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Comparison of *in vivo* organic matter digestion of native Australian shrubs by sheep to *in vitro* and *in sacco* predictions☆

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ABSTRACT

There is increasing interest in selection of genotypes of Australian perennial shrubs such as saltbush (*Atriplex nummularia*, *Atriplex amnicola* and *Atriplex semibaccata*), orange wattle (*Acacia saligna*), small-leaved bluebush (*Maireana brevifolia*) and rhagodia (*Rhagodia preissii*) for extensive grazing systems with sheep or cattle. A major limitation to cultivar development is that determining the *in vivo* organic matter digestibility of forage is expensive, time consuming and requires substantial amounts of biomass. A number of *in vitro* and *in sacco* techniques are available to predict *in vivo* digestibility of grasses and legumes however none of these prediction methods have been calibrated to *in vivo* data for Australian native shrubs. The aim of this study was to determine the *in vivo* digestibility of native shrub forage and compare these data to predictions using *in vitro* and *in sacco* methodologies. The hypothesis tested was that there is a linear relationship between *in vivo* organic matter digestibility (OMD) of Australian perennial shrub forage and a number of commonly used prediction methods. Of all the methods used, *in vitro* gas production from microbial fermentation showed the best relationship with *in vivo* OMD ($r^2 = 0.904$). This method appears to be suitable for broad screening and ranking of genotypes. The *in vitro* pepsin-cellulase technique did not provide a good first estimate of *in vivo* OMD across all plant species but may be suitable to rank genotypes if data are calibrated with internal standards to manage intrinsic assay variation (step 1) followed by a correction to account for the high salt content of some plants (step 2) and a final linear correction to account for systematic overestimation of OMD of native shrubs (step 3). Further testing and refining of this third calibration step is required. The prediction of *in vivo* OMD using a 72 h *in sacco* digestion was adequate for the saltbush species but not for the orange wattle and small-leaved bluebush accessions. Prediction of *in vivo* OMD using the Daisy^{II} rumen fluid digestion system was not satisfactory and there was not a statistically significant relationship between the acid detergent fibre, neutral detergent fibre or the acid detergent lignin content of the shrubs and *in vivo* OMD. The differences between the various laboratory-based methods to predict *in vivo* OMD are discussed and we hypothesise that the presence of plant secondary compounds may interfere with rumen microbial fermentation.

1. Introduction

Climate change and salinity are major challenges for Australian agriculture. In southern Australia, it is predicted that climate change will lead to marked reductions in rain-

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fall, higher temperatures, higher evapotranspiration rates and a high probability of increased seasonal variability leading to lower and more variable pasture production and possibly shorter growing seasons (Crimp et al., 2002; Howden et al., 2008). In Western Australia, dryland salinity has already removed up to 1.2 million ha of agricultural land from crop production with a further 2.8–4.4 million ha of land at risk (Macfarlane et al., 2004). Dryland salinity is brought about by rising watertables, following clearing of perennial vegetation for agriculture. The watertables rise because infiltration of rainfall below the root zone of exotic annual crops and pastures is much greater than leakage below the native, perennial vegetation that grew on the land prior to cropping (Turner and Asseng, 2005). As watertables rise they bring salts from deep in the soil profile to the surface. Australian perennial shrubs such as old man and river saltbushes (*Atriplex nummularia* and *A. amnicola*), orange wattle (*Acacia saligna*), rhagodia (*Rhagodia preissii*) and small-leaved bluebush (*Maireana brevifolia*) offer an opportunity to reduce the feed shortages associated with climate change and to improve the productivity of salt-affected land. These shrubs are noted for tolerance of both salinity and drought. The shrubs may also play a role in reducing future salinity through use of rainfall in summer and autumn when annual crops and pastures have senesced. This reduction in water table recharge can lead to rehabilitation of saline land through abatement of salt scalding and waterlogging at the soil surface (Silberstein et al., 2008).

The nutritive profiles of these native Australian shrubs are unique when compared to other sources of on-farm feed due to very high salt and antioxidant levels (Norman et al., 2005) and this is a partial consequence of the significant need for drought tolerance and osmotic-regulation in the plant. A general observation is that these shrubs have low to moderate levels of digestible organic matter (OM), moderate to high crude protein (CP) and, particularly in the case of saltbushes and bluebushes, high levels of minerals (Masters et al., 2009). Previous studies have revealed that there is variation in the nutritive value within saltbush (Tiong et al., 2004) and orange wattle species (George et al., 2007).

The majority of native shrubs planted in Australia are sourced from 'wild' material. A significant opportunity exists for selecting species and genotypes with higher nutritive values as this will improve the profitability of shrub-based systems. Whole farm economic modelling by O'Connell et al. (2006) suggests that the most critical factor influencing profitability of shrubs in saline land is their digestible and metabolisable energy (ME) values. They predict that a 10% increase in digestibility of shrubs can lead to a doubling of profitability from the shrub enterprise (in this case saltbush planted on saline land). The economic model indicates that improving digestibility by 10% is three times more profitable than increasing biomass production by 10% or reducing the cost of establishment by 10%. Researchers are therefore increasingly interested in the variation in digestibility of forage from a range of Australian native genera.

Determining the *in vivo* organic matter digestibility (OMD) of forage is expensive, time consuming and requires many kilograms of biomass. A number of *in vitro* and *in sacco* techniques are used to predict *in vivo* digestibility (Coleman and Henry, 2002; Mould, 2003). These prediction methods need to be calibrated to *in vivo* data, usually using standard calibration samples. For most traditional types of feed (for example cereal hays and annual pasture species) these calibrations are widely available and accurate. To date, few native Australian shrub samples have been through both the animal and laboratory measurement of digestibility. In the case of saltbush, this has led to considerable confusion in industry due to variation in prediction of saltbush digestibility (thus energy) by commercial and research laboratories using methodologies that are not calibrated for these shrubs.

The aim of this study was to develop a range of shrub forage standards with known *in vivo* digestibility and then to test these forages with a range of *in vitro* and *in sacco* methodologies. The hypothesis tested a linear relationship between *in vivo* OMD of shrub forage and a number of digestibility prediction methods including *in sacco* digestibility, *in vitro* gas production, *in vitro* digestion with rumen fluid, *in vitro* pepsin-cellulase digestion and *in vitro* fibre profiles; neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). In addition, the volatile fatty acid (VFA) profiles resulting from the digestion of these shrubs in the rumen were also assessed.

2. Materials and methods

The research complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (Australian Government National Health and Medical Research Council, 2004), following approval by the CSIRO Centre for Environment and Life Sciences Animal Ethics Committee.

2.1. Shrub accessions

Over 100 kg of dry matter (DM) of each of 11 shrub accessions were sourced from a range of species and environments. The species were selected because they are used in commercial grazing systems or they have been identified as promising by pasture agronomists. Table 1 lists the accessions used in the experiment. In addition, a single batch of commercially sourced lucerne chaff was used as a comparison diet. The 'edible' shrub component (generally leaves and stems with a diameter of < 3 mm) of the accessions were picked by hand and dried at 65 °C for 48 h then stored in dry conditions for up to 2 months before use. In the case of bluebush, creeping saltbush and tagasaste, wet material was cut into 5 cm lengths with a commercial mulcher prior to drying. Each accession was thoroughly mixed prior to feeding and subsampled on a daily basis during the *in vivo* feeding experiment. The representative subsampled material was bulked and used for *in sacco* and *in vitro* analyses.

The representative subsamples were used to determine the concentrations of OM, total ash and soluble ash according to the methods of Faichney and White (1983). Total nitrogen was determined by combustion using a Leco FP-428N Analyser (Sweeney and Rexroad, 1987). Phosphorus, potassium, sulphur, sodium, calcium, magnesium, copper, zinc, manganese, iron and boron were measured by ICP-AES (McQuaker et al., 1979). Chloride and nitrate were measured using a Lachat Flow Injection Analyser by the method of Zall et al. (1956).

2.2. Measurement of in vivo OMD

Seventy five, 3-year-old Merino wethers of similar weight were selected and housed in individual pens in an animal house. Immediately prior to introduction to the animal house, the wethers were given an oral anthelmintic drench (Cydectin at the recommended dose level of 0.2 mg moxidectin/0.2 mg/kg body weight) and an injection of vitamins E and B_{12}.