

Estimation of Effective Protein Degradability of Fresh Forage *in situ*: Correction for Microbial Contamination using ¹⁵N-Labelled Plant Material

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It is difficult to make field measurements of the effective degradability (ED) of fresh forage ingested by grazing ruminants. The *in situ* or nylon bag technique is often used for estimating ruminal protein digestibility of forage samples, but microbial attachment to feed residues in the bags causes true degradability coefficients to be underestimated (Nocek and Grant 1987). In addition, the forage samples are often dried or frozen before being placed in the bags and this may affect microbial attachment. Internal and external microbial markers have been used to correct for microbial 'contamination' (Nocek and Grant 1987). In this study, we have used an alternative approach, i.e. labelling the plant material (perennial ryegrass, *Lolium perene* L.) with ¹⁵N to determine the extent of microbial ¹⁴N contamination *in situ* and, from this, the errors in predicting ED.

Perennial ryegrass was grown in 4-L pots in a glasshouse and watered with ¹⁵NH₄¹⁵NO₃ solution twice weekly to produce labelled forage that was placed in porous nylon bags in 3 rumen cannulated sheep (~3 y old wethers, 45.3±1.73 kg). The sheep were housed in individual metabolism crates in a controlled environment under continuous lighting, given free access to water, and offered chopped lucerne hay (600g, 92% DM, 19% CP) and oaten chaff (400 g, 91.5% DM, 7.5% CP) in a daily meal at about 0900h. Eighteen nylon bags were filled with about 5g of freshly cut, chopped ryegrass. Five bags were placed in the rumen of each sheep; the 3 other bags were 'zero time' samples. Bags in the rumen were removed progressively after 3, 7, 12, 21 and 33 h of incubation and washed according to the method of Nandra *et al* (2000). Loss of N from the bags over time was fitted using the model of Ørskov and McDonald (1979) and ED was calculated from $a+(bc/(c+k))$ where a, b and c are the model parameters (see Figure 1) and k is the rumen outflow rate (/h).

We found that residues from the washed zero time bags had lower ¹⁵N enrichments (7.7% enriched) than the original fresh samples (8.3% enriched). Accordingly, the microbial N fraction present in the residues was calculated from the enrichment ratio, i.e. N in residues after incubation: N in washed residues at time zero (rather than using 'N enrichment in the fresh sample' as the denominator). Microbial N was assumed to be unlabelled.

In Figure 1, (A) shows both apparent and corrected proportional N disappearance when the fresh ryegrass was incubated *in situ* in sheep, and (B) shows the predicted ED of forage N (crude protein) before and after correction for microbial contamination, for various outflow rates (and periods of digestion in the rumen).

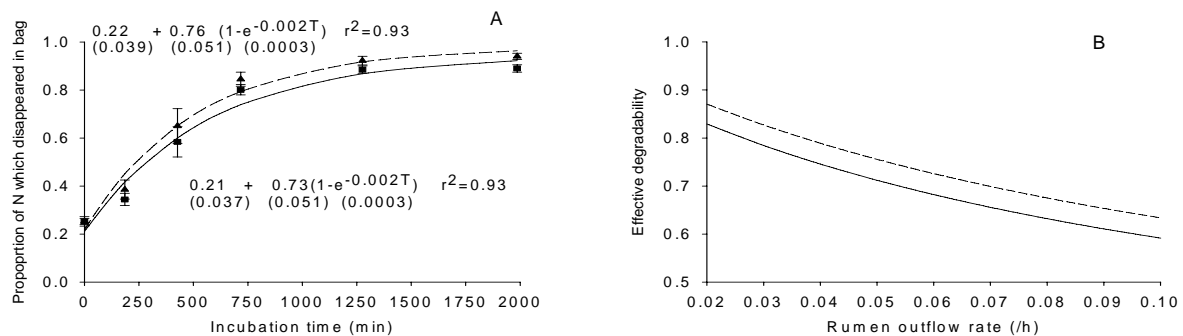


Figure 1. (A) Mean (± se) fresh ryegrass N disappearance (apparent ■, — fitted; corrected ▲, ---- fitted) from nylon bags in sheep and (B) effective degradability estimates at increasing rumen outflow rates

Under-estimation of ED of protein in fresh forages by about 4% would have potential consequences for predictions of ruminally fermentable and 'escape' protein and thus for dietary protein feeding management. However, because the correction assumes contaminating microbial N is unlabelled, but microbes attached to labelled ryegrass would become labelled to some extent, the true error (and ED) may still be underestimated. Studies with two markers would help us to better understand the errors associated with the *in situ* technique.

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