Technical note: Comparison of dry matter measurements from handheld near-infrared units with oven drying at 60°C for 48 hours and other on-farm methods

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ABSTRACT

This study compared dry matter (DM) predictions of 3 handheld near-infrared spectrophotometer (NIRS) units (Moisture Tracker, Digi-Star Inc., Fort Atkinson, WI) to conventional oven drying at 60°C using 2 alfalfa and 2 corn silages. In addition, on-farm DM methods [microwave, Koster tester (Koster Moisture Tester Inc., Brunswick, OH), and food dehydrator methods] were also compared. Corn and alfalfa silages (1,600 g) obtained from the University of Wisconsin Dairy Cattle Center (DCC) and the Arlington Research Station (ARS) were analyzed for DM daily for 20 d. Two NIRS calibration methods were also tested within each unit. The DM predicted from the factory-preset calibrations was NIR_f . The adjusted DM prediction was NIR_a , where the average difference between oven-dried and NIR_f determined on duplicate forage samples for 3 d before the experiment was used as a bias adjustment for all subsequent DM determinations. The average predicted DM from the 20 scans was recorded as the forage DM. The process was repeated 3 times with each NIRS unit. Two 100-g subsamples of each forage were also oven-dried for 48 h at 60°C daily in a forced-air oven. Oven DM of ARS and DCC alfalfa silages were $37.3 \pm 1.1\%$ and $48.5 \pm 1.9\%$, respectively (mean $\pm$ standard deviation). Oven DM of ARS and DCC corn silages were $34.7 \pm 1.2\%$ and $37.4 \pm 0.5\%$, respectively (mean $\pm$ standard deviation). Dry matter determinations from NIR_f were on average 3.5 units higher than the oven DM values. The NIR_a DM predictions were on average 1.7 DM units lower than the oven DM values. Additionally, differences among the 3 NIR_f probe results were detected (43.1, 40.7, and 41.3% DM, respectively), but all other results were similar between probes. Determinations of DM by the microwave and food dehydrator were also similar with the 60°C, 48-h oven method, whereas the

Koster tester was lower than the oven. The handheld NIRS units more accurately predicted DM content of the alfalfa silage but were not as accurate with corn silages when the factory preset calibrations were corrected for bias.

Key words: dry matter, near infrared, dairy cattle

Technical Note

Near-infrared spectroscopy (NIRS) has been used for determining DM of forages since 1987, when one of the first calibrations for forages was created and validated (Windham et al., 1987). With recent advancements in technology, handheld NIRS instruments have become another option for determining DM on farm. Using NIRS could be a fast and effective way of evaluating forage DM, if a tool can be effectively calibrated. This study evaluated an adjustment feature of a handheld NIRS unit (Moisture Tracker, Digi-Star, Fort Atkinson, WI) and how the NIRS results compared with other methods for determining forage DM on farm. Our hypothesis was that the NIRS units would calculate similar DM results for both sets of alfalfa silage and corn silage as those obtained from oven drying at 60°C for 48 h in a forced-air oven once the units were each corrected for bias.

Four fresh forage samples of approximately 1,600 g were collected daily from 2 upright silos and 2 bunker silos from the University of Wisconsin Dairy Cattle Center (DCC) and the Arlington Research Station (ARS), respectively. One sample of corn silage and one sample of alfalfa silage from the face of each silo were collected daily for 20 d in January and February of 2016. Average DM for corn silage was $37.9 \pm 1.6\%$ DM and was $44.4 \pm 5.2\%$ DM for alfalfa silage (mean $\pm$ SD). Samples were collected and sealed in plastic resealable bags and transported immediately to the laboratory for analysis. Dry matter analysis commenced for all samples within 2 h of on-farm collection, according to Figure 1. Bags containing each sample were placed in a white plastic tub and mixed before scanning by

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Figure 1. Diagram of sequence of events involved in 20-d trial. NIRS = near-infrared reflectance spectroscopy; UW = University of Wisconsin. Koster tester = Koster Moisture Tester Inc., Brunswick, Ohio. Color version available online.

the NIRS units. Three NIRS units were used for this analysis. Forage thickness was at least 7.6 cm within the tub to ensure that the white surface of the tub was not exposed to the NIRS. The original factory calibration was based on drying reference samples by microwave, and then subsequently determining residual DM by oven drying at 105°C for 4 h.

The scanning procedure used was designed so that each NIRS probe analyzed the forage in a sequential manner. Each NIRS probe was checked to ensure no debris was obscuring the lens and calibrated with the calibration disk provided from the manufacturer. The probe was then used to scan 20 times in different places in the forage pile. The device then produced a DM reading based on the average of the results of these spectra, thus yielding 1 DM result per each set of 20 individual scans. The forage was then remixed and scanned with the next probe in the same manner and repeated a third time for the third probe. Once all 3 units had been used to scan the forage, they were used to scan the forage in 2 additional rounds in the same order and manner as described. For each day, the total number of NIRS scan DM outputs per feed was 9, totaling 36 outputs per day with the units for the 4 feeds.

The NIR_f values (NIR_f) were initially from the factory calibration settings. Following the completion of each scan, the spectral data are stored on a flash drive. The NIRS calibrations were then adjusted for

each sample based on a 3-d pretest (whose readings are classified as NIR_a ; NIR_a) using DM values obtained from the oven and calibrated before each scan (Figure 1). This 3-d pretest consisted of gathering fresh forage daily for 3 d and scanning with all 3 NIRS units as described above. Each silage was then subsampled, and 100-g duplicates were dried in a forced-air oven for 48 h at 60°C. These DM values were then used to calculate the NIR_a adjustment to correct for bias in the NIR_f values.

After scans were completed, the 1,600-g sample was mixed again and dumped onto a flat surface. A plastic straight edge was then used to cut the pile into quarters. Two sections diagonal to each other were discarded, whereas the other 2 were combined and mixed together thoroughly. Samples were then split into 8 subsamples of each approximately 100 g, which were then used for the other DM methods. Once subsampling was complete, DM determinations by other methods were started immediately. When a sample could not be analyzed via the microwave or Koster tester immediately, samples were weighed, placed into sealed containers, and analyzed as soon as possible, typically within 2 h.

Time from the start of scanning to weighing of samples for comparative DM analysis was approximately 15 to 25 min. Four Koster testers (Koster Moisture Tester Inc., Brunswick, OH) were used to run 2 different samples in duplicate at one time. Residue weights were

measured after 50 min and every 5 min thereafter until the sample weight did not change by more than 0.1 g. Microwave dry-down was conducted by weighing approximately 100 g of sample on a large paper plate and placing into a microwave (1,000 wattage, R309YK, Sharp, Osaka, Japan) without water for 5 min at 70% power. A cup of water was then placed in the microwave and samples were microwaved for 1-min intervals at 70% power until the gross weight did not change by more than 0.1 g. Two food dehydrators (American Harvest, Nesco, Mayfield Heights, OH) were used to evaluate the dehydrator method. Samples of each forage were placed on a large fruit roll sheet at 71°C for 24 h. For oven drying, duplicate 100-g subsamples were weighed into aluminum pans and then oven-dried for 48 h at 60°C in a forced-air oven. Daily replications were averaged before statistical analysis.

Following dry down during the third and fourth week of this trial, 5 sets of samples from all methods were collected on random days and placed into a forced-air oven for 24 h at 100°C (method 2.1.2, NFTA, 1993) to measure residual moisture content of samples.

Data were evaluated by the MIXED Procedure of SAS (v9.3, SAS Institute Inc., Cary, NC), with feed, method, day, day $\times$ feed, and day $\times$ method set as fixed effects and feed $\times$ method (whole plot error) set as random effect and repeated measure. The covariance structure compound symmetry (CS) was used. Statistical significance was declared if $P < 0.05$. A Tukey test was used to compare treatment means for overall DM results as well as for residual DM.

Dardenne (2009) pointed out the importance of reporting repeatability and reproducibility when models using spectral data are developed. In the current study, we calculated the standard error of the laboratory (SEL or reproducibility; referring to the error of the reference data) using the duplicates that were analyzed for the reference method (oven). In addition, 3 combinations were used (triplicate 1 vs. triplicate 2, triplicate 1 vs. triplicate 3, triplicate 2 vs. triplicate 3) to calculate overall SEL for each NIRS probe for each day. Repeatability is also important but is often not reported when evaluating models derived from NIRS data (Dardenne, 2009). Repeatability of NIRS predictions is high in samples that are relatively homogeneous, but repeatability decreases in relatively heterogeneous samples, such as corn silage (Dardenne, 2009). In this study, DM predictions from corn silage and alfalfa silage were measured. Thus, we calculated the repeatability across NIRS probes for each type of forage for each research station according to Decruyenaere et al. (2015).

To assess the quality of the NIRS prediction, the daily average results from NIRS (NIR_a and NIR_f) were compared with the observed values (oven). The

goodness of fit of the NIRS predictions were evaluated using the coefficient of determination (R^2) between the predicted and observed values. The accuracy was calculated based on the concordance correlation coefficient (CCC), mean bias, and root of mean square error prediction (RMSEP).

The CCC is the multiplication of the correlation coefficient (r) between observed and predicted values by the bias correction, which indicates how far the regression line deviates from the slope of unity (Lin, 1989). The RMSEP equation consists of the square root of mean square error prediction (MSEP), where MSEP is the sum of the squared difference between observed values and model-predicted values divided by the number of data points (n ; Bibby and Toutenburg, 1977). The MSEP was decomposed into MB, slope bias, and random errors (Theil, 1961). The NIRS predictions were assessed using the software Model Evaluation System (MES, College Station, TX; <http://nutritionmodels.tamu.edu/mes.html>) as described by Tedeschi (2006).

A separate model was used to compare the 3 NIRS probes as well as the 2 adjustment methods. The MIXED procedure of SAS was used, with method $\times$ unit, day, and day $\times$ method $\times$ unit set as fixed effects. Random effects were feed and method $\times$ unit $\times$ feed, with method $\times$ unit $\times$ feed set as a repeated measure. Compound symmetry (CS) was used as a covariance structure. A Dunnett test was used to determine differences between NIRS probes and calibrations versus oven DM results, which was used as our control.

The third model used was conducted on the residual DM results from a small subset of the original data set ($n = 5$) to determine if there was a difference in residual DM results from each method. For this data set, equipment represents the different units used in each DM analysis (for example, 2 microwaves were used). The data were analyzed using the MIXED Procedure of SAS, with method, equipment, equipment $\times$ method, and feed as fixed effects. Day was set as a repeated measure, with nested effect of place(feed) and day set as random. The covariance structure AR(1) was used in this analysis. All superscripts for all 3 models were determined by using the pdmix800 code according to Saxton (1998).

Summarized in Table 1 are the 20-d average DM determinations for all feeds. Significance was found for the method, day, day $\times$ method, feed, and day $\times$ feed effects ($P < 0.05$; Table 1). Method $\times$ feed is a repeated measure and a random effect in the model. Overall, NIR_f DM results differed from all other DM methods and NIR_a was different from all other methods except for the Koster tester (Table 1). The food dehydrator and microwave determinations for DM were not different from those of the oven DM method.

Table 1. Comparison of various DM methods over feed type

Item	Method ¹						<i>P</i> -value ²		
	Oven	NIR _f	NIR _a	Dehy	Koster	MW	SEM	M	D × M
DM (% as is)	39.6 ^b	43.1 ^a	37.9 ^c	39.5 ^b	38.4 ^c	39.9 ^b	0.24	<0.01	<0.01

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹NIR_f represents values obtained from calibrated near-infrared spectrophotometer (NIRS) unit with no adjustment factor applied; NIR_a represents values obtained from adjusting the NIRS units based on a 3-d pretest; Dehy = dehydrator; Koster = Koster Moisture Tester Inc., Brunswick, Ohio; MW = microwave.

²M represents the method effect; D represents the day effect; D × M represents the day × method interaction; feed and day × feed interactions were also significant ($P < 0.01$).

Overall, the repeatability of the measurements taken from corn silage samples were lower when compared with alfalfa silages (Table 2). As reported by Dardenne (2009), the main reason for this may be explained by the greater heterogeneity of corn silage compared with alfalfa silage. The morphological composition of corn silage includes leaves, cob, husk, stalk, and the kernels (ground, cracked, or whole), whereas alfalfa silage is normally stem and leaves.

The SEL (reproducibility) of the NIRS method was similar or less than the oven method (Table 2). The SEL for NIR_a and NIR_f are the same in Table 2 because the difference between replicates were calculated within probe and NIR_a is only a bias adjustment of NIR_f, thus the difference is the same. Repeatability should differ between the NIRS methods since it is calculated as

the mean square of the difference between the NIRS measurement of a single probe and the average of 3 different probes.

Figures 2 and 3 and Table 3 show the relationship between oven DM results and those determined by the 2 NIRS calibration methods. The range in forage DM was small and the correlations are not only affected by the NIRS calibrations, but also errors associated with the oven drying method. Within the limitations of this set of data, both NIRS predictions had low goodness of fit but good accuracy when compared with oven values. The NIRS predictions for both NIR_f and NIR_a had higher R^2 and greater slope for alfalfa silage compared with corn silage (Table 3, Figures 2 and 3). The NIRS probes had lower R^2 and lower slope for corn silage. The poorer fit for corn silage could be due to the

Table 2. Mean repeatability and reproducibility in samples from oven (reference method) and near-infrared spectrophotometer (NIRS) methods

Item	Mean DM (%)	Mean DM range (%)	Repeatability	Repeatability range	Reproducibility ¹
ARS farm ²					
Corn silage (n = 20)					
Oven	34.7	31.8–43.1	0.99	0.09–6.96	2.49
NIR _f ³	38.1	36.2–40.2	0.94	0.45–1.63	0.93
NIR _a ⁴	33.9	32.2–35.2	0.43	0.08–0.77	0.93
Alfalfa silage (n = 20)					
Oven	37.4	33.9–39.1	0.18	0.01–1.01	0.40
NIR _f	40.6	35.8–47.7	1.88	0.86–2.84	1.09
NIR _a	35.6	31.4–41.2	0.85	0.16–1.29	1.09
DCC farm ⁴					
Corn silage (n = 20)					
Oven	37.4	36.3–38.9	0.26	0.01–0.57	0.43
NIR _f	38.8	36.6–41.7	1.37	0.77–2.32	0.57
NIR _a	35.8	34.5–37.5	0.54	0.14–1.36	0.57
Alfalfa silage (n = 20)					
Oven	48.6	45.0–51.2	0.21	0.01–2.53	0.81
NIR _f	49.1	43.7–55.1	2.89	2.25–3.66	0.85
NIR _a	46.1	42.0–50.0	0.48	0.08–1.14	0.85

¹Reproducibility results between NIR_f and NIR_a are the same due to the only difference between them being a bias correction.

²Arlington Research Station, Arlington, Wisconsin.

³NIR_f represents values obtained from calibrated NIRS unit with no adjustment factor applied.

⁴NIR_a represents values obtained from adjusting the NIRS units based on a 3-d pretest.

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limited DM range of the corn silages relative to alfalfa (Figures 2 and 3) as well as the more heterogeneous nature of corn silage, with possible differing DM in the husk, stalk, cob, and kernel material. The factory preset NIR_f calibration had the poorest predictions for ARS results, with the highest mean bias, RMSEP, and the lowest CCC. The NIR_a adjustment increased the intercept for all feeds except for the corn silage from the DCC. The NIRS units did a better job of predicting DM for alfalfa silage compared with corn silage for both ARS and DCC (Figures 2 and 3).

When comparisons were made between individual NIRS probes and the 2 adjustment methods versus the

Figure 2. Relationship between near-infrared spectrophotometer (NIRS) adjustments and oven DM for Arlington Research Station (Arlington, WI). (A) For corn silage, the relationship between the oven and NIRS factory calibration (NIR_f; ■) was $y = 0.63x + 10.9$, with $R^2 = 0.11$. The relationship between the oven and from adjusting the NIRS units based on a 3-d pretest (NIR_a; ▲) was $y = 0.62x + 13.6$, with $R^2 = 0.11$. (B) For alfalfa silage, the relationship between the oven and NIR_f was $y = 0.47x + 18.1$, with $R^2 = 0.63$. The relationship between the oven and from NIR_a was $y = 0.47x + 20.5$, with $R^2 = 0.63$. Color version available online.

Figure 3. Relationship between near-infrared spectrophotometer (NIRS) adjustments and oven DM for the University of Wisconsin Dairy Cattle Center (Madison, WI). (A) For corn silage, the relationship between the oven and NIRS factory calibration (NIR_f; ■) was $y = -0.07x + 40.3$, with $R^2 = 0.01$. The relationship between the oven and from adjusting the NIRS units based on a 3-d pretest (NIR_a; ▲) was $y = -0.07x + 40.1$, with $R^2 = 0.01$. (B) For alfalfa silage, the relationship between the oven and NIR_f was $y = 0.89x + 5.11$, with $R^2 = 0.87$. The relationship between the oven and from NIR_a was $y = 0.89x + 7.56$, with $R^2 = 0.88$. Color version available online.

oven DM results, the interaction between method and unit, and the effect of day were significant ($P < 0.01$), the interactions between method, unit, and day were not significant ($P > 0.05$; Table 4). The NIR_f results from probes 1 and 3 were significantly different from the oven DM results, whereas probe 2 had similar DM results to the oven with the factory settings (Table 4). The NIR_a results for all 3 probes were significantly lower than the results of the oven.

Residual DM were evaluated by running 5 sets of duplicates from each drying method in the oven at 100°C for 24 h. Approximately 3 to 4.5% residual water remained after the completion of the other drying methods as shown in Table 5, which is similar to what was discussed in Oetzel et al. (1993). We found a significantly lower residual DM for the microwave method compared with the other methods (Table 5). This may

Table 3. Evaluation of near-infrared spectrophotometer (NIRS) predictions compared with observed values from oven

Item	R ²	Mean bias	CCC ¹	RMSEP ² (%)	MSEP decomposition ³ (%)		
					Mean bias	Slope	Random errors
ARS farm ⁴							
Corn silage (n = 20)							
NIR _f ⁵	0.10	-3.42	0.04	3.67	87	1	12
NIR _a ⁶	0.10	0.83	0.21	1.55	28	3	69
Alfalfa silage (n = 20)							
NIR _f	0.64	-3.21	0.22	3.41	88	8	4
NIR _a	0.63	1.74	0.43	2.10	68	22	10
DCC farm ⁷							
Corn silage (n = 20)							
NIR _f	0.01	-1.37	0.02	1.59	73	17	10
NIR _a	0.01	1.64	0.01	1.83	80	12	8
Alfalfa silage (n = 20)							
NIR _f	0.87	-0.52	0.90	0.86	37	6	57
NIR _a	0.87	2.47	0.51	2.55	93	1	6

¹CCC = concordance correlation coefficient.²RMSEP = root mean square errors of prediction.³MSEP = mean square errors of prediction.⁴Arlington Research Station, Arlington, Wisconsin.⁵NIR_f represents values obtained from calibrated NIRS unit with no adjustment factor applied.⁶NIR_a represents values obtained from adjusting the NIRS units based on a 3-d pretest.⁷University of Wisconsin Dairy Cattle Center, Madison.

have been due to differing microwave methods in our study and the study of Oetzel et al. (1993), which had water in the microwave throughout drying and power setting at 600 W, whereas we did not add water for the first interval and used 700 W of power throughout. Our results indicate that DM measured from the food dehydrators were similar to oven drying at 60°C for 48 h.

Data collection on farm to make management decisions has been implemented in many areas (Borchers and Bewley, 2015), and using NIRS could be another tool that producers could use to improve precision in their operation. Variation in daily silage DM on farm due to events such as rain and field conditions is a major source of error in ration formulation (St-Pierre and Weiss, 2015). The speed and precision of the hand-held NIRS unit would be desirable if used daily when mixing rations. Results suggest that the NIRS units

could be as accurate as oven drying at 60°C for 48 h if the factory-set calibrations were corrected for bias. We also found that food dehydrators were an accurate and consistent way of determining DM, similar to what was found by Mertens et al. (2004).

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Table 4. Comparison of near-infrared spectrophotometer (NIRS) probes and calibrations to oven DM results

Item	Oven ¹	NIRS 1		NIRS 2		NIRS 3		SEM	P-value ⁴		
		F ²	A ³	F	A	F	A		M × U	D	M × U × D
DM (% as is)	39.6 ^a	43.1 ^b	37.9 ^b	40.7 ^a	37.8 ^b	41.3 ^b	37.9 ^b	2.85	<0.01	<0.01	0.99

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).¹Analysis was conducted using a Dunnett test, where all methods were compared with control (oven).²F represents values obtained from calibrated NIRS unit with no adjustment factor applied.³A represents values obtained from adjusting the NIRS units based on a 3-d pretest.⁴M × U represents the method × unit interaction; D represents the day effect; M × U × D represents the method × unit × day interaction.

Table 5. Comparison of various DM (mean $\pm$ SD) methods after dried samples were dried at 100°C for residual moisture (n¹)

Item	Oven ²	Microwave	Koster	Dehydrator	RMSE ³	Method ⁴
DM	97.1 (± 0.32) ^a	95.5 (± 0.25) ^b	97.3 (± 0.25) ^a	97.1 (± 0.3) ^a	0.132	<0.01

^{a,b}Means within row with different superscripts differ ($P < 0.05$).

¹Observations for each method are as follows: oven: n = 11; microwave: n = 18; Koster (Koster Moisture Tester Inc., Brunswick, OH): n = 18; dehydrator: n = 13.

²Oven results were first dried at 60°C for 48 h before residual DM drying.

³RMSE = root mean square error.

⁴Represents the method effect.

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