Title: There's An App for That! Validating Real-Time Assessment of Alfalfa Nutritive Value

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Abstract: Having the power of a hand-held near-infrared reflectance spectroscopy (NIRS) would allow for immediate, in-field analysis of forage nutritive values and could provide a critical piece of technology for the forage and livestock sectors. However, no hand-held NIRS technologies marketed for farmers are currently programmed to assess alfalfa forage nutrient values. Therefore, the objectives of this project were to develop equations for prediction of nutritive values of fresh, or green chopped, alfalfa forage using a hand-held NIRS unit. Samples from an established alfalfa field in Minnesota were collected by hand harvest starting on 10 June 2019 when alfalfa vas vegetative and continued every 7 days until full flower. Samples were collected from second cutting starting when the alfalfa was vegetative and continued every 7 days. A total of 100 samples were collected. Individual fresh samples, chopped to 2.5 (n=80) and 1 cm (n=20) lengths were scanned by a portable NIRS unit (NIR4 Farm, AB Vista, Marlborough, England). After scanning, samples were dried, ground, thoroughly mixed, and sent to a commercial laboratory for wet chemistry analysis to determine crude protein, acid detergent fiber, neutral detergent fiber (NFD), and NDF digestibility (NDFD48). Laboratory wet chemistry analyses were used with spectra collected by the NIR4 Farm unit to develop calibration equations for fresh or green chopped alfalfa. Based on commonly used calibration statistics (R^2, SEC, and SECV), the NIR4 Farm unit was able to accurately predict all major forage nutritive value components except non-fiber carbohydrates and NDFD48. The NIR4 Farm unit has the capacity to be effectively used on farm, potentially saving livestock owners and forage producer both time and money while providing accuracy and flexibility in the field or barn.

Introduction: We live in an age of rapidly changing technology, with information available at our finger tips. The agricultural sector is a technology leader, and farmers are well-known as early adaptors and innovators. For example, remote sensing technologies can be used to predict alfalfa yield and some forage nutritive value components. Specifically, remote sensing technology combined with growing degree day units explained most of the variability in several forage nutritive values (Noland et al., 2017), and visible and near-infrared reflectance spectroscopy (NIRS) has been used to predict nitrogen, neutral detergent fiber (NDF), acid detergent fiber (ADF), and the relative forage value (RFV) based hay grade classifications (Starks et al., 2015). Nutritive value is a general term that describes the feeding value of alfalfa (and other forages) to livestock. Of the forage nutritive value components, several are commonly used to predict animal performance, including NDF, ADF, and NDF digestibility (NDFD48), which are predictors of intake, digestibility, and fiber digestibility, respectively (Beauchemin, 1991; Coleman and Moore, 2003; Oba and Allen, 1999). These nutritive value components are typically determined using laboratory NIRS which can be costly, labor intensive, and require several days from sampling to return of data.

Having the power of a portable NIRS would allow for immediate, in-field analysis of forage nutritive values and would provide a critical piece of technology for the forage and livestock sectors. Currently, morphological development of alfalfa (e.g. 10% bloom) or days between cuttings (e.g. 28 days) are traditionally used to schedule harvest times and estimate the nutritive value of alfalfa. However, visual appraisal of alfalfa maturity can be challenging, and both approaches can be poor predictors of alfalfa nutritive value due to seasonal variability and weather impacts on plant growth. A refined strategy based on numerically staging alfalfa growth for a set number of stems using mean stage by weight (Kalu and Fick, 1981) or mean stage by count (Mueller and Fick, 1989b) improves the accuracy of prediction, but is impractical for farmers in the field because of the time requirements and field variability. 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 nutritive values are critical to aid farmers in decision making regarding harvesting, feeding, and marketing alfalfa.

Updated Project Direction: The goal of this project was originally to evaluate the accuracy of SCiO technology (Tel Aviv, Israel), a hand-held NIRS unit, for predicting the nutritive value of fresh alfalfa. The SCiO device was advertised as an affordable, hand-held, lightweight sensor that communicated with smartphones using Bluetooth wireless technology to estimate dry matter (DM), crude protein (CP), energy, fat, oil, starch, and sugar concentrations of various feed stuffs. After the grant was received, unfortunately, it became apparent that the SCiO technology was not refined to the level necessary for accurate prediction of nutritive value of alfalfa forage.

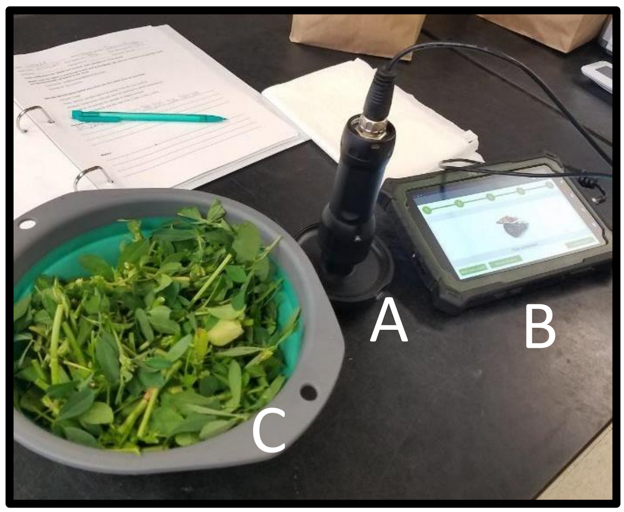

Figure 1: NIR4 Farm hand-held NIR unit (A, NIRS probe; B, NIRS tablet; C, scanning bowl). Photo credit: Jessica Prigge, UMN.

After further evaluation of additional hand-held NIRS technologies marketed for farmers, it became apparent that none were currently programmed to assess alfalfa forage nutrient values. Therefore, the direction of the project changed to focus on working with AB Vista (Marlborough, England) to develop fresh, or green chopped, alfalfa equations for their hand-held NIRS technology, NIR4 Farm (Figure 1). The NIR4 Farm technology had been successfully used for the analysis of nutritive value of grasses in Europe and was emerging in the U.S.

Objectives: Develop equations for prediction of nutritive values of fresh or green chopped alfalfa forage using a hand-held NIRS unit.

Materials and Methods: Alfalfa was harvested from a University of Minnesota – St. Paul field planted in 2017 that contained three varieties, 'Magnum 7', '440HVX.RR', and 'SW4107' planted in 2 m x 6 m plots. Samples from first cutting alfalfa were collected starting on 10 June 2019 when alfalfa was vegetative and continued every 7 days until 50 samples were obtained. The field was then mechanically harvested with a flail harvester (Carter Manufacturing Company, Inc., Brookston, IN) when the alfalfa reached the late flowering stage (Kalu and Fick, 1981; Fick and Mueller, 1989). An additional 50 samples were collected from second cutting starting when the alfalfa was approximately 30.5 cm and vegetative and continued every 7 days. At each harvest, samples were evenly collected among the three cultivars throughout the harvest windows.

All alfalfa herbage was harvested by manually cutting a0.6 m^2 area to a 2.5 cm stubble height to obtain a sample with a minimum wet weight of 400 grams. Immediately after harvest, samples were sorted by maturity (vegetative or bud/flowering; Fick and Mueller, 1989). Eighty samples were cut into 2.5 cm lengths and 20 samples were chopped to 1 cm lengths using a Master Prep® Ninja® blender. Individual samples were placed in the NIR4 Farm scanning bowl with a minimum 5 cm depth and scanned using the handheld unit (Figure 2). Individual samples were scanned six times following the recommended NIR4 Farm protocol. Samples were mixed in the bowl between repeated scans, and the probe was calibrated as directed on the tablet provided by AB Vista. Additionally, the probe was cleaned between scans with a clean cloth. Spectra generated from scans were automatically sent to a cloud file system accessible to AB Vista. While scanning, the number of samples, time spent using the device, and battery life were recorded.

After scanning, samples were dried at 60°C until no weight change occurred (~84 h). Dried samples were ground through a 0.5 cm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) followed by a 0.1 cm screen in a Cyclotec (Foss, Hillerød, Denmark). Samples were thoroughly mixed and 10 g were sent to a commercial laboratory (Dairy One, Ithaca, NY) for wet chemistry analysis to determine crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), and neutral detergent fiber 48 hour digestibility (NDFD48). Laboratory wet chemistry analyses were used with spectra collected by the NIR4 Farm unit to develop calibration equations for fresh or green chopped alfalfa.

Figure 2: Scanning a dried alfalfa samples using the NIR4 Farm hand-held NIR unit. Photo credit: Liz Patton, UMN.

Results and Discussion: General observations were made regarding chopping time, scanning time, and battery life. Chopping samples to a 2.5 cm length took approximately 3 minutes per sample, while chopping to the 1 cm length took approximately 10 minutes per sample. It took approximately 1 minute to scan each sample. The battery lasted between seven to eight hours on a full charge, and recharged within two hours.

Equations were developed to predict the nutritive value components of fresh alfalfa, or green chopped alfalfa forage, using the NIR4 Farm. Calibration statistics include the coefficient of determination (R^2), standard error of calibration (SEC), and standard error of cross validation (SECV). R^2 represents the proportion of the total variation among the samples for a component that is explained by the calibration. The SEC is the standard error of the difference between the laboratory value and the NIRS predicted value for the same sample, while the SECV is the standard error of the difference between the predicted and laboratory values when samples are sequentially removed from the calibration process. The best NIRS calibration equation should consider all three statistics, but preferably have R^2 values greater than 0.60 with small SEC and SECV values.

Based on these values, the NIR4 Farm unit was able to accurately predict all major forage nutritive value components except non-fiber carbohydrates and NDF48 (Table 1). Therefore, this research showed that the NIR4 Farm unit has the capacity to be effectively used on farm, potentially saving livestock owners and forage producer both time and money. These results are not surprising since laboratory NIRS units have been utilized to assess forage nutritive values for decades, and have increased in their precision to accurately measuring these values (Sørensen, 2002). The addition of a portable NIRS unit will likely maximize the efficiency of determining forage nutritive values, but there is a tradeoff between the ability to rapidly predict forage nutritive value in the field, and precision, flexibility, and speed of use. However, this unit appears to provide accuracy and flexibility.

These calibrations were developed using a standardized, controlled procedure to sample research plots which minimized variability. Additional samples collected from production fields are needed to increase the prediction power of the unit. Additionally, all calibrations were developed from scanning wet or fresh alfalfa herbage. Therefore, there are opportunities to expand the application of this technology to harvested and stored alfalfa forages including hay, silage and baleage.

Table 1. Summary of NIR4Farm calibration statistics for prediction of moisture and forage nutritive values of fresh or green chopped alfalfa.

Components[1]	N[2]	Mean	SD	SEC	R^2	SECV	Acceptable
Moisture	100	9.58	0.66	0.341	0.733	0.374	Y
DM	100	90.43	0.66	0.348	0.718	0.376	Y
CP	100	23.11	3.63	1.449	0.841	1.559	Y
NDF	100	36.63	4.07	2.320	0.676	2.456	Y
NFC	100	28.13	2.35	1.591	0.542	1.689	N
TDN	100	65.48	2.08	1.312	0.603	1.373	Y
NEL	100	0.69	0.03	0.020	0.638	0.020	Y
NEM	100	0.66	0.04	0.021	0.650	0.022	Y
NEG	100	0.39	0.03	0.020	0.619	0.021	Y
IVTD48	100	81.23	3.28	2.055	0.607	2.200	Y

NDFD48	100	49.15	4.62	3.649	0.375	3.846	N
ME_lb	100	1.10	0.05	0.022	0.789	0.024	Y
ME_kg	100	2.43	0.11	0.048	0.796	0.054	Y
TDN1X	100	60.95	1.49	0.877	0.653	0.915	Y

[1]DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; NFC, non-fiber carbohydrates; TDN, total digestible nutrients; NEL, net energy for lactation; NEM, net energy for maintenance; NEG, net energy for gain; IVTD48, in vitro digestibility at 48 hours; NDFD48, neutral detergent fiber digestibility at 48 hours; ME lb, metabolizable energy in pounds; ME kg, metabolizable energy in kilograms; and TDN 1X, total digestible nutrients at one times maintenance level.
[2]N, number of samples; SD, standard error; SEC, standard error of calibration; and SEVC, standard error of cross validation.

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