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Research Article

Tropical Forage NIRS Database: Tropical Dairy Cattle Forage Accuracy Detection Using NIRS Dry and Wet Calibrations

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Abstract

Background and Objective: Daily fluctuation in the supply of nutrients from fresh forage offered by dairy farmers should be detected and adjusted to guarantee a consistent supply of nutrients for dairy cows. Currently, available dried forage detection using NIRS requires sample preparation. This study aimed to develop a wet forage NIRS database and compare its accuracy with a dry database.

Materials and Methods: A total of 133 NIRS spectra were collected for fresh and dried forage, including napier grass, natural grass, rice straw, corn stover and corn husk. Chemical analysis was conducted using proximate and Van Soest methods to analyse dry matter (DM), ash, crude protein (CP), crude fiber (CF), neutral (NDF) and acid (ADF) detergent fibers. The chemical data were used to calibrate the spectrums to produce the NIRS prediction model. **Results:** The wet spectrums varied considerably compared to dry spectrums. Higher reflectance of the dry spectrum showed higher nutrient density in the dried forage. All nutrient contents can be detected accurately using dry or wet NIRS database ($R^2C > 0.5$ and $RPD > 1.5$). However, a dried database is still more accurate ($R^2C > 0.78$) than a wet database ($R^2C > 0.63$). However, external validation of the dry database showed a significant difference in CP and ADF with the chemical analysis ($t\text{-test} < 0.05$). **Conclusion:** The wet and dry NIRS databases can predict daily variation of fresh forage nutrient content. For higher accuracy, the prediction model for CP and ADF of the dry database is suggested to be improved.

Key words: Fresh forage, fibre fraction, wet calibration, dry calibration, NIRS

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Dairy cattle ration, on average, consisted of 50% forage to produce sufficient milk fat¹, providing nutrients for rumen microbes and the host animal². Forage is also used to give bulkiness and maintain rumen health³. Adequate fiber in dairy ration triggers sufficient chewing activity and produces enough saliva to maintain rumen pH⁴. However, a high fiber feed proportion in a dairy ration, especially a low-digestibility fiber feed source, resulted in an insufficient nutrient supply for the animal³. Therefore, adequate fiber that triggers chewing activity maintains ruminal pH, provides precursors for milk fat synthesis and should be provided consistently in daily dairy ration⁵.

Dairy cattle require a temperature humidity index below 72 to achieve optimum performance⁶. Unfortunately, such conditions can only be fulfilled by the high mountains of tropical areas⁷. The situation is also favorable for tourism and horticulture. It makes the land competition and price unavoidable⁸. Limited availability of land for forage cultivation forces dairy farmers to use natural grass and agricultural byproduct⁹. During the dry season, the percentage of agricultural byproducts used in dairy farms increased due to the slow growth of cultivated and natural grass¹⁰.

Dairy farmers in tropical areas, mainly smallholder farmers, used fresh forage, which varies in daily quality⁹. The variation of fresh forage incredibly natural grass was high depending on the species¹¹ and region¹⁰. To provide consistent nutrients for dairy cattle, adjustments of concentrate and supplement used should be made by reformulating the ration¹².

To reformulate the ration, farmers need information on the nutrient content of the fresh forage timely¹³. Conventional methods of gathering nutrient content information of feeds involve chemical analysis, which takes a long time to produce the data¹⁴. The technique fails to provide real-time data and requires a lot of chemical substance and skillful labor¹⁵. An alternative assessment method is needed to provide real-time nutrient content information.

The NIRS has been used in many areas due to its fastness and no chemical is required¹⁶. The NIRS can gather nutrient content information in real time. According to Despal *et al.*¹⁷ the accuracy of NIRS in estimating nutrient content depends on their database. There is a need to create more precise local databases. The current local database was developed based on dry forage^{10,15-19}, which still needs lengthy procedure sample preparation. Therefore, wet forage calibration needs to be developed, which measures forage

nutrients instantly. This study compared dry and wet calibration of tropical dairy cattle forage using NIRS.

MATERIALS AND METHODS

Study area: The research was conducted at the Dairy Nutrition Laboratory within the Department of Animal Nutrition and Feed Technology and the Integrated Laboratory, housed in the Faculty of Animal Science at IPB University. The study commenced on February 1st and concluded on December 15th, 2023.

Sample collection: A 3300 fiber feed samples consisting of Napier grass, natural grass, rice straw, corn stover and corn husk have been collected from a dairy farming area in Bogor. About 2 kg of each fresh sample was collected and scanned for their wet spectrum. The samples were sun-dried for three days and subsequently sent to an oven at 60°C for 2 days. The dried forages were ground using a laboratory hammer mill equipped with a 1 mL sieve. The ground samples were put in plastic containers for further analysis.

Chemical analysis: The proximate analysis of dry matter (DM), ash and crude protein (CP) was conducted according to Horwitz and Latimer²⁰. Dry matter was determined after drying the sample in an NDO 400 Eyela oven (made in Japan) at 105°C for 48 hrs. The remaining ash was measured after incinerating the dried sample in a 500°C Nabertherm N50 (made in Germany) for 6 hrs. For CP analysis, a Gerhardt Kjeldahl system was used. The CF, NDF and ADF were analyzed using the fiber technique of Ankom 200 (made in the USA). The CF, NDF and ADF analyses followed AOCS procedures as Despal *et al.*²¹ used.

NIRS spectrum collection: Fresh and dry samples were collected using modular FT-NIR Spectrometer Solids Cell (BUCHI, NIRFlex N-500 made in Switzerland). The instrument warming up and system suitability test (SST) were conducted before spectrum collection to check if the instrument worked properly. The SST lasted for 15 min. Completing SST, external and internal references were run using the application of the NIRSwave operator. Before running the reference tests, an external reference (provided by BUCHI) had been inserted into the external reference holder. The spectrum was ready to be collected after completing the reference measurement.

The spectral collection process involved placing a 50 g fiber-feed sample in a Petri dish. After ensuring a homogeneous distribution and the sample covered the entire bottom of the dish, the dish was positioned in a Petri dish

holder for spectrum measurement. Near-infrared light was then directed into the sample and absorbance was measured across a range of wavelengths (800-2500 nm or 12500-4000 cm^{-1}), facilitating sample identification by penetrating several millimeters into the sample. Each sample underwent three scanning repetitions.

NIRS database development: The collected spectra were input with chemical analysis data using the NIRS were Management Console. The NIRCal V5.6 facilities were used to calibrate and validate the model developed. A new set of independent data samples validated the developed model (external calibration). A model can be accepted if the R^2C and RPD produced higher than 0.5 and 1.5, respectively. The external calibration succeeded if the t-test between chemical analysis data and NIRS data were insignificantly different ($p>0.05$) with minimum residual (SEP/SEL).

Statistical analysis: An explorative study and predictive studies were used in this research. Descriptive statistics were used to describe the center and distribution of the data. A t-test was used to compare chemical analysis data from NIRS. Database development used partial least squares from NIRCal V5.6.

RESULTS

Original spectra graphics: Comparisons between the wet and dry spectrum of tropical dairy cattle fiber feed sources were shown in Fig. 1. The figure shows that most of the spectrums overlapped, showing slight variation in nutrient content.

The wet spectra have lower reluctance than the dried spectra. The wet spectra were more diverse than the dried spectra. The peak of wet spectra reflectance only reaches up to 0.55, while the dried spectra are more than 0.7.

Nutrient content of fibre feed: Nutrient content of fiber feed used in this study was shown in Table 1. Nutrient content of the fibre feeds vary greatly within and between the forage. The DM content of the fiber feed ranged from 15 to 26.55%. Ash content of corn stover was the lowest (3.84%), while rice straw was the highest (20.49). Variation of CP content was also observed in the forage, ranging from 5.67% in corn stover to 13.64% in natural grass. The CF and ADF content were similar in all the fiber feeds (25.03-29.86% and 29.46-34.13%, respectively). The NDF fraction varied greatly. The highest NDF content was found in corn stover (71.47%) and the lowest in natural grass (56.13%).

Database calibration: The internal and external calibrations of wet spectra were shown in Table 2. It can be seen that the R^2C values more than 0.63. An R^2C of more than 0.80 was found in Ash's and ADF's parameters. However, the RPD for DM, CF and NDF is lower than 1.5. It can not be categorized as an accurate model. Validation failed to improve the accuracy. The R^2V values below 0.5 were found for CF parameters. Low RPD values also accompany the lower R^2V values. The $RPD>1.5$ is only found in ash and ADF. External validation of the model using an independent dataset did not significantly differ between chemical analysis and NIRS results (t-test >0.05).

The calibration results for nutrient content using dried forage spectra can be seen in Table 3. The R^2C values

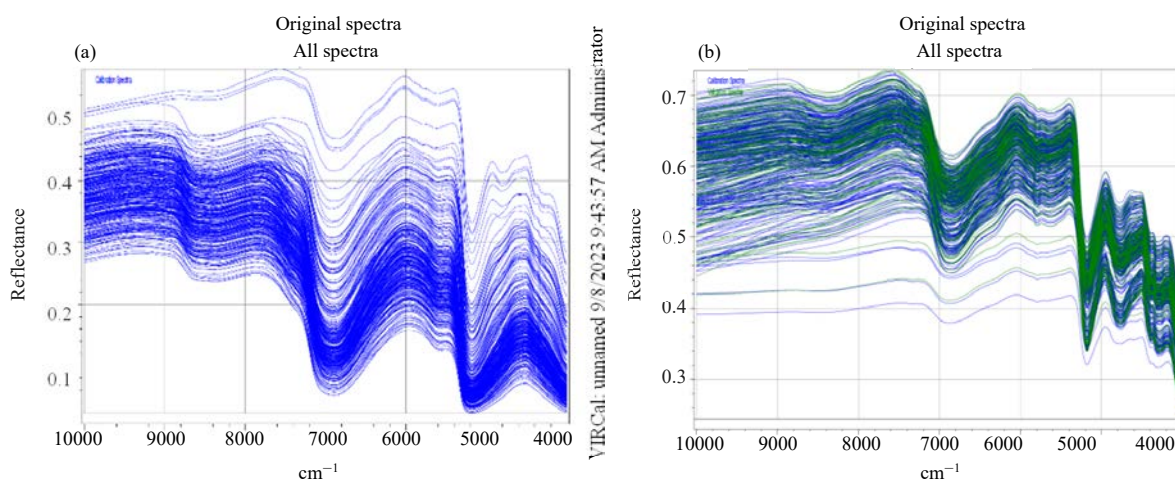


Fig. 1(a-b): Spectrum of tropical dairy cattle fiber feed, (a) Wet and (b) Dry

Table 1: Nutrient content of fiber feed used

Nutrient content	Forages				
	Napier grass	Natural grass	Corn husk	Corn stover	Rice straw
Dry matter (%)	26.55±1.55	23.12±2.22	15.00±0.44	21.75±0.96	18.21±1.45
Ash (% DM)	12.41±2.28	12.28±2.80	8.37±1.90	3.84±1.38	20.49±2.91
CP (% DM)	11.28±4.53	13.63±4.43	11.34±2.11	5.67±0.80	7.41±1.32
CF (% DM)	29.86±2.66	25.23±2.73	26.67±1.92	25.03±2.48	26.10±1.94
NDF (% DM)	65.09±3.35	56.13±8.18	58.94±3.29	71.47±4.64	60.93±4.26
ADF (% DM)	34.13±3.38	31.90±5.03	29.80±2.56	29.46±3.88	33.50±3.15

CP: Crude protein, CF: Crude fiber, NDF: Neutral detergent fiber and ADF: Acid detergent fiber

Table 2: Wet spectra calibration of fiber samples

Internal calibration								
Range								
Parameter	N	Average	Min	Max	STD	SEC	R ² C	RPD
Dry matter	142	91.91	89.30	93.58	1.06	0.75	0.67	1.42
Ash	176	9.82	0.73	19.44	4.09	1.49	0.88	2.75
CP	182	9.90	3.41	15.36	2.84	1.73	0.73	1.64
CF	74	27.17	24.81	28.96	1.01	0.78	0.63	1.30
NDF	132	63.69	53.48	72.11	3.93	2.90	0.68	1.36
ADF	121	31.84	25.94	39.30	3.18	1.34	0.85	2.38
Internal validation								
Range								
Parameter	N	Average	Min	Max	STD	SEV	R ² V	RPD
Dry matter	71	91.86	89.67	93.44	0.98	0.80	0.62	1.22
Ash	88	9.37	-0.05	18.46	4.24	1.79	0.84	2.37
CP	91	9.77	3.15	14.56	2.78	2.01	0.64	1.38
CF	37	27.14	24.82	28.74	1.06	1.05	0.38	1.01
NDF	66	63.85	54.08	73.02	3.90	2.96	0.70	1.32
ADF	61	31.93	26.52	39.16	3.25	1.64	0.78	1.98
External validation								
WCA								
Range								
Parameter	Average	STD	Average	STD	t-test	SEL	SEP	SEP/SEL
Dry matter	92.16	1.18	91.95	1.11	0.19	1.00	0.81	0.81
Ash	11.24	5.02	11.82	5.09	0.18	0.90	2.12	2.34
CP	9.79	3.55	9.730	2.75	0.90	2.55	2.28	0.90
CF	27.50	3.64	27.16	0.75	0.53	3.67	3.60	0.98
NDF	63.92	7.99	63.20	4.70	0.39	5.25	6.12	1.17
ADF	32.85	4.18	31.87	2.92	0.30	5.18	4.99	0.96

CF: Crude fiber, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, CP: Crude protein, SEC: Standard error calibration, R²C: Coefficient of determination calibration, RPD: Residual standard deviation, SEP: Standard error procedure. R²V: Coefficient of determination validation, WCA: Wet chemical analysis, NIRS: Near-Infrared spectroscopy, STD: Standard deviation, Significance t-test and SEL: Standard error laboratory

exceeding 0.78 were found for all forage nutrient parameters. The highest R²C value, more than 0.9, was found in ash and CP parameters. Validation slightly improves NDF and ADF parameters, as the higher R²V and RPD values show. Testing the model using an independent dataset found a significant difference between NIRS and chemical analysis results in CP and ADF parameters, although the SEP/SEL is less than 1.

DISCUSSION

The NIRS spectra represent the concentration of nutrients in the feed tested. The dried spectra showed a higher reflectance in comparison to the wet. This higher reflectance showed a higher nutrient concentration in the dried sample¹². The dried fiber feed spectra reflectance found in this research

Table 3: Dry spectra calibration of fiber samples

Internal calibration								
Range								
Parameter	N	Average	Min	Max	STD	SEC	R ² C	RPD
Dry matter	196	92.08	88.49	94.30	1.15	0.58	0.80	2.00
Ash	238	11.39	1.89	26.27	5.44	0.81	0.98	6.74
CP	230	9.90	4.23	23.54	3.45	1.04	0.92	3.32
CF	228	27.10	20.88	35.46	2.43	1.13	0.82	2.14
NDF	218	63.05	51.24	73.57	5.04	2.67	0.78	1.89
ADF	184	32.00	24.80	37.83	2.62	1.30	0.80	2.01
Internal validation								
Range								
Parameter	N	Average	Min	Max	STD	SEV	R ² V	RPD
Dry matter	98	92.09	88.66	94.24	1.14	0.59	0.79	1.94
Ash	119	11.40	2.070	26.13	5.42	0.81	0.98	6.73
CP	115	9.87	4.150	23.73	3.42	1.04	0.92	3.28
CF	114	27.10	21.25	36.12	2.44	1.16	0.81	2.11
NDF	109	63.11	52.14	72.57	5.05	2.64	0.79	1.91
ADF	92	32.10	26.52	38.00	2.62	1.23	0.82	2.13
External validation								
WCA			NIRS		t-test	SEL	SEP	SEP/SEL
Parameter	Average	STD	Average	STD				
Dry matter	92.10	1.17	92.21	0.91	0.26	0.55	0.48	0.86
Ash	11.12	4.93	11.34	5.16	0.30	0.97	0.97	1.00
CP	9.570	3.42	8.830	3.18	0.01	1.65	1.50	0.90
CF	27.89	3.37	27.29	2.37	0.17	1.91	1.84	0.96
NDF	64.11	8.04	63.91	4.71	0.70	4.89	4.78	0.98
ADF	32.93	4.22	31.66	3.22	0.01	3.01	2.28	0.76

CF: Crude fiber, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, CP: Crude protein, SEC: Standard error calibration, R²C: Coefficient of determination calibration, RPD: Residual standard deviation, SEP: Standard error procedure, R²V: Coefficient of determination validation, WCA: Wet chemical analysis, NIRS: Near-infrared spectroscopy, STD: Standard deviation, Significance t-test and SEL: Standard error laboratory

is almost similar to that reported in earlier studies²¹ due to the similar fiber feed used, although the origins were different. The wet spectra have a different pattern than the dry spectra¹⁷. The dried spectra were more concentrated, while the wet is more spread out. The broader spread of wet spectra represents the more varied concentration of nutrients²². Some of the spectrums separated significantly from others¹¹. These showed a significant difference in nutrient content from most fiber feed samples²³. Only 6 separate spectra from most spectra were found in the dried samples. Foskolos *et al.*²² also reported similar dry spectra. The significant difference was found at wavenumber 10000-7000 cm⁻¹. At wavenumber 4000-5200 cm⁻¹, only a few spectra were separated from most fiber feed spectra. According to Workman and Weyer²⁴, a sharp nonbonded OH peak near 6993 cm⁻¹ and the lack of a water peak near 5154 cm⁻¹ in sucrose. It was reported that multiple quantitative calibrations of cellulose and lignin were determined using the regions from 4348 to 4237 cm⁻¹ and 6042 to 5865 cm⁻¹.

Table 1 shows the variation of nutrient content in the fiber feeds. The nutrient content varies within and between the species. Variation of DM content in the fiber feeds determines the availability of nutrients. The higher the DM, the more nutrient content in the fiber feeds. Many factors influence DM content in forage, such as species, age of plant and time of harvesting²⁵, weather, climate and soil conditions²⁶ and plant composition²⁷. Variation of ash content among different types was also high. Rice straw, a byproduct of rice plants, has the highest ash content, while corn stover, part of the corn plant, has the lowest mineral content. Natural grass and Napier grass have nearly the same mineral content, around 12%. Similar natural and Napier grass ash contents were found in previous studies using the same feed²¹. As a late-mature plant byproduct, rice straw is lignified extensively and bound in high silica concentrations.

Moreover, the dried rice straw used in this study also resulted in more lignified and silica content in the rice straw. Gummert *et al.*²⁸ reported rice straw ash content is around

18.67-29.1%, which includes noncombustible residues. The rice straw ash content found in this study was 20.49 ± 2.91 , which is in the range of the report.

The protein content of the forages used by dairy farmers also varies. Corn stover, rice straw and corn husks have less than 10% CP content. The low CP content is not only due to the maturity of the plants and passing through the generative phase but also because these forages are cellulose-rich agricultural residues that contain high fiber and are more difficult to digest. Meanwhile, natural grass has the highest CP content (13.63%), slightly higher than Napier grass. The CP content of natural grass used in this study was higher than in previous research by Mtengeti *et al.*⁹. Variations in the nutrient content of natural grass can be attributed to various factors such as botanical composition, maturity stage¹⁰, season, land use and sunlight intensities²⁹. In the first lactation, a dairy cow weighing 400 kg, producing milk 13 L/day, requires a ration with a protein content of 12.6%. It indicates that Napier grass and natural grass can be used as the primary forages in the diet of dairy cows. Despite the higher CP content in natural grass, its availability was low due to the limited natural land space. Therefore, Napier grass remains the primary forage used in dairy farming in Indonesia^{1,30,31}. Rice straw, corn husks and corn stover require protein supplements if used as forages in dairy cattle rations³. If these supplements come from concentrate, it will increase the feed cost significantly. In the dry season, when forage growth slows down, the use of agricultural waste such as rice straw, corn stover and husks is often unavoidable. Previous studies have shown an increase in the use of agricultural waste during the dry season⁹.

Fiber is the primary source of energy for ruminants. The highest fiber content was found in Napier grass. Meanwhile, the fiber content in natural grass, corn stover, rice straw and corn husk was similar. Fiber digestion significantly impacts dairy cows' milk production more than other feed nutrient components³². Crude fiber (CF) alone is insufficient to describe the forage quality. Specifically, the quality of dairy cow forage is determined based on fiber fractions, such as NDF and ADF content. Therefore, an analysis of fiber fractions in NDF and ADF was performed. Unlike fiber content, NDF and ADF values vary more among forages. Forages with ADF <40% are categorized as high-quality forage, while those >40% are classified as poor-quality forage³³. The ADF content was directly related to the lignin content of feed material, the lower the ADF content, the higher the digestibility³⁴. High NDF content can limit dry matter intake due to a negative correlation between the two³⁵. The NDF has a bulky physical trait, making it slow to digest³². Rohweder *et al.*³⁶ classified the quality of forage based on its NDF content. Forage containing NDF <45% is classified as high quality, between 45-65% as

medium quality and >65% as low quality. Among CF, ADF and NDF, the NDF content has a more significant impact on digestibility. Based on its NDF content, it can be seen that the forage in this study falls into the medium to low-quality category.

Table 2 shows the wet spectra calibrations produced R^2 values >0.63. It was lower than the dry spectra calibration (R^2 >0.78). The data shows that the calibration model using dry spectra produced a more accurate prediction of the nutrient content of the fiber feeds. The RPD <1.5 found in wet spectra calibration for DM, CF and NDF shows that the model can not be categorized as accurate. The RPD values found in dry spectrum calibration were more than 1.89 for all parameters, indicating that the models were accurate. Validation of the wet spectra model failed to improve the accuracy. The R^2 values below for CF parameters fell below 0.5, accompanied by low RPD values representing an accurate model. Although external validation resulted in a non-significant difference between chemical analysis and NIRS results (t-test >0.05), calibration improvements, such as outlier removal and spectra pre-processing, are still needed. Samadi *et al.*³⁷ compared several pre-processing spectra and found that de-trending (DT) spectra correction was better than standard normal variate (SNV) and baseline shift correction (BSC) in improving ADF prediction.

The R^2 value is used to explain the accuracy of the prediction model, so the closer the R^2 value is to 1, the higher the accuracy of the prediction³⁸. Meanwhile, RPD is used to determine the effectiveness of the calibration model produced. Although wet spectra calibration produced a less accurate prediction than dry spectra, the R^2 produced for all parameters was more than 0.5. The prediction was considered valid when R^2 >0.5 and RPD >1.5³⁹. The RPD value for DM, CF and NDF using wet spectra calibration shows that the model can not be used to estimate the fresh fiber feed's DM, CF and NDF content. The model still needs improvement by increasing the sample number, outlier selection, or pre-processing spectra. Therefore, the calibration and internal validation results of forage samples with dry spectra in this study can be considered sufficiently accurate.

External validation was used to perform the prediction model⁴⁰. Larger prediction errors might occur due to the different samples used between calibration and external validation⁴¹. The external validation results show a significant difference between wet chemical analysis results and prediction models for ADF and CP nutrient parameters (p <0.05). The PRL values (SEP/SEL) <2 were found for all nutrient parameters. The PRL calculation is done to assess the accuracy of the created model²². The smaller the SEP/SEL, the more accurate the data.

Based on the internal calibration and validation results, it can be assumed that the model can only predict forage samples with wet spectra well and reasonably accurately for ADF and ash parameters. External validation results show no significant difference between wet chemical analysis and prediction models for all nutrient parameters. However, PRL values (SEP/SEL)>2 were found for ash nutrient parameters. Therefore, there was a potential excess of wavelength in the model that does not represent the substrate being modeled²².

Based on the above results, it can be seen that the best prediction model was found in a database with dry spectra. This result was in line with the broader variety of wet spectra compared to dry spectra. However, there was potential for database development using fresh spectra, as evidenced by R² values of mostly>0.5 and SEP/SEL values of mostly relatively low.

CONCLUSION

Based on the distance between spectra, dry spectra exhibited higher reflectance and nutrient concentration. The resulting prediction model from dry spectra was more accurate, with values of R²C and R²V exceeding 0.5, RPD surpassing 1.5 and SEP/SEL less than 1. Although, the wet spectrum was less precise than the dry spectrum, it can be improved to take full advantage of the NIRS technology in providing rapid information on dairy cattle nutrient supply fluctuation due to daily fresh forage utilization.

SIGNIFICANCE STATEMENT

This study aims to optimize the sample preparation process for forage nutrient content analysis to acquire real-time data. The sample preparation requires a laborious two-day procedure involving drying and grinding, necessitating the plant's harvest before analysis. Current research has effectively developed a wet database calibration, facilitating the immediate analysis of fresh forage. Furthermore, this calibration can be applied to analyze standing plants, offering a vital decision-making tool before harvesting.

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