

SCIENTIFIC PERSPECTIVE

Forage & Grazinglands

Reporting forage nutritive value using near-infrared reflectance spectroscopy

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Abstract

Despite the well-documented merits of near-infrared reflectance (NIR) spectroscopy for forage nutritive value analysis, recent studies reveal inconsistencies in accuracy of NIR-predicted values. These findings underscore the critical need for robust validation efforts to ensure reliability. Employing visual tools, such as scatter plots comparing laboratory-measured with NIR-predicted values, enhances the interpretation and qualification of data. Standardized reporting of validation outcomes, including key metrics and best practices, is essential for ensuring data quality and fostering broader adoption of NIR spectroscopy across research and industry. In this article, we suggest guidelines for reporting NIR spectroscopy predictions and emphasize the need for independent validation as a required procedure to enhance the credibility and application of NIR spectroscopy for forage analysis.

Plain Language Summary

The accuracy and precision of near-infrared reflectance (NIR) spectroscopy predictions cannot be assumed because a NIR solution produced a value. Using scatter plots to compare laboratory-measured versus NIR-predicted values, in addition to fit statistics such as standard error of prediction, bias, and the slope, intercept, and coefficient of determination of the linear regression of laboratory-measured versus NIR-predicted values, can reveal inconsistencies often overlooked. We provide guidelines and rationale for developing independent testing to enhance the credibility and application of NIR spectroscopy for forage analysis.

1 | USE OF NIR SPECTROSCOPY FOR FORAGE ANALYSIS

The merit and validity of near-infrared reflectance (NIR) spectroscopy as a tool for rapid determination of forage

nutritive value are well-documented in the literature. A comprehensive review of these contributions is beyond the scope of this article. Nevertheless, we refer the reader to Batten (1998), Shenk and Westerhaus (1994), and Burns and Ciurczak (2007) as starting references for further exploration on the topic.

The use of NIR spectroscopy to estimate nutritive value and digestibility of forages can be traced in US literature to the

Abbreviations: CP, crude protein; DM, dry matter; NIR, near-infrared reflectance; SEP, standard error of prediction.

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seminal study by Norris et al. (1976). Recognizing the importance of validation for NIR-predicted values, Norris et al. (1976) indicated that “to provide a better test of the merits of such measurement (referring to NIR-predicted values), we used the odd-numbered samples to develop calibration equations, which were then used to predict crude protein (CP), neutral detergent fiber (NDF), and in vivo dry matter digestibility (DMD) for the even-numbered samples.” By comparing the NIR-predicted values versus the laboratory-measured values, they reported that forage nutritive value and digestibility could be predicted within standard errors of $\pm 9.5 \text{ g kg}^{-1}$ for CP, $\pm 31.2 \text{ g kg}^{-1}$ for NDF, and $\pm 51 \text{ g kg}^{-1}$ for DMD. In 1978, the United States Department of Agriculture Near-Infrared Spectroscopy (USDA NIRS) Forage Network was started to develop software, coordinate further research, and prepare the technology to be transferred to the private sector (Marten et al., 1989; Shenk & Westerhaus, 1994; Templeton et al., 1983).

Technological advancements have greatly improved accessibility and affordability of NIR spectroscopy technology. A variety of spectrometers is now available, including desktop and portable handheld types (Berzaghi et al., 2021; Crocombe, 2018; Zhu et al., 2022). Additionally, multiple software platforms with diverse methodologies have been developed to support NIR spectroscopy applications (Breunig et al., 2000; Ng et al., 2018; Xiaobo et al., 2010). The adoption of NIR spectroscopy applications is growing rapidly among researchers and non-researchers, and this technological tool is particularly being portrayed as a “turnkey” solution. However, it is essential that all users, especially scientists, remain cognizant of the potential limitations of erroneous NIR-predicted estimates when reporting response variables and treatment effects from experimentation, particularly when relying on third-party or pre-loaded NIR calibrations.

2 | CONTEXTUALIZATION OF THE ISSUE

Shenk and Westerhaus (1991) introduced a method for defining population boundaries and selecting samples for calibration of NIR models based on Mahalanobis distances. Their approach demonstrated the feasibility of selecting a small, structured subset of samples for calibration of NIR spectroscopy models from larger datasets. However, they acknowledged uncertainties regarding the relative importance of neighborhood size, sample quantity, and the number of model terms in achieving accurate calibrations.

An essential step in the development of application of NIR solutions is the measurement of the similarity or distance between samples in the NIR space. We refer to an NIR solution as the integration of software, hardware, and sampling procedures that result in NIR-predicted values.

Core Ideas

- Near-infrared reflectance (NIR) spectroscopy is a well-documented technology for determination of forage nutritive value.
- Technological advancements have greatly improved accessibility and affordability of NIR spectroscopy technology.
- Recent studies reveal inconsistencies in accuracy of NIR-predicted values.
- Conducting an independent validation is the ultimate standard for assessing the quality of NIR-predicted values.

Mahalanobis distance has become the standard population-structuring methodology and is used to identify spectral outliers in commercial applications of NIR solutions (Pérez-Marín et al., 2005; Shenk et al., 1997). Different software packages use different variants of the same Mahalanobis distance (Garrido-Varo et al., 2019). Nevertheless, Williams et al. (2017) noted that “it is possible to obtain high errors in prediction of samples with Mahalanobis distances well within the acceptable range.” Working with soil samples and various distance metrics to investigate the relationship between soil compositional similarity and soil vis-NIR, Ramirez-Lopez et al. (2013) reported that the least accurate results were obtained using the Mahalanobis distance method. Therefore, relying solely on spectral data does not ensure accuracy of NIR predicted values.

3 | JUSTIFICATION FOR INDEPENDENT VALIDATION

Estimates of forage nutritive value from handheld NIR spectrometers can be of comparable performance to those derived from benchtop-type spectrometers (Acosta et al., 2020; Berzaghi et al., 2021; Digman et al., 2022). However, accuracy of NIR predictions cannot be assumed because a “number is produced” by the NIR solution. Cherney et al. (2021) reported the results of an independent validation test for three commercially available NIR solutions and concluded that none of the three NIR solutions predicted dry matter (DM) concentration with enough accuracy. Additionally, the authors reported that NIR-predicted forage nutritive value estimates were not accurate for fiber components (i.e., acid detergent fiber [ADF] and aNDF) of haylage, corn silage, and total mixed rations, while CP concentration results were mixed. Le Cocq et al. (2022) also reported results of an independent validation of four commercially available NIR solutions to determine nutritive value of haylage samples and concluded that the

results were not reliable for on-farm applications. The lack of publicly independent evaluations of the relative effectiveness of NIR solutions currently on the market, particularly for field applications, is a significant concern. Additionally, the unvalidated use of handheld NIR instruments in forage research studies, which was noted by Cherney et al. (2021), highlights an important gap that must be addressed to ensure the reliability and accuracy of reported findings.

Conducting an independent validation—comparing laboratory-measured to NIR-predicted values—is the ultimate standard for assessing the accuracy and reliability of NIR solutions. This process not only verifies the utility of the application but also enhances the interpretation of results. Independent validation is essential not only during calibration development but, most importantly, for end-users who rely on NIR predictions from service providers or resulting from pre-loaded calibrations in NIR solutions. Validation can be accomplished by comparing laboratory-measured to NIR-predicted values using samples that meet two requirements. First, the samples for the independent validation exercise were not included in the calibration of the NIR model and, second, the samples represent the population being analyzed and cover the range of NIR-predicted values.

4 | PERFORMANCE METRICS

Eleven items were proposed by Williams et al. (2017) when reporting the results of an independent validation. Among these were the global and average neighborhood Mahalanobis distances, metrics used to assess spectral outliers and to define the population boundaries. However, the application of these two metrics is limited to specific software packages, and their inclusion is not necessarily indicative of the overall performance of the validation exercise, nor are they universally applicable across all contexts as was acknowledged by Shenk and Westerhaus (1991).

Fearn (2002) discussed the utility of various statistical metrics for evaluating the performance of NIR calibrations, including standard error of prediction (SEP), ratio of standard error of performance to standard deviation, range error ratio, and coefficient of determination (r^2). He highlighted SEP as a straightforward and reliable measure of a model's predictive accuracy for future samples and emphasized that ensuring linearity in predictions and reporting SEP alone is sufficient for evaluating the performance of an NIR solution in validation exercises. The magnitude of the SEP will depend on how well the independent validation samples are represented by the samples that were used in the calibration step.

Windham et al. (1989) suggested that SEP values within two times the standard error of the laboratory analysis (SEL)—which represents the standard error of replicate analysis by the laboratory—are acceptable. The authors also indicated that if the SEP in validation is too large, then the

source of the variation should be identified and potentially removed. As a reference, SEL varies by constituent; for example, $\leq 3, 6, 12, 9, 15$, and $20\text{--}37 \text{ g kg}^{-1}$ for DM concentration, CP, ADF, acid detergent lignin, NDF, and in vitro true DMD, respectively, and is preferably half of these values (Shenk et al., 1981; Templeton et al., 1983; Windham et al., 1989). Across several portable and benchtop-type NIR solutions, reported fit statistics in validation include: for CP, r^2 values as high as 0.98, SEP values as low as 7.9 g kg^{-1} , and bias as low as $\pm 0.3 \text{ g kg}^{-1}$; for in vitro true digestibility, r^2 values as high as 0.97, SEP values as low as 23.8 g kg^{-1} , and bias as low as $\pm 1.8 \text{ g kg}^{-1}$; for an NDF and ADF, r^2 values as high as 0.96 and 0.93, SEP values as low as 19.2 and 14.2 g kg^{-1} , and bias as low as ± 0.3 and $\pm 0.9 \text{ g kg}^{-1}$, respectively (Acosta et al., 2020; Berzaghi et al., 2021).

Based on the previous information, NIR-predicted estimates of forage nutritive value are expected to be nearly identical or highly similar to laboratory-measured values, with performance fit statistics such as r^2 approaching 1, low SEP, bias close to 0, and a slope of the regression line near 1 with an intercept close to 0 (Abrams et al., 1987; Acosta et al., 2020; ISO, 2017; Windham et al., 1989). However, acceptable fit statistic metrics appear to be specific to the field of study. For instance, working with soil data and using r^2 as a fit statistic to evaluate NIR calibration performance, Malley et al. (2004) proposed four categories: excellent ($r^2 > 0.95$), successful ($r^2 = 0.90\text{--}0.95$), moderately successful ($r^2 = 0.8\text{--}0.9$), and moderately useful ($r^2 = 0.7\text{--}0.8$), while noting that even calibrations with $r^2 < 0.7$ may still be useful for screening purposes. Chang et al. (2001), also working with soil data, categorized calibration success into three groups: A ($r^2 = 0.8\text{--}1.0$), B ($r^2 = 0.5\text{--}0.8$), and C ($r^2 < 0.5$). Nduwamungu et al. (2009) summarized guidelines proposed by seven different authors for assessing NIR calibration accuracy for soil data. Considering these findings, NIR-predicted values must be “sufficiently” correlated and unbiased relative to laboratory-measured values to ensure their practical utility. However, the achievable and acceptable level of sufficiency varies across fields of application. While there appears to be a general desire for a single statistic that measures in some absolute way how “good” a calibration is (Fearn, 2002), no universally accepted standard exists. Instead, a comprehensive assessment of multiple factors is required.

A scatter plot (biplot) of the laboratory-measured versus NIR-predicted values (Figure 1) provides a visual assessment for evaluating the (expected) linearity and overall agreement between the two datasets, enhancing the interpretation and qualification of results (Dardenne, 2010; Fearn, 2002; ISO, 2017). This visualization helps identify trends, deviations, or inconsistencies, serving as a useful internal quality assurance measure for researchers. However, such plot is typically not to be included in the final publication, as it is more suited for internal validation and ensuring data reliability.

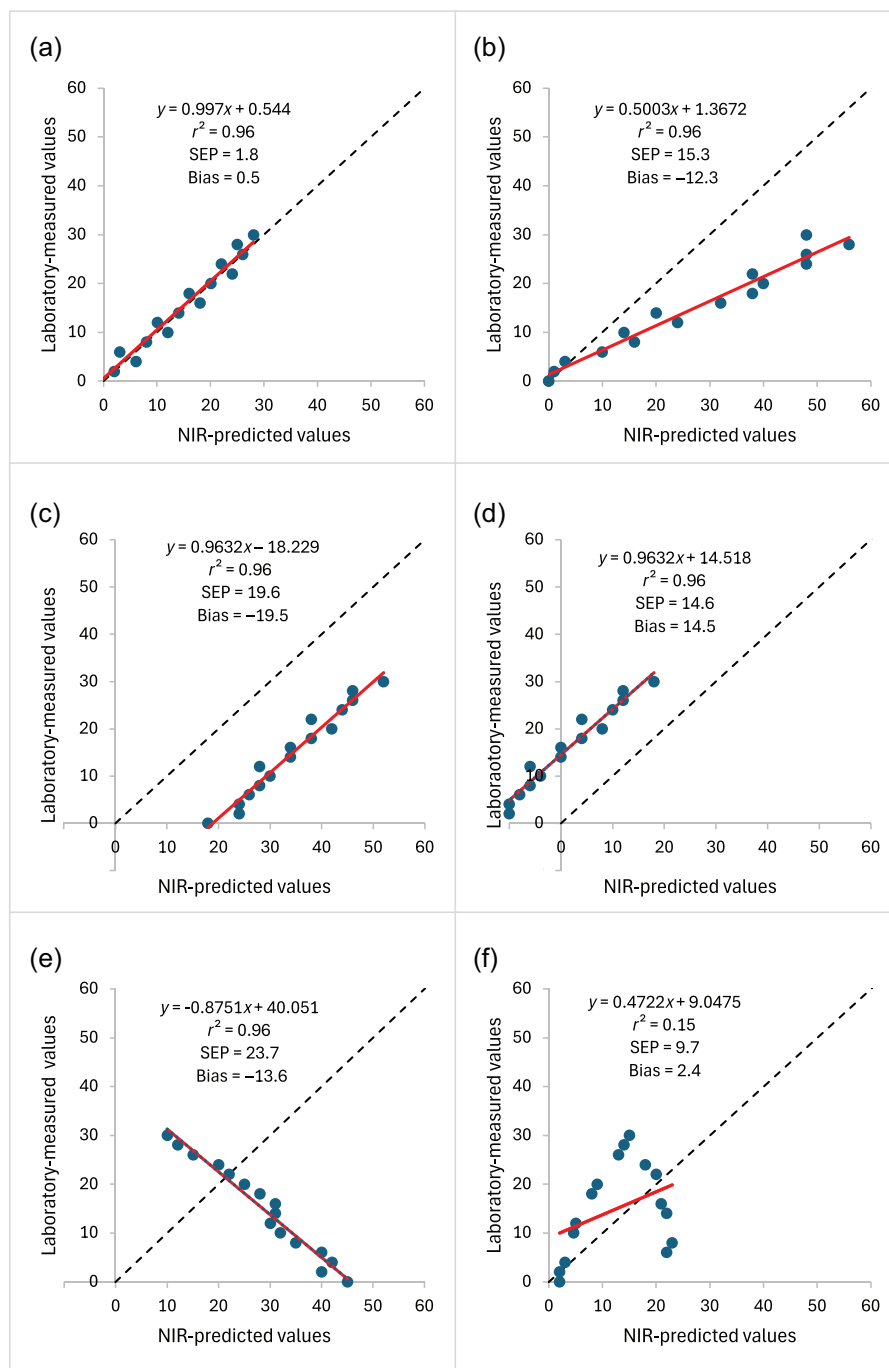


FIGURE 1 Laboratory-measured versus near-infrared reflectance (NIR)-predicted values (a–f). Dotted line represents a reference line of slope 1. The red (solid) line is the fitted linear regression. SEP, standard error of prediction.

Using simulated data, Figure 1 demonstrates how a visual assessment of the relationship between laboratory-measured and NIR-predicted values can reveal inconsistencies overlooked if relying on isolated fit statistics. Notably, panels b, c, d, and e share identical r^2 values; however, the NIR-predicted values differ substantially from the laboratory-measured values with bias ranging from -19.5 to 14.5 . Although panel f has a lower SEP value compared to panels b, c, d, and e, the relationship is not linear. Panel a represents a desirable outcome.

5 | SUGGESTED GUIDELINES FOR REPORTING NIRS-PREDICTED VALUES

When reporting NIR spectroscopy as the methodology for forage nutritive value, it is suggested that the following items should be included:

- (1) A description of the reference analytical methods used to develop NIR models. This information should always

accompany the description of the NIR-predicted response variables as it is critical to contextualize the results. For example, if the NIR solution determines concentration of N in the plant tissue, the authors could include: “Concentration of N was analyzed by dry combustion using XYZ equipment and XYZ protocol after samples were ball milled” and provide the appropriate references. Ideally, SEL for each reference method should be provided.

- (2) Fit statistics of the independent validation exercise includes the following:
 - (a) The number of samples (n) used in the validation exercise. At least 10–20 independent validation samples that represent the population and cover the range of NIR-predicted values should be used in the determination of bias, slope, and SEP (ISO, 2017; Windham et al., 1989).
 - (b) The SEP and bias are estimated as follows:

Let d_i be the difference between the laboratory-measured values minus NIR-predicted values for the i th sample. Then,

$$\text{SEP} = \sqrt{\frac{\sum (d_i)^2}{n}},$$

$$\text{Bias} = \frac{\sum (d_i)}{n},$$

where the sum, like all those that follow, is over all samples, that is, from 1 to n (Fearn, 2002).

- (c) The r^2 , slope, and intercept values of the fitted linear regression from laboratory-measured values (y -axis) and NIR-predicted values (x -axis) (Fearn, 1998).

The five fit statistics of the independent validation exercise (i.e., SEP, bias, r^2 , slope, and intercept of the linear regression), including the description of the validation set (n), can be reported in the text of the article in the materials and methods section where the use of the NIR technology is described.

Statistical analyses of treatment effects from experimentation are warranted only after the researcher verifies the usability and accuracy of the NIR-predicted values by collectively evaluating the fit statistics from the independent validation exercise, with emphasis on the word “collectively.” Emphasizing a collective assessment helps prevent cases like the higher r^2 values seen in panel e (Figure 1), or lower SEP values seen in panel f (Figure 1), from being mistakenly prioritized over the other fit statistics. This approach helps researchers make well-informed interpretations of results.

6 | SUMMARY AND CONCLUSIONS

NIR spectroscopy has been extensively validated as a rapid tool for assessing forage nutritive value, with its roots in the foundational work of Norris et al. (1976) and subsequent advancements through the USDA NIRS Forage Network. Despite technological progress, including the development of handheld portable devices and diverse software platforms, the accuracy of NIR predictions cannot be assumed because a NIR solution produces a number. Validation by comparing laboratory-measured versus NIR-predicted values, using samples outside the calibration dataset, remains essential to identify potential anomalies and assess the accuracy of NIR-predicted values. This step is critical for users who receive results from service providers or who use pre-loaded calibrations. We provided specific guidelines and justification for what items should be reported in research projects when using NIR technology. Adherence to these practices ensures robust NIR data quality, paving the way for valid experimental analyses and broader adoption of this promising technology.

AUTHOR CONTRIBUTIONS

Miguel S. Castillo: Conceptualization; data curation; formal analysis; methodology; project administration; visualization; writing—original draft; writing—review and editing. **Thomas C. Griggs:** Conceptualization; writing—review and editing. **Matthew F. Digman:** Conceptualization; writing—review and editing. **João M. B. Vendramini:** Conceptualization; writing—review and editing. **Jose C. B. Dubeux Jr.:** Conceptualization; writing—review and editing. **Carlos G. S. Pedreira:** Conceptualization; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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