Validating Real-Time Assessment of Alfalfa Nutritive Value Using a Portable NIR Unit

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Abstract: Near-infrared spectroscopy (NIRS) is a standard technology in laboratories used to determine forage quality in an affordable and efficient manner compared to wet chemistry analysis. The use of portable NIRS technology has not been thoroughly developed but could potentially be a tool to measure forage quality. The objective of this research was to validate NIRS predictive equations for fresh alfalfa (*Medicago sativa* L.) using a hand-held NIRS device (NIR4 Farm, AB Vista, Marlborough, England). In 2020, alfalfa was harvested from existing stands, sorted by maturity, and chopped to 2.5-cm lengths. Samples were scanned wet after harvesting and after drying using the hand-held NIRS device. Samples were also analyzed using wet chemistry and prediction equations developed. Based on coefficients of determination (R^2) values of .70 and above, it appears the portable NIRS could estimate the concentration of some forage quality components in wet and dried alfalfa. However, based on RPD, a non-dimensional statistic for the evaluation of a NIR calibration equation, the portable NIR is not suitable to routinely predict nutrient constituents of wet or dried alfalfa.

Introduction: Near-infrared reflectance (NIRS) is routinely used in laboratories to predict nitrogen (crude protein, CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and relative forage value (RFV) (Starks et al., 2015). CP, NDF, ADF, and NDF digestibility (NDFD) are commonly used to predict animal performance (Coleman and Moore, 2003). Laboratory or benchtop NIRS is utilized to replace wet chemistry and to determine nutrient concentration, however, the process requires sampling, drying, and delivery of samples to a laboratory with results returned several days after sampling.

In the last decade, the rise of portable or hand-held technology has increased accessibility and efficiency in many sectors, including the agricultural community. Having an accurate and portable NIRS unit would allow for immediate, in-field analysis of forage nutrient composition and would provide a critical piece of information for forage and livestock growers. Currently, morphological development of alfalfa (e.g., 10% bloom; Fick and Mueller, 1989) or days between cuttings (e.g. 28 days) are used to schedule harvest times and estimate the nutrient composition of alfalfa. However, visual appraisal of alfalfa maturity can be challenging, and can be a poor predictor of alfalfa nutrient composition due to seasonal variability and weather impacts on plant growth. Another common approach is to use predictive equations for alfalfa quality (PEAQ) based on maturity and height of the most mature stems to predict RFV (Sanderson, 1992). Although this approach provides a low-cost and rapid method of prediction, numerous samples are needed for improved accuracy, and sampling may be time prohibitive. Having access to in-field, real-time alfalfa nutrient composition values is critical to aid farmers in decision making regarding harvesting, feeding, and marketing alfalfa. Therefore, the objective of this research was to validate nutrient prediction equations for fresh and dried alfalfa using a hand-held NIRS device (AB Vista, Marlborough, England; Figure 1).

Materials and Methods: The experiment was conducted using an alfalfa field at St Paul, MN, in 2020. The alfalfa was harvested from a 1-year-old stand containing 112 0.9-m by 6.1-m plots with multiple cultivars represented. Random 1-m^2 areas were hand-harvested from three of the cultivars ('SW 5511', '54VR10', and '55VR08') using randomly selected replicates. Samples were harvested bi-weekly until alfalfa reached an early seedpod maturity when they were mowed to a 5-cm stubble height and allowed to regrow. This process was repeated four times between June and October and resulted in collection of nearly 200 samples.

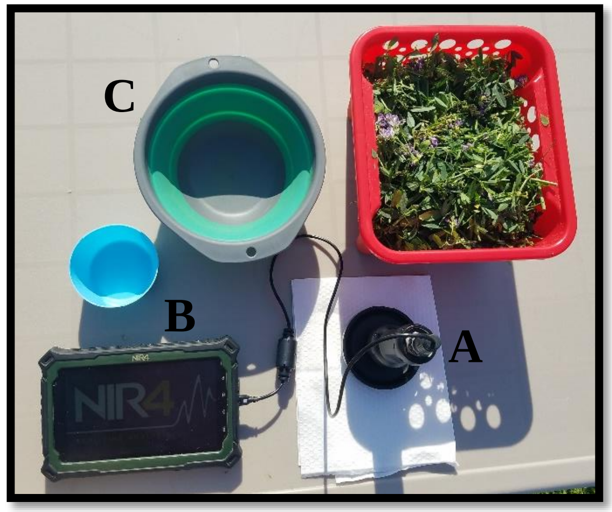

Figure 1: NIR4 Farm hand-held NIR unit (2019 model; A, NIRS probe; B, NIRS tablet; C, scanning bowl). Photo credit: Jessica Prigge, UMN.

Samples were sorted by individual stems for maturity in the field and immediately chopped to 2.5-cm lengths. Samples were then scanned with the hand-held NIRS device using a manufacturer recommended protocol. Specifically, fresh samples were placed in the scanning bowl with a minimum 5-cm depth and scanned six times. Each sample was mixed in the bowl and the NIRS probe was wiped and recalibrated between scans. Data was stored on the tablet provided with the hand-held NIRS device and was instantly shared to a cloud file system accessible to AB Vista, Marlborough, England.

Fresh samples were then dried in a forced-air oven at 60˚C until they maintained a constant mass. Once dry, samples were scanned following the same protocol outlined above. The dried samples were ground through a 5-mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) followed by a 1-mm screen in a Cyclotec (Foss, Hillerød, Denmark). After grinding, samples were thoroughly mixed and scanned (Figure 2). Samples were also, analyzed for forage nutrient composition using standard wet chemistry procedures. Specifically, samples were analyzed for CP, DM, ADF, NDF, ADL, IVTD48, and NDFD48.

Statistical analysis of all data was conducted by AB Vista in the UK. Validation statistics include RSQ (R^2, coefficient of determination) and SEP and RPD. The RSQ represents the proportion of the total variation that is explained by the linear regression while the SEP represents the accuracy of the predicted values considering the difference between the NIRS and the wet chemistry values. Both parameters should be considered when evaluating the quality of the prediction equations. Preferably, R^2 values should be greater than 0.60 and the SEP values should be smaller, representing more accurate prediction equations. RPD is a non-dimensional statistic for the evaluation of a NIR calibration equation. It is the ratio of the standard error of prediction (SEP) to standard deviation (SD). RDP=SD/SEP (or SECV). Because of similar responses, calibrations for dried whole and dried ground samples were merged.

Results and Discussion: General observations were made regarding unit performance in the field and scanning time. While the unit tolerated light rainfall while in use outdoors, output was irregular when used during extreme heat (> 29.4˚C) and was inoperable during cool temperatures (< 4.4˚C). Therefore, only inside scanned wet samples were used in equation development. Including calibration time, it took 6 to 7 minutes to scan each sample. Scanning time was not affected by climate, battery level, or the length of time the unit had been actively scanning.

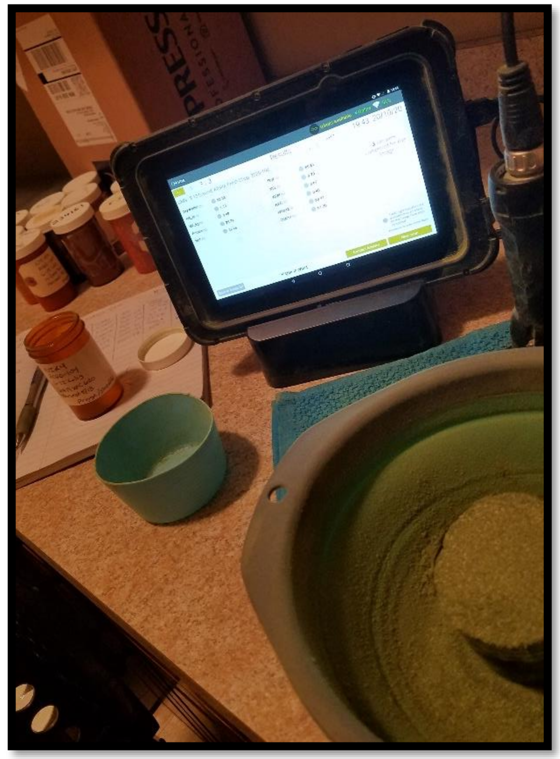

Figure 2: Scanning a ground alfalfa sample in the laboratory using the hand-held NIRS device (2020 model). Photo credit: Jessica Prigge, UMN.

Statistics for prediction of dried and wet alfalfa forage samples are shown in Table 1 and 2. Predicted means for wet and dried alfalfa samples were similar. RSQ values for prediction equations for nutrient value components were above .710 for dried samples and above .619 for wet samples. RSQ explains that part of the variation that is predicted by an equation. i.e., an RSQ of 1 would be 100% of the variation explained by the prediction equation. Overall, dried samples had RSQ values above those for wet samples.

Table 3 shows the RPD evaluation statistic used by AB Vista to appraise NIR equations developed for each constituent. RPD is a non-dimensional statistic for the evaluation of an NIR calibration equation. It is the ratio of the standard error or prediction (SEP) to standard deviation (SD). RDP= SD/SEP (or SECV). Unfortunately, RPD values of 2 or 3 were calculated for most variables except for dry matter in both wet and dry samples and for protein in dried samples (RDP=4). RDP values of 2 or 3 indicate that NIR4 farm technology is of low value in predicting constituents. An RDP of 4 indicates the technology is useful but would need more monitoring with wet chemistry.

While laboratory NIRS units have been utilized to determine forage nutrient composition for decades with precision and accuracy (Sørensen, 2002), they scan over a broader wavelength than the portal NIRS unit evaluated. Compared to laboratory NIRS units, hand-held NIRS devices have the potential for added flexibility and speed but may be challenging to use in the field in adverse weather.

Table 1. Prediction statistics for dried alfalfa forage samples.

Constituent	N	Mean	SD	SEC	RSQ	SECV	RPD
Dry_Matter	198	87.9	5.75	0.264	0.998	0.355	22
Moisture	200	9.2	0.70	0.375	0.715	0.394	2
Protein	196	23.1	2.98	0.752	0.936	0.816	4
NDF	197	37.5	3.86	1.585	0.831	1.745	2
IVTD_48	198	78.6	4.25	1.778	0.825	1.980	2
NDFD_48	197	49.3	4.44	2.408	0.710	2.482	2
ADF	101	27.2	2.97	0.897	0.909	1.023	3
ADL	98	6.3	0.71	0.352	0.752	0.372	2

Table 2. Prediction statistics for wet alfalfa forage samples.

Constituent	N	Mean	SD	SEC	RSQ	SECV	RPD
Dry_Matter	191	26.3	9.33	1.010	0.988	1.178	9
Moisture	198	9.2	0.70	0.410	0.622	0.445	2
Protein	200	23.1	3.05	1.060	0.854	1.285	3
NDF	198	37.5	3.81	2.150	0.619	2.588	2
IVTD_48	198	78.6	4.22	2.072	0.735	2.383	2
NDFD_48	198	49.4	4.39	2.532	0.613	3.113	2
ADF	102	27.2	2.97	1.326	0.735	1.888	2
ADL	101	6.4	0.72	0.269	0.736	0.422	3

N=number of samples used in equation development, Mean=average predicted value, SD=standard deviation, SEC=standard error of calibration, RSQ=coefficient of determination, SECV=standard error of cross validation, RPD= an equation evaluation statistic.

Table 3. RPD evaluation statistic for prediction of alfalfa forage constituents.

RPD	Key	Comment
<1		Very poor of little or no use
<2		Poor but could be used as a screen for expensive tests
<3		Successful will provide useful QC trending information
<4		Good can be used for QC with limited wet chemistry support (1 in 10 samples)
<5		Good can be used for QC with occasional wet chemistry support (outliers)
<6		Very good can be used in QC and form reject/accept criteria
>7		Excellent can replace wet chemistry

RPD is a non-dimensional statistic for the evaluation of a NIR calibration equation. It is the Ratio of the standard error or prediction (SEP) to standard deviation (SD). RDP= SD/SEP (or SECV).

Summary: The portable NIR4-Farm unit was used to predict forage nutritive constituents in wet and dried alfalfa of varying nutrient content. It provided RSQ (coefficient of determination) values above .70 for many nutritive value constituents. However, results using RFD were less promising.

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