

Prediction of Laboratory and In Situ Protein Fractions in Legume and Grass Silages Using Near-Infrared Reflectance Spectroscopy¹

P. C. HOFFMAN,^{*,2} N. M. BREHM,^{*} L. M. BAUMAN,[†]
J. B. PETERS,[†] and D. J. UNDERSANDER[‡]

^{*}Department of Dairy Science,

[†]Department of Soil Science, and

[‡]Department of Agronomy, University of Wisconsin, Madison 53706

ABSTRACT

Legume and grass silage samples (n = 121) were collected from commercial forage testing laboratories (trial 1). Samples were dried at 55°C for 48 h, ground, scanned on a near-infrared reflectance spectrophotometer, and analyzed for crude protein (CP), soluble CP, acid detergent fiber (ADF) CP, and neutral detergent fiber (NDF) CP by wet chemistry methods. Sixty samples were selected for calibration development, and the remaining samples were used for equation validation. Near-infrared reflectance spectroscopy accurately predicted the CP content of the silages ($R^2 = 0.96$), but prediction of soluble CP, ADF CP, and NDF CP was markedly less accurate. The coefficients of determination and standard errors of calibration for CP, ADF CP, NDF CP (percentage of DM), and soluble CP (percentage of CP) were as follows (0.96 and 0.80, 0.77 and 0.24, 0.72 and 0.71, and 0.82 and 4.40). In a second study, legume and grass silage samples (n = 32) were dried at 55°C and ground (2 mm). Duplicate dacron bags containing 5 g of silage were incubated in the ventral rumen of three ruminally cannulated cows for 0, 3, 6, 12, 24, 48, and 72 h. In situ protein fractions, including rapidly degraded protein, slowly degraded protein, undegradable protein, degradation rate, and rumen-undegradable protein, were determined. Original samples were reground (1 mm) and scanned. Previously defined near-infrared spectroscopy calibration procedures were conducted. Coefficients of determination for in situ CP fractions were $R^2 > 0.92$ with the exception of degradation rate ($R^2 = 0.87$). Data suggest that in situ protein fractions are better predicted by near-infrared reflectance spectroscopy than by laboratory protein fractions.

(**Key words:** silage, near-infrared, in situ, protein degradation)

Abbreviation key: CNCPS = Cornell Net Carbohydrate and Protein System, CPR = CP remaining, k_d = degradation rate, NIRS = near-infrared reflectance spectroscopy, SEC = standard error of calibration, SEP = standard error of performance.

INTRODUCTION

Dairy cattle rations require (19) specific amounts of RDP and RUP. Researchers (8, 12) have proposed enhancements in ruminant protein nutrition by further subdivision of protein fractions. The Cornell Net Carbohydrate and Protein System (CNCPS) (8, 12) partitions protein fractions among NPN, soluble true protein, rapidly degradable true protein, slowly degradable true protein, and undegradable protein (C). The protein fractionization scheme as proposed by the CNCPS (8, 12) has been adopted into the 1997 Nutrient Requirements of Beef Cattle (20), which also requires knowledge of the degradation rates (k_d) of each protein fraction. Changes in ruminant protein nutrition offer great potential in understanding or predicting animal performance, but they create extreme challenges in routine feedstuff analysis. Protein fractions defined by the CNCPS and the 1997 Nutrient Requirements of Beef Cattle (20) are determined by measuring total CP and the CP content of ADF and NDF. In addition, a borate phosphate buffer solution (8) and a tungstic acid solution (8) are used to determine soluble CP and soluble true protein fractions. To fractionate protein in accordance with the CNCPS (8), five preparatory lab procedures (CP, ADF, NDF, borate buffer soluble protein filtration, and tungstic acid insoluble protein filtration) are required followed by five Kjeldahl (3) procedures. As a result, determination of protein fractions defined by the CNCPS are arduous and expensive. To increase efficiency and decrease expense, many commercial forage testing laboratories (M. Van Amburgh, 1997,

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²To whom correspondence should be addressed.

personal communication) have developed near infrared reflectance spectroscopy (NIRS) equations for CNCPS protein fractions. There is, however, a paucity of published research information that describes the accuracy of NIRS prediction equations for CNCPS protein fractions. Further, there are no commercial tests available to predict the k_d of CNCPS protein fractions. One resolution to these problems may be use of NIRS to predict directly the protein fractions and k_d from in situ studies employing full kinetics protocols. Tremblay et al. (28) was successful in predicting ($R^2 = 0.90$; $SE = 2.41 \text{ g}/10^{-1} \text{ kg}$ of CP) the RUP content of roasted soybeans using NIRS. Halgeron et al. (10) accurately predicted ($R^2 = 0.95$; $SE = 1.01 \text{ g}/10^{-1} \text{ kg}$ of CP) the CP remaining (CPR) in alfalfa after 24 h of ruminal incubation but failed to predict accurately the CPR in perennial grass samples using NIRS. Those studies (10, 28) used the inhibitor in vitro system of Broderick (5) or a single 24-h ruminal incubation to determine RUP or CPR; therefore, prediction of soluble protein, slowly degraded protein (B), C, and k_d of the B fraction by NIRS was not possible. The following studies were implemented to explore these problematic areas in protein fraction determination. Studies were conducted to explore the accuracy of NIRS in predicting protein fractions defined by the CNCPS and to explore the potential of NIRS to predict protein fractions and degradation rates common to in situ protocols. Legume and grass silages were chosen as the evaluatory feedstuffs because, in the upper Midwest of the United States, they commonly supply and can be the largest variant of CP fractions in the diet of dairy cows.

MATERIALS AND METHODS

Trial 1

Legume and grass silage samples ($n = 128$) were collected between September 1 and December 31, 1996 from commercial forage testing laboratories in Wisconsin. Samples were dried at 55°C for 48 h and then milled through a Udy mill (Udy Corp., Boulder, CO) fit with a 1-mm screen. Final DM determination was conducted by AOAC procedures (3). Samples were evaluated for CP (3) and soluble CP in a borate phosphate buffer (13). The soluble true protein fraction as determined by tungstic acid insolubility (8) was not determined because previous research had demonstrated a negligible amount in legume and grass silages (6). The ADF CP and NDF CP content of the silages was ascertained by a preliminary nonsequential determination of ADF or NDF using the

procedures of Goering and Van Soest (9) with modifications by Mertens (16).

Because the procedures of Mertens (16) recommend the use of sodium sulfite to remove CP from the NDF fraction, no sodium sulfite was added, thereby maintaining CP in the NDF fraction. Final determination of the CP content of ADF and NDF was determined by Kjeldahl procedures (3).

Samples were packed into a cylindrical sample holder equipped with a quartz window and scanned according to the procedures of Marten et al. (15) on a near-infrared reflectance spectrophotometer (model 6500; NIR System, Perstop Analytical, Silver Spring, MD) with a spinning cup holder. Spectra were saved with center and select procedures implemented using Infrasoft International[®] software [version 3.0 (24)]. Spectra from 60 samples (calibration set) were used to develop calibration equations for CP, soluble CP, ADF CP, and NDF CP. Calibrations were made using a modified partial least squares regression method. The number of terms in the equation was varied until no significant or relevant improvement in the coefficient of determination or standard error of calibration (SEC) could be determined. Different math transformations (24) were explored, and the 2, 10, 10, 1 math transformation offered the best prediction for all protein fractions (CP, soluble CP, ADF CP, and NDF CP). The remaining samples ($n = 68$) were used as a validation set to evaluate the bias and standard error of performance (SEP) of the equations.

Trial 2

Forage sampling procedures. Upon completion of trial 1, legume and grass silage samples ($n = 32$) of sufficient size to conduct an in situ evaluation were collected from the same sources described in trial 1. Samples were dried at 55°C for 48 h and ground through a Wiley mill (2-mm screen; Arthur H. Thomas, Philadelphia, PA). A subsample was reground through a Udy mill fit with a 1-mm screen. Samples were scanned on a near infrared reflectance spectrophotometer using the procedures described in trial 1, and spectra were saved. Silage samples were evaluated for CP, ADF, NDF, ADF CP, NDF CP, and soluble CP by the procedures described in trial 1.

An in situ evaluation of each silage sample was conducted over eight 3-d periods. Dacron bags ($18 \times 9 \text{ cm}$; $52\text{-}\mu\text{m}$ pore size) were filled with approximately 5 g of dried silage. Bags were placed in a mesh net secured to the ruminal cannula via a nylon cord. Duplicate sample bags at each time point were incubated in the ventral rumen of three midlactation ruminally cannulated cows for 0, 3, 6, 12, 24, 48, or 72

h and removed simultaneously. In addition, a standard forage (bud alfalfa silage) was incubated in duplicate for 24 h in each cow and evaluated for DM and CP disappearance to assess period effects. If DM or CP disappearance of the standard forage varied more than 5% for any incubation, the period was repeated. One period was repeated during the study. Zero-hour bags were soaked in tepid water for 0.5 h prior to removal of all bags for the period. All bags were immediately immersed in ice water upon removal from the rumen to stop microbial activity. Bags then were washed according to the procedures of Cherney et al. (7).

Bags were dried at 55°C for 48 h and weighed. The remaining DM was determined. Residue from the bags was removed, and duplicates at each time point were composited for chemical analysis.

Bag residue was analyzed for CP (3) that was adjusted for microbial CP contamination using an NIRS calibration (4), which estimated ($R^2 = 0.81$) milligrams of RNA CP per gram of DM. Base chemistries for the NIRS calibration (4) were the procedures of Zinn and Owens (29) with modifications by Aharoni and Tagari (1). An NIRS (4) equation was used to predict microbial CP contamination of the bag residues for the following reasons. First, use of diaminopimelic acid as a microbial marker has been demonstrated to overestimate microbial CP contamination of forages (21). Second, enrichment of silage samples used in this study with ^{15}N or ^{35}S was not possible. Third, use of RNA microbial CP marker procedures (1, 29) were possible but would have been extremely arduous given the number of bag residue samples involved ($n = 1392$). As a result, we converted RNA microbial CP maker procedures (1, 29) to an NIRS equation (4) to provide efficacious analyses of microbial CP contamination of bag residues when large numbers of samples were involved.

The CP degradation data were analyzed using the NLIN procedure of SAS (23) and were fitted to the model of Mertens and Loften (17):

$$\text{FR} = \text{Pe}^{-k(t-L)} + \text{U} \text{ when } t > L$$

and

$$\text{FR} = \text{P} + \text{U} \text{ when } 0 < t < L$$

where

- FR = CP remaining at time t,
- P = potentially digested fraction ($100 - \text{U}$ at fractional rate k , $k > 0$),
- U = fraction undigested at 72 h,
- L = discrete lag time, and
- t = incubation time (hours).

The RUP was estimated using the equation of NRC (18):

$$\text{RUP} = 100 - \left\{ \text{rapidly degraded protein (A)} + \frac{\text{B}}{[k_d/(k_d + k_p)]} \right\}$$

where k_p = ruminal passage rate (0.06/h).

Cow care. Three ruminally cannulated Holstein cows housed in tie stalls during the in situ study were fed a TMR once daily at 0600 h. The TMR contained 55% alfalfa silage; 43% grain mix containing shelled corn, oats, and soybean meal (17% CP); 0.9% mineral supplement; and 0.5% salt and vitamins of ration DM. Nutrient composition of the ration DM was 17.3% CP, 19.0% ADF, 28.0% NDF, 0.75% Ca, and 0.50% P.

NIRS and statistical analysis. The number of samples ($n = 32$) was chosen to minimize the intensive effort required to conduct an in situ study with a kinetics protocol while providing enough samples to yield a useful exploratory NIRS evaluation. Spectra from the 32 silage samples were used to develop calibration equations for A, B, C, k_d , degradation lag time, and RUP calculated by the equation $100 - \{A + B [k_d/(k_d + k_p)]\}$ where k_p = ruminal passage rate (0.06/h). Calibration equations were also developed for CPR at each incubation time (3, 6, 12, 24, 48, and 72 h) except 0 h. Because this was an exploratory NIRS evaluation, only 1, 4, 4, 1 and 2, 10, 10, 1 math transformations were considered, and the number of terms used in the equations was limited to no more than six to avoid overfitting the data. The number of terms in the equation was varied until no significant or relevant improvement in the coefficient of determination or SEC could be determined. All calibrations were made using a modified partial least squares regression method. Correlations between laboratory and in situ protein fractions of trial 2 silages were made using the correlation procedures of SAS (23).

RESULTS AND DISCUSSION

Sample Composition

Laboratory and in situ protein fractions for trial 1 and 2 silages are presented in Table 1. For trial 1, the CP of silages ranged from 9.4 to 24.3% with a mean of 17.7%, indicating that a wide compositional range was available for NIRS equation development. Wide compositional ranges were also present for soluble CP, ADF CP, and NDF CP in trial 1. For trial 2, the composition ranges of in situ and laboratory CP fractions were similarly wide, indicating a robust data

TABLE 1. Laboratory and in situ protein fractions of trial 1 and 2 legume and grass silages.¹

Item	Minimum	Maximum	Mean	SD
Trial 1 (n = 21)				
Laboratory protein fractions				
CP, % of DM	9.4	24.3	17.7	3.27
Soluble CP, % of CP	17.9	74.1	49.5	10.46
ADF CP, % of DM	0.74	3.2	1.7	0.49
NDF CP, % of DM	1.6	8.5	4.2	1.34
Trial 2 (n = 32)				
In situ protein fractions				
A, % of CP	51.4	81.8	67.4	8.70
B, % of CP	10.5	37.1	23.4	7.92
C, % of CP	4.7	13.5	9.1	2.52
k _d , 1/h	0.05	0.20	0.11	0.038
Lag, h	0.36	11.1	2.9	2.07
RUP, ² % of CP	9.0	26.7	17.6	4.81
CPR After				
3 h, % of CP	16.5	46.8	30.0	8.29
6 h, % of CP	11.2	42.6	26.5	8.29
12 h, % of CP	7.9	32.5	18.9	6.99
24 h, % of CP	5.6	18.2	12.1	3.79
48 h, % of CP	5.3	14.3	9.9	2.78
72 h, % of CP	4.9	13.9	9.3	2.48
Laboratory protein fractions				
CP, % of DM	12.6	26.3	20.5	3.36
Soluble CP, % of CP	43.3	79.9	62.2	9.37
ADF CP, % of DM	0.91	2.4	1.5	0.423
NDF CP, % of DM	1.4	7.6	3.5	1.84

¹A = Rapidly degraded protein, B = slowly degraded protein, C = undegradable protein, k_d = fractional degradation rate of B, lag = protein degradation lag time, and CPR = CP remaining.

²Calculated as $100 - \{A + B [k_d / (k_d + k_p)]\}$, where A, B, and k_d are as previously defined, and k_p = ruminal passage rate of 0.06/h.

set. Although not statistically evaluated, trial 2 silages contained more CP and soluble CP and less ADF CP and NDF CP than did trial 1 silages. Because NIRS evaluations were made independently on trial 1 and 2 silages, differences between silage data sets are not discussed.

NIRS Results for Trial 1

Calibration and validation statistics for NIRS analysis of trial 1 legume and grass silage laboratory protein fractions are presented in Table 2. The CP content of trial 1 legume and grass silages was accurately predicted by NIRS as evidenced by the high coefficient of determination ($R^2 = 0.96$) and low SEC (0.80% of DM).

The NIRS CP equation values observed in this study were nearly identical to R^2 and SEC values determined in other studies (11, 25), reconfirming that the estimation of CP content of silages by NIRS is accurate. Prediction of soluble CP in trial 1 silages by NIRS was notably less accurate ($R^2 = 0.82$) than NIRS CP prediction. We can find no previous study in which NIRS was used to predict soluble CP in silages

via borate phosphate buffer and, as a result, cannot verify or negate our findings based on previous literature. Reeves et al. (22) estimated the inverse of a fraction similar to soluble CP (hot water-insoluble N) of undried silages using NIRS. Prediction of hot water-insoluble N content of silages by NIRS was more accurate ($R^2 = 0.95$; SEC = 0.68% of DM) than our NIRS soluble CP prediction equation. Although calibration statistics suggest high accuracy, the SEP of the hot water-insoluble protein equation of Reeves et al. (22) was considerably less accurate ($r^2 = 0.82$; SEP = 1.28% of DM), which compares with our validation observations (Table 2). Because of procedural differences between soluble CP and hot water-insoluble N determination, complete comparison between data of Reeves et al. (22) and our data cannot be made.

Prediction of ADF CP in trial 1 silages by NIRS was considerably less accurate than NIRS CP prediction. Reeves et al. (22) observed R^2 and SEC values of 0.71 and 0.65% of DM, respectively, for NIRS determinations of ADF CP in undried silages. Those values are similar ($R^2 = 0.77$; SEC = 0.24% of DM) to our ADF CP predictions by NIRS. Shenk and Westerhaus

TABLE 2. Calibration and validation statistics for near-infrared reflectance spectroscopy (NIRS) analysis of laboratory protein fractions in legume and grass silages (trial 1).¹

Item	NIRS Analysis					
	R ²	SEC	PLS	Transformation ²	SEP	Bias
Calibration (n = 60)						
CP, % of DM	0.96	0.80	6	2, 10, 10, 1
Soluble CP, % of CP	0.82	4.4	5	2, 10, 10, 1
ADF CP, % of DM	0.77	0.24	6	2, 10, 10, 1
NDF CP, % of DM	0.72	0.71	6	2, 10, 10, 1
Validation (n = 61)						
CP, % of DM	0.96	0.79	-0.12
Soluble CP, % of CP	0.82	2.79	-0.14
ADF CP, % of DM	0.42	0.28	-0.01
NDF CP, % of DM	0.84	0.43	0.14

¹SEC = Standard error of calibration, PLS = partial least squares (terms), and SEP = standard error of performance.

²Order of derivative function, segment length (nanometers), segment length (nanometers) of first smoothing, and segment length (nanometers) of second smoothing.

(25) observed even higher SEC values when determining ADF CP using NIRS. Prediction of NDF CP by NIRS was the least accurately predicted laboratory protein fraction with an R² of 0.72 and an SEC of 0.71% of DM. Although no confirming literature is available for silage NDF CP determination by NIRS, it would be logical to assume similar prediction accuracies as ADF CP because reference procedures and dynamics of NIRS physics involved should fundamentally be the same. Our data support this assumption.

There are two logical reasons why prediction of ADF CP or NDF CP by NIRS would be less accurate than CP prediction. First, determination of ADF CP or NDF CP requires a dual assay (3, 9), each with laboratory error. As a result, the laboratory error of the reference method is higher for ADF CP or NDF CP compared with CP, which is known (2) to decrease NIRS prediction accuracy. Secondly, prediction of CP by NIRS needs only quantification of N bonds (26). To predict ADF CP or NDF CP, NIRS must quantify and qualify ADF-N or NDF-N bonds, which decreases NIRS prediction accuracy (2).

NIRS Results for Trial 2

Calibration statistics for NIRS analysis of in situ CP fractions in trial 2 legume and grass silages are presented in Table 3. In general, NIRS equations were developed for trial 2 silage in situ CP fractions using 2, 10, 10, 1 or 1, 4, 4, 1 math transformations with five or six terms in the equation. The A fraction of trial 2 silages was accurately predicted (R² = 0.96; SEC = 1.8% of CP) by NIRS. Our data support the data of Swift et al. (27) who observed similar prediction accuracy (R² = 0.94; SEP 1.3% of CP) for the A fraction in silages. It is unclear why NIRS accurately

predicted the A fraction in trial 2 silages and prediction of soluble CP in trial 1 silages was markedly less accurate. Conceptually, A and soluble CP are simple solubility measurements and are highly correlated (Table 4); NIRS logically should provide similar prediction accuracies.

Our data, however, suggest that NIRS predicts in situ A in silages with a greater degree of accuracy than it does soluble CP. We, however, cannot com-

TABLE 3. Calibration statistics for near-infrared reflectance spectroscopy (NIRS) analysis of in situ protein fractions in legume and grass silages (trial 2).¹

Item	R ²	NIRS Analysis		
		SEC	PLS	Transformation ²
A, % of CP	0.96	1.8	6	2, 10, 10, 1
B, % of CP	0.96	1.5	6	2, 10, 10, 1
C, % of CP	0.92	0.69	6	2, 10, 10, 1
k _d , 1/h	0.87	1.4	6	2, 10, 10, 1
Lag, h	0.84	0.83	6	1, 4, 4, 1
RUP, ³ % of CP	0.94	1.2	5	2, 10, 10, 1
CPR After				
3 h, % of CP	0.94	1.8	5	2, 10, 10, 1
6 h, % of CP	0.95	1.8	5	2, 10, 10, 1
12 h, % of CP	0.95	1.6	6	1, 4, 4, 1
24 h, % of CP	0.96	0.80	6	2, 10, 10, 1
48 h, % of CP	0.92	0.79	6	2, 10, 10, 1
72 h, % of CP	0.92	0.69	6	2, 10, 10, 1

¹SEC = Standard error of calibration, PLS = partial least squares (terms), A = rapidly degraded protein, B = slowly degraded protein, C = undegradable protein, k_d = fractional degradation rate of B, lag = protein degradation lag time, and CPR = CP remaining.

²Order of derivative function, segment length (nanometers), segment length (nanometers) of first smoothing, and segment length (nanometers) of second smoothing.

³Calculated as $100 - \{A + B[k_d/(k_d + k_p)]\}$, where A, B, and k_d are as defined previously, and k_p = ruminal passage rate of 0.06/h.

pletely support or reject this hypotheses because trial 1 and 2 NIRS analyses were conducted on different silages. Although desirable, it was impossible to conduct in situ evaluations and subsequent NIRS analysis in trial 1 silages because a large number (more than 100) of diverse silage samples were needed to maximize NIRS accuracy, but samples submitted to laboratories for routine analysis do not contain enough material to conduct kinetic in situ analysis. The B and C fractions of trial 2 silages were similarly predicted with acceptable degrees of accuracy ($R^2 > 0.92$) by NIRS. The coefficients of determination and proportionate SEC for the B and C in situ CP fractions were similar to those for A. The degradation lag time of trial 2 silages was predicted less accurately by NIRS than were A, B, or C. The RUP content of trial 2 silages was also predicted with a similar degree of accuracy by NIRS as compared with A, B, and C by NIRS prediction. These data are encouraging and suggest that if a robust set of in situ RUP values could be generated, NIRS could predict RUP content with similar precision as CP (Table 2). The CPR after 3, 6, 12, 24, 48, or 72 h of ruminal incubation was also predicted by NIRS with a similar degree of accuracy as CP or RUP.

Laboratory Versus In Situ Relationships

The laboratory CP fractions measured in trial 1 silages were also measured in trial 2 silages. No NIRS evaluation was made on laboratory CP fractions of trial 2 silages because trial 1 was designed to facilitate maximum NIRS accuracy for CP, soluble CP, ADF CP, and NDF CP. Laboratory CP fractions were determined on trial 2 silages to explore relationships between laboratory CP fractions and in situ CP fractions. Correlations between laboratory and in situ protein fractions in trial 2 silages are presented in Table 4. Crude protein was negatively correlated ($P < 0.05$) with C and CPR after 24, 48, or 72 h of ruminal incubation. The data support the literature base (18), which suggests CP content of silages is not a good indicator of ruminal CP degradation kinetics. Soluble CP was positively correlated ($P < 0.05$) with A and k_d and negatively correlated ($P < 0.05$) with B, C, RUP, and CPR after 3, 6, 12, 24, 48, and 72 h of ruminal incubation. The strong correlations ($r > \pm 0.84$) among A, B, RUP, and soluble CP are logical. First, the soluble CP and in situ A assay differ in solvents (borate phosphate buffer vs. water) and filtration mechanics but fundamentally measure the same protein fraction; therefore, a high correlation is expected. Second, because in situ RUP content is so

TABLE 4. Correlations of laboratory and in situ protein fractions in legume and grass silages (trial 2).¹

In situ protein fraction	Laboratory protein fraction			
	CP	Soluble CP	ADF CP	NDF CP
	r =			
A, % of CP	0.24	0.90*	-0.48*	-0.72*
B, % of CP	-0.08	-0.84*	0.43	0.65*
C, % of CP	-0.57*	-0.48*	0.32	0.45*
k_d , 1/h	0.24	0.54*	-0.50*	-0.19
Lag, h	-0.09	-0.29	0.60*	0.36
RUP, ² % of CP	-0.40	-0.91*	0.56*	0.65*
CPR After				
3 h, % of CP	-0.32	-0.93*	0.57*	0.75*
6 h, % of CP	-0.33	-0.93*	0.61*	0.76*
12 h, % of CP	-0.28	-0.85*	0.64*	0.60*
24 h, % of CP	-0.46*	-0.78*	0.63*	0.57*
48 h, % of CP	-0.57*	-0.60*	0.43	0.45*
72 h, % of CP	-0.60*	-0.50*	0.37	0.39

¹A = rapidly degraded protein, B = slowly degraded protein, C = undegradable protein, k_d = fractional degradation rate of B, lag = protein degradation lag time, and CPR = CP remaining.

²Calculated as $100 - \{A + B[k_d/(k_d + k_p)]\}$, where A, B, and k_d are as defined previously and k_p = ruminal passage rate of 0.06/h.

* $P < 0.05$.

heavily influenced by the amount of A, and soluble CP is correlated with A, then soluble CP would logically be strongly (negatively) correlated with RUP. Danish workers (14) reported good correlations ($r = 0.64$) between soluble CP and in situ RUP values but cautioned that the relationship between soluble CP and in vivo CP degradation was poor. The ADF CP content of trial 2 silages was negatively correlated ($P < 0.05$) with A and k_d and positively correlated ($P < 0.05$) with degradation lag time, RUP, and CPR after 3, 6, 12, and 24 h of ruminal incubation. All correlations between in situ protein fractions and ADF CP were relatively weak ($r < \pm 0.65$), and, interestingly, ADF CP was not correlated with C, which, in the CNCPS, it is supposed to partially represent. The NDF CP content of trial 2 silages was correlated with in situ protein fractions in the exact same manner as the soluble CP and in situ protein fraction relationships. The NDF CP and in situ protein fraction correlations carry the opposite sign and, in general, are weaker than the soluble CP and in situ protein fraction correlations. Our data suggest that NDF CP content of silages is a weak measure of insoluble CP in silages and is not strongly related to any in situ protein fraction.

CONCLUSIONS

Our data reconfirm that CP content of silages can be accurately measured using NIRS. Our data also

suggest that NIRS measurement of soluble CP, ADF CP, and NDF CP is markedly less accurate than NIRS measurement of CP and that measurement of these protein fractions using NIRS should be approached with caution. Further, our data suggest that ADF CP and NDF CP are not strongly related to any in situ protein fraction, raising the larger question of the relationship between ADF CP and NDF CP and in vivo CP degradation. In this study, we had good success in predicting in situ protein fractions in silages using NIRS. Our data set, however, was very small, and a larger data set would be required to develop robust NIRS equations for in situ protein fractions in silages. As with laboratory CP fraction, the greater question of the relationship between in situ protein fractions and in vivo CP degradation still remains.

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