Comparative near Infrared (NIR) spectroscopy calibrations performance of dried and undried forage on dry and wet matter bases

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HIGHLIGHTS

- Findings indicate that dried feed samples yield more accurate predictions than wet sample calibrations.
- Prediction errors increase with moisture level and when above 50%, moisture may hinder the ability to accurately predict nutrition profile of feed.
- For wet samples, the development of predicting models on the wet or dry basis doesn't seem to have an effect on the accuracy of predictions.

GRAPHICAL ABSTRACT

Evaluation for the accuracy for NIR spectroscopy calibrations for wet and dry forage samples which chemical values expressed with dry matter basis and wet matter basis.

ARTICLE INFO

ABSTRACT

The application of Near Infrared (NIR) spectroscopy for analyzing wet feed directly on farms is increasingly recognized for its role in supporting harvest-time decisions and refining the precision of animal feeding practices. This study aims to evaluate the accuracy of NIR spectroscopy calibrations for both undried, unprocessed samples and dried, ground samples. Additionally, it investigates the influence of the bases of reference data (wet vs. dry basis) on the predictive capabilities of the NIR analysis. The study utilized 492 Corn Whole Plant (CWP) and 405 High Moisture Corn (HMC) samples, sourced from various farms across Italy. Spectral data were acquired from both undried, unground and dried, ground samples using laboratory bench NIR instruments, covering a spectral range of 1100 to 2498 nm. The reference chemical composition of these samples was analyzed and presented in two formats: on a wet matter basis and on a dry matter basis.

The study revealed that calibrations based on undried samples generally exhibited lower predictive accuracy for most traits, with the exception of Dry Matter (DM). Notably, the decline in predictive performance was more pronounced in highly moist products like CWP, where the average error increased by 60–70%. Conversely, this reduction in accuracy was relatively contained (10–15%) in drier samples such as HMC. The Standard Error of Cross-Validation (SECV) values for DMres, Ash, CP, and EE were notably low, at 0.39, 0.30, 0.29, 0.21% for CWP and 0.49, 0.14, 0.25, 0.14% for HMC, respectively. These results align with previous studies, indicating the reliability of NIR spectroscopy in diverse moisture contexts.

The study attributes this variance to the interference caused by water in 'as is' samples, where the spectral features predominantly reflect water content, thereby obscuring the spectral signatures of other nutrients. In terms of calibration development strategies, the study concludes that there is no significant difference in predictive performance between undried calibrations based on either 'dry matter' or 'as is' basis. This finding emphasizes the potential of NIR spectroscopy in diverse moisture contexts, although with varying degrees of accuracy contingent upon the moisture content of the analyzed samples. Overall, this research provides valuable insights into the calibration strategies of NIR spectroscopy and its practical applications in agricultural settings, particularly for on-farm forage analysis.

1. Introduction

In numerous dairy regions globally, corn (Zea mays L.), harvested for silage, is a pivotal feed and energy source in dairy diets, offering higher Dry Matter (DM) and digestible nutrient yields compared to many other feed crops [25,52]. As per United States Department of Agriculture (USDA) statistics, corn also constitutes the largest portion of global grain production [49].

As highlighted by Castro Campos [8], crop quality is susceptible to various factors, including weather conditions, plant diseases, and insect infestations, all of which can significantly impact farm profitability. Consequently, the precision and timeliness of feed quality monitoring become imperative to optimize feeding systems effectively. This necessity can be addressed by adopting rapid and economically viable technological solutions, as advocated by Modroño et al. [31] and da Silveira et al. [48].

Near Infrared (NIR) spectroscopy is recognized as a rapid, valid, and accurate analytical technique, meeting the growing demand for quick quantification of feed nutrients [43]. Its application spans from large feed industries to small farms, where the evaluation and quantification of feed nutrients predominantly depend on NIR instruments [7]. The availability of rapid NIR analysis directly at farms, particularly for undried feed materials without the need for sample preparation, offers substantial convenience for field testing. This approach enables swift assessment of compositional changes [11] and facilitates feed payments based on quality [15]. NIR techniques have been employed for many years to analyze and predict the composition of undried samples [39,35,16,53]. However, the accuracy of these predictions can be compromised by high moisture content [27], primarily due to the strong absorption bands of water [54,39]. These bands can overlap and obscure spectral features of other nutrients, and may also lead to non-linear relationships between spectra and chemical properties [1]. While NIR analysis of wet forages is increasingly popular for its practical benefits, the specific impact of high-water content on the accuracy of NIR analysis remains somewhat unclear. This is due to the limited number of studies [46,34,42] that have compared the performance of calibrations developed for the same feed using both wet and dried, ground samples.

The composition of feed nutrients is commonly reported on a Dry Matter (DM) basis, a practice particularly relevant for feeds like silages, which exhibit significant moisture variability. Consequently, it is imperative that calibrations are evaluated based on their performance with data expressed on a DM basis, aligning with the reporting format predominantly utilized by nutritionists. While previous studies, Park et al. [35] have employed NIR spectroscopy for the analysis of undried silages, these have predominantly reported performance metrics solely on a wet basis. However, there is a scarcity of literature that delves into the application of NIR in analyzing undried samples when expressed in varying forms [37,39]. This gap in research underscores the importance of evaluating calibrations for undried, unground products with reference values expressed on both DM and wet bases. This evaluation is essential for a thorough understanding of the calibration accuracy across diverse contexts.

The principal objective of this research was to evaluate the accuracy of Near-Infrared (NIR) spectroscopic predictions pertaining to two widely used corn-based products, namely Corn Whole Plant (CWP) and High Moisture Corn (HMC). This evaluation focused on comparing calibrations developed using undried samples against those using dried and ground samples. The secondary objective was to investigate, specifically for undried products, the variations in NIR spectroscopic performance when calibrations are developed with reference values expressed either on a wet matter or a dry matter basis. This dual-focused approach aims to provide a comprehensive understanding of the efficacy of NIR spectroscopy in different states of sample preparation and varying moisture content contexts.

This is a retrospective study based on samples and data collected at the CNX laboratory of the University of Padua from 2010 and 2014.

1.1. Samples collection

The study used 492 corn whole plant (CWP) and 405 high moisture corn (HMC) samples, collected either for commercial or research purposes. Samples arrived either fresh or frozen at the lab and in this case, they were thawed before any analysis. In order to create an independent validation data set, samples received in 2010——2013 were used to develop calibration sets (CWP = 456; HMC = 364) and those received in 2014 (CWP = 36; HMC = 41) were used to validate prediction models. This methodological design, leveraging historical samples for calibration alongside unseen samples from future period for validation, was intentionally chosen to demonstrate our potential for real-world application in NIR, ensuring our findings are translatable to feed analysis practices.

Despite the initial concern over the size of validation set, our thorough review of the literature and subsequent statistical analyses affirm its adequacy. Some findings [19,47,5] demonstrate successful calibration and validation outcomes with a similar validation set size or even smaller than our validation set (n = 36).

1.2. Sample preparation and spectra collection

Undried and unground samples were scanned using a Foss NIRSystem 5000 (1100–2498 nm, 2 nm, Foss Analytical Hillerød, Denmark) scanning monochromator equipped with a sample transport attachment. Each sample was thoroughly mixed and packed into a natural product cup (215 × 56 × 43 mm) in order to allow scanning of an extended surface by sliding up and down in front of the scanning window. The instrument was set to first scan the white reference (16 scans) and then the sample, collecting and then averaging 32 scans. Spectra was collected as absorbance in logarithmic scale (log 1/R).

Samples of about 200 g of CWP and 100 g of HMC were oven drying at 60 °C for 48 h, in pre-tared aluminum pans. After drying, samples were allowed to cool down for at least 2hrs, then they were ground in a laboratory blade mill (Fritsch Pulverisette 19, Fritsch Gmbh, Germany) through a 1 mm screen, and stored in a sealed plastic cup for consecutive NIR scanning and chemical analysis.

Dried and ground samples were then packed into ring cups (diameter 50 mm) and scanned on a second Foss NIRSystem 5000 instrument equipped with drawer system with rotating ring cups. Scanning setting was similar as for the wet samples with 16 scans of the white reference and 32 scans of the sample.

1.3. Chemical analysis

Samples were analyzed for residual dry matter (DM) by oven at 105 °C (AOAC International, 2003); Ash as gravimetric residue after incineration at 550 °C; Crude protein (Nitrogen × 60.25) by the Kjeldahl method [4]; EE [4]; Neutral detergent fiber (aNDF) with amylase and sodium sulphite used in the NDF procedure and tared F57 bags with 25 µm pore size [2,18], Acid detergent fiber (ADF) (ANKOM Technology. 2015b); WSC [9] and Starch [3].

Overall DM was calculated from the partial DM recorded drying at 60 °C and the residual DM of the ground samples. Nutrients values were expressed on a DM and also on wet basis.

1.4. NIR calibrations and statistics

Spectra of undried samples were associated to wet chemistry values expressed on a wet and DM basis, while spectra of dried and ground samples where combined only with wet chemistry on DM basis. Common pretreatments used in NIR applications were tested, like smoothing (15pt, first order interpolating polynomial) [40,41], in combination with Autoscale or Mean Center [21,33] or the combination of Smoothing at 15pt and Standard Normal Variate (SNV) [6], de-trending (DET) (second order interpolating polynomial) and first derivative (second order interpolating polynomial) [41], with a window size of 7 points and quadratic polynomial smoothing applied before calibration. The pretreatments were based on previous experience with the same dataset. Calibration performance was evaluated based on the standard error of cross validation (SECV; Shenk & Westerhaus [44], the coefficient of

determination for cross validation (RSQCV), the ratio of performance to deviation (RPD) (Williams, 2001). For the validation set, predicting performances were evaluated base on standard error of prediction (SEP), the coefficient of determination (r^2val), bias and slope and standard error of prediction corrected for bias (SEPc).

All calculation procedures were computed by R software (R version x64 40.1.0 & R studio version 1.30.1093) with pls prospectr [50], metrics [22], e1071 [30] and Spectracus (https://github.com/mariofajardo/Spectracus) packages. Calibration and validation were developed and computed based on Partial Least Square (PLS) regression selecting the optimal numbers of PLS factor based on SECV.

2. Results and discussion

2.1. Chemical composition of samples for major components

Table 1 presents the descriptive statistics of the chemical composition for this study, expressed both on a dry and wet basis. Data on a wet basis accurately reflect the chemical composition of samples, incorporating the dilution effect of moisture. As a result, nutrients expressed on a wet basis are invariably lower in magnitude than those expressed on a dry matter (DM) basis. For example, the average DM of Corn Whole Plant (CWP) samples was 34.6 %, implying that mean concentration values on a wet basis are roughly one-third of those on a DM basis. This phenomenon also leads to smaller standard deviations (SDs) for wet basis data compared to those on a DM basis, primarily due to the water dilution effect. However, the coefficient of variation (CV), which indicates relative variability, tends to be higher for wet basis measurements. This is because CV is influenced by variations in both nutrient and moisture concentrations.

The data sets for both products demonstrate a wide range of variations, attributable to the samples being collected over a four-year period and encompassing different corn hybrids, locations, and growing conditions. Such diversity ensures that the calibrations comprehensively represent the variation in the products. Greater variation within a product is advantageous for the development of NIR calibrations, as it lays the groundwork for creating accurate and robust predictive models [10].

When compared with the composition statistics of Italian samples, as detailed in [26], the concentrations of chemical components in this

Table 1
Chemical composition of CWP and HMC silage samples.

| | Range | | | Mean | | | CV | | | SD | |
| | dry basis | wet basis | | dry basis | wet basis | | dry basis | wet basis | | dry basis | wet basis |
	%DM	%		%DM	%		%DM	%		%DM	%
CWP											
DM		190.41–570.68			340.59		–	180.07		–	60.25
DMres	890.13–950.76	–		920.69	–		10.67	–		10.55	–
Ash	20.26–60.35	0.77–20.24		40.06	10.37		190.46	170.52		0.79	0.24
CP	40.30–90.73	10.46–40.50		70.04	20.43		10.37	20.58		0.73	0.50
EE	10.57–30.71	0.36–10.61		20.49	0.86		150.26	260.74		0.38	0.23
aNDF	260.70–640.73	10.05–270.19		440.54	150.32		120.17	180.02		50.42	20.76
ADF	120.52–420.61	50.14–150.37		240.94	80.51		160.60	180.33		40.14	10.56
WSC	0.03–140.95	0.01–40.98		30.54	10.20		660.67	650.83		20.36	0.79
Starch	150.62–460.33	20.26–240.76		310.52	110.05		170.61	310.04		50.55	30.43
HMC											
DM	–	420.55–850.75		–	640.90		–	110.39		–	70.39
DMres	890.55–960.75	–		920.52	–		10.60	–		10.48	–
Ash	10.09–20.48	0.58–10.65		10.63	10.05		140.11	150.24		0.23	0.16
CP	50.72–120.01	20.89–80.72		80.94	50.80		110.30	190.31		10.01	10.12
EE	20.36–40.98	10.14–30.51		30.62	20.32		130.81	190.40		0.50	0.45
aNDF	30.89–40.26	20.86–230.62		150.14	90.63		510.12	450.38		70.74	40.37
Starch	360.24–750.35	170.78–570.78		60.08	390.07		130.05	210.68		70.84	80.47

Dry basis: as a dry matter basis; wet basis: as a wet matter basis; CWP: corn whole plant; HMC: high moisture corn; Mean: conventional chemical analysis mean; SD: Standard deviation; CV = Coefficient of Variation; DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; WSC: water-soluble carbohydrate.

research for both CWP and HMC samples align well with typical values. This comparison underscores the representativeness of our study's findings within a broader geographical and agricultural context.

2.2. Spectra comparison of dried and undried silages

The raw spectra of CWP and HMC samples were averaged, and the resultant mean spectrum is depicted in Fig. 1(a) and Fig. 2(a). As

illustrated, moisture exerts a significant influence on the spectra of undried samples, characterized by two prominent and broad peaks around 1450 nm and 1970 nm. These peaks are associated with the O–H overtones of water, as identified by Murray [32]. It was observed that the average absorption for both wet products was consistently higher than that for dried products. This difference is attributed to the combined effects of the larger particle sizes and higher water content in undried products compared to their dried and milled counterparts [24].
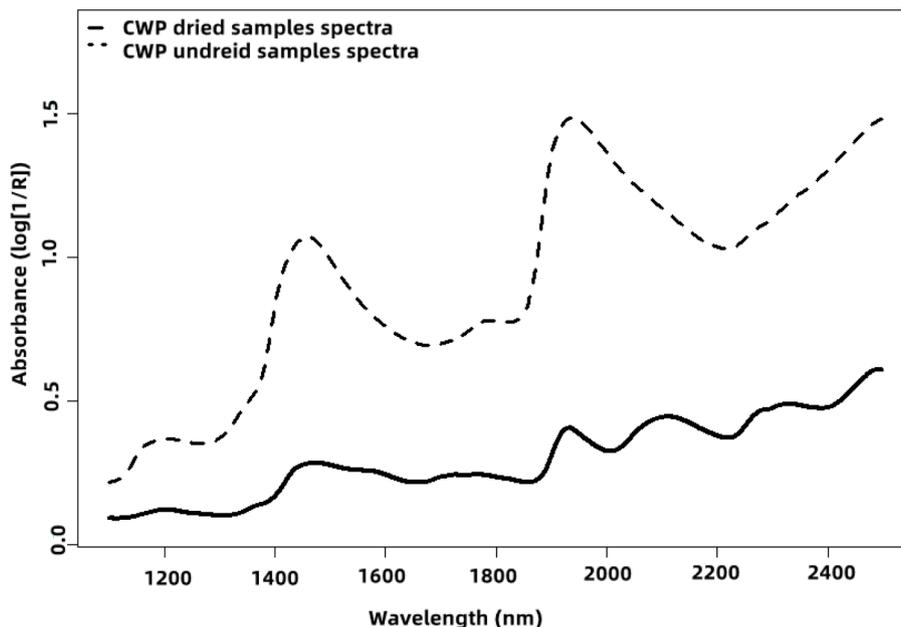
(a) Raw spectra
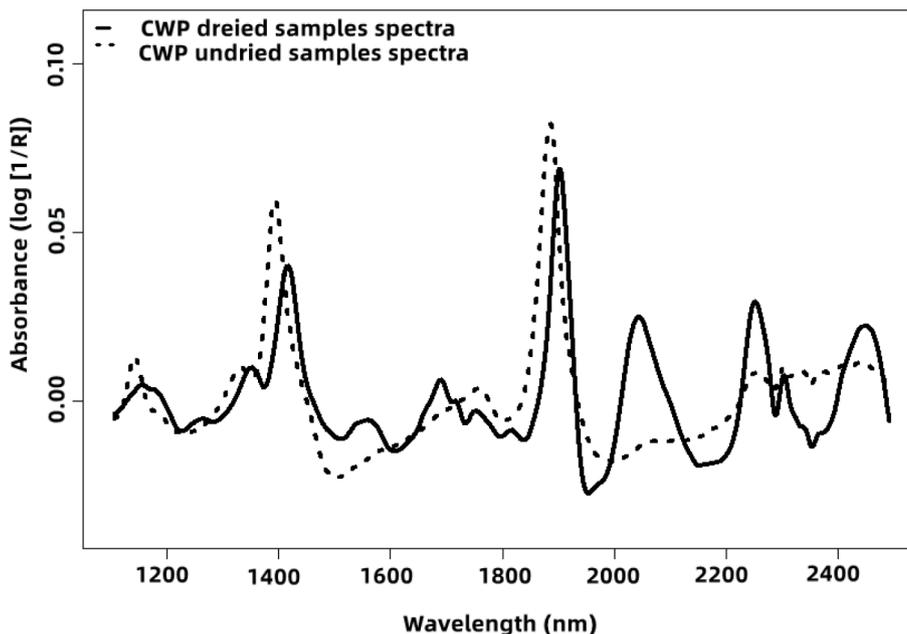
(b) Preprocessed spectra

Fig. 1. CWP dried and undried samples spectra: a) Results obtained before spectral processing; 2b) Results obtained after applying (SNV + Detrend + Savitzky Golay) preprocessing methods.
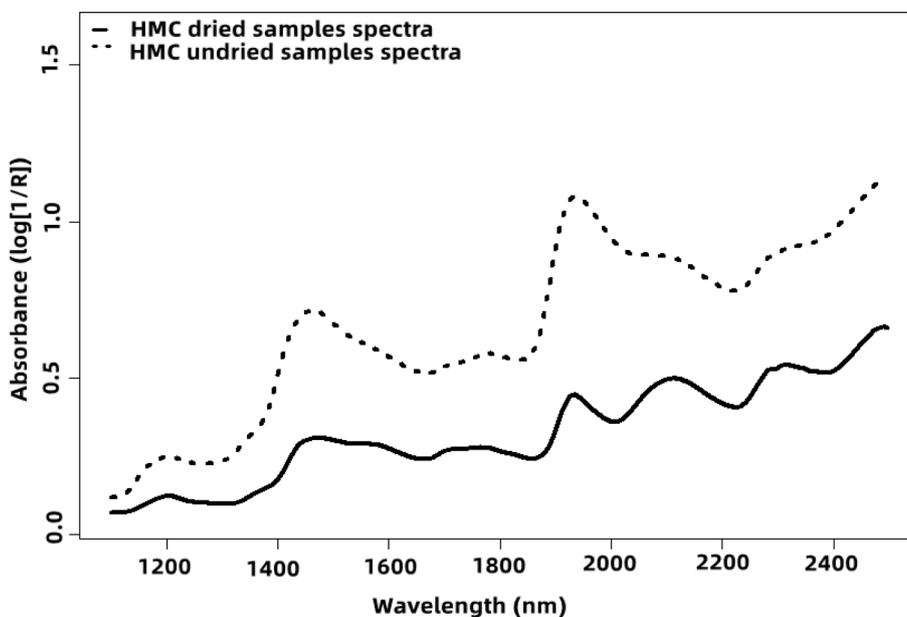
(a) Raw spectra
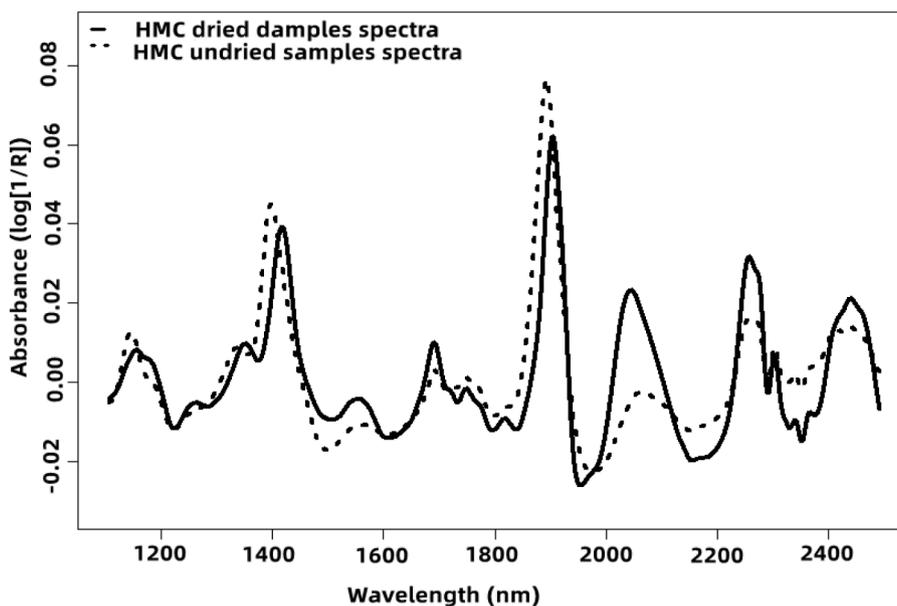
(b) Preprocessed spectra

Fig. 2. HMC dried and undried samples spectra: a) Results obtained before spectral processing; 5b) Results obtained after applying (SNV + Detrend + Savitzky Golay) preprocessing methods.

Upon comparing the average absorption spectra of the two wet products, a notable distinction emerges. Specifically, the average spectrum of wet CWP, as shown in Fig. 1(a), exhibits higher absorbance than that of wet HMC in Fig. 2(a), primarily attributed to the larger particle size and higher moisture content in CWP, as discussed by Philippeau & Michalet-Doreau [38]. In contrast, the dried samples of both products revealed more distinct spectral features. Notably, major characteristic peaks are observable in the 2000–2100 nm and 2250–2400 nm regions. These peaks correspond to the combination of CHO-H wavebands (carbohydrates) and C–H chemical bonds (fats), as identified by Hoffman et al. [23] and Givens et al. [20], respectively. The substantial water content

in undried samples appears to obscure e certain spectral information that is distinctly visible in dry products. This observation suggests that the high moisture content in undried silage samples may pose limitations to the accuracy of spectral prediction.

Mathematical treatments, aimed at reducing the effects of particle size, resulted in the average spectra of both wet and dry samples largely overlapping post-treatment. An additional observation of interest is the slight shift in the water peaks for both products. Concerning the shifts in the first derivative of NIRS spectra, as presented in Fig. 1(b). Specifically pertains to the water absorption bands at 1450 nm and 1930 nm in samples with varying moisture content. The absorption peaks in undried

samples are not merely elevated but also exhibit broader dispersion when compared to their drier counterparts. This phenomenon manifests as a bifurcated shift where the peak's most pronounced increase in absorption (on its left side) shifts leftward, while its most pronounced decrease (on the right side) shifts rightward. This dual shift reflects a broader and more intense absorption feature, indicative of the increased moisture content's effect on both the intensity and the spectral width of these absorption bands.

This nuanced interpretation has been informed by a reexamination of the spectral data in conjunction with established spectroscopic principles, notably those discussed by Workman & Weyer [55]. It highlights the intricate relationship between physical properties of the samples and its spectral signatures in NIR spectroscopy, emphasizing the necessity for accurate data interpretation.

2.3. Calibration and validation statistics

2.3.1. NIR analysis of dried samples for major components

Table 2 delineates the performances of the optimal calibration models developed for dried samples. The calibration equations for Dry Matter Residue (DMres) of Corn Whole Plant (CWP) and Crude Protein (CP), Ether Extract (EE), and Neutral Detergent Fiber (NDF) of High Moisture Corn (HMC) exhibited a Cross-Validation Coefficient of Determination (R^2cv) greater than 0.90. According to Shenk & Westerhaus [45], such values are indicative of excellent model performance. A comparative analysis of the calibration performance between these two distinct dried samples revealed that the R^2_{CV} for the main nutrients (CP, EE, aNDF, and Starch) was generally lower for CWP than for HMC. This discrepancy can be attributed to the lesser variability in CWP composition, as documented by Reeves et al. [39] and evidenced in Table 1.

The robustness of our models, as evidenced by our results, aligns with the findings from both the feed and broader agricultural sectors, demonstrating that a validation set of approximately 36 samples is sufficient for rigorous NIR spectroscopy analysis. This decision mirrors the methodological rigor observed in studies such as those conducted by [47] and [5], where smaller validation sets did not compromise the quality or applicability of NIR spectroscopy calibrations.

The SECV values for DMres, Ash, CP and EE were specifically 0.39, 0.30, 0.29, 0.21 % for CWP and 0.49, 0.14, 0.25, 0.14 % for HMC, respectively, results which are consistent with studies reported by Fassio et al. [16] and Marchesini et al. [29]. In the case of dried and ground CWP, Cozzolino et al. [12] reported generally higher R^2cv values and

lower SECV compared to this study. This difference is primarily due to the fact that our calibration datasets exhibited less variability.

As delineated in Table 2, HMC generally exhibited superior predictive accuracy compared to CWP in validation. For HMC, all nutrients, with the exception of Ash, demonstrated a validation coefficient of determination (r^2val) equal to or greater than 0.84. In contrast, for CWP, only Crude Protein (CP) and Starch achieved an r^2val above 0.80. Additionally, the Standard Error of Prediction (SEP) values were consistently lower for HMC than for CWP, excluding Starch.

Notably, the bias values were relatively large. The bias test, as outlined by Windham & Coleman [54], indicated significant values for most nutrients in both CWP and HMC. Consequently, bias correction was implemented to reduce the SEP, following the methodology of Davies & Fearn [13]. The resulting SEPc (Table 4 and Table 5) values were generally consistent with the SECV (Table 3) for both CWP and HMC. However, exceptions were observed for Ash and CP in CWP, where the errors nearly doubled post-correction.

The observed larger biases in the validation sets can be attributed to the fact that these sets comprised samples collected in different years. This approach was deliberately chosen to mimic the real-world scenario of NIR analytical routines, where established calibration models are applied to predict new and unknown samples. This scenario underscores the necessity of continuous monitoring of NIRS prediction performances, even when using well-established calibration models. Such monitoring can be effectively achieved through regular internal controls, which may include conducting wet chemistry analyses routinely or participating in proficiency testing. Proficiency tests often involve ring test samples provided by recognized organizations such as the National Forage Testing Association (NFTA), the Association of American Feed Control Officials (AAFCO), or Bipea. These measures are crucial for ensuring the ongoing accuracy and reliability of NIR analysis in varying sample conditions.

In their study, Fatemi et al. [17] developed a NIR calibration equation using a well-known and publicly accessible corn dataset [14]. When compared to our results, their calibration exhibited a higher R^2cv and a lower SECV for CP and Starch, specifically 0.93 versus 0.84. This difference can be attributed to the lower standard deviation (SD) in their study compared to ours. Furthermore, when comparing the performance of the NIR equation for CP in HMC samples with a previous study by Fassio et al. [16], our research demonstrates better performance. Our R^2cv is higher and our SECV is lower than those reported by **Fassio et al. (2022)**, with values of 0.94 versus 0.85 and 0.25 versus 0.52, respectively.

Table 2

Accuracy of near infrared spectroscopy (NIRS) in analyzing CWP and HMC dried samples for nutrient parameters.

	Calibration			Validation					
	PLS f	R^2cv	SECV	r^2val	SEP	SEPc	Slope	Bias	RPD
CWP	n = 456			n = 36					
DMres	6	0.94	0.39	0.73	0.83	0.23	0.32	0.80*	0.54
Ash	10	0.85	0.30	0.47	0.97	0.71	0.44	0.65*	10.03
CP	10	0.82	0.29	0.86	0.62	0.46	0.81	−0.4*	10.88
EE	9	0.69	0.21	0.56	0.26	0.26	0.68	0.04	10.53
aNDF	8	0.87	10.98	0.78	30.53	20.39	0.72	−20.60*	10.44
ADF	7	0.83	10.75	0.72	10.63	10.56	0.81	0.45	10.85
WSC	4	0.51	10.66	0.76	20.91	10.05	0.47	−20.72*	0.73
Starch	5	0.84	20.16	0.86	20.27	20.27	0.89	−0.11	20.68
HMC	n = 364			n = 41					
DMres	3	0.88	0.49	0.93	0.58	0.53	0.92	−0.22*	30.22
Ash	8	0.64	0.14	0.71	0.28	0.26	0.64	−0.11*	10.49
CP	10	0.94	0.25	0.91	0.38	0.36	0.92	−0.12*	30.26
EE	8	0.92	0.14	0.84	0.28	0.27	0.82	−0.10*	10.46
aNDF	6	0.94	10.84	0.95	30.09	20.56	0.92	10.72*	20.86
Starch	5	0.87	20.80	0.95	30.00	20.61	0.93	−10.47*	30.27

PLS f: the PLS factors used to develop the best partial least square regression; R^2cv: multiple correlation coefficient of cross validation; R^2: multiple correlation coefficient of validation determination; SECV: standard error of cross validation; SEP: standard error of prediction; SEPc: standard error of performance corrected for bias; RPD: ratio of performance to deviation; Bias: average difference between reference method and NIRS analytical values; * Significant difference ($P < 0.05$).

Table 3

Calibration performance of NIRS in analyzing CWP and HMC undried samples for nutrient parameters based on wet matter basis (%) and dry matter basis (%).

Calibration	PLS f		R^2_{cv}		SECV		RPD	
	dry basis	wet basis	dry basis	wet basis	dry basis	wet basis	dry basis	wet basis
CWP	n = 456							
DM	–	5	–	0.78	–	20.69	–	20.16
Ash	7	7	0.41	0.54	0.59	0.16	10.30	10.48
CP	7	6	0.33	0.71	0.56	0.25	10.22	10.86
EE	4	4	0.19	0.51	0.34	0.15	10.11	10.44
aNDF	9	5	0.45	0.76	40.03	10.17	10.35	20.06
ADF	10	9	0.54	0.73	20.83	0.72	10.48	10.92
WSC	5	6	0.41	0.53	10.82	0.54	10.31	10.46
Starch	10	9	0.47	0.68	30.97	10.90	10.38	10.76
HMC	n = 364							
DM	–	5	–	0.98	–	10.07	–	60.78
Ash	4	5	0.55	0.66	0.15	0.09	10.49	10.72
CP	8	5	0.71	0.81	0.54	0.47	10.86	20.30
EE	8	5	0.73	0.82	0.27	0.19	10.92	20.34
aNDF	8	8	0.86	0.83	20.79	10.73	20.69	20.45
Starch	5	6	0.72	0.89	40.00	20.70	10.90	30.04

PLS f: the PLS factors used to develop the best partial least square regression; R^2cv: multiple correlation coefficient of cross validation; SECV: standard error of cross validation; RPD: ratio of performance to deviation.

2.3.2. NIR calibration performances of undried samples using reference data expressed on dry or, wet matter basis

Calibrations conducted on a wet matter basis exhibited superior performance compared to those on a dry matter basis for both CWP and HMC, as evidenced by higher R^2cv, greater RPD, and lower SECV values (refer to Table 3). Specifically, on a wet basis, most nutrients for both products were predicted with an R^2cv greater than 0.70, with the notable exceptions of Ash (0.54 for CWP and 0.66 for HMC), WSC (0.53), and Starch (0.68). These findings align closely with those reported by Parrini et al. [36] in their study on fresh pasture samples, where they observed an R^2cv for CP and NDF of approximately 0.89. However, it is noteworthy that their SECV for CP was higher than that observed in our study. Additionally, Park et al. [35], in one of the earlier studies on NIRS calibration for undried silages, reported SECV values for NDF and ADF of 0.53 % and 0.41 %, respectively. These values are lower than those found in our current study, highlighting the variability in calibration performance across different sample types and study conditions.

In the study conducted by Park et al. [35], grass silage samples were preprocessed using liquid nitrogen and subsequently pulverized to enhance homogeneity by reducing particle size. In contrast, our Corn Whole Plant (CWP) samples were scanned in their received state, with a notable variation in length of cut ranging from 5 to 20 mm. Although spectral preprocessing techniques such as derivatives and SNV were employed to minimize the effect of particle size, it is conceivable that the substantial variability in CWP particle size may have influenced the calibration performance in our study. This hypothesis is supported by Lowett et al. [28], who indicated that increased particle size can detrimentally affect calibration performance, potentially explaining our results. In our study, HMC exhibited a smaller particle size compared to CWP and consequently demonstrated better predictive accuracy for the same nutrients. Additionally, HMC had a higher DM content (64.9 % vs. 34.6 % for CWP), which suggests that the pronounced water peaks in HMC may have obscured some spectral information, as illustrated in Fig. 1. The results for Ashes and WSC in this study are in line with those reported by Thomson et al. [51] for fresh grass calibration. The limited absorption of inorganic substances like ashes in the NIR region could account for the lower performance observed in Ashes prediction.

The lower concentration of nutrients in wet basis data, as shown in Table 1, typically leads to an expectation of reduced SECV for wet basis calibrations. Additionally, higher R^2cv and RPD could be anticipated, primarily due to the larger CV associated with wet basis data. However, a significant contributing factor to these improved metrics, particularly for R^2cv and RPD, is the observed strong correlation between all nutrients and DM content on a wet basis, as detailed in the supplementing material. This correlation is noteworthy because DM content was accurately predicted in our study, thereby enhancing the overall calibration performance for nutrients on a wet basis.

While feed analyses are universally reported on a DM basis, as per the guidelines in feeding recommendations (NASEM, 2021), this necessitates the conversion of predictions initially made on a wet basis to a DM basis. This conversion process and its outcomes are detailed in the validation tables (Tables 4 and 5). Notably, results obtained on a wet matter basis exhibited higher validation coefficients of determination (r^2val) and lower SEP and SEPc compared to those on a DM basis, aligning with the observed calibration performances. However, a significant deterioration in validation statistics was observed when wet matter predictions were converted to a DM basis, resulting in performances similar to those of direct dry basis calibrations. For instance, the r^2val for CP in CWP was 0.50 on a wet basis (Table 4), but this value dropped to 0.09 when converted to a DM basis, performing worse than the 0.30 r^2val obtained with direct dry basis calibrations. Similarly, the accuracy of CP prediction, as indicated by SEP, was 0.50 on a wet basis, but increased to 1.11 when converted to a DM basis, closely mirroring the SEP of dry basis predictions. This trend of deteriorating accuracy upon conversion from wet to dry basis is also evident in HMC across all nutrients (Table 5). For example, the SEP for aNDF in HMC was 1.52 on a wet basis, but increased to 2.46 when converted to a DM basis, closely approximating the 2.53 SEP observed in dry basis calibrations.

When comparing the calibration performances of dried and ground samples (as shown in Table 2) with those of wet samples (Tables 4 and 5), distinct effects are observed for CWP and HMC. Using the SEPc of dry ground samples as a benchmark, the prediction errors for CWP undried and unground samples (Table 4) were, on average, 60 % and 73 % higher for the dry and wet converted basis, respectively. The most significant increase in prediction error was observed for CP (0.46 vs. 1.0 and 1.13 % DM) and for Starch (2.27 vs. 4.27 and 5.09 % DM), where the error approximately doubled with calibrations on wet samples compared to the dry and ground ones. Conversely, for HMC, the increase in prediction error was more contained, averaging 10–15 %, with the largest discrepancy observed in the prediction of CP. An analysis of the error distribution in undried calibrations reveals a correlation with nutrient concentration, particularly for CWP, as detailed in the supplementing material. This trend was somewhat expected, given the slope observed in the validation results, which indicated greater prediction errors for samples with compositions at the extreme ends of the distribution. This phenomenon could be attributed to the fact that water peaks in the spectra may obscure information related to individual nutrients, thereby reducing sensitivity, especially at nutrient

Table 4
NIRS validation performance of CWP undried samples based on dry matter basis, wet matter basis and with calibration equations corrected to a dry basis.

Validation n = 46	r^2val			SEP			SEPc			Slope			Bias			RPD		
	dry	wet	wet converted to dry	dry	wet	wet converted to dry	dry	wet	wet converted to dry	dry	wet	wet converted to dry	dry	wet	wet converted to dry	dry	wet	wet converted to dry
DM	–	0.95	–	–	20.09	–	–	20.07	–	–	0.94	–	–	–0.32	–	–	40.44	–
DMres	0.37	–	0.39	30.07	–	30.07	10.02	–	10.04	0.07	–	0.22	20.90	–	–20.90	0.15	–	10.28
Ash	0.18	0.38	0.31	0.92	0.30	0.87	0.91	0.27	0.79	0.41	0.49	0.84	0.16	0.12	–0.38	10.08	10.19	10.20
CP	0.30	0.50	0.09	10.09	0.50	10.11	10.00	0.50	10.12	0.27	0.69	0.86	–0.43	0.07	–0.14	10.08	10.28	10.05
EE	0.06	0.73	0.05	0.40	0.16	0.44	0.40	0.15	0.42	0.25	0.82	0.30	–0.03	–0.05	0.14	10.02	10.85	10.02
aNDF	0.38	0.94	0.31	70.04	10.92	40.73	30.90	10.60	40.30	0.27	0.88	10.19	–50.86	–10.06	20.09	0.72	20.59	10.20
ADF	0.30	0.93	0.36	50.03	0.86	20.69	20.48	0.79	20.61	0.21	0.92	0.92	–40.37	–0.36	0.78	0.60	30.29	10.25
WSC	0.67	0.69	0.64	40.13	10.37	30.57	10.17	0.44	10.28	0.26	0.33	0.97	–30.96	–10.30	30.34	0.51	0.60	10.67
Starch	0.47	0.74	0.56	50.14	20.72	60.70	40.27	20.08	50.09	0.56	0.77	10.09	20.85	10.74	–50.39	10.18	10.55	10.50

dry: dry matter basis; wet: wet matter basis; wet converted to dry: wet matter basis using calibration equation corrected to a dry matter basis; DM: dry matter; DMres: dry matter residual; Ash: Ash in a wet matter basis; CP: Crude protein; EE: Ether Extract; aNDF: Neutral detergent fiber; ADF: Acid detergent fiber; WSC: Water-soluble carbohydrate.

Table 5
NIRS validation performance of HMC undried samples based on dry matter basis, wet matter basis and with calibration equations corrected to a dry basis.

Validation n = 41	r^2_{val}			SEP			SEPc			Slope			Bias			RPD		
	dry	wet	wet converted to dry	dry	wet	wet converted to dry	dry	wet	wet converted to dry	dry	wet	wet converted to dry	dry	wet	wet converted to dry	dry	wet	wet converted to dry
DM	–	0.97	–	–	10.54	–	–	10.48	–	–	0.95	–	–	–0.41	–	–	50.43	–
Ash	0.79	0.76	0.82	0.13	0.10	0.14	0.11	0.09	0.11	0.76	0.81	10.28	–0.06	–0.04	0.09	10.81	10.91	20.36
CP	0.65	0.90	0.57	0.68	0.47	0.73	0.65	0.47	0.73	0.73	0.91	10.04	–0.20	–0.04	0.09	10.65	30.02	10.52
EE	0.76	0.93	0.76	0.27	0.15	0.24	0.22	0.12	0.23	0.78	0.92	0.96	–0.15	–0.08	0.09	10.74	30.20	20.03
aNDF	0.95	0.93	0.95	20.53	10.52	20.46	20.51	10.49	20.36	0.93	0.92	10.17	–0.32	–0.33	0.50	20.63	30.27	40.42
Starch	0.89	0.93	0.85	30.74	30.00	30.90	30.66	20.98	30.97	0.87	0.92	10.15	–0.76	–0.33	–0.12	20.61	30.46	20.58

dry: dry matter basis; wet: wet matter basis; wet converted to dry: wet matter basis using calibration equation corrected to a dry matter basis; DM: dry matter; Ash: Ash in a wet matter basis; CP: Crude protein; EE: Ether Extract; aNDF: Neutral detergent fiber.

concentrations that deviate significantly from the mean.

In the context of related research, Thomson et al. [51] conducted a study involving the collection of spectra from undried, unground mixtures of grasses and clover silages obtained from commercial farms in the UK. They developed calibrations on both a dry and wet basis, observing generally better accuracy and robustness for calibrations based on the wet basis. However, it is important to note that Thomson et al. reported prediction errors on a mean relative basis. Consequently, their wet basis predictions were not converted to a dry basis, which limits direct comparability with the findings of our study. In a separate study, Pérez-Marín et al. [37] analyzed 121 TMR using NIR instruments to predict DM, CP, and NDF on both an "as DM basis" and an "as is basis". Their results were found to be similar to those of our study, further validating our findings and underscoring the relevance of the chosen basis for calibration in NIR spectroscopy analysis.

3. Conclusion

Optimal and most accurate predictions using NIR spectroscopy were observed with samples that had been dried and milled. However, in field or farm applications, there is often a necessity to scan undried samples, where preprocessing steps such as moisture removal and particle size reduction are not feasible. This study, utilizing laboratory NIR instruments, highlights potential limitations in the accuracy of NIR predictions when conducted on undried and unground samples.

When analyzing undried and unground samples in their natural state, deciding whether to develop calibrations based on data expressed on a 'wet matter' or 'dry matter' basis presents a challenge, as neither approach offers a clear, overarching advantage. Generally, calibrations based on wet matter appear to yield slightly better results, particularly with very wet materials like CWP, as demonstrated in this study. Therefore, it becomes imperative to report actual prediction performances on a dry matter basis, as these more accurately reflect the true precision of the calibrations.

For drier products such as HMC, which are analyzed in their natural state, calibrations developed on a dry matter basis are effective. This approach has the added benefit of eliminating the need for result conversion when used for diet formulation, streamlining the process.

CRediT authorship contribution statement

Xueping Yang: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Alejandra Arroyo-Cerezo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Paolo Berzaghi:** Writing – review & editing, Visualization, Validation, Resources, Project administration, Investigation, Funding acquisition. **Luisa Magrin:** Visualization, Software, Methodology, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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