Mid-Infrared and Near-Infrared Calibrations for Nutritional Parameters of Triticale (*Triticosecale*) and Pea (*Pisum sativum*)

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The objective of this study was to develop Fourier transformed mid-infrared (MidIR) and near-infrared (NIR) calibrations for acid detergent fiber (ADF), neutral detergent fiber (NDF), and total nitrogen in triticale, peas, and triticale/pea mixtures. Heterogeneous calibration–validation combinations were also tested for calibration quality. The forage samples were collected from forage plots grown following millet or wheat. Other factors included population density, forage mixtures, and nitrogen fertilizer rate. Total N always achieved a better validation R^2 than ADF and NDF, regardless of the sample set or spectral range. The ADF and NDF could not be predicted well with heterogeneous calibration/validation sets, with the exception of ADF predicted by the pea/triticale mixture in the MidIR. Using whole sample sets resulted in better predictive calibrations for the fiber analytes for both the MidIR and the NIR. This study shows that MidIR compares well with NIR for the development of ADF and total N calibrations in forages. The NIR and MidIR are both useful as quick methods for measuring total N, and they show promise for measuring ADF and total N in forage samples, but performance with NDF was less satisfactory.

KEYWORDS: Diffuse reflectance; ADF; NDF; nitrogen; Fourier transformed; infrared; mid-infrared; near-infrared; NIR; FTIR

INTRODUCTION

The successful adoption of a forage for animal husbandry requires knowledge of the nutritional quality of the forage for maintaining animal health. This then requires forage quality analysis and monitoring for proper feed rationing development. Forages will vary greatly in chemical composition and nutritional value (*1*). Forage quality is mainly determined by its dry matter, fiber, crude protein, and energy content (*2*). Forage testing laboratories analyze total nitrogen, acid detergent fiber (ADF), and neutral detergent fiber (NDF) to calculate a series of important nutritional parameters. For example, the total nitrogen content of a forage is used to calculate the available crude protein, which indicates the amount of amino acids available for energy and growth.

Both the ADF and the NDF determinations rid the sample of proteins, soluble carbohydrates, and lipids. The ADF is the cell wall material that is insoluble in an acid detergent solution and is composed mainly of cellulose and lignin. The ADF has an inverse relationship with the total digestible nutrients, net energy for lactation, net energy for maintenance, and net energy for gain. The NDF consists of the indigestible and slow-to-digest fiber portion of the feed, composed of the ADF fraction plus the hemicellulose-like constituents. The NDF is used to estimate the

potential dry matter intake or the amount of forage that can be consumed by an animal before the rumen is full. For example, dairy cows can consume up to 1.2% of their body weight in NDF per day (*3*). The relative feed value is an additional hay quality index calculated from the ADF and NDF that indicates how well the material will be consumed and digested.

The value of near-infrared diffuse reflectance spectroscopy (NIR) for predicting the nutritive value of forage species was first reported by Norris et al. (*4*). Since then, NIR has become a common method for quantitative analysis of protein and fiber in plant material (*5*). However, diffuse reflectance Fourier transformed mid-infrared spectroscopy (MidIR) has yet to be commonly adopted for the analysis of agricultural products because of the notion that materials need to be diluted with KBr to avoid specular reflection and spectral distortion (*6*). Parameters are frequently optimized to achieve a linear relationship between band intensities and analyte concentration. However, radiation that has been reflected from the front surface of the sample can cause nonlinear responses. These specular reflections are rarely observed in the NIR. Few research studies of nondiluted grain samples have compared NIR and MidIR directly, but MidIR can perform as well or better than NIR (*7–11*). Recently, the fatty acid composition was predicted using MidIR and NIR, which is another important nutritional aspect of forages (*11*). An additional advantage of MidIR over NIR is that while NIR spectra may seem featureless and hard to interpret, the MidIR is rich in

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information with specific bands that are attributable to different chemical compounds or functional groups.

The conventional approach for NIR analysis is to develop a broadly applicable equation that can be used to predict other similar samples, thus minimizing the time spent on further calibration development (*12*). Recently, Foster et al. (*13*), and Calderón et al. (*11*) demonstrated that both the NIR and the MidIR can be used to quantify fatty acids in a heterogeneous set of forage samples, including triticale. Both studies showed that one set of calibrations developed with different forage species was able to predict fatty acids of individual forages. Snyman and Joubert (*14*) successfully calibrated for protein, ADF, and NDF in triticale—oat—fescue mixtures using NIR data. However, no studies to date have tried to relate MidIR spectra to fiber composition in triticale, and the performance of MidIR vs NIR remains unknown. It is a commonly held opinion that reliable infrared spectroscopic calibrations are based in a homogeneous and representative set of calibration samples. However, work with soils (*15*) and forages (*13, 11*) has shown that it is possible to build robust calibrations with heterogeneous sample sets as long as they adequately bracket the variability in the target samples. It follows then that it would be most convenient if a single calibration could be developed for mixed crops such as the aforementioned legume—triticale systems, as opposed to having to develop separate calibrations for the legume and triticale.

The objectives of this study were to (1) test if a calibration developed using triticale samples could be used to quantitatively predict the ADF, NDF, and total N contents of pea samples and vice versa and (2) compare MidIR with the more commonly used NIR in terms of calibration performance for ADF, NDF, and total N.

MATERIALS AND METHODS

Safety. Operators of Fourier transform infrared spectrometers need to exercise caution to avoid looking at the laser unit because it can cause serious injury to the eyes. Forage fiber analysis utilizes solvents as well as strong acids. Use a fume hood when handling solvents and avoid inhalation or skin contact by wearing a lab coat, safety glasses, and gloves. A lab coat, acid resistant gloves, and safety glasses should be used when handling sulfuric acid. Always add sulfuric acid to water and not water to concentrated sulfuric acid. If acid contacts the skin, rinse with abundant amounts of water.

Description of the Study Site. The study was located at the Central Great Plains Research Station, Agricultural Research Service, U.S. Department of Agriculture (45° 09′ N, 103° 09′ W, altitude 1384 m), 6.5 km east of Akron, CO, on a Weld silt loam soil (fine, smectitic, mesic Aridic Paleustoll). The average annual precipitation for the site has been 419 mm over 100 years. The field part of the study was carried out in the 2005/2006 and 2006/2007 growing seasons. Varieties used were NE422T winter forage triticale (*16*) and Austrian winter field peas (*Pisum sativum* subsp. *arvense*).

Experiment Design. To achieve a robust set of calibrations, the field experiments included different agronomic treatments that encompassed common variations likely to be encountered in future triticale/pea crops. The study was planted in fields that had millet or separate fields that had wheat the previous year. This resulted in two different crop residue conditions at planting and introduced variables including starting soil—water and residue levels. At each crop residue site, there were experiment plots that included three factors: seeding rate/species, planting date, and fertilizer rate. For each crop residue treatment, there were the following treatments: seeding rate, species and fertilizer rate. There were four levels of seeding rate/species treatments: (1) 45 kg ha⁻¹ of triticale, (2) 90 kg ha⁻¹ of triticale, (3) 90 kg ha⁻¹ of peas, and (4) 45 kg ha⁻¹ of triticale mixed with 45 kg ha⁻¹ of peas. The fertilizer treatments applied were no fertilizer added and 68 kg ha⁻¹ applied as ammonium nitrate with a drop spreader. The experimental design was a randomized complete block with a split plot

arrangement. The seeding rate/species was the main plot, while the fertilizer treatment was the subplot. There were a total of 96 samples per seeding rate/species treatment, and 192 samples per fertilizer or residue treatment. The total number of samples in the data set was 384, but 383 were used in the calibrations due to a single missing sample. The whole plots measured 4.6 m by 30.5 m, and the subplots measured 4.6 m by 15.25 m. The plots were planted with a single disk drill model 750 (John Deere, Moline, IL) with the openers set at 19 cm row spacing. Seventeen kg ha⁻¹ of phosphorus (P) as monoammonium phosphate (11-52-0) was applied at planting to all plots.

Field Sampling. Areas measuring 1 m² were randomly selected for biomass sampling. The growth stage for the primary sampling date was heading 1.2 complete for the triticale and flowering for the peas. Each biomass sample was cut at ground level and collected in a bag. The samples were then dried at 60 °C for 2—4 days until weight loss ceased. The samples were ground using a Wiley mill (Thomas-Wiley, Swedesboro, NJ) through a 2 mm screen and then using a Retsch grinder (Retsch GmbH, Haan, Germany) through a 0.5 mm screen before analysis.

NDF, ADF, and Total Nitrogen Analysis. The NDF, ADF, and total nitrogen were analyzed by the Soil—Water—Plant Laboratory at Colorado State University. The ADF was determined from 0.5 g samples using the ADF method 4.1, determination of ADF by refluxing (*17*). The NDF was quantified on 0.5 g samples with the NDF method 5.1, determination of amylase NDF by refluxing (*17*). Total nitrogen was determined on 0.1 g samples using the nitrogen (crude protein) method 3.3, nitrogen determination by combustion method (*17*). The range of NDF was 183.7—847.7 g kg⁻¹, the range of ADF was 130.4—537.3 g kg⁻¹, and the total N range was 4.3—43.7 g kg⁻¹. To document the precision of the wet chemistry analyses, we analyzed two triticale and two pea samples, each one in triplicate. Each one of the triplicate assays was carried out from the same homogenized sample. The percent relative standard deviation for the samples, calculated as 100 × (standard deviation) × (mean)⁻¹ was, on average, 4.9 for NDF, 6.0 for ADF, and 5.2 for total N.

Spectroscopy. All of the samples were composed of the aboveground biomass of triticale at the heading stage and/or the peas at the flowering stage. The spectra of the dried and ground samples were obtained in diffuse reflectance mode using a Pike AutoDIFF autosampler (Pike Technologies, Madison, WI) in line with a Digilab FTS 7000 Fourier Transform spectrometer (Varian, Inc., Palo Alto, CA). Sulfur and KBr were used as background samples for the NIR and MidIR, respectively. A lead selenide detector and a quartz beam splitter were used for the NIR range, and a deuterated triglycine sulfate detector and KBr beam splitter were used for the MidIR. To minimize noise, the data were collected as 64 coadded scans per spectrum. Resolution was set at 4 cm⁻¹ from 10000 to 4000 cm⁻¹ for the NIR and from 4000 to 400 cm⁻¹ for the MidIR. Each NIR spectrum contained 3113 data points, and each MidIR spectrum contained 1868 data points. **Figure 1** shows the average MidIR and NIR spectra of all of the forage samples.

Multivariate Analyses and Calibrations. Spectra were examined qualitatively using the Principal Components Analysis feature of the PLSPlus software in GRAMS/AI 7.02 (Thermo Galactic, Salem, NH). A Mahalanobis distance analysis of the spectral residuals showed that only a few samples (a maximum of two) for each of the different factors and the different spectral ranges were identified as outliers (data not shown). We chose to include all samples in our calibrations.

Data pretreatments during calibration development are necessary to obtain the best information possible from the spectra while removing spectral variation unrelated to analyte composition, such as spectral responses to particle size. Reeves and Delwiche (*18*) designed a program using SAS (SAS, Institute, Inc., Cary, NC), which can test various combinations of derivatives and scatter corrections (*18, 19*). This program identifies the best calibrations according to the best pretreatments, while avoiding overfitting. The program minimizes the number of pretreatments to the most useful for the calibration procedure and then uses the best pretreatments for performing partial least-squares (PLS) regression calibrations. This technology permits the rapid testing of thousands of possible calibrations and identifies the best calibrations with the highest R^2 and smallest root mean squared deviation (RMSD). The RMSD, also known as the standard error of prediction, is the standard deviation of the residuals due to differences between the wet chemistry values and the predictions using spectral data. It is used as a measure of the predictive
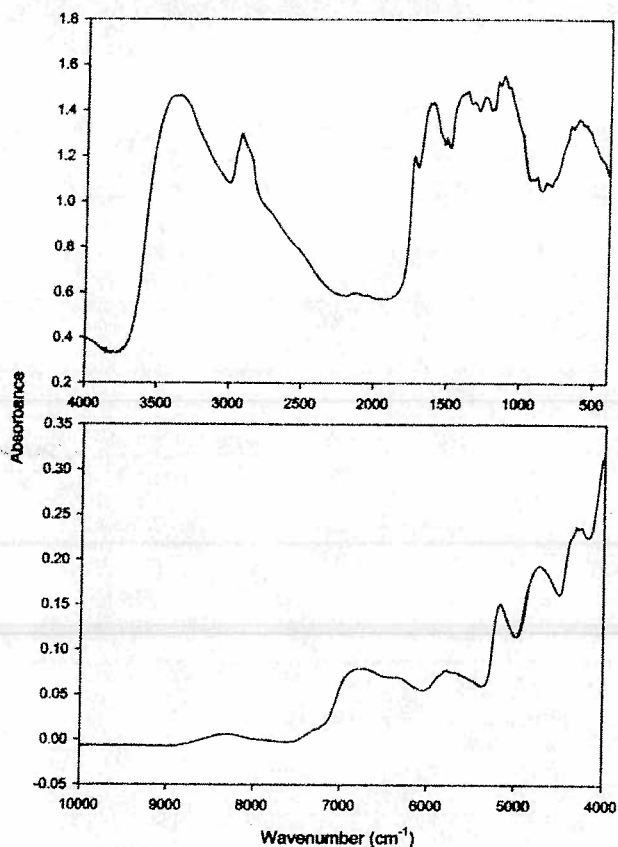
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Figure 1. Average mid-infrared spectrum (top) and near-infrared spectrum (bottom) of the forages; $n = 383$.
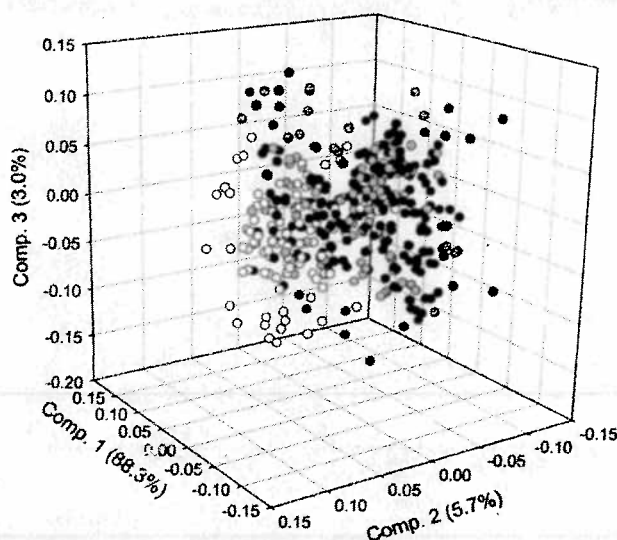

Figure 2. PCA results of the mid-infrared spectra of the forage samples, coded by forage species. The triticale samples are in black, the triticale/pea mixtures are in gray, and the pea samples are in white. The variance accounted by each component is in parentheses.
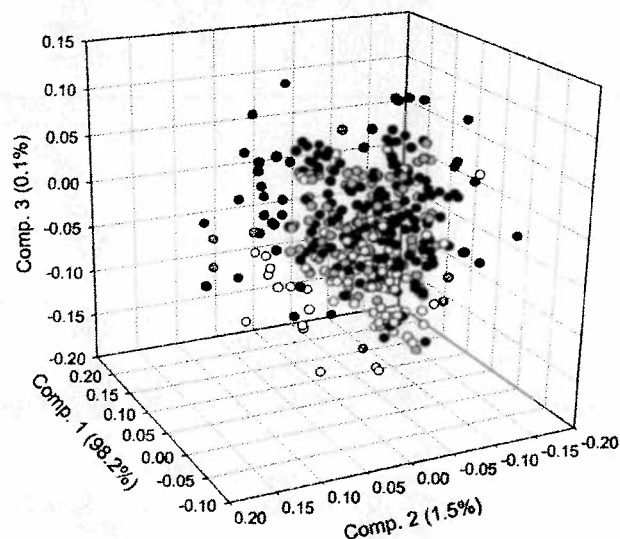

Figure 3. PCA results of the near-infrared spectra of the forage samples, coded by forage species. The variance accounted by each component is in parentheses. The triticale samples are in black, the triticale/pea mixtures are in gray, and the pea samples are in white.

power of a calibration model. All spectra were variance-scaled and mean-centered before the PLS analysis.

We carried out four types of calibrations according to the type of sample used in the calibration set. The results from the different calibrations allowed us to determine how the best possible results compare for calibrations using all of the samples or specific sets. The first set of calibrations was done with all 383 samples (the whole sample set) as one calibration and no validation set. The next three calibration types are the heterogeneous calibration/validation sets, in which a different kind of sample was used to develop the calibration from the type of sample that was used in the validation. The second type of calibration was done using the mixed pea/triticale samples to predict the rest of the samples of pure triticale and pure pea samples (95 calibration and 288 predicted samples). In the third set of calibrations, the pure pea samples were used to calibrate for the triticale and the triticale/pea mixtures (96 calibration and 287 predicted samples). The fourth type of calibration was done using the pure triticale samples as the calibration set and the rest of the pure pea samples and mixed pea/triticale samples as the validation set (192 calibration and 191 predicted).

Analysis of Variance (ANOVA). To determine significant effects on the fiber and total N, we performed ANOVA with the proc GLM procedure of SAS version 9.2. Forage type, stubble, and nitrogen rate were the fixed effects, and the blocks in the randomized complete block design were the random effects. Mean separations were determined with the least significant difference (LSD) according to a Student's t test.

RESULTS AND DISCUSSION

Principal Components Analysis (PCA) of the Spectral Properties of the Whole Sample Set. The PCA of both the NIR and MidIR shows that the pea and triticale sample set forms a cohesive pattern with no distinct groupings (**Figures 2** and **3**). The pea samples tend to group closer to each other than to the triticale and the mixed samples on both the MidIR and the NIR, but they do not form separate clusters. The spectral properties of the pea/triticale mixture are indistinguishable from the pure triticale. The level of nitrogen fertilizer, the population density, or the type of residue did not affect the distribution of the samples in the PCA of either the NIR or the MidIR spectral ranges, suggesting that these management and environmental parameters do not have a large influence on the MidIR and NIR spectral properties of the samples (data not shown). The PCA results suggest that the sample set as a whole forms a cohesive set of samples amenable to calibration development.

Agronomic Effects on the Constituents. The agronomic treatments had significant effects on the chemical composition of the forage samples. This has the desirable effect of diversifying the composition of the sample chemistry for the spectroscopic calibrations. The addition of N fertilizer resulted in a significant increase in total N content. The plots receiving 68 kg ha^{-1} had a

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Table 1. Average NDF, ADF, and Total Percent N of the Forage Samples in the Added N and 0 N Treatments[a]

added N	NDF	ADF	total N
0 kg ha⁻¹	445.6 (7.5)	307.9 (5.9)	17.3 (0.5)
68 kg ha⁻¹	442.0 (7.7)	304.0 (5.7)	20.9 (0.6)
LSD	16.3	15.1	1.4

[a] All data are in g kg⁻¹. The samples were averaged across residue type and forage type. $n = 191$ for the 0 N treatment and 192 for the 68 kg ha⁻¹ treatment. The standard error of the mean is in parentheses. The LSDs for the means according to a Student's t test are shown ($P < 0.05$).

Table 2. Average NDF, ADF, and Total Percent N of the Forage Samples Planted in Millet or Wheat Residue[a]

residue type	NDF	ADF	total N
millet	442.4 (7.2)	308.4 (5.8)	17.9 (0.5)
wheat	445.1 (7.9)	303.4 (5.7)	20.3 (0.6)
LSD	16.2	15.1	1.4

[a] All data are in g kg⁻¹. The samples were averaged across fertilizer treatment and forage type. $n = 191$ for the wheat treatment and 192 for the millet treatment. The standard error of the mean is in parentheses. The LSDs for the means according to a Student's t test are shown ($P < 0.05$).

Table 3. Average NDF, ADF, and Total Percent N of the Forage Samples According to Forage Type[a]

forage type	NDF	ADF	total N
pea and triticale mixture	474.5 a (7.8)	317.0 a (8.1)	19.0 a (0.8)
pea	327.2 b (9.6)	256.3 b (7.2)	23.7 c (0.7)
triticale, high population	487.0 a (6.7)	329.4 a (7.3)	16.1 b (0.7)
triticale, low population	486.8 a (8.4)	321.2 a (8.0)	17.7 ab (0.8)

[a] All data are in g kg⁻¹. The samples were averaged across fertilizer treatment and residue type. $n = 95$ for the pea and triticale mixture, and $n = 96$ for the rest of the forage treatments. The standard error of the mean is in parentheses. Means not sharing a letter within a column are significantly different according to a Student's t test ($P < 0.05$).

21% increase in total N relative to the plots receiving no fertilizer (**Table 1**). The addition of fertilizer did not affect the ADF or NDF content of the forages. The preceding crop's residue also had a significant effect on the total N content but no effect on the ADF or the NDF (**Table 2**). The forages planted on the wheat residue had 13% higher total N content than those planted on the millet residue (**Table 2**). We hypothesize that the lower total N after the millet crop was due to higher residual N levels at planting in the wheat stubble than the millet stubble (data not shown). It is possible that there might have been greater N tied up via immobilization with the millet residue than with the wheat residue. A comparison of the different forage species and mixtures shows that peas had significantly less NDF and ADF and up to 47% more total N than the triticale and the triticale—pea mixture (**Table 3**). The population level also caused differences in the total N content of the triticale, with the triticale grown under high population having less total tissue N than the triticale grown under low population (**Table 3**), possibly because the reduced number of plants per unit area allowed for a higher fertilizer N per plant. The pea and triticale mixture had a significantly higher total N content than that of the triticale high population and a higher, albeit nonsignificant difference, to the triticale low population.

Overall, our results indicate that NDF and ADF are not responsive to fertilizer, residue, or population management practices in this pea/triticale system, but the fiber levels can be reduced by including the pea in the forage. Total N

(and consequently crude protein) can be increased by the addition of fertilizer, by managing the residue left by the previous crop, and by reducing the population levels at planting.

Calibration Results Using Triticale Spectra as a Calibration Set To Predict the Rest of the Samples. Tables 4 and 5 show the calibration quality indicators for the NIR and MidIR data. The MidIR performed slightly better than NIR in the ADF, NDF, and total N constituents as indicated by the calibration R^2 (CALR2). However, calibrations for NDF were poor for both the NIR and the MidIR, with validation R^2 (VALR2) below 0.60. Calibrations for ADF were not acceptable, with VALR2 of 0.70 and 0.76 with the NIR and MidIR data, respectively. Acceptable calibrations for total N were achieved with the NIR and MidIR data, with a VALR2 of 0.86 and 0.88 (**Tables 4 and 5 and Figure 4**). We used the Reeves and Delwiche (*18*) program to select the best combinations of pretreatments and number of factors for the PLS model. **Tables 4 and 5** show the best derivative, gap for derivative, scatter correction pretreatments, as well as the number of factors for each calibration.

Results Using Pea Data as a Calibration Set To Predict the Rest of the Samples. The CALR2 values of the NIR were better than those of the MidIR when predicting for NDF and total N, but the ADF calibrations were good for both spectral ranges with CALR2 of 0.92—0.94 (**Tables 4 and 5**). The VALR2 values, however, were very poor for ADF and NDF regardless of the spectral range. Calibrations for total N achieved VALR2 values of 0.82 and 0.84 for MidIR and NIR, respectively. It is possible that the difference in the range of the pea analyte values relative to the rest of the samples could have limited the performance of these calibrations.

Results Using the Pea/Triticale Mixtures Data as a Calibration Set To Predict the Rest of the Samples. The CALR2 values show that the NIR was better than the MidIR for NDF and ADF, while calibrations for total N were very good for both spectral ranges with CALR2 values of 0.97 and 0.98, even better than using all samples as one calibration (see below). The VALR2 values were poor for ADF and NDF, resulting in relatively important differences between actual and predicted values (**Figure 4**). Total N achieved a VALR2 of 0.87 with the MidIR data, which was better than the VALR2 of 0.78 achieved with the NIR data (**Tables 4 and 5**).

Results Using All Samples as One Calibration. The calibrations developed with the NIR data resulted in very similar CALR2 values than those developed with the MidIR data (**Tables 4 and 5**). The CALR2 values for the fiber constituents were unacceptable regardless of the spectral range used for the calibration, with the possible exception of the NIR calibration of ADF, which achieved a CALR2 of 0.87 (**Table 4 and Figure 5**). Total N achieved a CALR2 of 0.94 with the MidIR data, which was the highest CALR2 achieved in both spectral ranges with the whole sample set as calibration set (**Figure 5**).

Overall Calibration Observations and Conclusions. The VALR2 values show that total N always achieved a better predictive value than ADF and NDF, regardless of the sample set or spectral range used to develop the calibrations (**Tables 4 and 5**). In turn, the ADF usually achieves better calibrations than the NDF as indicated by the VALR2, and this is true for both NIR and MidIR. In general, the ADF and NDF cannot be predicted well with heterogeneous calibration/validation sets, with the exception of ADF predicted by the pea/triticale mixture in the MidIR, which achieved a VALR2 of 0.82. Similarly, Minson et al. (*20*) found that NIR often gave biased estimates of the nutritive value of tropical forages when equations were used on sample populations not included in the calibration set. The NDF has an overall unacceptable VALR2 when heterogeneous calibration sets are

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Table 4. Quality Indicators of Calibrations Developed with Near-Infrared Spectra for NDF, ADF, and Total Nitrogen Content (Total N)[a]

analyte	DER	MSC	GAP	factors	RMSD	VRMSD	CALR2	VALR2
using triticale as a calibration set to predict the rest of the samples (192 calibration and 191 predicted)								
NDF	2nd	STR	4	2	4.84	10.25	0.57	0.47
ADF	2nd	MSC	8	3	2.85	4.42	0.85	0.70
total N	2nd	MSC	16	3	0.23	0.32	0.90	0.86
using pea spectral data as a calibration set to predict the rest of the samples (96 calibration and 287 predicted samples)								
NDF	2nd	STR	2	4	2.44	10.95	0.93	0.20
ADF	1st	STR	8	5	1.99	4.25	0.92	0.75
total N	1st	MSC	16	5	0.15	0.59	0.95	0.84
using the pea/triticale mixtures data as a calibration set to predict the rest of the samples (95 calibration and 288 predicted samples)								
NDF	2nd	STR	16	3	2.75	7.02	0.87	0.65
ADF	2nd	STR	4	3	2.41	5.05	0.90	0.63
total N	2nd	STR	8	3	0.14	0.36	0.97	0.78
all samples as one calibration (383 samples)								
NDF	1st	MSC	4	3	4.86	N/A	0.78	N/A
ADF	2nd	STR	4	5	2.84	N/A	0.87	N/A
total N	2nd	STR	16	4	0.24	N/A	0.91	N/A

[a] DER, derivative (1st, 2nd, or none); MSC, scatter correction (STR, no correction or straight spectra; MSC, multiplicative scatter correction); GAP, number of gap for derivative; FACTORS, number of factors; RMSD, root mean squared difference; and VRMSD, root mean squared difference for the validation set.

Table 5. Quality Indicators of Calibrations Developed with Mid-Infrared Spectra for NDF, ADF, and Total Nitrogen Content (Total N)[a]

| analyte	DER	MSC	GAP	factors	RMSD	VRMSD	CALR2	VALR2
using triticale as a calibration set to predict the rest of the samples (192 calibration and 191 predicted)								
NDF	1st	MSC	1	2	4.78	12.11	0.59	0.38
ADF	1st	MSC	1	3	2.73	4.00	0.87	0.76
total N	1st	MSC	2	4	0.19	0.32	0.94	0.88
using pea spectral data as a calibration set to predict the rest of the samples (96 calibration and 287 predicted samples)								
NDF	2nd	MSC	1	2	4.29	6.04	0.79	0.53
ADF	2nd	MSC	32	7	1.74	6.93	0.94	0.62
total N	2nd	MSC	2	3	0.21	0.36	0.89	0.82
using the pea/triticale mixtures data as a calibration set to predict the rest of the samples (95 calibration and 288 predicted samples)								
NDF	2nd	STR	1	1	3.95	7.20	0.73	0.65
ADF	2nd	MSC	4	2	3.21	3.41	0.83	0.82
total N	2nd	STR	16	6	0.11	0.28	0.98	0.87
all samples as one calibration (383 samples)								
NDF	2nd	STR	4	3	4.63	N/A	0.80	N/A
ADF	1st	STR	1	4	3.09	N/A	0.85	N/A
total N	2nd	MSC	8	6	0.20	N/A	0.94	N/A

[a] DER, derivative (1st, 2nd, or none); MSC, scatter correction (STR, no correction or straight spectra; MSC, multiplicative scatter correction); GAP, number of gap for derivative; FACTORS, number of factors; RMSD, root mean squared difference; and VRMSD, root mean squared difference for the validation set.

used, never achieving a VALR2 higher than 0.65 in either the MidIR or the NIR. Furthermore, the NDF, with the exception of peas as the calibration set in the NIR, has a low CALR2, underscoring the difficulty for the development of NDF calibrations with this sample set.

Our data indicate that to obtain a reasonably good predictive calibration for the fiber analytes, it is better to use the whole sample set in the calibration, and this is true for both the MidIR and the NIR (**Tables 4** and **5**). Using the full sample set, the MidIR had slightly better CALR2 than the NIR for NDF and total N, while the NIR did slightly better for ADF. Snyman and Joubert (*14*) also found that NDF did not calibrate as well as crude protein and ADF using the NIR. In their study, they found that crude protein, with R^2 ranging from 0.92 to 0.96, achieved better calibration quality than ADF, which in turn had better R^2

than NDF across a variety of forage species. The NDF quality may vary between different forages because of compositional differences in the cell walls. Han et al. (*21*) show that the NDF in different grass populations can vary because of different lignin contents in the cell walls, because lignin is part of the NDF. This means that calibrations for NDF may be difficult because we are in effect trying to calibrate not for one chemical entity but for a diverse set of chemical entities. It is important to note, however, that vibrational spectroscopy has been used to determine complex chemical parameters in forages. We hypothesize that good NDF calibrations were difficult to obtain in this project not only because of the complex nature of NDF but also because the NDF quality might have varied between the different forage types. While the ADF and NDF assays have a similar relative error in the analytical procedure, the NDF error terms should be
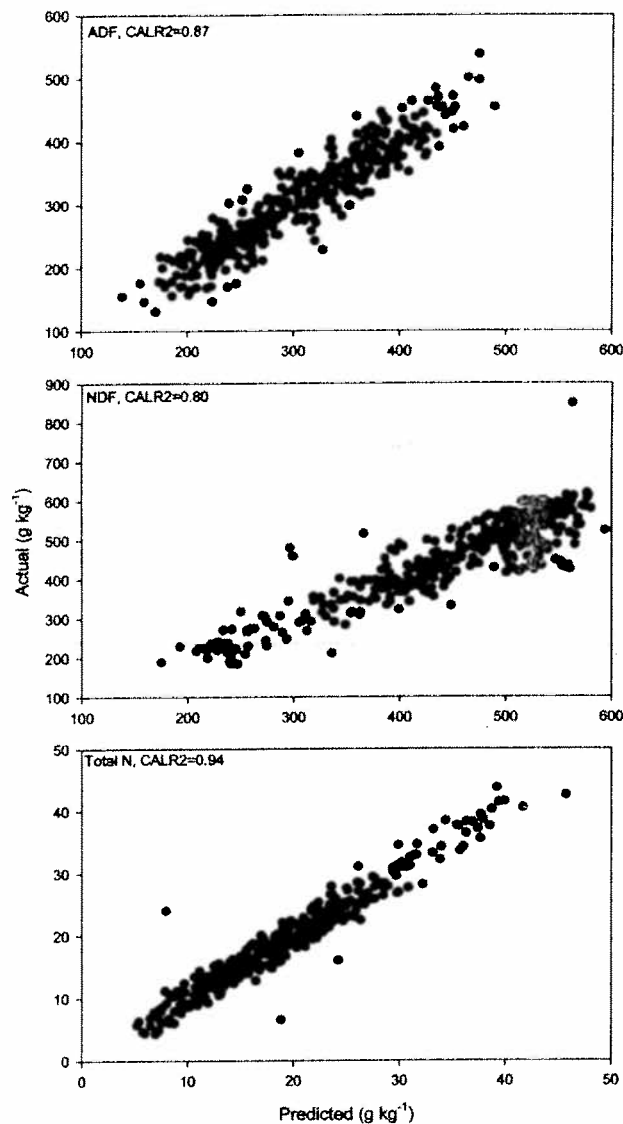
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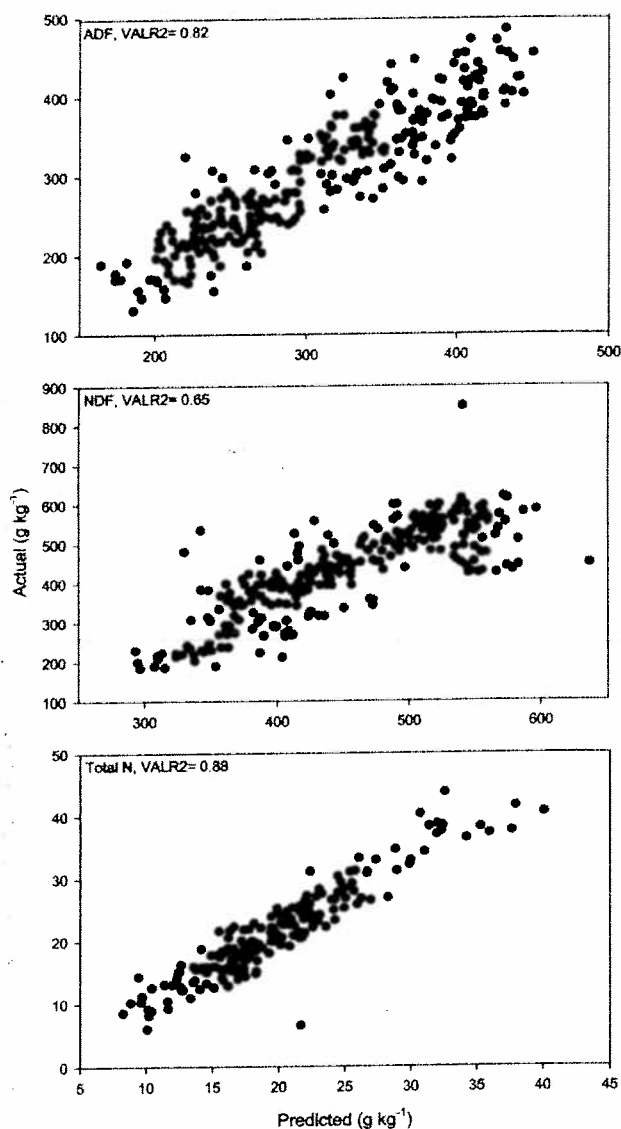
Figure 4. Selected actual vs predicted values for ADF, NDF, and total N. The calibrations were developed with pea/triticale mixtures spectra for ADF and NDF and with triticale spectra for total N. The validation set samples were different from the calibration set. The ADF calibration was developed with mid-infrared data, the NDF calibration was developed with near-infrared data, and total N calibration was developed with mid-infrared data.

Figure 5. Actual vs predicted values for ADF, NDF, and total N. All samples were used as one calibration (n = 383 samples). The near-infrared spectra were used to calibrate for the ADF, while the mid-infrared spectra were used for the NDF and total N. CALR2, calibration R^2.

somewhat better because the error promulgates into the ADF during the sequential chemical extractions. However, we hypothesize that the ADF fiber is more likely to be similar across species as almost all of the hemicellulose and any cell wall proteins have been removed during the analysis.

The total N was less sensitive to sample heterogeneity in the calibration sets, and our data show that a calibration built on a smaller set of pea/triticale mixture samples is adequate for calibrating and predicting total N. The calibration for total N with triticale NIR data for the calibration set yielded a VALR2 of 0.86 (**Table 4**), and using the pea/triticale mixture and the triticale alone for the calibration set on the MidIR yielded VALR2 values of 0.87 and 0.88, respectively (**Tables 4** and **5**). Using the MidIR spectra of the pea/triticale mixtures yielded the highest CALR2 of the experiment of 0.98 (**Table 5**). The calibrations with heterogeneous samples for total N with MidIR did slightly better than NIR as given by the VALR2, which averaged 0.83 for the NIR

and 0.86 for the MidIR, mainly due to the better performance of the MidIR with the pea/triticale mixtures.

This is the first report of the use of the MidIR range to predict fiber and nitrogen quality in triticale. In addition, this work shows how the MidIR compares with NIR in terms of calibration performance for forage quality. This is important because while NIR is a more established technology, the MidIR can be used as a research tool for spectral interpretation. This research also shows that a single calibration can perform well for pea and triticale samples, and we illustrate how whole sample sets are usually better than heterogeneous sets for the development of calibrations for fiber and nitrogen in both the MidIR and the NIR. Finally, we confirm what has been shown by others, that calibrations for NDF tend to be problematic, possibly because of the complex chemical nature of NDF.

ABBREVIATIONS USED

ADF, acid detergent fiber; CALR2, calibration R^2; MidIR, Fourier transformed mid-infrared spectroscopy; NDF, neutral

detergent fiber; NIR, Fourier transformed near-infrared spectroscopy; PCA, principal components analysis; VALR2, validation R^2.

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LITERATURE CITED

(1) Murray, I. Near infrared reflectance analysis of forages. In *Recent Advances in Animal Nutrition*; Heresign, W., Cole, D. J. A., Eds.; Butterworths: London, 1986.

(2) Ball, D. M.; Collins, M.; Lacefield, G. D.; Maitin, N. P.; Mertens, D. A.; Olson, K. E.; Putnam, D. H.; Undersander, D. J.; Wolf, M. W. Understanding forage quality. In *Publ. 1-01. Am. Farm Bur. Fed.*; Am. Farm Bur. Fed.: Park Ridge, IL, 2001.

(3) Mertens, D. R. Nonstructural and structural carbohydrates. In *Large Dairy Herd Management*; Van Horn, H. H., Wilcox, C. J., Eds.; Am. Dairy Sci. Assoc.: Champaign, IL, 1992.

(4) Norris, K. H.; Barnes, R. F.; Moore, J. E.; Shenk, J. S. Predicting forage quality by near infrared reflectance spectroscopy. *J. Anim. Sci.* **1976**, *43*, 889–97.

(5) Shenk, J. S.; Workman, J. J.; Westerhaus, M. O.; Burns, D. A.; Ciurczak, E. W. Application of NIR spectroscopy to agricultural products. In *Handbook of Near Infrared Analysis*, 2nd ed.; Taylor & Francis: United States, 2001; Vol. 27.

(6) Reeves, J. B. Mid-infrared diffuse reflectance spectroscopy: Is sample dilution with KBr neccesary, and if so, when? *Am. Lab.* **2003**, *35*, 24–28.

(7) Reeves, J. B., III. Near- versus mid-infrared diffuse reflectance spectroscopy for the quantitative determination of the composition of forages and by-products. *J. Near Infrared Spectrosc.* **1994**, *2*, 49–57.

(8) Reeves, J. B., III. Improvement in Fourier near- and mid-infrared diffuse reflectance spectroscopic calibrations through the use of a sample transport device. *Appl. Spectrosc.* **1996**, *50*, 965–969.

(9) Reeves, J. B., III; Delwiche, S. R. Determination of protein in ground wheat samples by mid-infrared diffuse reflectance spectroscopy. *Appl. Spectrosc.* **1997**, *51*, 1200–1204.

(10) Reeves, J. B., III; Zapf, C. M.; Simovik, I.; Delwiche, S. R. Mid-infrared versus near-infrared diffuse reflectance spectroscopy for quantitative and qualitative analysis of agricultural products. *Recent Res. Dev. Agric. Food Chem.* **1999**, *3*, 201–222.

(11) Calderón, F. J.; Reeves, J. B.; Foster, J. G.; Clapham, W. M.; Fedders, J. M.; Vigil, M. F. Comparison of diffuse reflectance fourier transform mid-infrared and near infrared spectroscopy with grating-based near infrared spectroscopy for the determination of fatty acids in forages. *J. Agric. Food Chem.* **2007**, *55*, 8302–8309.

(12) Smith, K. F.; Flinn, P. C. Monitoring the performance of a broad-based calibration for measuring the nutritive value of two independent populations of pasture using near infrared reflectance (NIR) spectroscopy. *Aust. J. Exp. Agric.* **1991**, *31*, 205–210.

(13) Foster, J. G.; Clapham, W. M.; Fedders, J. M. Quantification of fatty acids in forages by near-infrared reflectance spectroscopy. *J. Agric. Food Chem.* **2006**, *54*, 3186–3192.

(14) Snyman, L. D.; Joubert, H. W. Prediction of the chemical composition and in vitro dry matter digestibility of a number of forages by near infrared reflectance spectroscopy. *Suid-Afrikaanse Tydskrif vir Veekunde* **1993**, *23*, 20–3.

(15) Reeves, J. B., III; Follett, R. F.; Mccarty, G. W.; Kimble, J. M. Can near- or mid-infrared diffuse reflectance spectroscopy be used to determine soil carbon pools? *Commun. Soil Sci. Plant Anal.* **2006**, *37*, 2307–2325.

(16) Baenziger, P. S.; Vogel, K. P. Registration of 'NE422T' Winter Triticale. *Crop. Sci.* **2003**, *43*, 434–435.

(17) Undersander, D.; Mertens, D. R.; Thiex, N. *Forage Analysis Procedures*; National Forage Testing Association: Omaha, NE, 1993.

(18) Reeves, J. B., III; Delwiche, S. R. SAS partial least squares regression for analysis of spectroscopic data. *J. Near Infrared Spectrosc.* **2003**, *11*, 415–431.

(19) Reeves, J. B., III; Delwiche, S. R. Using SAS for PLS calibrations of spectroscopic data. *NIRS News* **2004**, *15*, 10–15.

(20) Minson, D. J.; Butler, K. L.; Grummit, N.; Law, D. P. Bias when predicting crude protein, dry matter digestibility and voluntary intake of tropical forages by near infrared reflectance. *Anim. Feed Sci. Technol.* **1983**, *9*, 221–237.

(21) Han, L. X.; Casler, M. D.; Grau, C. R. Responses to divergent selection for fiber concentration at two disease potentials in smooth bromegrass. *Crop Sci.* **2001**, *41*, 30–39.