

# Comparison of sheep rumen liquor and Rusitec fluid as inoculum for determining the *in vitro* digestibility of hays

M.L. Tejido, M.J. Ranilla and M.D. Carro

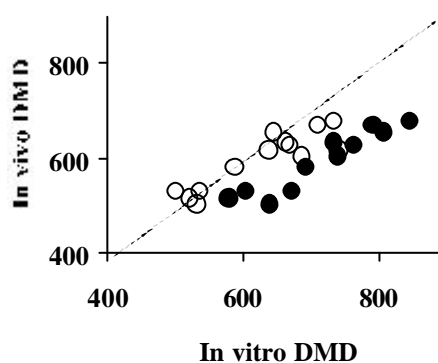
Departamento de Producción Animal I. Universidad de León. 24071 León. Spain

**Introduction** One practical disadvantage of some *in vitro* methods used to estimate the *in vivo* digestibility of forages is the need for fistulated donor ruminants to provide the rumen liquor. These are subject to restrictive legislation in many countries and are costly to prepare and maintain. The aim of this study was to investigate whether rumen liquor in the *in vitro* digestibility technique of Van Soest *et al.* (1966) could be replaced by microorganisms derived from Rusitec. This technique involves the incubation of samples with buffered rumen fluid for 48 h followed by an extraction with a neutral-detergent solution.

**Material and methods** Four rumen-fistulated sheep and a four-vessel Rusitec system were used as a source of microorganisms to determine the *in vitro* digestibility (Van Soest *et al.*, 1966) of twelve hays. The *in vivo* dry-matter digestibility (DMD) of the hays had been determined previously and ranged from 503 to 678 g/kg. Each sheep received 1 kg of 80:20 alfalfa hay:concentrate per day offered at 9.00 and 21.00 h for 15 days before starting the *in vitro* digestibility assays. The Rusitec system was inoculated with rumen contents from the same sheep, and each vessel was fed daily 16 g of the same diet for 12 days before doing the *in vitro* digestibility assays. Rumen fluid (from sheep and Rusitec vessels) was obtained 2 hours after morning feeding, filtered through four layers of cheese-cloth and mixed with the buffer solution of Goering and Van Soest (1970) in a proportion 1:4 (v:v). The ANKOM *in vitro* fermentation system Daisy II was used for the incubations. This system consists of a unit containing four glass flasks of 5 L of volume. Samples (500 mg) of each hay (ground to pass a 1.0 mm screen) were weighed in artificial fibre bags (50 x 40 mm;  $25 \pm 10$   $\mu$ m pore size), which were heat-sealed and placed in the flasks. Each flask was then filled with the buffered rumen fluid of one sheep or one Rusitec vessel. One bag of each hay was included in each flask, so that 4 values of *in vitro* digestibility were available for each feed sample. Duplicates of a reference hay of known *in vitro* digestibility were also included in each flask to detect possible irregularities during the fermentation process. All procedures were conducted under CO<sub>2</sub> with the solutions maintained at 39°C. Following 48 h of incubation, the bags were boiled in a neutral-detergent solution for 1 h using an ANKOM220 Fibre Analyser unit. Finally, the bags were washed with distilled water and dried at 60°C for 48 h to determine the *in vitro* DM digestibility. Two incubations were carried out, one with sheep rumen liquor and one with Rusitec fluid. Relationships between *in vivo* digestibility and data obtained with rumen liquor and Rusitec fluid were tested by linear regression procedures.

**Results** Rusitec fluid as a source of microorganisms resulted in significantly lower ( $P < 0.01$ ) DM digestibility values than sheep rumen liquor for all hays, with differences ranging from 52 to 162 g/kg. However, *in vitro* DMD (g/kg) using sheep rumen liquor ( $y$ ) was highly ( $R^2 = 0.858$ ;  $P < 0.001$ ) related to digestibility using Rusitec fluid ( $x$ ) by the following linear equation:  $y = 127 + 0.954$  (s.e. 0.1229)  $x$ ; RSD = 32.5. There were significant relationships ( $P < 0.001$ ) between the *in vivo* DMD of hays and the *in vitro* DMD determined with both types of inoculum (Figure 1). Judged by the size of the RSD (20.6 and 21.9 for rumen liquor and Rusitec fluid, respectively) and the coefficient of determination (0.899 and 0.896), the precision of the estimates of *in vivo* DMD were similar for both types of inoculum.

**Figure 1** Relationship between the *in vivo* DM digestibility (DMD; g/kg) of twelve hays and their *in vitro* DM digestibility (IVDMD; g/kg) determined using rumen liquor (●) or Rusitec fluid (○) as inoculum. Regression equations were:  $DMD = 83.2 + 0.713$  (s.e. 0.0756)  $IVDMD_{Rumen\ liquor}$  ( $R^2 = 0.899$ ;  $P < 0.001$ ; RSD = 20.6) and  $DMD = 144 + 0.729$  (s.e. 0.0829)  $IVDMD_{Rusitec\ fluid}$  ( $R^2 = 0.886$ ;  $P < 0.001$ ; RSD = 21.9).



**Conclusions** In spite of the scarce number of samples, the results of this study suggest that vessel fluid from Rusitec could be used as a source of microorganisms for determining the *in vitro* digestibility of hays. Use of Rusitec would obviate the need to use fistulated animals, as rumen liquor to commence Rusitec could be obtained at abattoirs.

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## References

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