Near Infrared Spectroscopy to Estimate Microbial Dry Matter and Nitrogen Contamination of In Situ Forage Residues

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Introduction

The in situ method is used widely to determine ruminal degradability of feed CP and DM. It is an attractive alternative to in vivo methods because it is simple, inexpensive, and rapid. However, several factors influence in situ results, the most important of which is microbial contamination of feed residues after incubation in the rumen. Degradation of forage CP and DM is systematically underestimated because of microbial contamination. Markers have been used to quantify microbial contamination of in situ residues. Microbial DM contamination ranged from 8 - 13% for forages and 8 - 25% for concentrates. Crude protein contamination of in situ residues was greater for forages than for concentrates. Microbial CP contamination of in situ forage residues was reported to be 15 - 50% when ^{35}S was used as the marker and 46 - 95% when diaminopimelic acid was used as the marker. Use of microbial markers to measure microbial contamination is time consuming and expensive. It may be possible to quantify microbial DM and N contamination in in situ forage residues using near infrared spectroscopy (NIRS). Our objectives were to: 1) determine the extent of microbial N and DM contamination of in situ residues of ruminally incubated ^{15}N labeled alfalfa hay, and 2) evaluate the use of NIRS to quantify microbial contamination of in situ forage residues.

Materials and Methods

Alfalfa was labeled with the stable isotope ^{15}N by growing plants on soil fertilized with $(^{15}NH_4)_2SO_4$. Plants were harvested at late bud stage, air-dried to prepare hay, and ground through a 2-mm screen. Enrichment of ^{15}N in alfalfa hay and in situ residues was determined by isotope-ratio mass spectrometry. Average ^{15}N enrichment in the alfalfa hay used in the study was .992 ± .01 (SD) atom % excess. In situ trials were conducted in a ruminally cannulated Holstein cow fed a total mixed diet containing forage from alfalfa silage and corn silage. About 7.5 g of alfalfa hay DM was weighed into 10 cm x 20 cm dacron bags with 40 µm pore sizes and incubated in the rumen for 0, 6, 12, 24, and 48 h. All bags were soaked in tap water to remove soluble or filterable material; 0-h bags were immersed in water only. After removal from the rumen, bags were rinsed, washed, then dried. Microbial N (RMN) contamination (% of total N) was calculated from the ^{15}N enrichment in the residue, relative to the enrichment of original feed:

$$\text{RMN, \%} = [1 - (^{15}\text{N in residual N} / ^{15}\text{N in original N})] \times 100$$

Contaminating microbial DM was calculated from RMN assuming 7.6% N in microbial DM. The quantity of microbial DM or CP contaminating in situ residues was subtracted from total DM or CP to obtain corrected degradabilities. An iterative least squares procedure was used to fit degradability data to the equations of Ørskov and McDonald (1979). An NIR System 4250 scanning monochromator was used for the NIRS analysis on the 96 samples of dried in situ residues; reflectance spectra were recorded over the 1618 to 2320 nm region and data were transformed and analyzed. Cross validation was used to estimate validation errors; validation errors were combined into a standard error of cross validation (SECV). The factors considered in the final selection of a calibration equation included: 1) high R^2, low SECV and low bias in the validation set; and 2) high r^2 and low standard error of calibration (SEC).

Results and Discussion

Degradation of alfalfa was very rapid and most of the DM and CP disappeared during the first 6 to 12 h of ruminal incubation (Table 1). At 48 h, apparent DM and CP recoveries were 20.1 and 43.8%, respectively. Higher apparent recoveries of DM and CP, compared to recoveries corrected for microbial contamination, reflected the considerable, increasing microbial attachment and growth on the in situ residues over the incubation. The data for uncorrected residual CP could not be fit to the degradation model; however, after correcting for microbial N contamination, residual CP data fit the model well

(Table 1). Apparent CP recovery was greater than corrected CP recovery ($P < .001$) and depended on the length of incubation (Table 1). Microbial N contamination of alfalfa residues increased continuously during the incubation, ranging from about 23% (6 h) to nearly 50% (48 h) of residual N (Fig. 1). The proportion of residual CP contamination was much higher than that observed for DM. This is probably due to the greater CP content of attached microbes relative to forage residues. Olubobokun and Craig (1990) reported that the CP content of firmly attached microbes was 45% of DM and undigested forage residues contained only 14.1% CP. Apparent and corrected CP degradations were 48.2 and 63.6%, respectively, ($P < .001$); apparent and corrected DM digestiblities were 70.9 and 72.7% and were not different ($P > .05$).

The range of residual microbial N, CP and DM recoveries, residual N and ^{15}N enrichment of the samples used for NIRS calibration, are in Table 2. Results from statistical analysis of NIRS calibrations and predictions of the degradation parameters are in Table 3. The results indicated that prediction of microbial N contamination using NIRS was successful: coefficient of determination (r^2) for calibration = .957, SEC = 2.34% and SECV = 2.39%. The coefficient of determination (r^2) was greater than .5 for each of the nine wavelengths used in the calibration. The NIRS data explained a large proportion of the variation in the degradation of CP and DM in alfalfa hay. The coefficient of determination (r^2) was higher than .97 (Table 3). The SEC and SECV of NIRS estimation were of similar magnitude to the SD of replicates for chemical analysis and ranged from .22 to 4.98% for CP recovery and from .11 to 3.03% for DM recovery. Highest correlations between corrected DM recovery and spectral data were found at approximately the same wavelengths as for corrected CP recovery. There was a strong correlation between DM recovery and CP recovery ($r^2 = .91$); therefore, the same spectral information was used for predicting both.

Conclusions

Microbial contamination of in situ forage residues leads to systematic underestimation of effective CP and DM degradabilities. Because it can be calibrated for rapid and accurate chemical analyses, NIRS represents an attractive alternative for estimating microbial contamination. These data showed that, following calibration using ^{15}N-labelled alfalfa, NIRS was an accurate and rapid method for determining microbial contamination of in situ forage residues. These results indicate that NIRS analysis can be used to correct in situ estimates of microbial DM and CP degradation.

References

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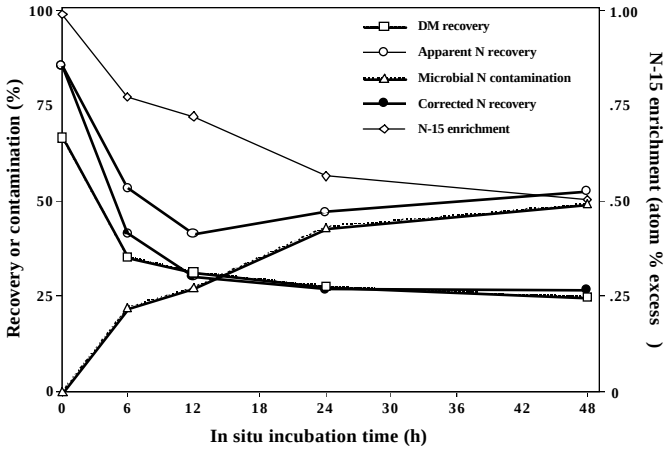

Figure 1. Apparent DM recovery, apparent and corrected N recovery, and microbial N contamination and N-15 enrichment of residues from alfalfa hay incubated in the rumen in situ.

Table 1. Apparent and corrected recovery of DM and CP in in situ residues of alfalfa hay and degradation parameters estimated using the model of Ørskov and McDonald (1979).

Incubation time, h	Dry matter		Crude protein	
	Apparent	Corrected[1]	Apparent	Corrected[1]
0	61.7 ± 3.2[a]	61.7 ± 3.2[a]	82.1 ± 7.4[a]	82.1 ± 7.4[a]
6	35.0 ± 4.9[b]	33.9 ± 2.1[b]	45.0 ± 6.1[b]	37.0 ± 4.1[b]
12	27.1 ± 7.7[c]	26.1 ± 2.4[c]	36.2 ± 3.6[c]	27.2 ± 2.2[c]
24	22.1 ± 3.4[d]	20.7 ± 2.8[d]	41.0 ± 4.3[d]	25.8 ± 1.3[d]
48	20.1 ± 4.2[e]	18.6 ± 3.7[e]	43.8 ± 5.4[bd]	24.5 ± 1.6[e]
Parameters[2]				
P, %	70.9	72.7	48.2	63.6
a, %	38.3	38.3	17.9	17.9
b, %	41.4	42.8	43.0	57.0
c, /h	.109	.117	NF[3]	.280
Lag, h	3.7	2.9	NF[3]	.5

[a,b,c,d,e]Mean recoveries (±SD) in the same column with different superscripts differ ($P < .05$).

[1]Differences between apparent and corrected data were significant for CP ($P < .001$) at all four incubation times, but not significant for DM ($P > .05$) at any incubation time.

[2]P = Effective degradability; a = rapidly soluble fraction; b = slowly degradable fraction; c = fractional rate at which b is degraded.

[3]Data did not fit the degradation model.

Table 2. Mean, range (minimum = MIN, maximum = MAX) and standard deviation (SD) of degradation parameters from alfalfa hay incubated in situ in the rumen and used in analysis of near infrared reflectance spectroscopy to determine microbial DM and N contamination.

Parameter	n	Mean	MIN	MAX	SD
Residual microbial N, % of total N	78	29.9	14.1	49.6	10.8
Apparent DM recovery, %	95	31.6	12.8	62.2	13.5
Corrected DM recovery, %	95	30.8	12.1	62.2	14.2
Apparent CP recovery, %	95	47.9	31.8	94.3	16.0
Corrected CP recovery, %	95	37.3	22.7	94.3	20.9
^{15}N enrichment, atom % excess	95	.75	.501	1.01	.15
Apparent residual N, % of total N	95	1.38	.89	2.65	.46
Corrected residual N, % of total N	95	1.07	.63	2.65	.59

Table 3. Results of near infrared reflectance spectroscopic determination of degradation parameters from alfalfa hay incubated in the rumen in situ.[1]

Parameter	Calibration		Validation		
	r^2	SEC	R^2	SECV	n
Residual microbial N, % of total N	.957	2.34	.951	2.39	6
Apparent DM recovery, %	.986	1.61	.984	1.70	4
Corrected DM recovery, %	.990	1.43	.988	1.54	4
Apparent CP recovery, %	.957	3.34	.950	3.55	4
Corrected CP recovery, %	.977	3.15	.973	3.40	6
^{15}N enrichment, atom % excess	.975	.023	.968	.026	7
Apparent residual N, % total N	.974	.074	.967	.084	6
Corrected residual N, % total N	.979	.086	.972	.097	6

[1] r^2 = Coefficient of determination; R^2 = coefficient of determination for cross validation; SEC = standard error of calibration; SECV = standard error of cross validation; n = number of outliers (samples with cross validation residuals > 2.5).