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Using selective NIR wavelengths in portable devices to evaluate the chemical composition of cattle feeds

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ABSTRACT

In modern cattle nutrition, the estimation of chemical composition must be accurate, rapid, and available throughout the farm feeding process. This goal can be achieved using portable near infrared instruments applied at several positions in the feeding chain. Although portable instruments are generally more affordable than benchtop equipment, the current costs of purchasing and maintaining these tools may still be prohibitive for small and medium-sized animal husbandry businesses. This makes their widespread adoption economically unsustainable for many farms. Reducing the spectral data helps prevent multicollinearity issues, enabling the use of a minimal–optimal problem approach and supporting the development of a targeted and cost-effective instrument. This was achieved by evaluating various cattle rations and silages (including rations for cows and bulls and grass and corn silage), where we selected the most significant wavelengths for fibre characterisation using the Random Forest (Boruta) algorithm. The number of identified features varied based on the spectral pre-treatments applied or the use of a batch effect reduction algorithm (ComBat function). External validation was accomplished with spectra collected by different instruments, origins and sample types, and the highest adjusted coefficients of determination in validation were 0.95 for dry matter and 0.84 for alpha-amylase and sodium sulphite-treated NDF. Results demonstrated that using a few spectral bands is suitable for instrument calibration.

HIGHLIGHTS

- A random forest algorithm effectively reduced the number of wavelengths.
- Selected wavelengths predicted fibre fraction composition with fair accuracy.
- A targeted NIR instrument is possible using reduced spectral data.
- Wavelength reduction must consider manufacturing cost, spectrum width, and potential customer base.

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Introduction

A specific and fundamental need in cattle production is the analysis of forage and ration fibre fractions. As well-known from the literature, fibre plays an essential role in a cow's nutrition, and its quality may affect feed intake and chewing activity (Dado and Allen 1995; Mertens 1997). As for dairy cows, the role of fibre from roughage sources in finishing beef cattle diets is to maintain rumen function, prevent digestive disorders, stimulate rumination, and maintain adequate ruminal pH, while the source and concentration of fibre can influence dry matter intake, ingestive behaviour, ruminal fermentation, and digesta kinetics (Goulart et al. 2020). One key prevention strategy for subacute ruminal acidosis (SARA) is maintaining an adequate fibre source and level in the diet, particularly physically effective fibre, to stimulate rumination and salivation and maintain a healthy rumen environment (Elmhadi et al. 2022), especially when considering fibre from forages (Grant 1997; Grant and Cotanch 2005; Fustini et al. 2017). Basically, fibre was characterised by its fractions, as proposed by Van Soest in the 1970s (VanSoest 1967; Van Soest et al. 1991), such as neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL). Further, the lignin content was used to

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characterise the indigestible fraction of fibre (Palmonari et al. 2016), and several equations based on the lignin content have been proposed to estimate the undigested fraction of alpha–amylase and sodium sulphite-treated NDF (aNDF) (Fox et al. 2004).

Another important parameter in cattle nutrition is dry matter (DM) intake, which plays a crucial role in calculating feed efficiency traits. Optimising DM intake is essential for improving feed efficiency in dairy cattle, either by reducing DM intake while maintaining production or by increasing production without increasing DM intake (Madilindi et al. 2022). Moreover, variations in feed moisture content can affect the actual nutrient intake if not adjusted for DM content.

A recent review (Evangelista et al. 2021) described how portable near infra-red spectroscopy (NIRS) instruments could impact dairy farming, accelerating the digital revolution. Using portable NIRS reduces response time and reduces analysis costs (Evangelista et al. 2021) compared with wet chemistry. Some of these instruments or their applications can be interestingly embedded in field harvesting equipment (Cherney and Cherney 2019) or mixer wagons (Evangelista et al. 2021; Serva, Magrin, Marchesini, et al. 2021). Recent technological advances have introduced affordable portable spectroscopy devices for *in situ* analyses (Acosta et al. 2020; Feng et al. 2023). A study (Berzaghi et al. 2021) comparing benchtop and portable NIRS instruments found that despite covering a smaller spectral range, portable devices performed similarly to benchtop instruments in calibration. The researchers recommend that portable instruments should cover specific spectral ranges, including the second overtone (1050–1650 nm) and possibly the first overtone (1500–2000 nm) with the combination band region (1900–2500 nm). However, the wavelengths range in portable instruments is typically determined by corporate strategy rather than targeted selection; therefore, these applications, so far, have been relying on a broad non-targeted wavelengths (Wvs) range.

NIRS spectrometers generate highly correlated spectral data and overwhelming information (Blanco and Villarroya 2002). This data abundance can paradoxically lead to decreased predictive performance (Andersen and Bro 2010). Additionally, processing large datasets can significantly slow down calibration algorithms (Bisutti et al. 2019). Therefore, a targeted reduction in wavelengths can potentially improve spectral analysis's accuracy and efficiency. Indeed, an attempt to choose a short range of close Wvs and avoid using those considered irrelevant can be a strategy against the so-called minimal-optimal problem (Nilsson et al. 2007). An efficient feature selection improves the model's predictive ability and reduces complexity (Edward 1994; García-Torres et al. 2016). Selecting a few helpful Wvs and avoiding those considered irrelevant may also yield considerable variation in test set prediction (Mehmood et al. 2012). For example, Leardi (2000) found that in a dataset of soy flour, gasoline, resorcinol, a mixture of non-Pt foodstuff, and wheat, very well-defined regions are selected mainly by genetic algorithm-partial least squares calculated under various conditions.

Variable selection methods were explored to identify informative spectral regions while eliminating noisy ones, aiming to minimise prediction errors by selecting an optimal subset of Wvs. Various studies have demonstrated that applying Wv selection can enhance the performance of partial least square (PLS) regression and principal component regression (PCR) methods. However, this is not always the case, as selecting only the most correlated Wv may eliminate those that account for the influence of interfering factors or compounds. In reality, a variable that may seem utterly unimportant on its own can lead to a significant performance improvement when used in conjunction with others (Xiaobo et al. 2010). Nevertheless, variable selection can offer quicker and more cost-effective predictions.

Application-driven spectrometers tailor Wvs selection to specific needs. These devices use either targeted emitters or a broadband emitter with filtered photodiodes (Malinen et al. 1998). While this approach offers advantages like scalable costs, compact design, minimal heat generation, and no moving parts, it has limitations. Low-cost options typically have a wide full width at half maximum (±50 nm) and may lose some spectral information compared to other technologies. Light-emitting diodes (LEDs) in these systems allow for frequency-modulated measurements, which can reduce noise - a crucial feature for field use. Some companies have developed cost-effective solutions for receiver-side wavelength selection, particularly in the visible (VIS) and near-infra-red (NIRS) ranges (Wang et al. 2022). This design balances specificity with affordability, making it suitable for targeted applications despite some spectral resolution and information density trade-offs. For example, Tsow et al. (2021) developed a low-cost ($215), lightweight (142 g), wireless, and wearable functional NIRS headband using inexpensive LED sources at optimal wavelengths (740 nm and 850 nm) for measuring brain hemodynamics, enabling affordable and scalable neuroimaging in naturalistic environments.

Due to the relevance of the role of DM and fibre in animal nutrition, in the present study, we want to find the most relevant bands or single Wv that are most important to calibrate the DM content and fibre characterisation in a variety of feeds, allowing a relevant decrease in instrument cost and, therefore, favouring their multi-application in several parts of the barn or the operating machines, coherently with the concepts of the Precision Farming. Despite its importance, crude fibre was not included in the analysis because no measurements for this parameter were available in the original datasets we used.

Materials and methods

Sample and NIRS data collection

A dataset of 339 samples (Table S1) was collected by the authors and analysed by the chemical-NIRS-XRF laboratory of the Department of Animal Medicine, Production and Health of the University of Padua (Padova, Italy) in the period 2016–2022. The dataset was composed of four feeds: cow's total mixed ration (TMRc, $n = 168$), bulls' total mixed ration (TMRb, $n = 85$), grass silage (GS, $n = 43$) and corn silage (CS, $n = 43$). Samples were collected from commercial farms in northern Italy, including the most representative Italian regions (Lombardia, Veneto, Emilia Romagna and Piemonte) for dairy and beef cattle farms. Samples (about 1 kg) were collected fresh from the silo bunker or the feeding alley, vacuum-packed in bags (with a portable device), refrigerated using a transportable refrigerator during transportation and a refrigerator (4 °C) for conservation until NIRS spectral acquisition (within 24 h) at room temperature with a benchtop instrument FOSS DS2500 (FOSS Analytical, Hillerød, Denmark) operating in a spectral range between 850 and 2500 nm gap 0.5 nm. Each sample was scanned twice (preparing and filling sample holders twice) as is (fresh sample without any preparation), using a rotating cup (100 mm diameter, 120 mm depth) in reflectance mode, and average spectra were recorded. The sample holder was cleaned with pure water and clean paper between any scan. For each of the two repetitions of the same sample, 32 scans were collected after 16 scans of the reference. The WinISI 4 software V4.10.0.15326 (FOSS Analytical A/S, Hillerød, Denmark) was used to record absorbance and data expressed as log (1/R), where R represented reflectance values. Spectra were exported in Microsoft Excel (Microsoft Corporation, Seattle, WA) spreadsheet format, allowing the data to be transmitted to other software for further analysis.

Reference analysis

Samples were promptly analysed for proximate composition after NIRS scan to ensure the chemical analysis's representativity, especially avoiding changes in humidity and fermentative profiles. Previous studies detailed and described the reference methods (Andrighetto et al. 2018; Serva, Magrin, Andrighetto, et al. 2021; Segato et al. 2022) and are further reported. The DM was determined according to AOAC #934.01 (AOAC 2000). The alpha-amylase NDF (aNDF) and ADF fibre fractions were determined using an AnkomFibre Analyser (Ankom Technology Corporation, Fairport, NY, USA). The aNDF was performed with sodium sulphite, heat-stable alpha-amylase, F57 bags with 25 μm pore size and included residual ash (Ferreira and Mertens 2007; Schlau et al. 2021; Gorla et al. 2022); non-sequential ADF was evaluated according to Vogel et al. (1999); finally, ADL was obtained placing the F57 bags after non-sequential ADF in sulphuric acid 72% (Auger and Shipley 2013; Thomas et al. 2013).

Experimental design

Calculations were performed using R version 4.1.3 (2022-03-10). All packages described in the data analysis were installed and executed using R software.

Spectra manipulation

The combat function is designed to adjust for known batches or sources of variation in high-throughput data, and it is used especially for omic science in genetic and biological data analysis. It uses an empirical Bayesian framework to estimate and remove batch effects by constructing a statistical model that includes terms for the batches and the outcome of interest.

In the context of NIRS data, each batch could represent a different experimental run, subject, or time point when the spectral measurements were collected. Batch effects can arise due to systematic differences in experimental conditions, instrument calibrations, or other technical factors between these batches.

In our experiment, in order to reduce the batch effects from spectra of different feed groups, an empirical Bayes frameworks-based ComBat (Cb) function (Johnson et al. 2007) from the 'sva package' was tested (Leek et al. 2012).

Furthermore, non-Cb treated or Cb spectra were used as non-pre-treated (non-Pt) or pre-treated (Pt), as reported in Figure S1. In non-Pt spectra only the Mean Centre and Autoscale were applied. In Pt spectra, the pre-treatments were applied as follows: the Mean Centre and Autoscale, the Standard Normal Variate

(SNV) (Barnes et al. 1989), De-trending (DET) (second order interpolating polynomial), smoothing (15 pt, first order interpolating polynomial) (Savitzky and Golay 1964; Rinnan et al. 2009) and Savitzky–Golay first derivative (second order interpolating polynomial) (Savitzky and Golay 1964).

Dataset splitting

Further, but before any other calculation (feature reduction, and reduced dataset calibration, cross-validation and validation), the original dataset was randomly split into training (Tr) and testing (Ts) sets (Tr, $n = 240$, Ts, $n = 99$), ensuring the same ratio of the sample type (TMRc, TMRb, GS, CS), by the use of the function 'creatDataPartition' of the package 'caret'.

Explorative analysis

On the Tr set, a principal component analysis (PCA) was used as an exploratory approach to better describe the spectral dataset. The PCA (calculated on non-Cb) or the PCA-Cb (calculated after Cb) algorithms were performed only on non-Pt spectra (Antonucci et al. 2011; Oliveri et al. 2011; Serva et al. 2023).

Calibration of the original dataset (850–2500, gap 0.5 nm)

Moreover, four PLS models (Abdi 2010), by the use of 'pls' package (Mevik and Wehrens 2007), were applied to the original Tr dataset's spectra with the four combinations of non-Cb or Cb and non-Pt or Pt spectral manipulation. The four calibrations have been validated in the corresponding Ts set.

Feature selection, calibrations and validations using selected Wvs

The spectral datasets obtained from the four combinations of non-Cb or Cb and non-Pt or Pt manipulations were used one at a time. For each of the four datasets, a random forest (RF) algorithm performed the feature selection procedure based on the Boruta algorithm (Bisutti et al. 2019; Currò et al. 2021) using the 'Boruta' package (Kursa and Rudnicki 2010) with a wrapper approach applied to select the most informative Wvs and to remove unrelated and noisy data (Bisutti et al. 2019; Ghidini et al. 2019; Ayyıldız and Arslan Tuncer 2020). The Boruta algorithm is a wrapper method built around the random forest classification algorithm, implemented in the package 'randomForest' (Liaw and Wiener 2002). In the random forest (RF) process of variable splitting, the modelling results are influenced by changes in the number of regression trees (n_{tree}) and the number of split variables (m_{try}) (Liaw and Wiener 2002; Chen et al. 2018). In the present study, we used the default number for $n_{tree} = 500$ and $m_{try} = p/3$, where p is the number of predictors (Liaw and Wiener 2002; Breiman et al. 2022). The RF algorithms were tested on the non-Pt spectra or after Pt and combined with the use of Cb transformation or in a non-Cb transformed dataset.

After RF, a PLS (RF-PLS), was run on the selected Wvs and applied to the Tr set spectra.

In summary, four RF-PLS experiments were run (Pt/non-Pt spectra combined with Cb/non-Cb) and compared with the full spectra PLS of the original dataset. A schematic flowchart of the experiment is reported in Fig. S1.

External validation for RF-PLS algorithm with non-Pt and non-Cb

Only the RF-PLS algorithm with non-Pt and non-Cb was validated on three external datasets (dataset-1, -2, and -3) obtained from other instruments, laboratories and sample origin/type. Data of dataset-1 (total number = 619; 248 samples of corn whole fresh plant-CFWP-, 147 TMRb, and 224 TMRc) were obtained using a diode array PolispecNIR instrument ('Polispec', ITPhotonics, Fara Vicentina, Italy), with a spectral range of 902–1680, 2 nm increment, from samples collected in Europe (Table S2). Data of dataset -2 ($n = 25$ composed of various by-products) were obtained from an open-access dataset (Samadi et al. 2020) and collected with a Thermo Nicolet Antaris II TM ('Antaris', Thermo Fisher Scientific, Sunnyvale, CA) in a Wv range from 1000 to 2500 nm. Finally, data (total number = 201; TMRc = 51; TMRb = 50; GS = 50; CS = 50) of dataset −3 were obtained from a NIRSystem 5000 ("Nirs5000", FOSS Analytical, Hillerød, Denmark) operating in a spectral range between 850 and 2500 nm, 2 nm increment, from samples randomly collected in south Europe between the years 2000 and 2015.

As the spectral range differs between the original studied dataset and the external validation datasets, to calculate the RF-PLS, the closest Polispec, Antaris and Nirs5000 Wvs were chosen to match the original RF selected. RF-selected Wvs exceeding the external validation's spectral range were dropped from the calculation. In particular, the RF-selected Wvs for fibre fractions were out of the external validation's spectral range for Polispec. Therefore, the DM RF–selected Wvs were used for all Polispec validation.

Calibration and validation of external datasets using Wvs selected by RF for non–Pt and non-Cb

Finally, Polispec, Antaris and Nirs5000 (dataset-1, -2, and -3) were calibrated (Tr of external validation set = 70%)

and validated (Ts of external validation set = 30%) using exclusively the Wvs selected by RF (non-Pt and non-Cb) applied on the original studied Tr dataset. Tr and Ts partition of the external validation set ensured the same ratio of each sample type.

Metrics used

The standard error of the laboratory (SEL) was calculated as SEL = {[sum(d1 – d2)2]/2n}0.5, where d1 = duplicate 1, d2 = duplicate 2, and n = number of samples (Ariza-Nieto et al. 2018), and the maximum R2MAX was computed as R2MAX = (SDL2-SEL2)/SDL2 (Dardenne 2010; Digman et al. 2022), where SDL = standard deviation of laboratory.

The performance of NIRS calibration was reported as the root mean square error (RMSEcv) and the coefficient of determination (R^2_{cv}) in a Venetian blind 50 folders cross-validation (cv). Due to the low numerosity of some Tr datasets (< 50), calibrating for the single sample type, the cv was evaluated on a 30-folder Venetian blind. The PLS was tuned in the number of used Principal Components (PC), minimising the RMSEcv and maximising the R^2_{cv}. The performances of the NIRS calibration were evaluated on a validation (v) test set (Ts), reporting the root mean square error (RMSEv). Moreover, the ratio of performance to deviation RPDv was calculated as the standard deviation of the reference data divided by the standard error of prediction (SEPv) (Williams 2014). Predicting the Ts spectra and comparing results with the actual values, and using the 'lm function' (the 'stat package' version 3.6.2), the adjusted coefficient of determination (adj-R^2_v), and the residual standard error (RSE_v) of validation were calculated. The statistical significance was set at a p-value < 0.05.

Results

The results (and further, in the same order, the discussion) have been summarised and organised into the following sections: (3.1) explorative analysis using PCA and PLS on the original dataset, (3.2) RF-PLS without ComBat, divided into non-Pt and Pt, (3.3) RF-PLS with ComBat, also divided into non-Pt and Pt, (3.4) selected wavelengths (Wvs) identified across the four RF tests, (3.5) external validation of RF-PLS for the non-Pt and non-Cb datasets, and (3.6) calibration and validation of the three external datasets using the selected wavelengths for the non-Pt and non-Cb conditions.

Explorative analysis, PCA and PLS on the original dataset and spectral range (850–2500. 0.5 nm)

PCA for non-pre-treated spectra and after pre-treatments

The PCA preserved the complexity of the original dataset while reducing the variables into fewer new features called Principal Components. As an explorative analysis, the first ten PCs were computed, and the first two PCs for non-Pt spectra described 99.3% (PC1 = 92.3 and PC2 = 6.70%) and 98.8% of the original variability of the dataset (PC1 = 89.5 and PC2 = 9.31%) in the non-Cb and Cb treated spectra, respectively (Figure S2, panels a and c, respectively).

The Cb reduced the spectral variability among the first two PCs, and the original variability explained by the PC1 was reduced while increased by the PC2. In both cases, PCs higher than two gained irrelevant information (<1.00%). At a visual inspection, in PCA without Cb, sample types grouped for TMRs and silages, while after Cb, the four groups overlapped.

The average of the Global Mahalnobis distances for non-Pt and non-Cb spectra were 9.97, 9.96 and 9.90 for the 339 sample dataset, the Tr and the Ts set, respectively.

PLS applied to the original spectra dataset (850–2500, gap 0.5 nm)

The results for PLS algorithms performed in the whole spectral range for the Tr set, and its validation in the Ts set are reported in Table S3.

RF-PLS whitout ComBat (non-Cb)

The RF-PLS algorithms were assessed with or without spectra Pt, both cases without Cb (non-Cb).

RF-PLS without pre-treatments (non-Pt) and without ComBat (non-Cb)

The scree plots (panel a) for the first 10 PCs and individuals PC1–PC2 scatter plot (panel b) for the non-Pt spectra are reported in Figure S2. The RF selected 57, 12, 0, and 1, the most representative Wvs for DM, aNDF, ADF and ADL, respectively (Table S4). In Figure 1, the selected features are plotted against their importance, which is calculated by the RF algorithm and roughly represents their Z score (Kursa and Rudnicki 2010). The ADF had no one Wvs selected by RF and ADL, only one (1420 nm).

Fibre quality traits are chemically interrelated (Berzaghi et al. 2021). While the RF algorithm used for wavelength selection in this study did not identify any relevant Wvs for ADF and found only one for ADL, the

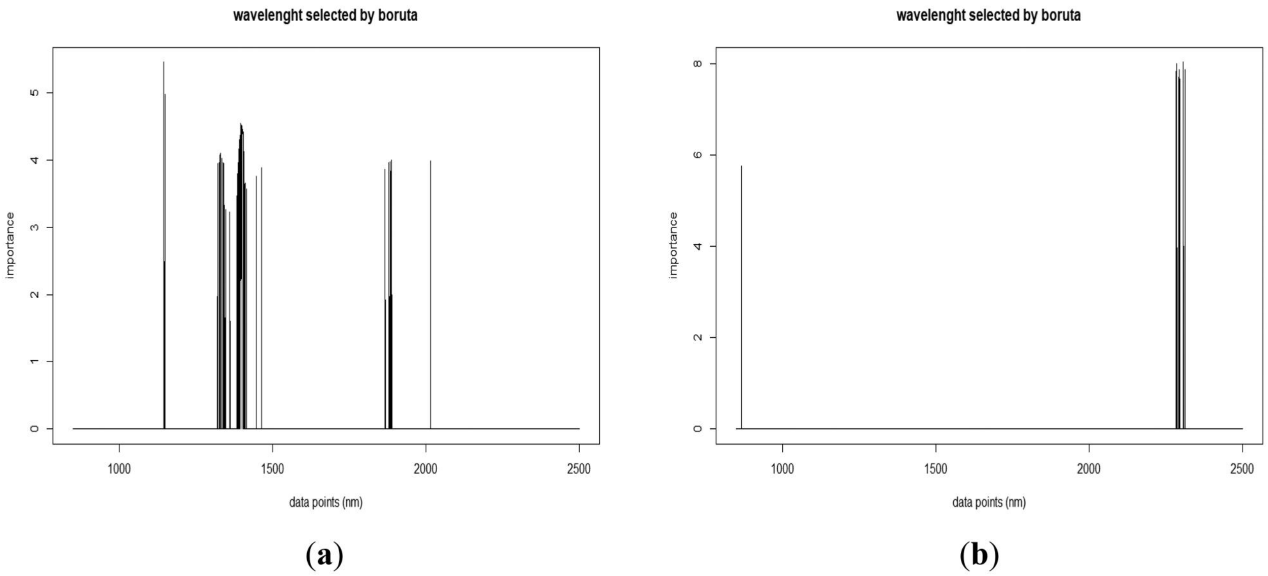


Figure 1. The most important wavelengths (Wvs) selected by the Random Forest algorithm on the non–pre-treated and non–ComBat spectra from the training dataset. The lines' height represents the selected feature's importance for dry matter (panel a), alpha–amylase neutral detergent fibre (panel b), while for acid detergent fibre and acid detergent lignin, less than two Wvs were selected as necessary.

Table 1. Cross-validation (CV) and validation (V) results for the random Forest (RF) partial least square (PLS) algorithm (RF-PLS) calculated in the four combinations of pre-treated, non-pre-treated, ComBat, and non-ComBat spectral datasets.

Traits	Ncomp[1].	% EV	R^2_{cv}	$RMSE_{CV}$	Adj–R^2_v	$RMSE_V$	RSE_V	RPD_V	*p*
non–pre-treated and non–ComBat									
DM	8	99.9	0.97	2.32	0.97	2.34	2.33	5.46	<0.001
aNDF	6	99.9	0.77	2.47	0.80	2.51	2.18	2.20	<0.001
ADF	5	99.9	0.71	1.74	0.77	2.02	1.45	1.97	<0.001
ADL	2	99.9	0.63	0.45	0.63	0.45	0.31	1.58	<0.001
pre-treated and non–ComBat									
DM[1]	5	98.2	0.97	2.30	0.97	2.02	2.00	6.32	<0.0001
aNDF[2]	3	93.5	0.87	1.82	0.86	2.14	1.69	2.57	<0.0001
ADF[3]	3	87.6	0.83	1.32	0.87	8.32	1.04	0.66	<0.0001
ADL[4]	4	95.2	0.60	0.40	0.82	0.31	0.24	2.31	<0.0001
non–pre-treated and ComBat									
DM[1]	6	99.9	0.50	9.48	0.47	9.46	7.78	1.35	<0.0001
aNDF[2]	3	99.9	0.42	3.89	0.64	3.45	2.25	1.60	<0.0001
ADF[3]	3	99.9	0.32	2.65	0.59	2.77	1.45	1.44	<0.0001
ADL[4]	5	99.9	0.15	0.58	0.46	0.55	0.28	1.29	<0.0001
pre-treated and ComBat									
DM[1]	2	76.4	0.42	10.3	0.62	7.94	5.45	1.61	<0.001
aNDF[2]	2	84.8	0.44	3.82	0.66	3.31	2.13	1.67	<0.001
ADF[3]	2	88.0	0.37	2.56	0.67	2.50	1.32	1.60	<0.001
ADL[4]	2	76.4	0.57	0.19	0.57	0.49	0.25	1.44	<0.001

[1] Dry matter (DM, %);
[2] Alfa-amylase neutral detergent fibre (aNDF, % DM);
[3] Acid detergent fibre (ADF, % DM);
[4] Acid detergent lignin (ADL, % DM).

Abbreviations: Ncomp: number of principal components used; RSE: residual standard error; RMSE: root mean square error; R^2: coefficient of determination; % EV: % of the explained variance; RPD_V: ratio of performance to deviation.

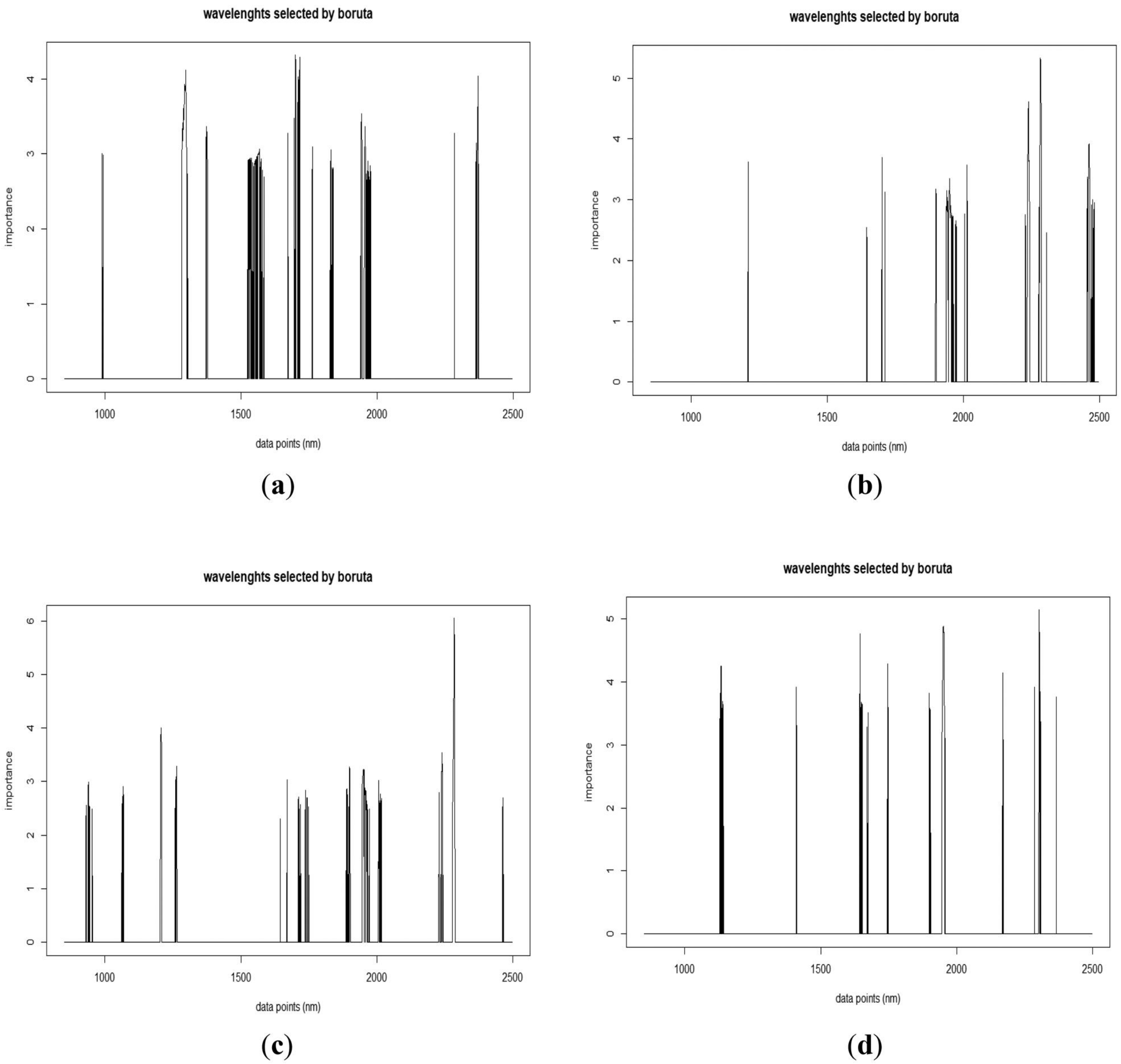
wavelengths selected for aNDF were utilised for PLS analysis of ADF and ADL. This approach was deemed the most practical under the experimental conditions, even though other algorithms might have yielded better results. The RF-PLS results for CV and validation are reported in Table 1.

By comparing the RMSEcv of the original dataset-PLS (Table 1) with the RMSEcv of RF-PLS non-Cb and non-Pt (Table S3), the ratios of the RMSEcv values were calculated. These ratios are 0.89, 1.08, 1.12, and 1.02 for DM, aNDF, ADF, and ADL, respectively, reflecting the relationship between the predictive performance of the two methods for each parameter.

The RF-PLS results (CV and V) from separate calibrations by sample types for the single traits (DM, aNDF, ADF, and ADL) are reported in Table S5. In the last calculation, the wavelengths used were those RF-selected (non-Pt-non-Cb) from the studied initially Tr dataset, which includes all samples without differentiating among sample types.

RF-PLS with pre-treatments (Pt) and without ComBat (non-Cb)

The Pt and non-Cb spectra for the Tr set are shown in Figure S3 (panel a). The Scree plots (panel a) for the first 10 PCs and individuals PC1–PC2 scatter plot (panel b) for the Pt spectra are reported in Figure S4 (panels a and b). The RF confirmed 190, 141, 167, and 79 as important Wvs for DM, aNDF, ADF and ADL, respectively (Table S4 and Figure 2). The RF-PLS results for CV and validation are reported in Table 1, showing values of Adj-R^2_v that are lower than 0.87 for fiber fractions, equal to 0.97 for DM. The DM showed equal results compared to those values reported for the non-Pt and non-Cb, while the fiber fractions demonstrated lower prediction capability.



(a) (b) (c) (d)

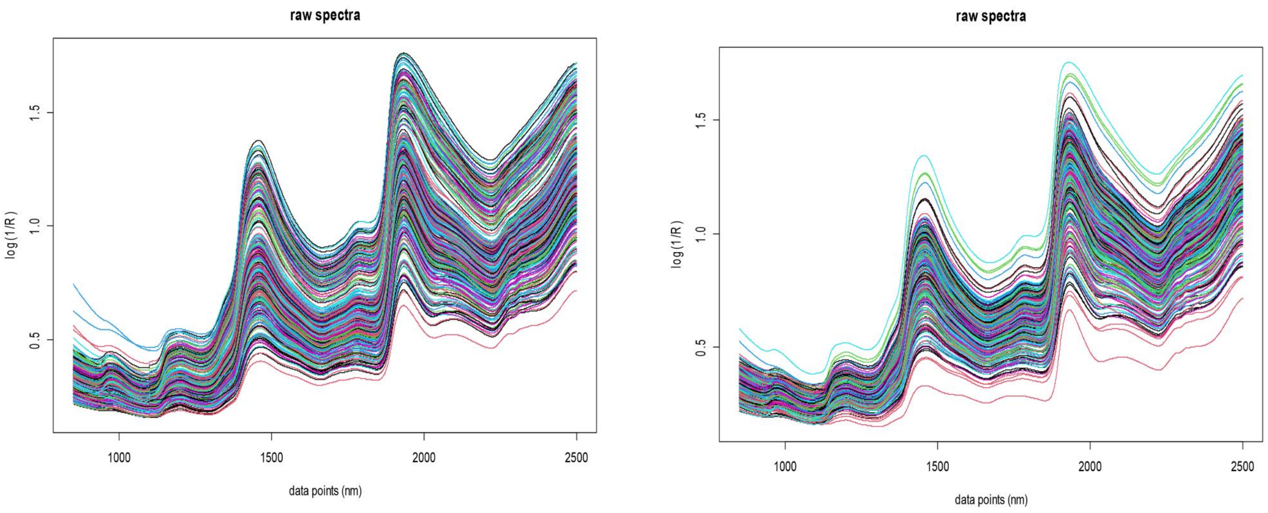
Figure 2. Most important wavelengths (Wvs) selected by the Random Forest algorithm on the pre-treated and non-ComBat from the training dataset the lines' height represents the selected feature's importance, for dry matter (panel a), alpha-amylase neutral detergent fibre (panel b), acid detergent fibre (panel c) and acid detergent lignin (panel d).

RF–PLS with ComBat (Cb)

The non-Pt spectra before (panel a) and after Cb (panel b) are presented in Figure 3. The algorithms were assessed on Pt or non-Pt, and both after using Cb. The most predictive features were calculated on the Tr dataset using the RF algorithm (Table S4). Furtherly, the PLS was calculated using only the RF-selected Wvs.

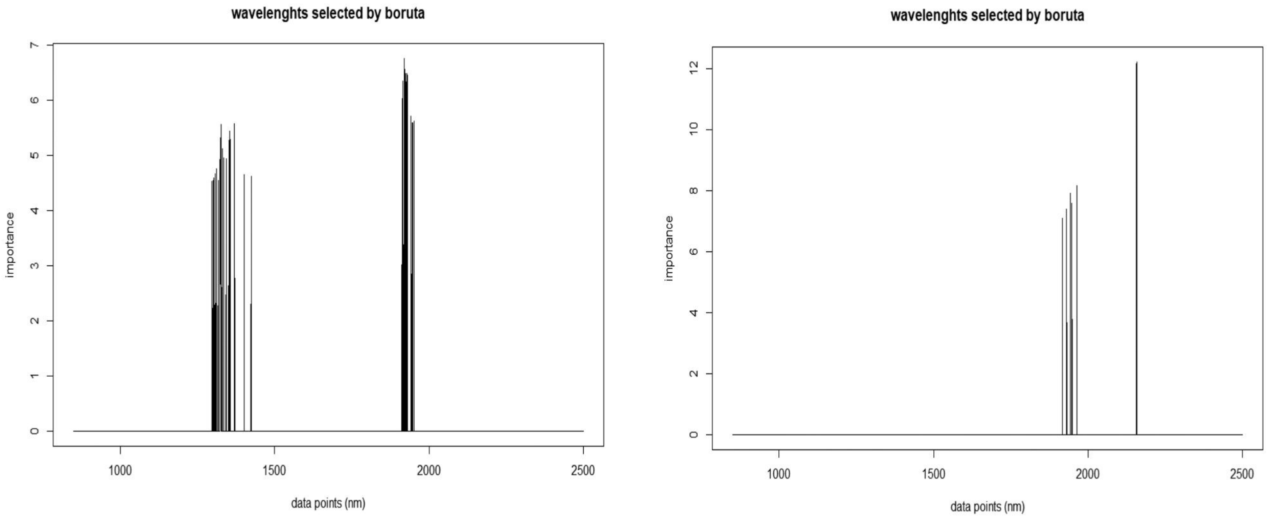
RF-PLS without pre-treatments (non-Pt) with ComBat (Cb)

The Scree plots for the first 10 PCs and individuals PC1–PC2 scatter plot for the non-Pt after Cb spectra are reported in Fig. S2 (panel c and panel d), respectively. The RF confirmed 44, 7, 2, and 2 as important Wvs for DM, aNDF, ADF and ADL, respectively (Table S4). The ADF and ADL had two Wvs selected by RF; therefore, those Wvs selected for aNDF were further adopted for ADF and ADL. As discussed in Section 3.2.1, fibre quality traits are chemically interrelated (Berzaghi et al. 2021), and while the RF algorithm failed to identify relevant Wvs for ADF and found only one for ADL, the wavelengths selected for aNDF were applied to PLS analysis for ADF and ADL as the most practical solution under the study's experimental conditions. In Figure 4, the selected features are plotted against their importance calculated by the RF algorithms. The RF–PLS results for CV and V are reported in Table 1, showing values of Adj-R^2_v that are lower



(a) (b)

Figure 3. Non–pre-treated spectra before (panel a) and after ComBat function.



(a) (b)

Figure 4. The most important wavelengths (Wvs) selected by the Random Forest algorithm on the non–pre-treated and ComBat from the training dataset. The lines' height represents the selected feature's importance for dry matter (panel a), alpha-amylase neutral detergent fibre (panel b), while for acid detergent fibre and acid detergent lignin only two Wvs were selected as necessary.

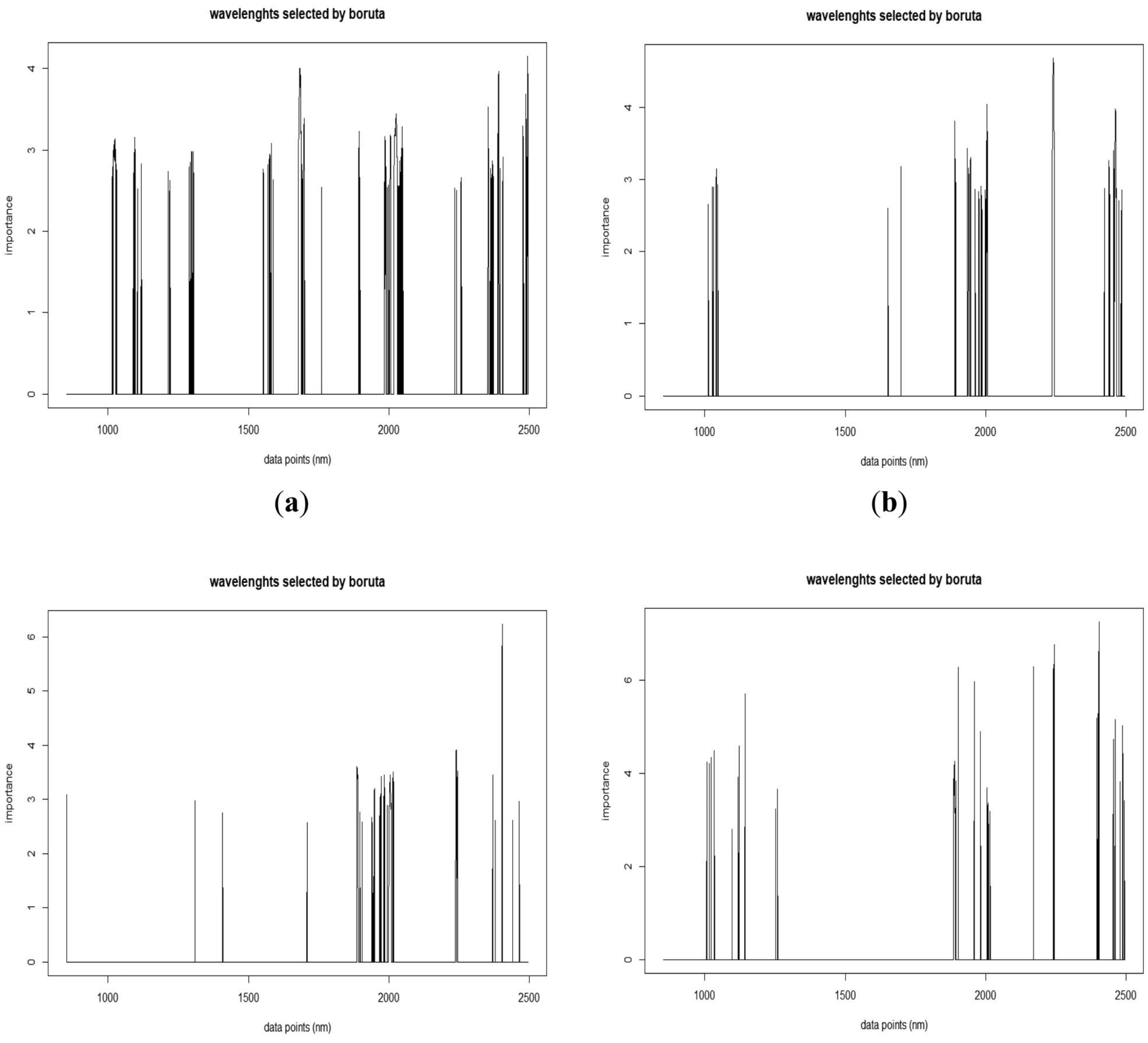
than 0.64 for fibre fractions, equal to 0.47 for DM, and lower to the correspondent observed in non-Pt and non-Cb test.

RF–PLS with pre-treatments (Pt) and with ComBat (Cb)

The scree plot of the first ten PC contributions and the first two PC score plots for Tr set after Pt and after Cb, are reported in Fig. S4 (panels c and d), and the spectra are shown in Fig. S3 panel a. The RF confirmed 231, 86, 85, and 54 as important Wvs for DM, aNDF, ADF and ADL, respectively (Table S4). In Figure 5, the selected features are plotted against their importance calculated by the RF algorithms. The RF–PLS results for CV and validation are reported in Table 1, showing values of Adj-R2v that are lower than 0.57 for fibre fractions, equal to 0.42 for DM, and lower to the correspondent observed in non-Pt and Cb test.

RF-selected Wvs in the four PT/non-Pt and Cb/non-Cb combinations

The count of selected Wvs by the four combinations of Pt, non-Pt, Cb, non-Cb spectra of the RF algorithms are reported in Figure 6 (panel a). The Spearman



(a) (b) (c) (d)

Figure 5. Most important wavelengths (Wvs) selected by the Random Forest algorithm on the pre-treated and ComBat spectra from the training dataset, the lines' height represents the selected feature's importance. The RF is reported for dry matter (panel a), alpha-amylase neutral detergent fibre (panel b), the acid detergent fibre (panel c), and acid detergent lignin panel d).

correlation (r) within the RF selected Wvs (the count of) for DM, aNDF, ADF and ADL is reported in Table 2 and Figure 6 (panel b).

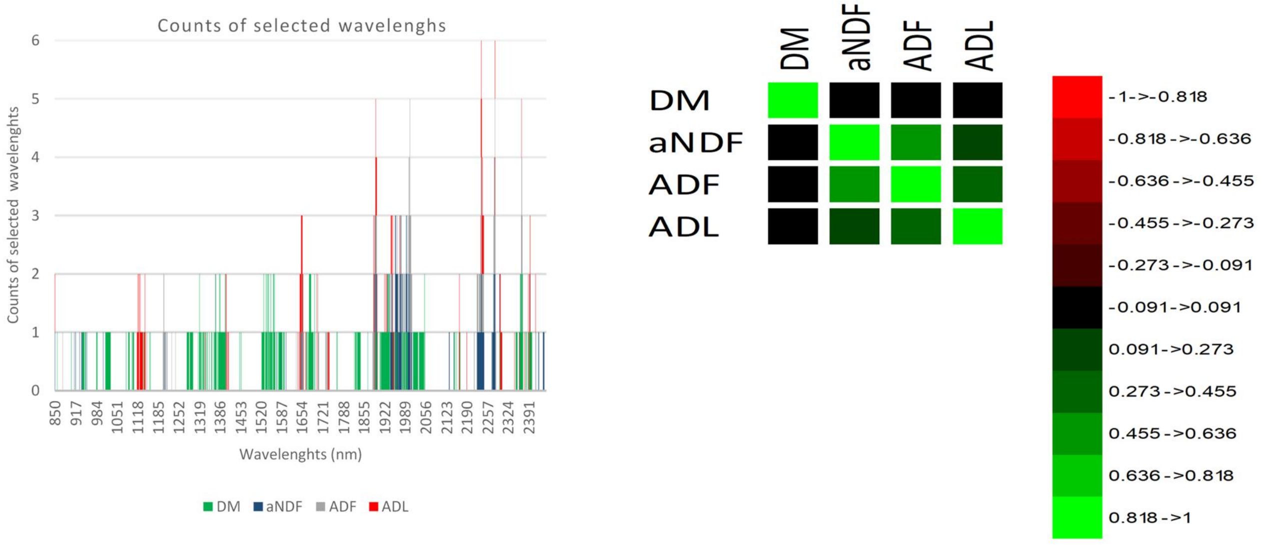
The Spearman correlation within the RF selected Wvs (the counts of), separately for DM, aNDF, ADF and ADL for the four combinations of non-Pt, Pt, Cb, non-Cb spectra, are reported in Table 3.

External validation for RF–PLS algorithm with non-Pt and non-Cb

Three validations were evaluated by applying a RF-PLS (non-Pt-non-Cb) calculated on the initially studied dataset to predict the external datasets -1, -2 and -3 distinctly for sample type. The spectral range of the four instruments did not align completely, necessitating adaptations to accommodate the original RF-selected Wvs. Specifically, the RF-selected Wvs for fibre fractions fell outside the spectral range available for Polispec in the external validation. Therefore, the DM RF-selected Wvs were used for all Polispec validation (Table S6), and results from the external validations are reported in Table S7.

Calibration and validation of external datasets using Wvs selected by RF for non-Pt and non-Cb

Finally, as described previously, the compatible RF-selected Wvs of the original non-Pt-non-Cb dataset



(a) (b)

Figure 6. The most important wavelengths (Wvs) selected by the Random Forest (RF) algorithm on the four combinations of non-Pt/pre-treated spectra and Combat (Cb) /non-Cb, the lines' height represents the sum of counts of the selected feature for dry matter (DM), the alpha-amylase neutral detergent fibre (aNDF), the acid detergent fibre (ADF) and the acid detergent lignin (ADL) (panel a). The Spearman (r) correlation coefficient within the selected Wvs (the counts of selected Wvs) (panel b). The single squares represent, in order, left to right, up to down, the DM, the aNDF, the ADF and the ADL. The RF selected for non-Pt and non-Cb spectra of ADF and ADL was considered null.

Table 2. The Spearman correlation (r) matrix for the counts of the selected wavelengths (Wvs) accounted altogether in the four algorithms by the combinations of pre-treated (Pt), non–Pt, ComBat (Cb), non-Cb spectra.

Traits	DM^1	aNDF^2	ADF^3	ADL^4
DM^1	1.00	0.08	0.03	0.01
aNDF^2	0.07****	1.00	0.45****	0.26****
ADF^3	0.03	0.45****	1.00	0.27****
ADL^4	0.01	0.26****	0.27****	1.00

[1] Dry matter (DM, %);
[2] Alfa-amylase neutral detergent fibre (aNDF, % DM);
[3] Acid detergent fibre (ADF, % DM);
[4] Acid detergent lignin (ADL, % DM).
*$p \leq 0.05$ **$p \leq 0.01$ ***$p \leq 0.001$ ****$p \leq 0.0001$.

were adopted to calibrate the external dataset from Polispec, Antaris and Nirs5000, and results for the new Tr and Ts set are reported in Table S8.

Discussion

It is recognised that good performance for NIRS calibrations might show RPD > 3 for quantitative analyses (Davies and Williams 1996). Consistently with Wiliams (2014), and referring to forages and feeds, the RPD value was graded as excellent (> 4.1, any application), very good (3.5–4.0, for process control), good (3.0–3.4, for quality control), fair (2.5 – 2.9, only for screening), while poor RPD usable only rough screening (2.0–2.4) or very poor (0.0 – 1.9), as not recommended. Moreover, according to Pérez-Marín et al. (2004), the R^2 could be graded as excellent ($R^2 \geq 0.9$), good ($0.7 \leq R^2 < 0.9$), or limited predictive ability ($0.5 \leq R^2 < 0.7$) for compound feedstuffs.

Table 3. The Spearman correlation (r) matrix for the counts of the selected wavelengths, accounted separately in the four algorithms by the combinations of pre-treated (Pt) or non-pre-treated (non-Pt) and ComBat (Cb) or non-Cb treated spectra; nw = not selected wavelengths by the Random Forest (RF) algorithm.

	Traits	Non-Pt- non-Cb	Pt-non-Cb	Non-Pt-Cb	Pt-Cb
		DM	DM	DM	DM
Non-Pt-non-Cb	DM^1	1.00	−0.03	0.04*	−0.04*
Pt-non-Cb	DM^1	−0.03	1.00	0.03	0.04*
Non-Pt-Cb	DM^1	0.04*	0.03	1.00	−0.01
Pt-Cb	DM^1	−0.04*	0.04*	−0.01	1.00
Non-Pt-non-Cb	aNDF^2	1.00	0.10	0.01	−0.01
Pt-non-Cb	aNDF^2	0.10****	1.00	0.06***	0.26****
Non-Pt-Cb	aNDF^2	0.01	0.06***	1.00	0.04*
Pt-Cb	aNDF^2	−0.01	0.26****	0.04*	1.00
Non-Pt-non-Cb	ADF^3	nw	nw	nw	nw
Pt-non-Cb	ADF^3	nw	1.00	0.05**	0.22****
Non-Pt-Cb	ADF^3	nw	0.05**	1.00	−0.01
Pt-Cb	ADF^3	nw	0.22****	−0.01	1.00
Non-Pt-non-Cb	ADL^4	1.00	0.01	0.01	0.01
Pt-non-Cb	ADL^4	0.01	1.00	0.08****	0.01
Non-Pt-Cb	ADL^4	0.01	0.08****	1.00	0.01
Pt-Cb	ADL^4	0.01	0.01	0.01	1.00

[1] Dry matter (DM, %);
[2] Alfa-amylase neutral detergent fibre (aNDF, % DM);
[3] Acid detergent fibre (ADF, % DM);
[4] Acid detergent lignin (ADL, % DM);
[5] Ncomp = number of principal components used.
*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$.

However, the use of both RPD and R^2 is reported as redundant (Minasny and McBratney 2013). RPD is graded differently by the matrix of interest, Minasny and McBratney (2013) stated that “there is no basis for this classification. It is all relative”. Furthermore, it is also known that forages are highly variable in composition, and inaccurate sampling or non-timely results can cause relevant differences from formulated rations to feed rations, causing possible consequences for the health and production of dairy cows (Sova et al. 2014; Miller-Cushon and DeVries 2017). Considering the error in the mean as the ratio of standard deviation to the root square of (n −1, where n is the number of repetitions), increasing the number of analyses will decrease the error associated with the variability and, therefore, allow for a more accurate ration preparation. The variability error is not accounted in the analytical error but is demonstrated to be relevant. Also, Martinez (Martínez et al. 1998) underlined the role of sample heterogeneity in the adopted number of replicates, which, using a portable NIRS, can virtually cover the complete feed ration or single feed (i.e. if mounted in an operating machinery). Therefore, using only RPD and R^2 metrics to evaluate NIRS implementation is a limitation when assessing on-field applications, as these metrics do not account for all potential errors in the feeding process, including those related to sample representativity and delays in analysis. Even if evaluating potential errors in the feeding process is not within the scope of the present study, this consideration might be considered in the discussion of the results.

Explorative analysis, PCA and PLS on the original dataset and spectral range (850–2500. 0.5 nm)

PCA for non-pre-treated (non-Pt) spectra and after pre-treatments (Pt)

Reducing the fraction of variability unrelated to the property of interest would help to avoid effects such as the light scattering, mainly related to the nature of the sample particle and the path length (Azzouz et al. 2003), and a strategy to eliminate systematic variations unrelated to analyte concentrations is Pt (Prieto et al. 2009); however, this approach could also remove useable information from the spectra (Azzouz et al. 2003). The right choice of the best Pt is an open-debate question, and a common approach is ‘trial-and-error’, in which results are somehow subjective (Gerretzen et al. 2015). Azzouz et al. (2003) tested several Pt combinations for a PLS performed on moisture and protein in alfalfa. They concluded that predictions were somewhat similar to whatever the data Pt used if enough PCs were considered. The use of Cb tentatively overcame the difficulties in the Pt applicability. The Cb algorithm is used when data suffer an unwanted effect associated with the experimental condition, which may result in relevant noise levels, commonly known as the ‘batch effect’, which can significantly reduce the power of the statistical algorithms (Ugidos et al. 2020). Our study’s batch effect was related to the different feed types used, but its use generally worsens the prediction ability of RF–PLS; nonetheless, the Pt-Cb increased the performances compared to non-Pt-Cb spectra, confirming the role of Pt.

PLS applied to the original spectra (850–2500, gap 0.5 nm)

Results from PLS of the original dataset (850–2500, 0.5 nm) showed ‘fair’ or better RPD_v grade for all traits in non-Cb spectra (both Pt or non-Pt, Table S3), except for ADL, while RF-PLS (Tables 1 and S9–S11) had “fair” or better RPD only for DM in non-Cb spectra (both Pt or non-Pt) and aNDF in non-Cb and Pt spectra and the use of Cb decreases the quality of the calibration significantly, probably due to a modification of the spectra shape with the transformation in an unexpected spectrum, as shown in Fig. S3– panel b. A shift in Cb RF-selected Wvs confirms these findings, and it can be inferred that this is the reason for the higher rate of explained variance from PC 2.

RF-PLS with or without pre-treatments (Pt/non-Pt) and ComBat (Cb/non-Cb)

The ratio of the RMSEcv of the RF-PLS (non-Pt-non-Cb) to the corresponding RMSEcv of the original dataset-PLS is slightly higher (aNDF, ADF, ADL) or even lower than one (DM), demonstrating that no significant errors have been added at the PLS algorithm by the RF compared to the PLS implemented on the whole spectral range of the original dataset. However, even though the single sample type calibration spectra may better simulate an actual in-field application, calibrating the RF-PLS for the individual sample type (non-Pt-non-Cb) showed a decrease in the quality of predictions, with $RPD_v > 2.5$ only for DM for TMR_c, TMR_b, and GS, while CS showed RPD_v of the DM = 1.90 (Table S5). However, the findings from the last calculations are probably due to the sample number for each RF-PLS algorithm being low (especially for GS and CS) to calibrate; furthermore, it is proven that RPD with a low sample number is highly inflated by a single high or low value (Williams 2014), such as one or more outliers.

Berzaghi et al. (2005) satisfactorily detected DM and NDF in CS with a 960 to 1700 nm diode portable NIRS, showing an R^2_{cv} of 0.88 and 0.87, respectively. Mertens and Berzaghi (2009), with a portable 960–1550 nm diode array instrument for DM found an $R^2_{cv} = 0.95$ and SECV = 2.2% for forages (CS, alfalfa, and feed rations). However, Patton et al. (2018) compared three portable instruments for DM and ADF, with a benchtop instrument used in an ISO 17025–certified laboratory (Patton et al. 2018), finding performances instrument-dependent and underlining that different manufacturing characteristics lead to different results. Marchesini et al. (2017) transferred a benchtop laboratory calibration for undried whole corn in an on-site spectrometer (960 1600 nm) but found different performances among two versions of the same instrument and, according to different transfer algorithms, the better-reported results were $R^2_{v} = 0.91$, 0.97 and 0.78 for DM, NDF and ADF, respectively., and the same authors reported, for the most performant portable instrument version, original (non-transferred) calibration with $R^2_{v} = 0.87$, 0.99 and 0.82 for DM, NDF and ADF, respectively. Moreover, Serva, Magrin, Marchesini, et al. (2021) reported that the metrics in TMRc, was $R^2_{cv} = 0.93$, 0.78, and 0.92 for DM, NDF, and ADF, respectively. Gorla et al. (2022) reported $R^2_{cv} = 0.78$ and 0.55 for DM and aNDF for dried and grounded alfa–alfa, analysed with a 1350–2550 nm range handheld spectrometer. Digman et al. (2022) used the same handheld Fourier transform instrument described by Gorla et al. (2022) in forage samples and, after applying Pt to the collected spectra, obtained $R^2_{cv} = 0.88$, 0.92, and 0.87 for NDF, ADF, and lignin, respectively. Moreover, Digman et al. (2022) reported R^2_{cv} for ground and dried samples of alfalfa and grass analysed with a benchtop monochromator (operating at a spectral range of 1100–2498, 2 nm, after applying Pt) which are comparable to our findings for the PLS performed in the original dataset (1100–2500, 2 nm, after applying Pt) except for ADL, which has been reported with $R^2_{cv} = 0.87$, therefore significantly higher than our. These comparisons let us speculate about the potential of the use of Pt, especially for undried-unground semple.

Generally, the reported literature results are comparable to our non-Cb calibrations findings. Nevertheless, calculating the Pt in the discontinuous and non-regularly distanced spectrum may not be applicable. Even though the RPD_v of our best R^2_{cv} for ADL in non-Cb experiments (Table 1) were less than fair, these results are better than those reported for the predictions of hay-based total mixed rations using a 900 to 2500 nm FT-NIR spectrometer and a combination of Pt and ten PC used (205 TMRs used) (Buonaiuto et al. 2021), confirming the difficulties in obtaining better results for this trait. Moreover, our results for ADL in non-Cb experiments showed results higher than those from an experiment conducted under typical laboratory conditions for a post-dispersive diode array (DA) 950–1650 nm, a digital light processing (DLP) 950–1650 nm, and an SCiO (SW) 740–1070 nm instruments (R^2_{cv} = 0.75, 0.56, and 0.48, respectively) (Berzaghi et al. 2021). The cited authors experimented with dried and ground GS, but better results for dried and grounded alfalfa. They applied pre-treatment (SNV, de-trend, and the first derivative) and concluded that the reference method is the real limit for ADL prediction with NIRS. Noticeably, these findings refer to instruments with a cost of around 20–24,000 United States Dollars (USD) for DA, 6–10,000 USD for DLP, all the way down to 400 USD for SW. Additionally, between the cited instruments, the cheapest (SW) performed with R^2_{cv} = 0.69 and 0.62 for NDF and ADF in grass silage. The latter results are consistently lower than our findings for non-Cb, probably due to the uninformative region used in SW (740–1070 nm). Conversely, our finding of PLS of ADL in the original dataset (1100 2500, 2 nm, after applying Pt) was even lower than those from a handheld instrument (Digman et al. 2022) for undried and ungrounded samples, leading to speculation about reference method precision.

RF-selected Wvs in the four PT/non-Pt and Cb/non-Cb combinations

NIRS spectra are formed of overtones and combination bands. Overtones are electron excitations occurring at multiple fundamental frequencies recorded in the middle infra-red region (Beć et al. 2022). The NIRS region covers four overtones. Indeed, the high absorption NIRS bands are typically in the combination bands (1900–2500 nm), whereas a decrease in absorption intensities continues until the third overtone (700–1100 nm) (Agelet and Hurburgh 2010), and Berzaghi et al. (2021) concluded that a range of 950–1650 nm can be a reasonable compromise and an alternative to the expensive laboratory equipment. Conversely, our results from non-Pt-non-Cb spectra show that several absorption peaks are located in the range 1100–2000 nm for DM and mainly in 2200–2350 nm for the evaluated fibres fractions (aNDF, ADF, ADL). Considering results from the four experiments (Figure 6, panel a), it can be visually noted that the DM-selected Wvs roughly fall in the whole spectral width, while fibre is mainly in the first overtone band. More detailed, the DM showed the densest region of RF-selected Wvs (non-Pt) between 1320.5 and 1463.5 nm (for both the Cb and the non-Cb) and between 1886.5 and 1951, confirming the role of the O–H bonds at 1410–1439 and 1949 nm, traditionally associated with water (Cozzolino and Labandera 2002; Williams 2020). The first cited band is in the second overtone (1050–1650 nm), while the second is in the first overtone (1500–2000 nm) coupled with a combination band (1900–2500 nm) region (Berzaghi et al. 2021). Regarding NDF, the RF-selected Wvs in non-Pt spectra fall in the first overtone and combination bands for non-Cb and Cb.

Besides, using non-Pt-Cb spectra, RF-selected ADF and ADL features were equally represented in the first and second overtones and combination bands. Moreover, our findings showed that Pt generally resulted in more RF-selected Wvs than non-Pt spectra. Overviewing the RF-selected features in the four experiments, it is clear that using non-Pt-non-Cb spectra allows the minimum number of features selected for DM and aNDF, while ADF and ADL require all spectra. Nevertheless, adopting the aNDF-selected Wvs for ADF led to discrete results in the validation for TMRs and GS. The use of Pt increased the calibration performances but, on the other hand, caused a higher number of selected Wvs, reducing the approach's effectiveness.

Fibre quality traits are related to each other; Berzaghi et al. (2021), considering GS and referring to NDF, reported $r = 0.93$ and 0.77 for ADF and ADL, and $r = 0.91$ between ADF and ADL. However, in our finding, the correlation between the RF most important selected Wvs showed a value of $r = 0.45$ among aNDF–ADF and $r > 0.26$ between ADL and aNDF or ADF, demonstrating a weak association between aNDF–ADF and ADL in terms of common Wvs. Finally, the correlation between RF-selected features in the four combinations of Cb/ non-Cb and Pt/non-Pt spectra was (in absolute value) $r < 0.04$, 0.26, 0.22, and 0.08 for DM, aNDF, ADF, and ADL, respectively, demonstrating the relevance of the strategy adopted to reduce unwanted noise effects.

External validation for RF-PLS algorithm with non-Pt and non-Cb

To validate the RF-PLS algorithm of the original dataset (non-Pt- non-Cb) was used to predict the external validation dataset -1, -2, and -3 (Table S7) and showed shallow results. However, these findings were expected as there can be consistent differences in spectra due to instrument architecture. In fact, most of the errors were attributable to a bias in prediction. Therefore, a good transfer algorithm that can standardise or clone large data collections of existing instrument data to handheld NIRS could allow excellent results to be reached (Pérez-Marín et al. 2022).

Calibration and validation of external datasets using Wvs selected by RF for non-Pt and non-Cb

A self-calibration of the external datasets -1, -2, and -3, singly, while adopting the Wvs selected by the RF-PLS (non-Pt- non-Cb) allowed at least 'fair' RPDv for DM, aNDF, and ADF for FOSS, and DM and aNDF for Polispec (Table S8). The good results for Nirs5000 are probably due to the likeness of the NIRS technologies adopted. In contrast, in the case of the Antaris, unacceptable results are possibly explainable by the relevant differences in the sample characteristics (various by-products in Antaris versus TMRs and silages in the dataset analysed in this study).

General comments

Data analysis based on spectral technology depends on the analytical methods. The total errors of a prediction by NIRS can be accounted as the sum of the errors, and SEL plays a crucial role in evaluating the performance of the chemometric model used. Our results show that average means and SEL are comparable to the literature. Digman et al. (2022) reports average means (SEL) values for fresh alfalfa and fresh grass as follows: NDF 87% DM (0.45), ADF 30 % DM (0.74), and ADL 3.7 % DM (0.29), Cherney et al. (2021) reports SEL values of 0.585, 0.547, 0.219 for NDF, ADF, and ADL in CS (mean 35.93, 21.84, and 2.47 % DM, respectively) and 0.497, 0.657, and 0302 for NDF, ADF, and ADL in TMR (mean 27.80, 17.61, and 2.81, respectively). Moreover, Cherney et al. (2021) reports SEL values of 0.657, 0.532, and 0.224 for NDF, ADF, and ADL in CS and 0.497, 0.657, and 0.302 for NDF, ADF, and ADL in TMR. Ariza-Nieto et al. (2018) report SEL values of 1.55, 1.45, and 1.23 (mean 54.1, 26.5, and 4.8 % DM) for NDF, ADF, and ADL in Colombian forages (grass, legumes, and other) with RPD of 2.14 for ADL, which is similar to those we found for Pt RF-PLS algorithms. However, the same Authors found a lower RPD (1.73) for ADL when using only the legumes for calibration. All these results indicate more ADF, especially ADL variation, than NDF. Therefore, the relatively poor results we observed with ADL may be due to too few samples and significantly higher laboratory error reference methods used for chemical determinations.

A limitation of the present study is the minor sample size for GS and CS and the limited number of measurements for ADF and ADL, which makes it less conclusive in determining whether the methods for generating selective wavelengths to represent specific parameters are effective. However, it must be considered that RF algorithms were developed in the overall sample without accounting for the differences in sample type. This is because the application should work independently from the fibre source. In this sense, the total sample numerosity is less imitating. Despite this, more studies are required to test RF tuning or different algorithms for Wvs selection.

Indeed, the results from dataset-2 underscore the critical role of sample homogeneity in identifying key wavelengths suitable for calibrating specific chemical components. This comparison also illuminates the technological disparities among various spectrometers, which introduce complexities due to differences in wavelength correspondence, spectral resolution, signal intensity, environmental sensitivity, and other factors (Tengstrand et al. 2024). Consequently, these findings delineate the boundaries of anticipated outcomes when attempting to select universal wavelengths (WVs) across highly diverse products or technologies. This insight is particularly valuable in understanding the limitations and challenges in developing broadly applicable calibration models across different sample types and spectroscopic instruments.

Moreover, many algorithms (other than RF) can be explored for variable selection (Cripps et al. 2005; Kahrıman and Liland 2021; Westad and Marini 2022), and better results could be obtained.

Conclusions

Portable NIRS instruments are increasingly relevant in agriculture, especially in fibre analysis of forages and TMRs. Nevertheless, factories designed their instruments for general purposes, making them rarely cost-effective, especially for small or medium farms and applications requiring several spots of data acquisition. Feature (Wvs) reduction may be a strategy that can be adopted to reduce the production costs of portable or embedded NIRS. Even if the RPD values do not always reach a 'good' grade, considering the applicability and the readiness of the analysis produced by a handheld or installed low-cost instrument allowing a massive screening of feed and ration, 'fair' is a satisfying result. However, the greater capability of analysing a large part of sample variability results in interesting in-field applications. As a feature selection algorithm, the RF was successfully tested in a dataset comprising a variety of forages and TMRs, even from external validation, allowing affordable PLS regression for fibre quality but with limited results for ADL. The use of Pt moderately improved the PLS performances, while the Cb algorithm did not concretely raise the usability of the RF-selected features.

However, the final choice of usable Wvs must consider other issues, such as the possible engineerable chance of the futuristic instrument, balancing manufacturing costs, spectrum width, and calibration performances.

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Author contributions

Conceptualisation, L.S. and M.M.; methodology, L.S and M.M. software, L.S., M.M. and D.P.; validation, L.S., M.M.; formal analysis, L.S., M.M.; investigation, L.S.; resources, I.A.; data curation, L.S.; writing–original draft preparation, L.S., M.M. and D.P.; writing–review and editing, L.S., L.M., M.M., G.M., G.C., and F.R.; visualisation, G.M. and L. M.; supervision, I.A., G.C., and R.V.; project administration, G.M. All authors have read and agreed to the published version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The data presented in this study are available on request from the corresponding author. The data are not publicly available because they are part of the calibrations datasets used in the commercial activity of the Department of Animal Medicine, Production and Health.

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