In vitro digestibility assessment of tropical shrub legumes using rumen fluid or faecal fluid as the inoculum source

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Abstract

In vitro dry matter digestibility (IVD) studies using a modified Tilley and Terry (1963) technique were conducted using either strained cattle rumen fluid or the liquid from fresh cattle faeces as sources of inoculum. The dried leaves of 14 tropical leguminous shrubs in the genera: *Acacia, Calliandra, Gliricidia, Leucaena* and *Zapoteca* were used as substrates together with 3 standard forages of known *in vivo* digestibility.

The IVD values from using faecal fluid were linearly correlated with those obtained with rumen fluid ($r=0.982$; $P<0.001$).

The faecal fluid gave significantly ($P<0.001$) lower digestibility values than those obtained with rumen fluid (by 3.5 percentage units).

Shrubs differed greatly ($P<0.001$) in IVD with a range of 22–80%. There was no significant interaction in IVD ($P>0.05$) between legume substrate and inoculum source.

On the basis of these results, it was concluded that the liquid fraction of cattle faeces provides the same precision as rumen fluid for *in vitro* digestion studies to rank tropical leguminous shrubs on the basis of their digestibility. In many situations, it may be easier to use faeces rather than to collect rumen fluid as an inoculum source for IVD studies, especially in developing countries.

Introduction

In vitro digestion techniques using rumen fluid as the inoculum (Tilley and Terry 1963) have proved useful in assessing the relative digestibility of many feedstuffs (Minson 1990). In tropical countries, maintenance of fistulated animals as a readily available source of rumen fluid can pose problems from infestations of insect larvae around the fistula and loss of fistula plugs, with ensuing dehydration of the animals. An alternative *in vitro* method to assess digestibility is the pepsin-cellulase technique. This has proved successful with a range of feedstuffs (Minson 1990). However, our use of this technique with tropical shrub legumes gave higher digestibility values than those obtained with the rumen fluid-pepsin technique. Furthermore, the differences varied greatly with species from 5–20 percentage units of digestibility (Balogun 1995).

Successful use of a liquid suspension of faeces from sheep to estimate digestibility of a range of temperate feedstuffs has been reported (El Shaer *et al.* 1987). With tropical grasses, faecal fluid gave far lower *in vitro* digestibility values than those obtained with rumen fluid (although the rankings were similar) and there was also a significant inoculant × forage type interaction (Manyuchi *et al.* 1991). The technique, however, was considered suitable for use in developing countries. It could also be valuable for assessing variation in the digestibility of shrub species by different wild ruminants without the need to immobilise or shoot them to obtain rumen fluid samples.

In the absence of appropriate *in vivo* standards for the tropical shrub species under study, we compared rumen fluid (RF) and the liquid fraction of faeces (FF) from cattle as inoculum sources for *in vitro* estimations of digestibility of 14 tropical shrub legumes, 13 of which were known to contain tannins.

Materials and methods

Leaves from 14 tropical browse legumes grown at the CSIRO Lansdown Research Station, 50km

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south of Townsville, and 3 samples of herbaceous legumes were used. These had been oven-dried and ground to pass through a 1mm screen before use. The shrubs were in the genera: *Acacia, Calliandra, Gliricidia, Leucaena* and *Zapoteca.* The 3 additional legume samples were of known *in vivo* digestibility, viz. lucerne (*Medicago sativa*) and 2 samples of *Stylosanthes hamata* cv. Verano.

Rumen fluid was obtained from a Droughtmaster steer maintained on a *Urochloa mosambicensis* pasture supplemented with about 2 kg of freshly cut shoots of *Calliandra calothyrsus* daily for 4 days before samples were taken. In previous months, the steer had also grazed plots of a range of leguminous shrub species.

Digesta were removed via the rumen fistula, squeezed through 3 layers of cheese cloth, and the liquid stored in a thermos flask which had been flushed with CO_2 gas, until used in the laboratory about 1h later.

Freshly voided faeces were obtained from the same steer, and from 4 other steers in the same group that had also received *Calliandra* supplementation. Samples were bulked, squeezed through cheese cloth and treated as for the rumen fluid sample.

Duplicate samples of the feeds (0.5 g) were weighed into digestion tubes, and separate samples weighed for oven-drying at 100°C for dry matter determination. A modification of the Tilley and Terry (1963) digestion technique was used. This was based on the method of Drew (1966), which, from experience with another tannin-containing species, *Desmodium intortum*, gave more consistent results with a 72h digestion and the addition of urea to provide ammonia.

One part of rumen fluid (or faeces extract) was mixed with 4 parts of artificial saliva (McDougall 1948) that had been saturated with anaerobic grade CO_2 gas, and the mixture adjusted to pH 6.7 and thoroughly stirred. Fifty ml was then added to each digestion tube, together with 0.6 ml of a freshly prepared solution of urea (30 g/l), the tube flushed with CO_2 and securely stoppered with a gas-release valve. Two control tubes with no forage sample were included in each batch.

Tubes were incubated at 39–40°C for 72h. During incubation they were gently swirled 3 times a day. Tubes were then centrifuged for 10min at 2000g, the supernatant liquid carefully poured off and 50ml of a freshly prepared pepsin solution [4g pepsin (1:1000) dissolved in one litre of 0.1N hydrochloric acid] added. The tubes were then incubated for another 24h and swirled 3 times. Digested samples were filtered in preweighed sintered glass crucibles of porosity 1, dried at 100°C for 24h and weighed.

The procedure was repeated a week later using freshly prepared media. There were thus 2 runs each with 2 replicates.

Data were analysed as a randomised block design with 2 inocula x 17 feedstuffs x 4 replicates. *In vitro* digestibility (IVD) estimates derived from the use of FF inoculum were regressed on the values derived from the RF inoculum.

Results

The effects of inoculum source and substrate on digestibility were highly significant ($P<0.001$). However, the interaction was not significant ($P=0.244$). The digestibility estimates of the feeds ranged from about 20–80% and ranked *Zapoteca* ≥ *Gliricidia* > *Leucaena* > *Acacia* > *Calliandra*. Overall, estimates from using RF inoculum were 3.5 percentage units higher than those from the FF inoculum. There was, however, a strong linear relationship between the 2 estimates (Figure 1) :

$$y = -5.819\ (\pm 2.922) + 1.042\ (\pm 0.052)\ x$$
$$(r = 0.982,\ P<0.001,\ RSD\ 3.69)$$

where y = dry matter digestibility (%) using faecal fluid and x = dry matter digestibility (%) using rumen fluid.

Although estimates of digestibility were lower with the FF inoculum, they were more consistent across replicates, giving an overall standard deviation of 3.65% units compared with 5.10% for the RF inoculum.

Discussion

The results show that the faecal fluid provided an inoculum source which produced results comparable with those of rumen fluid for *in vitro* digestion studies to assess the relative digestion of shrub legumes in evaluation studies. However, it should be noted that, for some shrubs, it would be relevant to use fresh rather than dried leaves for the digestibility estimates if the results are to relate to browsing stock. This is because drying, or prolonged wilting, can reduce the digestibility of some tanniniferous feeds (Palmer and Schlink 1992).

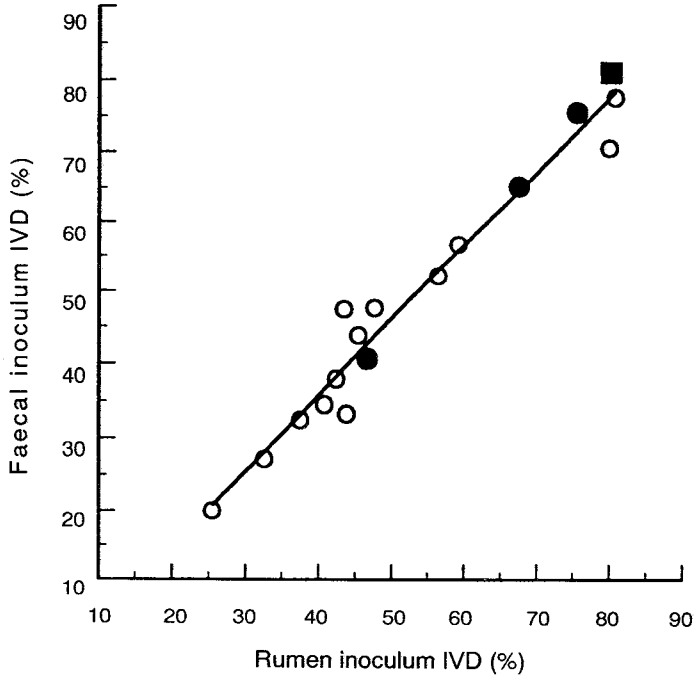

Figure 1. The relationship between *in vitro* dry matter digestibility (IVD) estimates determined using faecal fluid or rumen fluid as inoculum sources.
(○ tanniniferous browse species; ■ a non-tanniniferous shrub; ● non-browse standards)

The lower values when FF inoculum was used are not readily explained. It was noticed that digestion with RF appeared to commence earlier than with FF and had more active frothing. However, digestion appeared to have finished in all tubes after 72h.

In the study of El Shaer *et al.* (1987), there was no suggestion that the digestion of the various substrates using FF from sheep was lower than the *in vivo* values. The lower values obtained using FF with tropical grasses (Manyuchi *et al.* 1991) may have been due to the shorter digestion time (48 *vs.* 72 h) and to the fact that no urea was added to the digesta in their method. The possibility that the presence of tannins may be involved in the lower values obtained with FF in this study is unlikely, since 4 of the feeds used (one shrub legume and the three standards) had no tannins, yet there was no significant interaction of inoculum source x species (see Figure 1). It is possible that numbers of bacteria were lower in the FF or that it contained fewer species. The difference between our results and those of El Shaer *et al.* (1987) remains unexplained but it emphasises the need for the use of known *in vivo* digestibility standards, preferably of similar types of feed, in each run.

With drier sheep faeces (El Shaer *et al.* 1987), it was necessary to use buffer medium to extract a suitable liquid suspension. We did not need to use this approach with the cattle faeces. Squeezing the faeces in a muslin cloth 'bag' produced sufficient fluid for the inoculum. However, with drier cattle faeces produced when grazing dry season pastures, extraction with a buffer solution may be necessary.

The lower mean standard deviations associated with FF were unexpected, and indicate that the method did not have lower precision than when RF was used. This, coupled with the lack of a species x inoculum source interaction, suggests that the method could be as useful as the standard *in vitro* digestibility (IVD) technique.

Acknowledgements

We thank the Stapledon Memorial Trust for funding to enable Mr P. Barnes to visit Australia and to participate in the study. We also thank Mr R.P. LeFeuvre for skilled technical support and the statistical treatment of the data, Dr B. Palmer for access to the *Calliandra* collection grown at Lansdown, and Dr A.J. Ash for the provision of the *in vivo* standards.

References

BALOGUN, R.O. (1995) *Effect of tannins on the nutritive value of tropical shrubs.* M.Agric.Sci.Thesis. University of Melbourne.

DREW, K.R. (1966) The *in vitro* prediction of herbage digestibility. *Proceedings of the New Zealand Society of Animal Production*, **265**, 52–70.

EL SHAER, H.M., OMED, H.M., CHAMBERLAIN, A.G. and AXFORD, R.F.E. (1987) Use of faecal organisms from sheep for the *in vitro* determination of digestibility. *Journal of Agricultural Science (Cambridge)*, **109**, 257–259.

MANYUCHI, B., RUSIKE, E. and CHAKOMA, C. (1991) Comparison of the use of rumen fluid or dung as a source of microbial inoculum for the digestion of forages *in vitro*. *Zimbabwe Journal of Agricultural Research*, **29**, 17–25.

MCDOUGALL, E.I. (1948) Studies on ruminant saliva. I. Composition and output of sheep saliva. *Biochemistry Journal*, **43**, 99–109.

MINSON, D. J. (1990) *Forage in Ruminant Nutrition.* (Academic Press Inc.: San Diego, USA).

PALMER, B. and SCHLINK, A.C. (1992) The effect of drying on the intake and rate of digestion of the shrub legume *Calliandra calothyrsus. Tropical Grasslands*, **26**, 89–93.

TILLEY, J.M.A. and TERRY, R.A. (1963) A two–stage technique for the *in vitro* digestion of forage crops. *Journal of the British Grassland Society*, **18**, 104–111.

(Received for publication October 24, 1995; accepted February 12, 1996)