

# EFFECTS OF SAMPLE PROCESSING ON ASH CONTENT DETERMINATION IN SOLID CATTLE MANURE WITH VISIBLE/NEAR-INFRARED SPECTROSCOPY

S. L. P. Sakirkin, C. L. S. Morgan, B. W. Auvermann

**ABSTRACT.** Visible and near-infrared (VisNIR, 350-2500 nm) diffuse reflectance spectroscopy (DRS) may be a useful tool for determining crude ash content of solid cattle manure. However, the effect of sample preprocessing protocols on the predictive ability of the VisNIR-DRS models is unknown. In this study we explored the effects of drying and milling on the prediction of crude ash in feedyard manure using VisNIR-DRS. Samples ( $n = 120$ ) of beef manure from open lots were evaluated for ash content by dry oxidation and then subjected to four preprocessing treatment protocols: oven-dried and milled, air-dried and milled, oven-dried and not milled, and air-dried and not milled. Each treatment protocol was used to calibrate partial least squares regression models for prediction of ash content by VisNIR-DRS. Two thirds of the samples were randomly selected to build calibration models, and the remaining third was used for validation. The root mean squared deviation (RMSD) and the ratio of the standard deviation over the RMSD (RPD) for each treatment were assessed to determine the best pretreatment protocol for ash determination of manure. The first derivative of the reflectance from air-dried, unmilled samples consistently generated the best predictive models with an RMSD of 5% crude ash (d.b.), an RPD of 2, and a bias of 0% crude ash (d.b.).

**Keywords.** Ash content, Diffuse reflectance spectroscopy, Feedyard, Manure, Multivariate, Partial least squares, Post-processing, Preprocessing, Regression, VNIR, VisNIR.

Primary uses for cattle manure include land application as a fertilizer and gasification as a biofuel. Ash, a primary determinant of manure quality for both uses, is defined as the mass fraction of inorganic residue remaining after dry oxidation at  $575^{\circ}\text{C} \pm 25^{\circ}\text{C}$  relative to the oven-dried mass (ASTM, 2007). Contamination of manure by mineral soil particles may increase the ash content to a point where the value of the manure falls below both financial and net-energetic benchmarks. Processing manure to reduce ash is energy-intensive and impractical.

Producers and end users must know the ash content of manure to evaluate its quality and economic worth. Ash content is currently measured by standardized methods such as dry oxidation (ASTM Standard E1755-01) and bomb calorimetry (ASTM Standard D5468-02), both of which are time-consuming, expensive, and strictly lab-based procedures. A rapid, low-cost, and accurate means of determining ash content of manure in the field would provide producers and end users with a scientific basis, not only for determining manure quality, but also for improving manure-

management practices, optimizing the quality and value of harvested manure, controlling land-application costs, and increasing the suitability of manure for use as a biofuel.

Recently, visible and near-infrared (350-2500 nm) diffuse reflectance spectroscopy (VisNIR-DRS) was shown to have potential for determining crude ash in hand-made soil/manure mixes (Preece et al., 2009). VisNIR-DRS is faster ( $<100 \text{ ms scan}^{-1}$ ) than conventional ash determination, non-destructive, and field-portable, and lends itself to both laboratory and *in situ* analyses for proximal or remote sensing. In Preece et al. (2009), crude ash in proportionally mixed samples of soil and manure was predicted within  $\pm 5\%$  (d.b.) of the observed ash content as determined by dry oxidation. It is unknown how this prediction accuracy will change in VisNIR-DRS models created using more realistic grab samples from feedyards.

Rapid measurements of manure ash content *in situ* would dramatically increase the speed and convenience, and reduce the cost, of assessing manure quality. In a previous study, homogenized, oven-dried, and milled manure samples were mixed at different ratios with a single soil stock prior to being scanned by VisNIR-DRS (Preece et al., 2009). This study did not test the ability of a VisNIR-DRS model to predict crude ash in manure samples from different sources. Further, it is unknown what effect sample pretreatment processes, such as milling and drying, have on these models. Pretreatment processes increase the time and expense required for the determination of crude ash by VisNIR-DRS and may impede field measurements. This study investigated the effect of various pretreatment protocols, such as milling vs. no milling and air-drying vs. oven-drying, on the predictive ability of a VisNIR-DRS model. The manure samples in this study were composed of manure collected from several different pens at

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four different feedyards, as opposed to having a binary, artificial composition.

Particle size and moisture content have marked effects on the diffuse reflectance spectra of a material. By far, the most commonly applied sample pretreatment protocols involve oven-drying and milling the material to be analyzed to constrain particle size and moisture content (Kusumo et al., 2008; Viscarra Rossel et al., 2006; Malley et al., 2005). In soils, sediments, and manure, moisture can obscure important features of C, N, and organic constituents (Kooistra et al., 2003; Kusumo et al., 2008; Lobell and Asner, 2002; Malley et al., 2005; Morgan et al., 2009). Various combinations and overtones of deformations of the O-H bond in water saturate the spectrum at bands near 1400 nm and 1900 nm (Bruun et al., 2005; Dagnew et al., 2004; Huang et al., 2007; Malley et al., 2002; Malley et al., 2005; Xing et al., 2008). However, Brown et al. (2006) found no discernable difference in accuracy between oven-dried and air-dried samples in soil characterization by VisNIR-DRS. An alternative to drying samples to compensate for the negative effect of moisture content on spectral interpretation is to delete the strong water absorption bands from the spectra (Chang et al., 2001; Dagnew et al., 2004); however, deleting bands risks deleting useful information.

In general, the literature suggests that smaller, more uniformly sized particles facilitate the interpretation of spectra and determination of chemical composition. Uniform particles improve spectrally based interpretations because increasing particle size heterogeneity increases the variation in surface physical properties. This variation in particle surfaces can affect spectral analysis (Aucott and Garthwaite, 1988). For example, particle surface properties influence light scattering and path lengths. In soils, the smallest particles are the most spectroscopically active (Cabassi et al., 2008) because they contain clays and organic particles, and the reflectance spectra of a sample with varied particle sizes resemble the spectra of the small particles more closely than the large (Dahm and Dahm, 2007, as cited by Cabassi et al., 2008). However, some evidence exists that creating uniform particle size in samples does not affect prediction accuracy of clay in soil (Waiser et al., 2007), while a similar experiment looking at soil organic carbon showed improvement in prediction with more uniform particle sizes (Morgan et al., 2009). For manure ash determination, there are no studies reporting on the effect of sample particle size on spectra prediction accuracies.

How particle size and ash determination in manure will interact is unknown. It is known that transformations associated with the second derivative of reflectance can substantially increase the magnitude of spectral features, compensating for the effects of particle size and increasing the predictive values of major principal components in a regression model (Aucott and Garthwaite, 1988). Furthermore, scanning methods can be adapted for the effect of large, non-uniform particles in soil, such as by using a large (>3 cm<sup>2</sup>) scanning area (Brown et al., 2006) or a continuous scan of streaming matter on a moving turntable or belt. The spectrometer used in this study does not use a turntable or belt.

## RESEARCH OBJECTIVE

The overall research objective was to extend the work of Preece et al. (2009) by comparing predictions of ash content

in grab samples of actual feedyard manure to those in samples artificially mixed in the laboratory by identifying the best pretreatment protocol and elucidating methods for reporting model prediction accuracies. More specifically, our objectives were to (1) quantify the effect of milling and oven-drying on VisNIR-DRS predictions of crude ash content in grab samples of feedyard manure, and (2) quantify how altering the selection of the validation set changes prediction accuracies of VisNIR-DRS models.

## MATERIALS AND METHODS

### SAMPLE COLLECTION AND PREPARATION

Manure samples ( $n = 120$ ) were collected from randomly selected pens at four commercial cattle feedyards in October 2007. The feedyards were under independent management and ownership and were located in the Texas counties of Deaf Smith, Moore, and Randall. Pen capacity ranged from approximately 100 to 250 head of cattle. Samples were collected by hand from dry areas within the pens and bagged separately. Each sample was hand-mixed well to ensure maximum homogeneity prior to being divided into five subsamples. Table 1 presents the number of feedyards and pens sampled, the number of samples collected, and the number of subsamples created.

The five sets of subsamples were preprocessed as described in table 2. Air-dried samples were placed in open-topped tins in a greenhouse for 48 h, which was sufficient time to reduce the moisture content of the samples to near equilibrium atmospheric values. The greenhouse reached daily temperatures of approximately 50°C. This air-drying procedure resulted in manure samples ranging from 4.3% to 16.1% (w.b.) moisture content, gravimetrically measured (Hoskins et al., 2003). Oven-dried samples were placed in open-topped tins in a drying oven (Tru Temp model 214300, Hotpack Corp., Philadelphia, Pa.) for a minimum of 8 h at 105°C. Milled samples were milled in a No. 2 Wiley mill to

**Table 1. Summary of the number of feedyards and pens sampled, the number of samples collected, and the number of subsamples created. Subsamples were used to create the four pretreatments plus one sample for traditional laboratory analysis.**

Feedyard	Pens per Feedyard	Samples per Pen	Samples per Feedyard	Subsamples	
				per Sample	per Feedyard
1	9	3	27	5	135
2	8	3	24	5	120
3	10	3	30	5	150
4	13	3	39	5	195
Total	40	--	120	--	600

**Table 2. Summary of preprocessing and analytical methods for determination of crude ash imposed on five sets of identical subsamples of manure.**

Subsample Set <sup>[a]</sup>	Milled	Drying Method	Analytical Method
OdMn	No	Oven	Dry oxidation
OdMn	No	Oven	VisNIR-DRS
AdMn	No	Air	VisNIR-DRS
OdMy	Yes	Oven	VisNIR-DRS
AdMy	Yes	Air	VisNIR-DRS

<sup>[a]</sup> Od = oven dried, Ad = air dried, Mn = not milled, and My = milled.

reduce at least 80% of the manure particles to less than 850  $\mu\text{m}$ ; other samples remained unmilled.

The particle size distribution was measured in a motorized sieve shaker (Ro-Tap model RX-30, W. S. Tyler, Mentor, Ohio) according to ASTM Standard C136-06 (ASTM, 2006). The representative mean particle size for the milled samples was -8 mesh (850  $\mu\text{m}$ ) sieve, where a minus sign (-) before the mesh number indicates the particles pass through the sieve. For comparison, the representative mean particle size for the unmilled samples was -5/16 mesh (8.00 mm) sieve.

The preprocessed samples were stored in a freezer with a maximum temperature of 0°C to preserve them until they were scanned with the VisNIR spectrometer. One oven-dried, not milled (OdMn) set of samples immediately underwent crude ash determination by dry oxidation.

Dry oxidation was performed in an ashing furnace (model F-A1730, Argo Thermodyne Co., Bangalore, India) according to the procedures of Preece et al. (2009), with reference to ASTM Standard E1755-01 (ASTM, 2007). Differences in ash content between the feedyards were tested using Tukey's honestly significant differences (HSD) test in SPSS Statistics software (SPSS, 2008). Normality of the combustion ash values was tested using the Kolmogorov-Smirnov one-sample test in SPSS Statistics (SPSS, 2008).

The four remaining sets of subsamples were brought uniformly to room temperature from 0°C. A VisNIR spectrometer (AgriSpec, ASD, Inc., Boulder, Colo.) was used to measure sample reflectance in wavelengths from 350 to 2500 nm with spectral resolutions of 3 nm at 700 nm and 10 nm at 1400 and 2100 nm. Samples weighing approximately 20 g were placed on a muglight featuring a 1.2 cm diameter scanning area illuminated by an internal tungsten quartz halogen light source. The samples were scanned from underneath through a Duraplan borosilicate optical-glass puck. Each sample was scanned three times, rotating the puck 120° between each scan to compensate for any remaining heterogeneity within each sample. A Spectralon white reference panel was used in the puck to set reflectance to 100% prior to each scanning session, and the white reference was verified after every 25 samples.

The raw spectral data were processed in four steps using custom statistical computing code written in R (R Foundation for Statistical Computing, Vienna, Austria) following the procedures of Brown (2007) and described by Preece et al. (2009). First, because the spectrometer instrument was equipped with three sensors that detect reflectance over three distinct wavelength ranges, discontinuities created by splicing the reflectance data at the end ranges of the sensors were corrected (see Brown et al., 2006). Second, the three raw reflectance scans for each sample were averaged. Third, the average values were smoothed with a weighted cubic spline using 5 nm knots and a smoothing parameter of 0.05. Finally, the first and second derivatives of reflectance with respect to wavelength ( $\partial r / \partial \lambda$  and  $\partial^2 r / \partial \lambda^2$ ) were computed using the spline coefficients. The values for every 10 nm from 350 to 2500 nm for the smoothed raw data and for the first and second derivatives were recorded. Thus, the data processing yielded three sets of spectral data for each manure pretreatment protocol. The spectral data sets were 10 nm averages of reflectance, first derivative of reflectance, and second derivative of reflectance (Preece et al., 2009).

## CRUDE ASH MODEL CREATION AND VALIDATION

Partial least squares (PLS) regression models were developed in The Unscrambler software (CAMO, 2007) and validated with a test-set holdout. The calibration models were built on mean-centered data using a segmented cross-validation PLS method. The segments for cross-validation were randomly chosen and represented 4% of the calibration dataset. The Unscrambler used a standard non-linear iterative partial least squares (NIPALS) algorithm and chose the number of factors to include in each PLS model by minimizing the residual variance of the calibration cross-validation.

To investigate potential dependence within pens and within feedyards, three different groups of models were created. In the first group, 1/3 of the samples were randomly selected as a validation test set, which is a common method of creating a validation set. In the second group of models, samples were grouped by pen and approximately 1/3 of the pens were randomly selected equally from each feedyard, creating a more independent validation set. This stratified-random sampling design did not allow samples within a single pen to be split between validation and calibration data sets. Lastly, in the third group of models, all of the samples from each feedyard were held out of the calibrations and then used as validation sets, which were the most independent of the three groups. This third group of models was expected to be the most conservative estimate of prediction accuracy for VisNIR ash prediction.

In the first group of models, 80 samples from each of the three spectral data sets, reflectance ( $r$ ), first derivative ( $\partial r / \partial \lambda$ ), and second derivative ( $\partial^2 r / \partial \lambda^2$ ), were randomly selected to comprise the calibration set. The first derivative generally removes albedo effects and is a transformation commonly used in VisNIR spectroscopy (Viscarra Rossel et al., 2006). The second derivative has been shown useful to alleviate effects of heterogeneous particle size in spectroscopy (Aucott and Garthwaite, 1988). After calibration, the models were validated with the remaining 40 samples (validation set). This process was repeated five times for each treatment using different, randomly selected calibration and validation sets to verify the stability of the model creation and performance. For each of the 60 models in this first group, the software was permitted to determine the optimal number of PLS factors. This produced a range of 2 to 13 PLS factors among the models.

The PLS function in Unscrambler chooses the number of factors at which the prediction residuals are minimized using the calibration data; however, when three factors were chosen, the validation RMSD was consistently higher than when 13 factors were chosen. The occasional selection of 13 compared to 4 or 5 factors was a numerical problem created by the shape of the prediction residual curve; it had undulations and local minima. Because of this consistent instability, comparison between default models was difficult, and the instability suggested that using 13 factors was overfitting. To facilitate comparison, the models were reconstructed, but the number of PLS factors was limited to four. Four was selected because it was the maximum number of PLS factors among all of the models at which overfitting did not occur.

Again, the modeling process was repeated five times for each treatment with different, randomly selected calibration and

validation sets to verify the stability of model creation and performance. In addition, the models were recalculated with the water bands (1390-1410 and 1890-1910 nm) removed to determine if moisture was confounding the spectra (based on Chang et al., 2001, and Dagnew et al., 2004). The reflectance spectra were not used in the second and third model groups because they consistently produced the lowest prediction accuracies in the first modeling exercise.

In the second group of models, dependence among samples from the same pen was examined. Samples were grouped according to the pen from which they were collected, and the calibration ( $n = 81$ ) and validation ( $n = 39$ ) sets contained samples from randomly chosen pens with equal representation from each feedyard. In other words, samples collected from the same pen were not split between the calibration and validation sets. Further, each feedyard was equally represented in the calibration and validation sets. The number of PLS factors was limited to four, and eight models were built from the first and second derivatives of each treatment.

In the third group of models, all of the samples from each feedyard were held out of the calibration in turn. They were then used to validate their respective models. The four validation sets contained 39, 24, 20, and 27 samples, respectively. The software was permitted to determine the optimal number of PLS factors. Thirty-two models were built from the first and second derivatives of each treatment.

The root mean squared deviation (RMSD), coefficient of determination ( $r^2$ ), and bias of the validation data along with the number of PLS factors were considered in the evaluation of model performance. To investigate spectral similarity between the manure preprocessing treatments, biplots of the first two principal components of the first derivatives were created. Spectral similarities or differences between treatments can be visually identified with a convex hull biplot (Islam et al., 2005). Principal component decomposition of the first derivative spectra and convex hull calculations were performed in R (R Development Core Team, 2006).

## RESULTS AND DISCUSSION

Table 3 presents the minimum, maximum, mean, standard deviation, and skewness of measured crude ash (% d.b.) from

**Table 3. Summary statistics of crude ash (% d.b.) measured by dry oxidation from all manure samples together and by feedyard.**

Statistic	All	Feedyard			
		1	2	3	4
Minimum	19.2	19.2	22.4	23.8	22.9
Maximum	61.8	47.9	61.8	34.1	44.2
Mean	31.8	31.6	35.0	29.1	32.1
Standard deviation	7.68	7.37	11.63	3.13	6.47
Skewness	1.36	0.04	1.24	-0.18	0.29
Count	120	39	24	30	27

all manure samples together and by feedyard. Crude ash content of the manure samples was normally distributed ( $p < 0.05$ ), with a range of 19.2% to 61.8% (d.b.). For reference, the average ash content of manure excreted by cattle on standard finishing diets in the region was about 15% (d.b.) (Auvermann et al., 2007). All samples were above this value, indicating that they were a mixture of manure and soil, or that the cattle were not fed a standard finishing ration. The mean ash values between feedyards were statistically similar except that of feedyard 2, which yielded samples significantly higher in ash content than feedyard 3 ( $p < 0.05$ ). The mean 95% confidence limit for the ash results determined by dry oxidation was  $\pm 1.4\%$ .

Table 4 presents the mean results from the first group of models. Models built from the first and second derivatives of the spectra yielded slightly better prediction accuracies than the raw reflectance models in all four treatments. Prediction error (RMSD) in all of the models ranged from less than 4% to less than 6% ash (d.b.). This is similar to the prediction obtained in a previous study (Preece et al., 2009) in which crude ash was predicted within  $\pm 5\%$  ash (d.b.) from samples artificially manufactured from proportionate mixtures of composite stocks of manure and soil. The current study maintained prediction accuracy comparable to the previous study, despite using natural manure samples from multiple locations. A  $\pm 5\%$  ash (d.b.) prediction accuracy is useful for determining manure quality in commercial applications. Further, models built from the first and second derivatives had similar RPD values and very low biases.

The RPD is the ratio of the standard deviation over the RMSD and provides a numeric value for model comparison that represents the accuracy of the models with respect to the standard deviation of the population being predicted. General

**Table 4. Mean root mean squared error (RMSD<sub>cal</sub>) and mean number of PLS factors for the calibration ( $n = 80$ ) model using five unique VisNIR-DRS models. For the validation ( $n = 40$ ), the mean RMSD<sub>val</sub>, mean relative percent difference (RPD), and mean bias of five unique VisNIR-DRS models for each pretreatment and post-processing protocol are shown.**

Transformation	Treatment <sup>[a]</sup>	RMSD <sub>cal</sub> (ash % d.b.)	RMSD <sub>val</sub> (ash % d.b.)	RPD	Bias (ash % d.b.)	PLS Factors (count)
Raw spectra	AdMn	3.6	4.0	1.9	-0.1	3.6
	AdMy	4.1	4.6	1.7	-0.2	2.4
	OdMn	4.8	5.7	1.3	-0.1	2.4
	OdMy	4.0	4.3	1.8	0.1	3.8
First derivative	AdMn	3.2	3.6	2.1	-0.3	2.8
	AdMy	3.6	4.4	1.7	-0.4	2.6
	OdMn	4.5	5.7	1.4	-0.0	2.2
	OdMy	3.1	4.1	1.9	-0.2	3.2
Second derivative	AdMn	3.2	3.6	2.1	-0.1	2.2
	AdMy	3.4	4.9	1.8	-0.2	3.2
	OdMn	4.5	5.6	1.4	0.1	1.8
	OdMy	3.1	4.0	1.9	-0.2	3.2

<sup>[a]</sup> Od = oven dried, Ad = air dried, Mn = not milled, and My = milled.

guidelines for reporting the performance of calibrations for environmental or heterogeneous samples (such as manure) are based on five RPD levels (Huang et al., 2007; Malley et al., 2005; Saeys et al., 2005):

- RPD less than 1.5 means the calibration is not useful.
- RPD between 1.5 and 2.0 means that high and low values of the constituent being predicted can possibly be distinguished.
- RPD between 2.0 and 2.5 means approximate quantitative predictions are possible.
- RPD between 2.5 and 3.0 is good or successful.
- RPD above 3.0 is excellent.

Furthermore, an RPD above 4.0 indicates that the model is reproducible and can be used reliably in commercial applications (Williams, 2001). However, models with an RMSD below industry requirements, despite a low RPD, are also useful. For example, it may be useful to classify manure above or below an acceptable ash threshold, or into quality classes based on ash content. In these cases, knowing the ash value  $\pm 5\%$  (d.b.) may be sufficient, and a VisNIR-DRS model that can consistently predict ash within  $\pm 4\%$  (d.b.) is potentially useful.

Among the different pretreatment methods, the AdMn samples had the best prediction accuracies, while the OdMn samples had the worst prediction accuracies, with an increase in RMSD of almost 2.0% ash (d.b.) regardless of the data post-processing method. Oven-drying reduced the prediction accuracy among the samples that were not milled. In contrast, oven-drying reduced the prediction accuracy much less (less than 0.5% ash, d.b.) on models based on milled samples. Based on the RMSD values, milling reduced accuracy in air-dried samples by approximately 1.0% ash (d.b.) and improved prediction in oven-dried samples by approximately 1.5% ash (d.b.). Evidently, oven-drying decreased the prediction accuracy more strongly than milling, and clearly changed the spectral properties of the sample (fig. 1). The effect of oven-drying may be a result of reducing the number of organic carbon and water associated bonds (C-O, C-H), which are spectrally active (Williams, 2001).

Eliminating the water bands (1390-1410 and 1890-1910 nm) slightly increased prediction error in all AdMn models by less than 1% crude ash (d.b.). Eliminating the

visible range (350-1400 nm) also slightly increased prediction error in all AdMn models by less than 1% crude ash (d.b.). These results are inconsistent with other studies that found improvement in prediction accuracy for soil constituents with the elimination of water bands and/or wavelengths in the visible range (Chang et al., 2001; Dagnew et al., 2004). Manure has a tendency to be hygroscopic at the molecular level (despite being hydrophobic on a macro level), resulting in a greater proportion of adsorbed water as opposed to water in the vapor or free phase. Therefore, adsorbed water may contribute positively to spectral interpretation. Results reported by Lobell and Asner (2002) support this idea; they found that changes in reflectance in the 400 to 1100 nm range were caused solely by changes in relative refractivity of water adsorbed to particle surfaces, and changes in pore space water had little effect on reflectance.

Spectral similarities or differences between treatments can be visually recognized with a convex hull biplot (Islam et al., 2005). The distribution of the samples within the biplot area also provides a strong indication of their degree of variation. Figure 1 presents a convex hull analysis (Eddy, 1977) of the first derivatives of the reflectance for each treatment in the biplot of principal components 1 and 2. This chart offers a striking visual comparison of the four treatments and supports the conclusions that there is no compelling evidence to support milling samples and that the spectra of oven-dried samples were very different from those of air-dried samples.

Because three samples of manure were collected from each pen, samples from any single pen may have been represented in both the calibration and validation sets. To eliminate the potential for pen-wise pseudo-independence, a second group of models was constructed with stratified-random sampling in which all samples from one pen were grouped together and used either in the calibration or validation set. Results for this group of models are presented in table 5. The prediction accuracy of these models was approximately 1% crude ash (d.b.) lower than that of the first group of models, in which samples were not grouped by pen. This result indicates that some pen-wise dependency existed among the samples, and that the first group of models overestimated prediction accuracy by approximately 1% crude ash (d.b.). First and second derivative models performed similarly, and the biases were very small (-0.4 to 0.1). The AdMn treatment yielded RMSD and RPD values of 4.7 and 2.3, respectively, using first derivatives and 4.6 and 2.4, respectively, using second derivatives. Again, the RMSD of VisNIR-DRS for predicting crude ash in AdMn is within the accepted industry tolerance of  $\pm 5\%$ . Plots of the predicted versus measured ash content from models using stratified-random selection of calibration and validation sets and based on the first derivative of all treatments are presented in figures 2 through 5.

Results for the third group of models are presented in table 6. Prediction accuracy decreased by approximately 2% ash (d.b.) compared to random validation when whole-feedyard hold-out validation was used. The average ( $n = 4$ ) RMSD and RPD for the AdMn treatment were 4.3 and 1.7, respectively, using first derivatives and 4.6 and 1.5, respectively, using second derivatives. The prediction accuracies of the whole-feedyard hold-out validations were lower than those of the random and random-stratified hold-

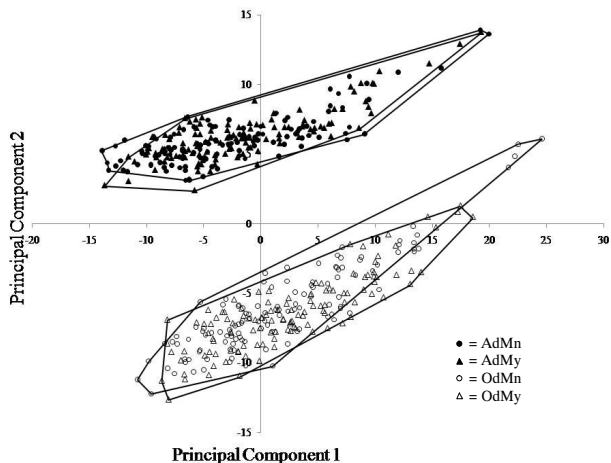
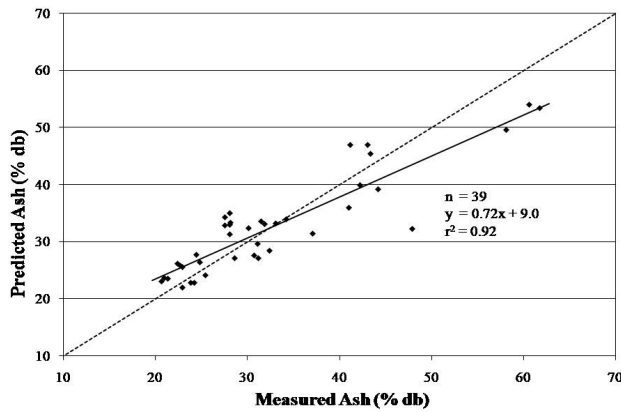


Figure 1. Distribution of samples for the first derivative of spectra from four different treatments in the convex hull biplot of principal components 1 and 2.

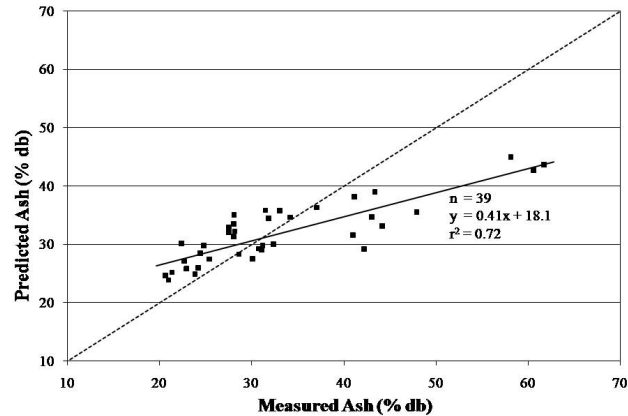
**Table 5. Root mean squared error (RMSD<sub>cal</sub>) and number of PLS factors for the calibration ( $n = 81$ ) VisNIR-DRS model. For the validation ( $n = 39$ ), the mean RMSD<sub>val</sub>, relative percent difference (RPD), and bias of VisNIR-DRS models created using stratified-random selection of calibration and validation sets for each pretreatment and post-processing protocol are shown.**

Transformation	Treatment <sup>[a]</sup>	RMSD <sub>cal</sub> (ash % d.b.)	RMSD <sub>val</sub> (ash % d.b.)	RPD	Bias (ash % d.b.)	PLS Factors (count)
First derivative	AdMn	2.4	4.7	2.3	-0.2	3
	AdMy	3.1	6.1	1.8	-0.6	4
	OdMn	3.9	6.9	1.6	-1.1	2
	OdMy	2.9	6.6	1.6	-1.2	2
Second derivative	AdMn	2.1	4.6	2.4	0.2	4
	AdMy	2.8	5.9	1.8	0.9	3
	OdMn	3.9	7.1	1.5	-0.8	2
	OdMy	2.5	6.2	1.7	1.7	3

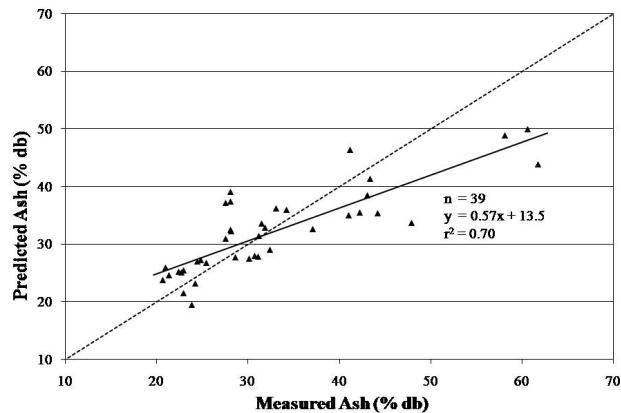
[a] Od = oven dried, Ad = air dried, Mn = not milled, and My = milled.



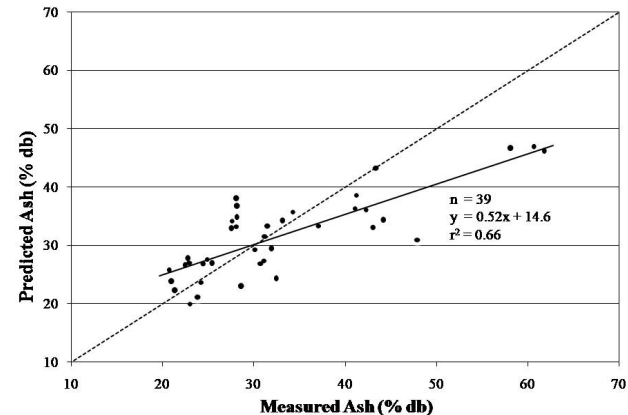
**Figure 2. Predicted ash (% d.b.) versus measured ash (% d.b.) from the validation of the AdMn model using stratified-random selection of the calibration and validation sets and using first derivative. A linear trend line is shown in bold, along with a dashed 1:1 line for reference.**



**Figure 4. Predicted ash (% d.b.) versus measured ash (% d.b.) from the validation of the OdMn model using stratified-random selection of the calibration and validation sets and using first derivative. A linear trend line is shown in bold, along with a dashed 1:1 line for reference.**



**Figure 3. Predicted ash (% d.b.) versus measured ash (% d.b.) from the validation of the AdMy model using stratified-random selection of the calibration and validation sets and using first derivative. A linear trend line is shown in bold, along with a dashed 1:1 line for reference.**



**Figure 5. Predicted ash (% d.b.) versus measured ash (% d.b.) from the validation of the OdMy model using stratified-random selection of the calibration and validation sets and using first derivative. A linear trend line is shown in bold, along with a dashed 1:1 line for reference.**

out validations. Increased error should be expected when using a spectral model to predict ash for a feedyard not included in the model calibration. First and second derivatives yielded similar prediction accuracies. Again, the AdMn treatment outperformed all other treatments, this time in 85% of the model comparisons. Biases ranged from -6 to 4 and were consistent with feedyard, regardless of treatment, confirming that some dependency existed between samples from within the same feedyard.

PLS factors were unrestricted when the whole-feedyard hold-out models were computed. The number of PLS factors among models built from the AdMn treatment ranged from 2 to 4, with an average ( $n = 4$ ) of 2.8, using first derivatives and from 2 to 4, with an average ( $n = 4$ ) of 3.3, using second derivatives. In contrast, PLS factors in models based on the remaining three treatments ranged from 2 to 9 using first derivatives and from 2 to 8 using second derivatives. This indicates that the spectral variation was adequately described

**Table 6. Root mean squared error (RMSD<sub>cal</sub>) and mean number of PLS factors for the VisNIR-DRS model calibration. For the validation (RMSD<sub>val</sub>), ratio of the standard deviation over the RMSD (RPD), and bias of VisNIR-DRS models using whole-feedyard hold-out selection of calibration and validation sets for each pretreatment and post-processing protocol are shown.**

Transformation	Holdout	Treatment <sup>[a]</sup>	Cal. (n)	Val. (n)	RMSD <sub>cal</sub> (ash % d.b.)	RMSD (ash % d.b.)	RPD	Bias (ash % d.b.)	PLS Factors (count)
First derivative	Feedyard 1	AdMn	81	39	2.2	5.6	1.3	2.5	4
		AdMy	81	39	2.3	5.4	1.4	0.7	7
		OdMn	81	39	4.5	6.1	1.2	2.7	2
		OdMy	81	39	1.9	7.2	1.0	4.4	6
	Feedyard 2	AdMn	96	24	3.1	4.5	2.6	1.6	3
		AdMy	96	24	2.8	6.6	1.8	0.0	7
		OdMn	96	24	3.6	7.9	1.5	2.8	3
		OdMy	96	24	3.7	6.1	1.9	1.3	2
	Feedyard 3	AdMn	100	20	3.3	3.4	0.9	-1.5	2
		AdMy	100	20	3.8	5.0	0.6	1.7	5
		OdMn	100	20	5.1	3.1	1.0	0.6	2
		OdMy	100	20	2.9	5.0	0.6	-2.7	5
	Feedyard 4	AdMn	93	27	3.3	3.7	1.8	-2.3	2
		AdMy	93	27	3.1	2.2	3.0	0.2	9
		OdMn	93	27	4.5	7.0	0.9	-5.5	2
		OdMy	93	27	2.9	5.0	0.6	-3.7	5
Second derivative	Feedyard 1	AdMn	81	39	2.3	5.0	1.5	2.2	4
		AdMy	81	39	2.4	6.8	1.1	3.1	5
		OdMn	81	39	4.5	5.4	1.4	1.8	2
		OdMy	81	39	2.5	7.1	1.0	3.2	5
	Feedyard 2	AdMn	96	24	2.8	4.8	2.4	2.9	3
		AdMy	96	24	2.3	6.5	1.8	3.3	8
		OdMn	96	24	3.5	8.7	1.3	3.0	3
		OdMy	96	24	3.2	6.6	1.8	3.0	3
	Feedyard 3	AdMn	100	20	3.5	3.1	1.0	-1.1	2
		AdMy	100	20	4.0	3.9	0.8	-1.7	5
		OdMn	100	20	5.1	3.0	1.1	1.2	2
		OdMy	100	20	3.9	4.2	0.7	-2.1	2
	Feedyard 4	AdMn	93	27	2.7	5.4	1.2	-4.4	4
		AdMy	93	27	3.9	5.1	1.3	-4.3	2
		OdMn	93	27	4.5	7.3	0.9	-6.1	2
		OdMy	93	27	4.1	4.8	1.4	-3.2	2

<sup>[a]</sup> Od = oven dried, Ad = air dried, Mn = not milled, and My = milled.

with fewer PLS factors in the AdMn treatment, and further, that the AdMy, OdMn, and OdMy treatments were more prone to overfitting or had more noise in the spectral data.

Samples collected at feedyard 3 were lower in ash content, and had less variance than samples from the other feedyards. When samples from feedyard 3 were held out of model calibration and were in turn predicted, those models yielded lower RMSD values. In contrast, when samples from feedyard 2 were held out and used as a validation set, those models produced higher RMSD values. Feedyard 2 had slightly higher average ash content and much higher variance than the other feedyards. Comparable observations were made in other studies (Brown et al., 2005; Waiser et al., 2007; Morgan et al., 2009). Specifically, Brown et al. (2005) applied first-derivative modeling to similar soils from six different sites. Using samples from all of the sites in both the calibration and validation sets produced good models. However, when samples from one field were predicted with models calibrated with samples from the remaining fields, three of the models failed. Brown concluded that pseudo-independent validation, which results from randomly selecting from non-independent samples, can result in an

overestimation of predictive accuracy relative to models validated with independent samples. With regard to feedyard manure, pseudo-independency may result from dependence between samples from the same feedyard and the same pen.

The software identified 58 wavelengths as significant ( $p < 0.05$ ), and they are listed in table 7. Ten were located in the visible range (350 to 700 nm), 30 in the IR-A near-infrared range (700 to 1400 nm), and 18 in the IR-B near-infrared range (1400 to 2500 nm).

**Table 7. Significant wavelengths ( $p < 0.05$ ) of a VisNIR-DRS model using stratified-random selection of calibration and validation sets for the AdMn treatment.**

Visible Range (350-700 nm)	IR-A Range (700-1400 nm)	IR-B Range (1400-2500 nm)
530-540	710-820	1410-1460
580-640	970	1540
700	1140-1160	1560-1570
	1180-1240	1680-1720
	1320-1370	1740
	1390-1400	1760
		2020-2030

## CONCLUSION

Crude ash in solid cattle manure was most accurately predicted by VisNIR-DRS using models built from scans of samples with a minimum of preprocessing, such as air-dried samples that were not milled. Furthermore, accuracy improved modestly when all wavelengths were considered in the models, including water bands and the visible region (by less than 1% ash, d.b.). Crude ash content was predicted with an accuracy of  $\pm 5\%$  (d.b.) of the observed ash content as determined by dry oxidation. The performance of models based on unprocessed samples was better by 1% to 2% ash (d.b.) when compared to processed samples, and similar to the prediction obtained in a previous study (Preece et al., 2009).

That unprocessed manure samples yield good VisNIR-DRS predictive models implies that there is potential for rapid, accurate, *in situ* analysis with portable spectrometers. This convenience increases the usefulness of VisNIR-DRS as a tool for determining manure quality on a commercial scale. This practical use can be taken advantage of by commercial manure producers and end users.

The estimated prediction accuracies of the VisNIR models decreased when the models were validated using stratified-random and whole-feedlot validation sets. Therefore, it is important to consider the assumptions under which prediction accuracy is estimated. If a commercial application using VisNIR to rapidly quantify crude ash in feedlot manure is developed, then care must be taken when introducing new samples that are not represented in the calibration data.

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