gas production but not methane production. All plants produced relatively low amounts of ammonia, while only 22 reduced the acetate: propionate ratio. Variability in response occurred at the genus and species level and plants with some favourable fermentative traits were identified. The screening of Australian woody perennial plants not previously considered for grazing systems illustrated the possibility of using some species to manipulate rumen fermentation, either as part of a mixed diet or to identify plant compounds associated with bioactivity in the rumen.

1. Introduction

Woody perennial plants of Australia have not been widely considered as forage because of their relatively low biomass productivity and nutritive value when compared to more commonly used pastures or crops (Revell and Sweeney, 2004). However, these plants may offer a supplementary feed for livestock, particularly in areas where or when conventional forages have low biomass, poor persistence or low nutritive value. It has been observed that inclusion of woody perennials can have multiple benefits in livestock nutrition, including increased feed intake, digestibility and rumen fermentation (Raghuvansi et al., 2007), reduced methane emission from ruminants (Soliva et al., 2008), stabilized shrub ecosystem (Aich,

Abbreviations: AB, antibiotic control; CP, crude protein; DM, dry matter; IVFT, *in vitro* fermentability test; NC, negative control; PC, positive control; PSC, plant secondary compounds; VFA, volatile fatty acids.

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1991), and improved feed availability during droughts and in areas of dryland salinity (Dynes and Schlink, 2002; Revell et al., 2006). However, consumption of Australian woody perennials is often associated with negative nutritional effects due to the presence of secondary compounds (PSC: Dynes and Schlink, 2002; Seigler, 2002; Revell et al., 2006). However, some recent reports indicate that PSC at appropriate concentrations may also have beneficial effects, similar to feed additives that are included in ruminant diets to reduce energy loses and increase useful end products of ruminal fermentation (Kamra et al., 2006b; Newbold, 2007). For example, flavonoid-rich plants (Broudiscou et al., 2000), saponins (Hu et al., 2005), essential oils (Mohammed et al., 2004; Calsamiglia et al., 2007), some culinary spices (Patra et al., 2006), medicinal herbs (Garcia-Gonzalez et al., 2004) and a range of forage plants (Kamra et al., 2006a; Bodas et al., 2008; Soliva et al., 2008) are capable of reducing methane produced by ruminal microbes *in vitro*. Feeding tannin containing plants can decrease ruminal protein degradation, promote microbial crude protein (CP) synthesis (Wallace et al., 1994; Getachew et al., 2000; Cardozo et al., 2004), and prevent excessive ruminal gas formation which can lead to bloat (Waghorn, 2003; Wina et al., 2004). Some Australian native plants are known to contain PSC with anti-microbial properties (Palombo and Semple, 2001; Ndi et al., 2007), some of which may affect specific ruminal microbial species (Hutton et al., 2009a). It has been recognised that, when investigating plants containing PSC, it is crucial to consider the effect of whole plants on rumen microbial fermentation (Broudiscou et al., 2000; McIntosh et al., 2003; Makkar, 2005) making the *in vitro* gas production technique particularly valuable when assessing the value of shrubs in ruminant nutrition (Norman et al., 2010).

The aim of this study was to examine the fermentative properties of Australian woody perennials to identify plants that produce favourable fermentation profiles and which may be considered as potential sources of feed for ruminant livestock.

2. Materials and methods

2.1. Plants

A total of 128 plant samples were collected during 2005–2008 (inclusive) from various locations in Australia (Table 1). All species were selected on the basis of having potential as forage plants, based upon their morphology, productivity, feeding value and palatability (Bennell et al., 2010). Samples were collected from numerous individual plants, and edible parts of the plants (*i.e.*, leaf and stems less than 3 mm in diameter) were harvested and the material was freeze-dried and ground to pass a 1 mm screen. Material was stored at room temperature (*i.e.*, 20–25 °C) in sealed containers until analysis.

2.2. In vitro fermentability test (IVFT)

The fermentability of plants was examined in an *in vitro* batch fermentation system which has been used to evaluate plants and their extracts (Cardozo et al., 2005; Busquet et al., 2006; Bodas et al., 2008) and it is a valuable tool for large-scale screening of plant bioactivity and complex interactions which may occur between the plant and the rumen microbes (Makkar, 2005). One day prior to the experiment, 0.1 g of plant material was weighed in Bellco tubes and transferred to an anaerobic chamber (Coy Vinyl Anaerobic Chamber; Coy Laboratory Products Inc., USA) maintained at 39 °C and supplied with 800 mL/L N_2, 100 mL/L CO_2 and 100 mL/L H_2, to expel the oxygen from the tubes. Inside the chamber, H_2 was maintained at 30 mL/L throughout the experiment and there was no detectable O_2, as monitored by Coy Oxygen and Hydrogen Analyser (Coy Laboratory Products Inc., USA). On the experimental day, rumen fluid was collected 2 h after feeding from two fistulated sheep acclimatized for two weeks prior to the sampling to a diet consisting of lupins and oaten chaff (*i.e.*, 1 kg oaten chaff + 250 g lupins + 25 g mineral mix). This aimed to assist adaptation of microbes to a forage-based substrate, which was the form of the plant material tested. Rumen fluid was removed using an electric vacuum pump fitted with a plastic tube and 1 mm metal screen on the sampling end to eliminate large feed particles during collection. After collection, rumen fluid was pooled, transferred into the anaerobic chamber, buffered to pH 7.2 (McDougall, 1948), and 10 mL of this mixture was dispensed into prepared Bellco tubes. A negative control (*i.e.*, buffered rumen fluid only; NC) and a positive control (*i.e.*, buffered rumen fluid and 0.1 g of oaten chaff; PC) were included in the assay as standards to detect differences in rumen fluid between runs. Oaten chaff was selected because it is commonly used in Australia as a supplementary feed for ruminants (Roberts, 2001) and does not contain substantive levels of PSC. Furthermore, an antibiotic treatment was included as a model for potential favourable effects in the rumen *(i.e.*, reduction in methane and proteolysis, increase in propionate: Wallace et al., 1980; Jalc et al., 1992). In the current study, the antibiotic control (AB) was oaten chaff and Rumensin 100 (100 mg/g monensin; Elanco, Advanced Feeds, Midvale, Australia) included at a level of 20 μg/0.1 g oaten chaff that corresponded to its recommended level of intake. Each treatment and the controls were prepared in triplicate. Inside the chamber, once the tubes were filled, they were sealed with a rubber stopper, crimped and incubated for 24 h at 39 °C, with constant shaking at 50 rpm. At the end of the incubation, tubes were placed in a water bath at 39 °C, and gas pressure was measured using a pressure transducer (Greisinger Electronic GmbH, Regenstauf, Germany). A total of four runs were required to analyse all plant samples. Controls were included in each run as standards and there was no difference between NC and PC among runs. Although other authors (Pell et al., 1998) have reported that gas pressures over 40 kPa can inhibit microbial activity *in vitro*, there was no evidence of inhibition in the current study, as gas pressure reached well above this value (*i.e.*, over 100 kPa). Consequently, it was unnecessary to release gas during fermentation.