



Prediction of Forage Energy Content by Near Infrared Reflectance Spectroscopy and Summative Equations

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Abstract

In livestock nutrition, summative models (SM) are displacing empirical models as a preferred method to predict energy content of forages. The objective of this study was to determine the effect of using near infrared reflectance spectroscopy (NIRS) to determine nutrient subcomponents required of SM on overall ability to predict energy content of corn and legume-grass silages. Corn ($n = 90$) and legume-grass ($n = 70$) silages were collected and analyzed for CP, ADF CP, NDF, NDF CP, in vitro (IV) digestible (d) NDF, ash, and fat by standard laboratory techniques. Samples were scanned on a Model 6500 NIRS, and calibration equations were developed for each nutrient. The TDN contents of corn and legume-grass silages were then estimated using a SM, where the model nutrients were determined by laboratory or NIRS methods. The predicted TDN content of corn and legume-grass silages was compared to IV d OM to assess overall utility. The NIRS calibrations were adequate ($R^2 > 0.90$) for CP and NDF for both corn and legume-grass silages with standard errors of calibration (SEC) < 0.55

for CP and < 1.09 for NDF. Near infrared calibrations for ADF CP, NDF CP, fat, and ash were less accurate in both corn and legume-grass silages with $R^2 < 0.75$. Calibrations for IV d NDF in corn and legume-grass silages had $R^2 = 0.87$ and 0.79 , respectively, but possible co-dependency with NDF is speculated. The relationship between corn and legume-grass silage SM TDN and IV d OM was excellent when model nutrients were determined by laboratory procedures. The TDN estimates when NIRS was used to determine all SM nutrients were superior to older empirical models, but SM TDN estimates using NIRS-determined nutrients were less accurate as compared with SM TDN prediction when model nutrients were determined by laboratory procedures. In particular, using NIRS to predict IV d NDF and ash for use in SM lead to the greatest challenge in TDN prediction in both corn and legume-grass silages.

(Key Words: Forage, Energy, Near Infrared Spectroscopy, Summative Model.)

Introduction

Accurate and precise estimates of forage energy content are required to formulate diets properly for lactating dairy cows and other ruminants. Al-

though important, development of accurate, rapid, and economical laboratory systems to predict forage energy content has been elusive. Commonly (Weiss, 1998), ADF has been used in first-order empirical equations to predict forage TDN content. Empirical equations account for one-half ($r^2 = 0.50$ to 0.60) of the variance in forage energy content. Of greater concern is the lack of accuracy using empirical equations, with prediction errors of 2 to 8 percentage units of TDN (Van Soest, 1965; McLeod and Minson, 1972). Chronologically, Ohio State researchers (Conrad et al., 1984; Weiss et al., 1992) developed a summative model (SM) to predict forage energy content. Summative models require laboratory evaluation of multiple nutrients to estimate the caloric contribution of each nutrient, followed by a final summation to estimate TDN at $1 \times$ maintenance (NRC, 2001). Summative models have been shown to be population independent and account for more variance in forage TDN than empirical models (Weiss, 1998). The SM adopted by the National Research Council (2001) requires laboratory determination of CP, ADF CP, NDF, NDF CP, ash, fat, and lignin or 48 h in vitro (IV) digestible (d) NDF. The SM adopted by the NRC has distinct advantages, but lab-

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oratory procedures are neither rapid nor economical to perform. As a result, there is interest among commercial forage testing laboratories to predict model nutrients of SM using near infrared reflectance spectroscopy (NIRS) to expedite forage energy prediction (D. J. Undersander, University of Wisconsin, 2002, personal communication). It is known that some SM nutrients, such as CP, NDF, and fat, can be accurately predicted by NIRS, while NIRS prediction of NDF CP, and ash can be problematic (Shenk and Westerhaus, 1994; Hoffman et al., 1999). The NIRS prediction of IV d NDF, which is also an integral component of SM forage energy prediction, has yet to be fully defined. This study was implemented to investigate the utility of using NIRS to predict the nutrients required of SM and determine the overall effect on predicting forage energy content.

Materials and Methods

Legume-grass ($n = 210$) and corn silages ($n = 300$) were collected from samples submitted for routine forage analysis to the Marshfield Soil and Forage Analysis Laboratory, Marshfield, WI. Dry matter, chemical, and digestibility determinations were conducted in duplicate and included the following. Samples were dried at 55°C for 48 h and then milled through a Wiley mill (Arthur A. Thomas Co., Philadelphia, PA) fit with a 1-mm screen. Residual DM determination was determined by drying at 135°C for 2 h. Samples were also evaluated for CP and ash by AOAC (1990) procedures. Crude fat was determined by acid hydrolysis (AOAC, 1990). The NDF content of forage was determined by the procedures of Goering and Van Soest (1970) with modifications by Mertens (1992). The ADF and NDF residues were evaluated for CP using AOAC (1990) procedures, resulting in ADF CP and NDF CP determinations. An estimate of non-fiber carbohydrate (NFC) content of forage is required of SM and was determined by the formula $NFC = 100 - [NDF +$

$CP + \text{ash} + \text{fat} - \text{NDF CP}]$ (NRC, 2001). The IV d OM and IV d NDF were determined by the procedures of Goering and Van Soest (1970). Basic IV procedures and apparatus consisted of incubating a 0.5-g forage sample in CO₂ back-pressured 125-mL Erlenmeyer flask containing rumen fluid, buffer media, and macro-mineral and micromineral solution (Goering and Von Soest, 1970) for 48 h in a water bath held at 39°C. Rumen fluid was harvested from a non-lactating dairy cow fit with a ruminal cannula fed alfalfa grass silage ad libitum, with supplemental minerals and vitamins. Rumen fluid was held at 39°C in CO₂-gassed vessels, mixed in a CO₂-gassed blender, and strained through three layers of cheesecloth prior to injection into the incubation flask. Critical conditions to maximize IV d NDF as defined by Grant and Weidner (1992) and Grant and Mertens (1992) were also adhered to. The 48-h IV incubations were terminated by a NDF determination (Goering and Van Soest, 1970; Mertens, 1992), yielding indigestible NDF. The IV d NDF content of each forage was determined as the difference between the original and indigestible NDF contents. The NDF digestibility (NDFD) as a percentage of NDF and IV d OM were also determined for each forage from the same assay by standard calculations (Goering and Van Soest, 1970).

The TDN content of legume-grass and corn silage samples was then calculated using a modified SM of the NRC (2001):

$$TDN_{IX} (\%) = (td \text{ NFC} + td \text{ CP} + (td \text{ fat} \times 2.25) + td \text{ NDF}) - 7$$

where

$$\begin{aligned} td &= \text{truly digested,} \\ td \text{ NFC} &= 0.98 (100 - [(NDF - NDF \text{ CP}) + CP + \text{fat} + \text{ash}], \\ td \text{ CP} &= CP \times \exp [-1.2 \times (ADF \text{ CP}/CP)], \\ td \text{ fat} &= \text{fat} - 1, \text{ and} \\ td \text{ NDF} &= \text{IV d NDF} (\% \text{ DM}). \end{aligned}$$

The primary SM of NRC (2001) that uses lignin to determine the NDF digestion coefficient was not considered in our evaluation because lignin is often not related to NDFD of C4 grasses such as corn silage (Jung et al., 1997). Because our evaluation contained a large corn silage database, IV d NDF was used to facilitate a universal measure of NDFD between the corn and legume-grass silage databases. In addition, the TDN contents of corn and legume-grass silages were estimated by the empirical equations of Adams (1980) and Rohweder et al. (1978), which use ADF to predict TDN. These TDN estimates were made to provide a base reference of forage TDN prediction systems.

A subsample of 1 mm of dried Wiley mill-ground forage was reground through a Udy mill (Udy Corp., Boulder, CO) fit with a 1-mm screen. The reground forage samples were then packed into cylindrical sample holders equipped with a quartz window and scanned between 400 and 2498 nm in duplicate according to the procedures of Marten et al. (1983) on a near-infrared reflectance spectrophotometer (Model 6500; FOSS-NIR System, Silver Spring, MD) fit with a spinning cup holder. Samples from 90 legume-grass silages and 70 corn silages were selected based on spectral diversity using CENTER and SELECT procedures implemented using Infrasoftware International® software [Version 3.0; (Shenk and Westerhaus, 1991)]. All samples included analysis for CP, ADF, ADF CP, NDF, NDF CP, fat, ash, IV d NDF, NDF, and IV d OM, for calibration development. Calibrations for the aforementioned nutrients were computed using partial least squares (PLS) regression methods, different math transformations, and different numbers of terms in the models. An optimum number of PLS terms were determined by Infrasoftware International software, using four cross-validation groups (Shenk and Westerhaus, 1991). The NIRS equations developed were then used to obtain predicted values of SM nu-

trients for validation sets of legume-grass (n = 120) and corn silage (n = 230) using the samples that were not selected for calibration development. Calibration performances were evaluated based on the coefficient of determination (COD), the standard error of prediction (SEP) corrected for bias, and the mean bias. The NIRS-predicted SM nutrients or SM nutrients as determined by laboratory methods were then used in the aforementioned various TDN models and were compared with IV d OM of the forages to assess overall utility of TDN estimation. The relationship of TDN to IV d OM of forages was chosen to evaluate the TDN models because collection of in vivo digestibility data on large sample bases is unrealistic and because IV d OM of forages has been demonstrated to reasonably predict ($R^2 > 0.80$) in vivo digestibility of forages (Weiss, 1994).

Results and Discussion

Nutrient composition of experimental corn and legume-grass silages is presented in Table 1. Based on minimum, maximum, mean, and standard deviations values, NIRS center and select procedures (Shenk and Westerhaus, 1991) yielded suitable and robust databases to conduct NIRS determinations. Specifically, IV d OM ranged from 60.3 to 83.4% DM for corn silage and from 46.3 to 78.1% DM for legume-grass silages. These data indicate a wide range of forage digestibilities were available to test the utility of NIRS in TDN prediction systems. As previously discussed, to predict forage energy content via SM, the NRC (2001) requires laboratory determination of CP, ADF CP, NDF, NDF CP, IV d NDF (or lignin), ash, and fat. Thus, to evaluate utility of NIRS in determining forage TDN contents, individual NIRS equations were developed for CP, ADF CP, NDF, NDF CP, IV d NDF, ash, and fat. Supplemental NIRS equations were also developed for ADF, NDFD (% of NDF) and IV d OM in corn and legume-

TABLE 1. Nutrient composition of experimental corn and legume-grass silages.

Item ^{a,b}	Minimum	Maximum	Mean	SD
Corn silage (n = 70)				
CP	5.0	13.8	7.8	1.4
ADF	16.5	49.4	26.5	6.1
ADF CP	0.14	1.17	0.39	0.15
NDF	34.2	78.3	47.2	8.4
NDF CP	0.57	2.65	1.33	0.38
IV d NDF	18.4	42.4	26.5	5.5
Fat	0.82	2.96	1.77	0.45
Ash	2.5	8.7	4.0	1.1
NFC	12.1	54.6	40.6	8.4
IV d OM, %	60.3	83.4	76.7	3.9
IV NDFD, % NDF	49.6	66.6	58.9	4.0
TDN _{1×} ^c	53.0	76.0	69.6	3.8
NE _{i, 3×} ^d , Mcal/kg	1.18	1.74	1.58	1.09
Legume-grass silage (n = 90)				
CP	8.6	23.9	18.4	3.2
ADF	22.6	47.2	34.9	5.0
ADF CP	0.20	2.68	1.22	0.57
NDF	30.9	65.5	43.8	7.6
NDF CP	1.08	7.85	2.93	1.35
IV d NDF	11.0	34.3	18.3	4.8
Fat	1.51	4.34	2.52	0.53
Ash	4.3	18.4	10.0	2.2
NFC	14.6	43.9	28.2	5.6
IV d OM, %	46.3	78.1	67.4	5.3
IV NDFD, % NDF	31.6	66.7	48.4	6.9
TDN _{1×}	38.4	67.5	57.1	5.4
NE _{i, 3×} , Mcal/kg	0.82	1.53	1.28	0.13

^aIV d NDF = In vitro digestible NDF, IV NDFD = in vitro NDF digestibility (% NDF), NFC = non-fiber carbohydrate, and IV d OM = in vitro digestible OM.

^bAll values are expressed as a percentage of DM unless otherwise listed. TDN and NE_i estimates were made using a summative model (NRC, 2001).

^cSubscript 1× = at maintenance.

^dSubscript 3× = 3× maintenance.

grass silages to facilitate additional interpretation of data.

Calibration and cross-validation statistics for NIRS analysis of CP, ADF CP, NDF, NDF CP, IV d NDF, ash, and fat in corn and legume-grass silages are presented in Table 2. For both corn and legume-grass silages, NIRS predicted CP content with a high degree of accuracy with relatively low SEP (<0.8% DM). The utility of NIRS to predict CP content of forages is well documented (Shenk and Westerhaus, 1994; Hoffman, et al., 1999). Similarly, NIRS predicted ADF and NDF contents of both corn

and legume-grass silages well, with calibration COD >0.90. For corn silage, the SEP for ADF and NDF were 2.19 and 1.67% DM, respectively. For legume-grass silages, SEP for ADF and NDF were 1.38 and 1.81% of DM, respectively. The NIRS COD and SEP for ADF and NDF for both corn silage and legume-grass silages were similarly consistent with previous reports (Reeves et al., 1991; Shenk and Westerhaus, 1994). The ADF CP content of corn silage was, however, not well predicted by NIRS, with a cross-validation COD of 0.70 and a SEP of 0.12% of DM. Prediction of ADF CP in le-

TABLE 2. Calibration and cross-validation statistics for near-infrared spectroscopy analysis of nutrients in corn and legume-grass silages.

Item ^{c,d}	Calibration ^a				Cross validation ^b	
	Transformation	PLS Terms	SEC	R ²	r ²	SEP
Corn silage						
CP	1,2,2,1	10	0.17	0.99	0.97	0.26
ADF	1,10,10,1	5	1.80	0.91	0.88	2.19
ADF CP	2,10,10,1	4	0.10	0.77	0.70	0.12
NDF	2,2,2,1	6	0.82	0.99	0.96	1.67
NDF CP	1,2,2,1	3	0.28	0.46	0.35	0.31
IV d NDF	1,2,2,1	9	1.25	0.95	0.87	1.95
Fat	2,10,10,1	3	0.29	0.59	0.52	0.31
Ash	2,20,20,1	8	0.42	0.77	0.51	0.61
IV NDFD, % NDF	3,4,4,1	3	2.91	0.47	0.19	3.62
IV d OM, % DM	1,2,2,1	4	1.28	0.88	0.85	1.47
Legume-grass silage						
CP	2,4,4,1	9	0.55	0.97	0.93	0.80
ADF	1,10,10,1	9	1.00	0.95	0.91	1.38
ADF CP	2,10,10,1	4	0.49	0.74	0.71	0.52
NDF	2,4,4,1	8	1.09	0.98	0.94	1.81
NDF CP	2,10,10,1	4	0.54	0.79	0.75	0.59
IV d NDF	1,20,20,1	6	1.81	0.83	0.79	2.04
Fat	2,10,10,1	5	0.28	0.68	0.53	0.34
Ash	3,4,4,1	7	0.52	0.93	0.73	1.01
IV NDFD, % NDF	1,4,4,1	6	3.49	0.75	0.69	3.93
IV d OM, % KM	1,4,4,1	5	1.74	0.87	0.82	2.07

^aPLS = Partial least squares; SEC = standard error of calibration.

^bSEP = Standard error of prediction.

^cIV d NDF = in vitro digestible NDF, IV d OM = in vitro digestible OM, and IV NDFD = in vitro NDF digestibility (% NDF).

^dAll nutrients were evaluated on a percentage of DM basis unless otherwise listed.

are as follows. The IV d NDF content of a forage is a simple mathematical function of NDF content and NDFD (% of NDF): IV d NDF = NDF × NDFD. If the range of NDFD (% of NDF) in corn silage is limited, then IV d NDF is simply and primarily dependent on the NDF content of the forage and could be determined by NIRS by predicting NDF content and simply co-dependently expressing NDF as IV d NDF. Although prediction of NDFD (% of NDF) is not essential for the NRC (2001) SM, we explored the utility of NDFD, as a percentage of NDF prediction by NIRS (Table 2) to provide inference. The NIRS could not predict ($R^2 = 0.19$) the NDFD content of corn silage, suggesting IV d NDF NIRS predictions are primarily dependent on the NDF content of corn silage. This rationale, however, does not fully explain our observations predicting IV d NDF in legume-grass silages with NIRS. The NIRS COD of IV d NDF in legume-grass silages was less ($R^2 = 0.79$ vs 0.87) than that for corn silage, but NIRS was able to modestly predict ($R^2 = 0.69$) NDFD, indicating the IV d NDF prediction in legume-grass silages might not have been co-dependent with NDF content as with corn silage IV d NDF.

Logically, the NDF of legume-grass silages would be more heterogeneous, containing both legume and grass NDF sources, as compared with a more homogenous NDF source in corn silage. Because NIRS can spectrally distinguish legumes and grass (Shenk and Westerhaus, 1994) and legumes and grasses have different IV d NDF potential (Hoffman et al, 1993), it is logical that NIRS could better distinguish NDFD differences in divergent legume-grass silages as compared with corn silage. In addition, as maturity of legumes and grasses advance, NDF content increases, which is negatively correlated to NDFD (Hoffman et al., 1993).

Correspondingly, advancing maturity in legumes and grasses creates predictable chemical changes in the plant such as increased lignin and cel-

lulose in legume-grass silages via NIRS was similar to corn silage, with a cross-validation COD of 0.71 and a SEP of 0.52. Our observations are similar to the observations of Marten and Linn (1989), who similarly observed low predictability ($R^2 = 0.59$) of ADF CP using NIRS. In a previous report (Hoffman et al., 1999), our laboratory also observed difficulty ($R^2 = 0.42$) in predicting ADF CP in legume-grass silages.

Results were similar for NIRS prediction of NDF CP in corn and legume-grass silages. Prediction of NDF CP in corn silage was most difficult, with a cross-validation COD of 0.35. The NDF CP content of legume-grass silages was better predicted ($R^2 = 0.75$) than NDF CP in corn silage and was

similar to our previous observations (Hoffman et al., 1999). The IV d NDF content of corn silage was well predicted ($R^2 = 0.85$) by NIRS. Our observations are similar to those of Jung et al. (1998), who observed a strong relationship ($R^2 = 0.92$) between NIRS and laboratory IV d NDF determinations. We believe these data should be interpreted with some degree of caution. First, Jung et al. (1998) evaluated IV d NDF on corn stem internode tissue and not whole-plant corn silage; therefore, direct comparison between our observations and those of Jung et al. (1998) may not be completely valid. Second, we believe there is a potential issue of co-dependency in NIRS IV d NDF determination. Reasons for our concern

lulose content, which have similarly been shown to be negatively correlated (Jung et al., 1997) to NDFD. These distinct chemical changes would logically influence NIRS absorption, reflection characteristics (Shenk and Westerhaus, 1994). This argument is not true for corn silage because grain (starch) content of corn silage can dilute or exaggerate actual composition of nutrients such as lignin or cellulose, making spectral relationships of these nutrients to NDFD difficult.

The NIRS calibration and cross-validation statistics for fat and ash contents in corn and legume-grass silages are also presented in Table 2. Fat content of corn and legume-grass silages was not well predicted ($R^2 < 0.53$) by NIRS. Fat content of wheat (Garnsworthy et al., 2000) has been accurately ($R^2 = 0.98$) predicted by NIRS, but comparative studies regarding NIRS fat prediction in forages are surprisingly absent from literature. Similarly, NIRS did not efficiently predict ($R^2 < 0.51$) ash contents of corn silage. Ash content of legume-grass silages was predicted ($R^2 = 0.73$) better than corn silage. The difficulty of NIRS to predict ash, especially exogenous ash, content of forages is not surprising because pure inorganics do not have NIRS absorption bonds (Shenk and Westerhaus, 1994).

The effect of analytical methods on corn silage TDN estimation was evaluated by the relationship between TDN and IV d OM, and results are presented in Table 3. Two older (Rohweder et al., 1978; Adams, 1980) empirical TDN predictions were included in the evaluation for comparative purposes. The empirical TDN prediction models of Adams (1980) and Rohweder et al. (1978), which predict TDN from ADF, are represented in Models 1 and 2. These models were evaluated because they have been extensively used by feed and forage testing laboratories for two decades and, thus, provide a historical reference point. When ADF was determined using laboratory methods (Goering and Von Soest, 1970), the empirical

TABLE 3. The effects of analytical method and TDN model on the relationship between corn silage TDN and in vitro digestible OM (IV d OM).

Model	Forage TDN model	Analytical method of model components ^a		Dependent variable	r ²	Intercept	Slope	SE
		Laboratory	NIRS					
1	Adams, 1980	ADF	—	IV d OM	0.61	47.9	0.43	2.5
2	Rohweder et al., 1978	ADF	—	IV d OM	0.61	32.3	0.65	2.5
3	Adams, 1980	—	ADF	IV d OM	0.57	48.4	0.42	2.6
4	Rohweder et al., 1978	—	ADF	IV d OM	0.57	33.0	0.64	2.6
5	NRC, 2001	CP, ADF CP, NDF, NDF CP, IV d NDF, ash, fat	—	IV d OM	0.98	10.8	0.96	0.6
6	NRC, 2001	—	CP, ADF CP, NDF, NDF CP, IV d NDF, ash, fat	IV d OM	0.78	20.5	0.82	1.8
7	NRC, 2001	CP, ADF CP, NDF, NDF CP, IV d NDF, ash	IV d NDF, ash, fat	IV d OM	0.98	10.8	0.96	0.6
8	NRC, 2001	CP, ADF CP, NDF, NDF CP, d NDF, fat	fat	IV d OM	0.92	7.7	1.00	1.1
9	NRC, 2001	CP, ADF CP, NDF, NDF CP, ash, fat	ash	IV d OM	0.80	18.7	0.85	1.8
10	NRC, 2001	CP, ADF CP, NDF, IV d NDF, ash, fat	IV d NDF	IV d OM	0.97	10.8	0.96	0.6
11	NRC, 2001	CP, ADF CP, NDF CP, IV d NDF, ash, fat	NDF CP	IV d OM	0.97	10.8	0.96	0.6
12	NRC, 2001	CP, NDF, NDF CP, IV d NDF, ash, fat	NDF	IV d OM	0.98	10.8	0.96	0.6
13	IV d OM	—	ADF CP	IV d OM	0.98	10.8	0.96	0.6
			IV d OM	NRC, 2001 TDN	0.83	-3.5	0.94	1.8

^aIV d NDF = In vitro digestible NDF; NIRS = near-infrared spectroscopy.

model of Adams (1980) and Rohweder et al. (1978) resulted in a COD of $r^2 = 0.61$ between TDN and IV d OM with standard errors of prediction of 2.5% DM of IV d OM. Using NIRS (Models 3 and 4) to determine ADF minimally increased SE of the TDN, IV d OM relationship from 0.10 to 0.50% of DM for corn silage and legume-grass silage, respectively (Rohweder et al., 1978; Adams, 1980). The slight increase in the SE is logical because NIRS prediction of ADF, although good (Table 2), will always slightly increase the determination error as compared with the original laboratory ADF determination (Shenk and Westerhaus, 1994).

Predicting the TDN content of corn silage using SM with all nutrients of the SM determined by laboratory methods related extremely well ($R^2 = 0.98$) to the IV d OM of corn silage (Model 5). It should be noted that the SM TDN and IV d OM relationship (Model 5) is somewhat confounded and likely over-expresses the true relationship because the ash and IV d NDF values used in the SM are a part of the IV d OM assay; therefore, comparisons are relative. Corn silage TDN as predicted by Model 5 had a superior relationship to the IV d OM content of corn silage as compared with Models 1 to 4. These data support the utility of SM as compared with empirical models to predict forage TDN content as proposed by Weiss (1998). The observed superiority of the SM to predict corn silage TDN as related to IV d OM was partially eroded when nutrients of the SM were determined using NIRS (Model 6). The COD between corn silage TDN and IV d OM was reduced from $r^2 = 0.98$ to $r^2 = 0.78$ when nutrients of the SM were determined by NIRS.

Despite shortcomings, the broad utility of NIRS in forage TDN prediction should not be underestimated. Using NIRS to predict all SM nutrients decreases TDN prediction error as compared with empirical models and has the ability to be standardized across laboratories. Although the rela-

tionship between SM TDN and IV d OM was superior when model nutrients were determined by laboratory procedures, laboratory procedures such as IV d NDF are especially arduous to standardize across laboratories, and it is unfeasible for many laboratories to conduct. In Models 7 to 12 in Table 3, a simplified matrix of analytical methods was employed to determine which SM nutrients when determined by NIRS were detrimental to the overall estimation of TDN in corn silage. In Model 7, fat was determined via NIRS, and all other SM nutrients were determined via laboratory methods. Determining fat content of corn silage by NIRS had no overall effect on the TDN IV d OM relationship as compared with Model 5, which contained laboratory fat determination. This is logical because fat content of corn silage is low (Table 1) and, thus, has a minimal effect on TDN content of corn silage. Similarly, determination of NDF CP, NDF, and ADF CP in corn silage by NIRS and using laboratory values for all other SM nutrients in Models 10, 11, and 12 resulted in only minor losses in ability to estimate TDN content of corn silage as compared with IV d OM. Explanations are similar to observations made for fat (Model 7) because NDF CP and ADF CP result in similar minor contributions to SM TDN (NRC, 2001). The NDF content of a forage does have a large influence on TDN, but NDF was well predicted by NIRS (Table 2), thus using NDF determined by NIRS had little effect on overall SM TDN estimation. Of all nutrients required of SM to estimate TDN, determining ash and IV d NDF using NIRS had the most detrimental effect on TDN estimations as evaluated by a TDN, IV d OM relationship. These effects are highlighted in Models 8 to 9 presented in Table 3. Our NIRS calibration and cross-validation statistics presented in Table 2 support this observation. The ash content of corn silage was poorly predicted ($R^2 = 0.51$) by NIRS, which results in a direct TDN prediction error when using SM. Although IV d NDF

was well predicted by NIRS ($R^2 = 0.87$), NDFD (% of NDF) was not ($R^2 = 0.19$). The loss of 0.16 COD units in the TDN, IV d OM relationship (Model 9, Table 3) when IV d NDF was determined by NIRS further raises suspicions that underlying problems existed when using NIRS to determine IV d NDF in corn silage.

Finally, we explored using NIRS to simply predict IV d OM (Model 13, Table 3) and evaluated the relationship to a SM TDN as determined by laboratory methods. The NIRS predicted IV d OM in corn silage with relative efficiency ($R^2 = 0.88$; SEC = 1.28% DM), (Table 2). The NIRS-predicted IV d OM was related to SM TDN with a COD of $r^2 = 0.83$. These data suggest corn silage TDN estimation via NIRS could be facilitated by a simple IV d OM NIRS equation with first-order conversion to TDN (Model 13, Table 3). This alternative method of predicting forage TDN has advantages and disadvantages. The principal advantage is only one NIRS calibration is needed as compared with seven required of SM to estimate TDN. The disadvantage is the first-order equation to convert IV d OM to TDN will be dependent on absolute values of IV d OM, which could vary from laboratory to laboratory (Weiss, 1994).

The effect of analytical method on TDN estimation as evaluated by the relationship between legume-grass silage SM TDN and IV d OM is presented in Table 4. Although numbers differ, fundamental issues associated with the effect of analytical method (laboratory vs NIRS) on legume-grass silage TDN estimation are nearly identical to those presented for corn silage. The SM (Model 5, Table 4) was superior in predicting TDN content of legume-grass silage as compared with empirical Models 1 to 4. Using NIRS to predict all SM nutrients (Model 6) resulted in a loss in the ability to estimate TDN content of legume-grass silages as evaluated by the TDN IV d OM relationship. Similar to corn silage, difficulty of NIRS to predict ash and IV d NDF in legume-

TABLE 4. The effect of analytical method and TDN model on the relationship between legume-grass silage TDN and in vitro digestible OM (IV d OM).

Model	Forage TDN model	Analytical method of model components ^a			Dependent variable	r ²	Intercept	Slope	SE
		Laboratory	NIRS	(% DM)					
1	Adams, 1980	ADF	—	IV d OM	0.61	27.3	0.70	3.3	
2	Rohweder et al., 1978	ADF	—	IV d OM	0.61	1.8	1.06	3.3	
3	Adams, 1980	—	ADF	IV d OM	0.49	30.9	0.64	3.8	
4	Rohweder et al., 1978	—	ADF	IV d OM	0.50	7.4	0.98	3.8	
5	NRC, 2001	CP, ADF CP, NDF, NDF CP, IV d NDF, ash, fat	—	IV d OM	0.90	17.4	0.88	1.7	
6	NRC, 2001	—	CP, ADF CP, NDF, NDF CP, IV d NDF, ash, fat	IV d OM	0.69	11.5	0.85	3.0	
7	NRC, 2001	CP, ADF CP, NDF, NDF CP, IV d NDF, ash	IV d NDF, ash, fat	IV d OM	0.90	11.2	0.87	1.7	
8	NRC, 2001	CP, ADF CP, NDF, NDF CP, IV d NDF, fat	fat	IV d OM	0.82	11.6	0.87	2.2	
9	NRC, 2001	CP, ADF CP, NDF, NDF CP, ash, fat	ash	IV d OM	0.78	17.8	0.74	2.7	
10	NRC, 2001	CP, ADF CP, NDF, IV d NDF, ash, fat	IV d NDF	IV d OM	0.90	10.8	0.88	1.7	
11	NRC, 2001	CP, ADF CP, NDF CP, IV d NDF, ash, fat	NDF CP	IV d OM	0.87	17.2	0.77	2.0	
12	NRC, 2001	CP, NDF, NDF CP, IV d NDF, ash, fat	NDF	IV d OM	0.89	11.8	0.87	1.7	
13	IV d OM	—	ADF CP	IV d OM	0.78	-7.4	1.06	2.8	

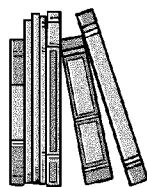
^aIV d NDF = In vitro digestible NDF; NIRS = near-infrared spectroscopy.

grass silages (Table 2) lead to the greatest negative influence on TDN estimation (Models 8, 9; Table 4). Similarly, because of their small influence on legume-grass TDN prediction of fat, ADF CP and NDF CP by NIRS had no major deleterious effect on TDN estimation (Models 7, 10, 12). Correspondingly, because NDF of legume-grass silages was well predicted by NIRS (Table 2), inclusion of NIRS NDF in the SM had no major effect on TDN estimation (Model 11). Finally, direct NIRS prediction of IV d OM in legume-grass silages by NIRS and first-order conversion to a SM TDN (Model 13) resulted in a better TDN to IV d OM relationships as compared with predicting all seven SM nutrients via NIRS (Model 6).

Implications

The following conclusions were made from our study. First, as compared to IV d OM, SM were superior in estimating the TDN content of corn and legume-grass silages vs compared to empirical models. Second, using NIRS to determine all nutrients required of SM lead to some erosion in the ability of SM to estimate TDN content of corn and legume-grass silages. Finally, of all SM nutrients, the inability or uncertainty of NIRS to determine ash and IV d NDF content of corn and legume-grass silage had the most deleterious effect on TDN estimation. It is our conclusion that NIRS can be used to derive CP, NDF, ADF CP, NDF CP, and fat for use in SM to estimate TDN either because NIRS prediction is good or because the particular nutrient contribution to TDN is small. However, because of their large contribution to TDN and NIRS determination uncertainty, ash and IV d NDF should be determined by laboratory methods to facilitate the best prediction of forage TDN when SM are used. Finally, we observed good utility in using NIRS to directly predict IV d OM and converting the absolute IV d OM values to TDN using a simple first-order equation. Some caution is advised,

however, because the first-order equation used to convert IV d OM to TDN is not a universal equation and would be dependent on the IV d OM procedures and absolute values of IV d OM from a given laboratory.



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