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PREDICTION OF CORRECTED IN SITU FORAGE PROTEIN DEGRADABILITY BY THE CORNELL METHOD

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ABSTRACT: *An experiment was conducted on eight fibrous feeds to compare the Cornell rumen degradable protein values with those of the in situ method that have been corrected for microbial contamination. Samples of hay, sugarbeet pulp, dried lucerne, maize silage, peahaulm silage, fermented whole crop wheat and two different grass silages were used for the Cornell method. A corresponding in situ experiment was carried out on the same samples to estimate their rumen degradable protein values. Regression was used to relate the Cornell rumen degradable protein to that of the in situ technique. Rumen degradable protein estimates observed using the Cornell method were, on average, 0.06 and 0.16 lower than their corresponding in situ uncorrected and corrected values, respectively, with the latter being statistically significant (P<0.01). However, regression analysis between the Cornell and the in situ uncorrected rumen degradable protein, using all eight feeds, was statistically significant (r^2 0.59; P<0.05). The relationship did not improve when the Cornell values were compared with the in situ corrected values for the eight feeds (r^2 0.55; P<0.05). On the basis of inadequate preparation of the peahaulm silage sample for the in situ experiment, it was removed from the data set and the ensuing equation accounted for 0.89 of the variability in the in situ uncorrected rumen degradable protein (P<0.01). A better agreement was observed between the Cornell and the in situ corrected rumen degradable protein (r^2 0.95; P<0.001). The Cornell method therefore significantly correlated with the in situ technique for fibrous feeds. Correlation between the methods could improve if microbial contamination was removed from the analysis. The in situ rumen degradable protein values appeared to be bigger than the associated Cornell values. The Cornell adopted rates of degradation therefore need to be evaluated.*

ORIGINAL ARTICLE

Key words: *Cornell, In situ, Protein, Forages, Degradability, Feeds*

INTRODUCTION

Experiments and data have been analyzed to evaluate the Cornell model against the in situ system for degradability (Shannak et al., 2000; Gosselink et al., 2004). For concentrates and by-products, estimates of protein degradability obtained using the Cornell model correlated well with the corresponding in situ estimates (Shannak et al., 2000). A lack of agreement between forages was observed and might be due to the narrow range of their degradability values and to the fact that nominally similar, rather than the same forages, were compared (Avornyo, 1999). In addition, the in situ values for forages may be affected by microbial contamination of bag residues (Shannak et al., 2000), which significantly reduces the apparent degradability (Alexandrov, 1997; Rodriguez and Gonzalez, 2006; Milis et al., 2007). Further studies may be needed to ascertain if the Cornell and the in situ methods disagree on their forage protein degradability estimates.

Experiments were conducted on eight fibrous feeds to compare the Cornell rumen degradable protein (RDP) values with the analogous in situ estimates that have been corrected for microbial contamination.

MATERIALS AND METHODS

The Cornell method

Samples of hay, sugarbeet pulp (SBP), dried lucerne (DL), maize silage (MS), peahaulm silage (PHS), fermented whole crop wheat (FWCW) and two samples of grass silage (Cambridge University Dairy Unit (DUGS), and a private farm (PFGS)) were weighed on dry matter basis. Wet samples were therefore oven-dried at 55^{0}C to steady state, and all samples milled through 1 mm dry sieve were used for the Cornell in vitro technique (Licitra et al., 1996).

The degradation rates applied to the fibrous feeds were those of the Cornell databank. A common rate of passage of feed of 0.05%/h was adopted for the study.

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The in situ method

A corresponding in situ experiment was carried out on hay, SBP, DL, MS, FWCW, PHS, DUGS and PFGS to estimate their protein degradability values.

The method provided by the AFRC Technical Committee (1992) for estimating protein loss from in situ bags was followed. It involved the use of a monofilamentous polyester cloth of 40-50 μ pore size. Dried lucerne pellets were crushed to achieve 4 mm size and sieved across 45 μm sieve. The rest of the samples were chopped to about 1 cm length. The few peas in PHS were therefore not crushed. Each bag contained approximately 5 g dry matter of sample.

Ruminal infusion of ^{35}S

A stock solution of 20 μCi/ml (^{35}S) containing 100 μg/ml anhydrous Na_2SO_4 was made. Some of the stock solution was further diluted with water in a large bottle to supply 190 μCi/sheep/day. Dosing of sheep with ^{35}S was enabled at 480 ml per sheep per day through tubes with the help of a peristaltic pump. Microbial protein formed in the rumen of sheep was marked with ^{35}S by continuous intra-ruminal infusion of $Na_2{}^{35}SO_4$ for a total of 28 days.

Preparation of solid associated microbes

The method of Whitehouse et al. (1994) was used to isolate solid associated microbes (SAM) for subsequent computation of the proportion of microbial non-ammonia nitrogen (NAN) in bag residual NAN.

Calculations and statistical analyses of data

The estimated in situ RDP, uncorrected and corrected using ^{35}S, were obtained for each feed. Regression was used to relate the Cornell RDP values of the eight feeds to the in situ RDP of the same feeds.

RESULTS

Chemical composition of experimental feeds

Table 1 shows the chemical composition of the experimental feeds.

Table 1 - Chemical composition[a] of the fibrous feeds[b]

Feed	DM, fr. of AR	OM, fr. of DM	CP, fr. of DM	NDF, fr. of DM	ADF, fr. of DM	ADIL, fr. of DM
Hay	0.88	0.91	0.21	0.62	0.24	-
SBP	0.85	0.90	0.12	0.40	0.12	-
DL	0.90	0.90	0.19	0.49	0.37	0.09
MS	0.40	0.95	0.09	0.35	0.18	0.02
DUGS	0.41	0.91	0.20	0.54	0.32	0.02
PFGS	0.30	0.90	0.18	0.54	0.33	0.03
PHS	0.56	0.93	0.17	0.51	0.38	0.07
FWCW	0.53	0.95	0.12	0.33	0.20	0.03

[a]DM, dry matter; fr., fraction; AR, as received; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADIL, acid detergent insoluble lignin; -, not determined. [b]SBP, sugarbeet pulp; DL, dried lucerne; MS, maize silage; DUGS, dairy unit grass silage; PFGS, private farm grass silage; PHS, peahaulm silage; FWCW, fermented whole crop wheat.

Cornell protein fractions and degradability

The fractions shown in Table 2 were determined from the Cornell method of protein partitioning. Fraction A was higher in the fermented feeds. Conversely, the dry feeds contained greater amounts of fractions B2 and B3. All feeds had lower than 0.1 of CP in the form of fraction C.

Table 2 - Cornell chemical protein fractions[a]

Feed[b]	A	B1	B2	B3	C
Hay	0.25	0.03	0.27	0.43	0.03
Sugarbeet pulp	0.44	0.03	0.12	0.36	0.06
Dried Lucerne	0.32	0.04	0.42	0.16	0.07
Maize silage	0.52	0.05	0.33	0.05	0.05
DUGS	0.59	0.02	0.16	0.18	0.05
PFGS	0.67	0.03	0.19	0.07	0.04
Peahaulm silage	0.33	0.08	0.37	0.16	0.06
FWCW	0.68	0.06	0.16	0.04	0.06

[a]A, soluble non-protein nitrogen; B1, quickly degradable true protein; B2, true protein of intermediate rate of degradability; B3, slowly degradable true protein; C, Undegradable protein. [b]SBP, sugarbeet pulp; DL, dried lucerne; MS, maize silage; DUGS, dairy unit grass silage; PFGS, private farm grass silage; PHS, peahaulm silage; FWCW, fermented whole crop wheat.

Cornell versus in situ method of protein degradability

The Cornell RDP ranged from 0.47 in hay to 0.86 of total crude protein in PFGS. Figures for the fermented feeds were high; 0.73, 0.76, 0.80, 0.85 and 0.86 for PHS, DUGS, MS, FWCW and PFGS, respectively. Sugarbeet pulp and DL degraded by 0.57 and 0.65, respectively. In situ RDP also varied, ranging from 0.62 in hay to 0.85 in

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PFGS for the uncorrected values. The fermented feeds, excluding PHS, recorded bigger RDP compared to the dry feeds (Table 3). Regarding RDP corrected for microbial contamination, the values varied from 0.72 in PHS to 0.94 of total protein in PFGS.

Table 3: Estimations of Cornell, in situ uncorrected and ^{35}S corrected protein effective degradabilities[a]

Feed[b]	Cornell RDP	Uncorrected in situ RDP	^{35}S corrected in situ RDP
Hay	0.47	0.62	0.75
Sugarbeet pulp	0.57	0.73	0.81
Dried Lucerne	0.65	0.74	0.81
Maize silage	0.80	0.77	0.93
DUGS	0.76	0.81	0.90
PFGS	0.86	0.85	0.93
Peahaulm silage	0.73	0.63	0.72
FWCW	0.85	0.84	0.93

[a]RDP, rumen degradable protein. [b]SBP, sugarbeet pulp; DL, dried lucerne; MS, maize silage; DUGS, dairy unit grass silage; PFGS, private farm grass silage; PHS, peahaulm silage; FWCW, fermented whole crop wheat.

Estimates observed using the Cornell method were, on average, 0.06 and 0.16 lower than their corresponding in situ uncorrected and corrected values, respectively, with the latter being statistically significant (P<0.01). However, regression analysis between the Cornell and the in situ uncorrected RDP, using all eight feeds, indicated statistical significance (Figure 1). The relationship did not improve when the Cornell was compared with the in situ corrected RDP for the eight feeds (Figure 2). There were a few whole peas in the peahaulm silage sample (chopped to approximately 1 cm length) for the in situ incubations, and none of them was degraded even after 72 hours of incubation. In contrast, the peahaulm silage samples and the peas in them were all ground through 1 mm sieve for the Cornell tests. Vanzant et al. (1998) have reiterated the importance of the influence of sample preparation on the in situ method. Mehrez and Orskov (1977) noted during their in situ incubations of barley that the barley samples, contained in in situ bags, which had more whole grains gave a lower DM degradability compared to those samples that contained fewer grains. On the basis of a few but variable amounts of intact peas in the in situ residues, the peahaulm silage was removed from the data set and the ensuing equation accounted for 0.89 of the variability in the in situ uncorrected RDP (Figure 3). In Figure 4, a better agreement is observed between the Cornell and the in situ corrected RDP for the feeds excluding PHS.

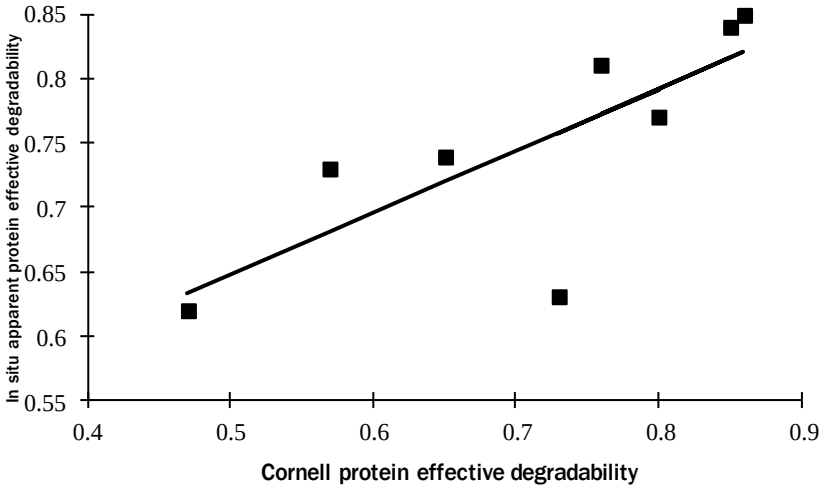

Figure 1 - The relationship between the in situ apparent and the Cornell protein degradabilities of eight fibrous feeds.
[in situ = 0.49 (s.e.=0.17)* × Cornell + 0.40 (s.e.=0.12)*; P<0.05; r^2 = 0.59; MSE = 0.06; n = 8; s.e., standard error of estimate; *, significant at P<0.05; MSE, mean square error.]

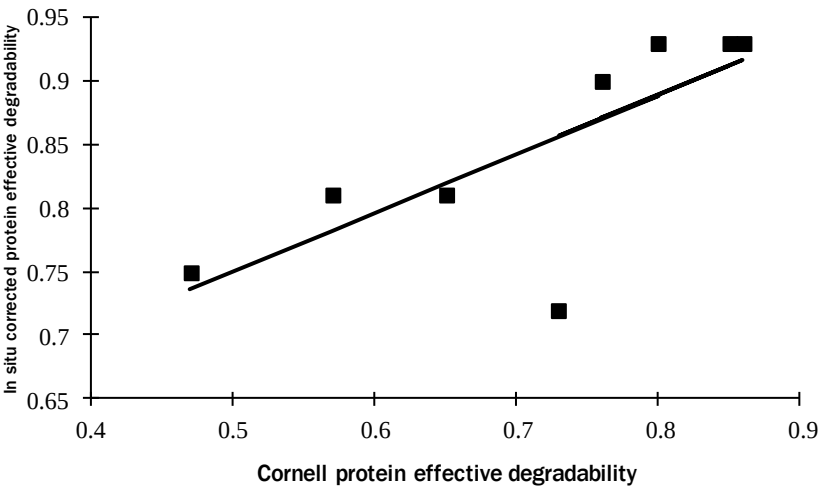

Figure 2 - Prediction of the in situ corrected protein effective degradability, with ^{35}S, by the Cornell protein effective degradability using eight fibrous feeds.
[in situ = 0.47(s.e.=0.17)* × Cornell + 0.51(s.e.=0.12)**; P<0.05; r^2 = 0.55; MSE = 0.06; n = 8; s.e., standard error of estimate; *, significant at P<0.05; **, significant at P<0.01; MSE, mean square error]

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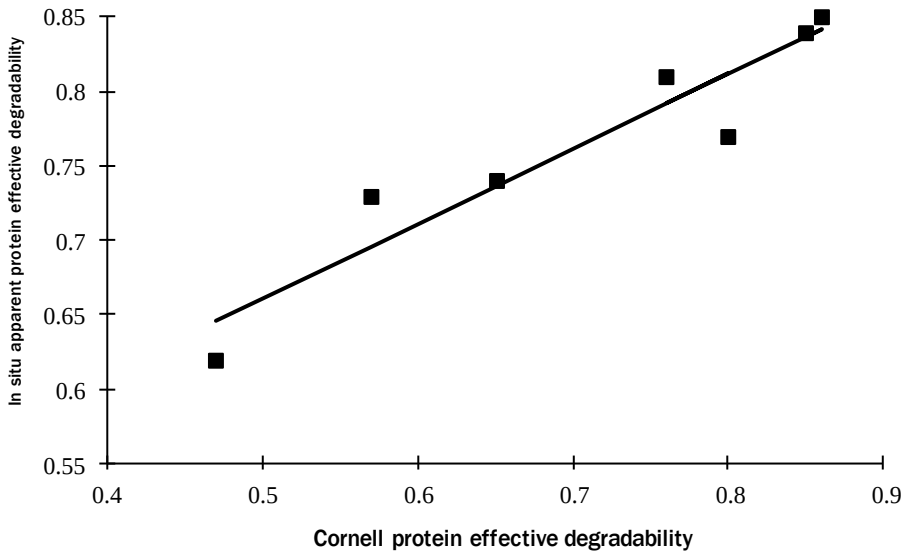

Figure 3 - The relationship between the in situ apparent and the Cornell protein degradabilities of the fibrous feeds excluding peahaulm silage.

[in situ = 0.51(s.e.=0.08)** × Cornell + 0.41(s.e.=0.06)***; P<0.01; r^2 = 0.89; MSE = 0.03; n = 7; s.e., standard error of estimate; **, significant at P<0.01; ***, significant at P<0.001; MSE, mean square error]

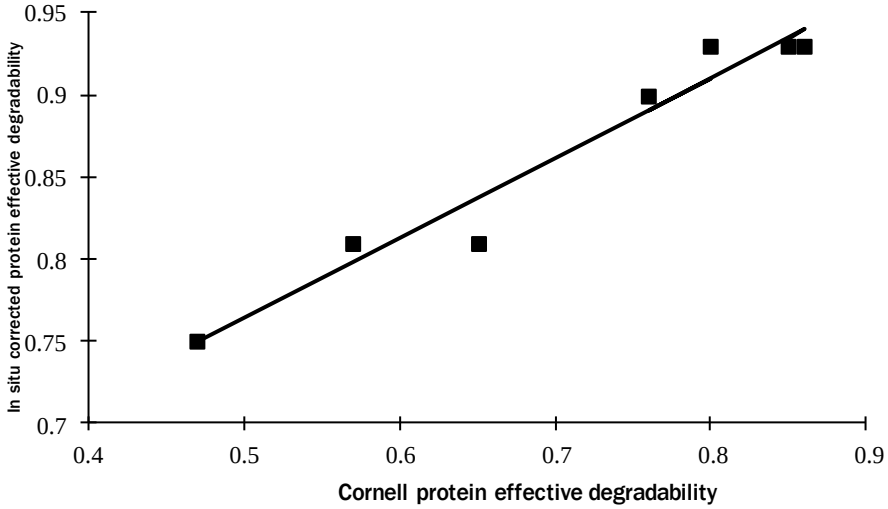

Figure 4 - Prediction of the in situ corrected, with ^{35}S, by the Cornell protein effective degradability of seven fibrous feeds, excluding peahaulm silage.

[in situ = 0.49(s.e.=0.05)*** × Cornell + 0.52(s.e.=0.03)**; P<0.001; r^2 = 0.95; MSE = 0.02; n = 7; s.e., standard error of estimate; **, significant at P<0.01; ***, significant at P<0.001; MSE, mean square error]

DISCUSSION

Cornell protein fractions and degradability

Ensiling of forage allows fermentation bacteria to degrade insoluble protein to non-protein nitrogen (NPN). In the experiment, the fermented forages contained a high amount of soluble protein (SP), much of which was NPN. Pichard and Van Soest (1977) also found that SP in silages and cut forages existed essentially as NPN. Fermentation of protein pools during ensiling would elevate A and C fractions while reducing true protein B. Therefore in damaged silage, nitrogen (N) would be present mainly as NPN and unavailable N. Since lowering of B fraction would be associated with damaged silages, DUGS appeared to be of good quality because of its higher true protein content, compared to the PFGS.

The observation that MS, PFGS and FWCW (Table 2) had a low amount of fraction B3 contrasts with the claim that forages contain significant amounts of pool B3 (Krishnamoorthy et al., 1982). Three reasons may account for this observation:

- variations in neutral detergent insoluble protein (NDIP) occur depending on the method of neutral detergent fibre (NDF) followed. There are about 14 published variations of NDF procedures (Mascarenhas-Ferreira et al., 1983; Van Soest et al., 1991),
- sometimes, neutral detergent solution degrades components that are precipitable in acid detergent solution and vice versa (Krishnamoorthy et al., 1982),
- the feeds mentioned have suffered substantial heat damage or are of poorer quality. However, the NDIP assay revealed very little indigestible N in the feeds analyzed.

Cornell versus in situ estimates

Soluble N estimated by the in situ method tended to be higher than the corresponding Cornell SP value (P<0.01). Lindberg and Varvikko (1982) observed that regardless of bag pore size, the quantity of feed residue left in the bag was about equal if incubation was long. It implied that undegraded feed particles flowing through the

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pores were potentially degradable. In addition to escape of fine particles, instantaneous solubility of some fraction B2 protein in in situ bags could be responsible for the higher in situ values. Pichard and Van Soest (1977) reported a rapidly degraded true protein with a half-life of 10 min, but which was not pool B1.

Peahaulm silage in situ RDP was comparatively low. Preparation of PHS sample for in situ incubation involved chopping only the peahaulm to 1 cm length and leaving the peas whole. Hydrolysable nutrients were confined as rumen actions failed to render the peas degradable. From the figures, it can be deduced that disparity between Cornell and in situ results could be due to major differences in the preparation of sample material (Trujillo et al., 2010). In their paper, Mehrez and Orskov (1977) reported that bags which contained more whole grains of barley yielded a lower DM loss, and vice versa.

Protein degradability in situ tended to be higher than the analogous Cornell value for the same feed. Undegraded fine particle loss from in situ bags has been associated with observed higher in situ values (Ghoorchi and Arbabi, 2010). Alternatively, lower degradation rates applied to Cornell pools would exaggerate the difference (Lanzas et al., 2008).

An agreement was affirmed between the Cornell and the in situ for forage protein degradability (Shannak et al., 2000). It was however noticed that if an abnormal value was included in the regression data (Shannak et al., 2000; Trujillo et al., 2010), in this case in situ PHS effective degradability value, correlation coefficient appreciably decreased. Correction for microbial contamination by the ^{35}S method would further improve the relationship between the Cornell and the in situ estimations.

CONCLUSION

The Cornell method significantly correlated with the in situ technique for fibrous feeds. Correlation between the methods could improve if microbial contamination was removed from the analysis. Method of preparation of feed for incubation would affect the correlation coefficient. The in situ protein effective degradability appeared to be bigger than the associated Cornell values. The Cornell adopted rates of degradation therefore need to be evaluated.

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