NIR FORAGE ANALYSIS: GLOBAL NETWORKS AND LOCAL CALIBRATION STRATEGIES

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Abstract

The authors review the methodological background of NIR forage analysis, with particular attention to database structures and size, inconsistencies among chemical reference methods, and the need for harmonization. The advantages and limitations of NIR spectroscopy and classic 'wet' chemical analysis are critically compared. Applications of NIR technology across harvesting, preservation, and feeding processes, including in situ measurements, are presented. The paper emphasizes a comparative evaluation of globalized NIR laboratory networks versus locally developed, customized NIR databases, highlighting their respective strengths, limitations, and interdependencies. The benefits and risks associated with reliance on global NIR networks are discussed in contrast to local calibration development.Furthermore, the study examines the relevance of specific NIR parameters to key agricultural domains, including crop production, machinery operation, livestock management, feed formulation and veterinarian aspects. The role of global NIR laboratory networks in improving efficiency across forage production, harvesting, storage, and feeding is highlighted. Parameter sets and performance outcomes of selected global NIR networks are compared.

Keywords: NIRS, forage, NIR calibration, handheld NIR device

INTRODUCTION

Forage quality is central to ruminant nutrition and determines the energy, protein and structural carbohydrate supply of dairy cattle, beef herds and small ruminants (Van Soest, 1994; Mertens, 1997). Despite being both accurate and well-validated, traditional analytical techniques like Kjeldahl, Dumas, Soxhlet extraction and the van Soest fiber system are time-consuming, laborious and costly (Van Soest and Wine, 1967; Thompson *et al*., 2002). Because they take a long time to complete, they are not widely employed in the daily feeding management. Near infrared spectroscopy (NIRS) is a fast alternative for investigating forages and total mixed rations (TMR). A standard NIR scan can be performed in only seconds with minimal sample preparation and negligible consumable costs (Shenk and Westerhaus, 1991; Roberts *et al*., 2004). Due to the fact that NIRS assesses chemical composition in terms of broad absorbing characteristics of organic molecules, sophisticated chemometric calibration models are indispensable in order to attain an accuracy similar to reference techniques (Martens and Næs, 1989; Cozzolino, 2014). In the last decades, global collaborative efforts, centralized calibration centers and extensive spectral databases have positioned NIRS as a critical technology in contemporary forage laboratories and during on-farm decision making (Williams and Norris, 2001; Givens *et al*., 1997; Decruyenaere *et al*., 2009).

DIFFERENCES IN NEAR-INFRARED (NIR) SPECTROSCOPY

NIR spectroscopy (NIR) spectroscopy is a technique for the detection of near infrared (NIR) light by the absorption of molecular overtones and combinations mainly with O–H, C–H, and N–H bonds. These vibrations are the dominant chemical forms of water, carbohydrates, lipids and proteins in plant matter. Shenk and Westerhaus (1976) state that NIR based methods (with high correlation coefficient) are possible in prediction of dry matter, crude protein and fibre fractions in forages. It does not need much sample preparation, typically only drying and grinding, and returns results in seconds, not hours or days. The near infrared range (780-2500 nm) preserves CH, OH and NH bond overtones and combination bands (Pasquini, 2018; Workman and Weyer, 2012). Spectral regions of significant importance for forage analysis are:

- 1450 nm – water (OH overtone).
- 1730 nm – lipid CH combinations.
- 1940 nm – strong water absorption band.
- 2100–2300 nm – combinations protein and fiber composition (amide, cellulose, hemicellulose) (Osborne *et al*., 1993; Williams and Norris, 2001).

NIR absorption bands are broad and overlapping, difficult for direct interpretation when compared with mid infrared (Pasquini, 2018). Chemometric modeling provides the link between the spectra and the composition (Martens and Næs, 1989; Cozzolino, 2014).

NIR SPECTROSCOPY TECHNICAL BACKGROUND

Strategies for NIR Instrumentation

Near-infrared spectroscopy (NIRS) techniques have become an important analytical technique in forage quality assessment, with advances in instrumentation, as well as with the development of global calibration networks, (Williams and Norris, 2001; Roberts *et al*., 2004; Givens *et al*., 1997). There are four broad classifications of near-infrared spectroscopy (NIRS) instrumentation for forage analysis:

- Fourier-transform NIR (FT-NIR).
- Diode array systems.
- Monochromator instruments.
- Emerging MEMS-based portable NIR devices (Pasquini, 2018; Crocombe, 2018; Beć *et al*., 2020a).

All the methods differ in optical configuration, spectral acquisition and operational suitability, all of which are directly relevant in the application of the technology in today's feed laboratory systems and on farm applications (Workman and Weyer, 2012; Pasquini, 2018). The efficacy of an NIR technology depends not only on the type of spectrometer used (e.g., Fourier transform NIR, diode array, monochromator, etc.), but also on calibration strategy on the spectral interpretation. These two dimensions are increasingly becoming intertwined in current analytical networks. Types of Instruments and their Function; NIR instruments applied for the analysis of forage include three main types that have different functional functions in analytical systems (Table 1).

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FT-NIR instruments employ an interferometer for simultaneous acquisition of all wavelengths, which could also be transformed mathematically into spectrum (Workman and Weyer, 2012; Pasquini, 2018). This method offers good spectral resolution, better wavelength reproducibility, and better signal-to-noise ratios (Burns and Ciurczak, 2007; Workman and Weyer, 2012). As such, these are ideal for developing calibration models using chemically-validated datasets in reference labs (Williams and Norris, 2001; Roberts *et al*., 2004).

Their stability facilitates a relatively reliable transfer of calibration between instruments and physical locations, which is vital for analytical networks of wide scale (Shenk and Westerhaus, 1991; Martens and Næs, 1989). It is stable enough to be especially useful for transfer of calibration in international laboratory networks. In worldwide forage laboratory networks (e.g. Eurofins Agro), FT-NIR is frequently used, for instance, in reference or master laboratories for:

- Calibration model development.
- Compositional analysis for high precision.
- Definition of reference spectra databases (Williams, 2001; Roberts *et al*., 2004).

Diode array instruments use a fixed detector array to measure multiple wavelengths simultaneously (Workman and Weyer, 2012; Pasquini, 2018). The ideal of a diode array is rapid and portable systems that allow near-instantaneous acquisition of spectral information without the need for moving parts. They remain lightweight, rugged, and low-pressure, as there is no moving optical component (Crocombe, 2018; Beć *et al*., 2020a) and the main reason to choose a diode array instrument. These instruments are applicable in on-farm settings (e.g., ASD, Büchi, AgriNIR) where accuracy of critical parameters such as dry matter and crude protein need to be quickly estimated (Roberts *et al*., 2004; Williams and Norris, 2001).

Their performance is significantly reduced by the need to rely on externally developed calibration models derived from centralized databases (Shenk and Westerhaus, 1991; Martens and Næs, 1989). These instruments are generally employed in forage systems:

- On-farm (i.e. silage pits, TMR analysis).
- For rapid evaluation of dry matter and crude protein.
- In real-time decision support systems (Decruyenaere *et al*., 2009; Givens *et al*., 1997).

More and more organizations, including Dairy One and Rock River Laboratory, also implement handheld diode-based NIR equipment in advisory service. However, their reduced spectral resolution and sensitivity to calibration transfer means high levels of reliance on centralized calibration models are needed (Williams and Norris, 2001; Pasquini, 2018).

Monochromator-based instruments use diffraction gratings or filters to filter out wavelengths in an ordered sequence of time (Workman and Weyer, 2012; Pasquini, 2018). These sensors are intermediate and are a widely accepted compromise between performance, cost, and robustness (Williams and Norris, 2001; Roberts *et al*., 2004). They are often used for regular laboratory assays of the subject (e.g. FOSS, Bruker, Unity) with standardized protocols and moderate throughput requirements. It is the consistent use in regional laboratories that is testament to their reliability and compatibility with established calibration procedures (Shenk, 1991; Martens and Næs, 1989). They are widely used in conventional laboratory forage analysis; industrial feed testing laboratories; and in state-of-the-art quality control environments (Givens *et al*., 1997; Decruyenaere *et al*., 2009). Monochromator systems are the basic standard for commercial or industrial use of monochromator (as in case of CVAS) based laboratory networks, where the monochromator is used for the daily high-throughput analysis, because of the consistency provided by the calibration of monochromators, the reproducibility in the output signal, and their intermediate maintenance requirements (Williams and Norris, 2001; Roberts *et al*., 2004).

Integration within NIR Networks in the World: Modern forage analysis systems (e.g. Eurofins Agro, Dairy One, Rock River Laboratory, CVAS) do not depend on one tool type but rather on a hierarchical and complementary tool type (Different tool types are integrated under a common calibration model). In such systems:

- FT-NIR instruments are developed in central or master laboratories to produce high quality reference spectra and calibration models (development and validation for calibration)
- Monochromator instruments in regional laboratories conduct standardized, high-throughput routine analyses (routine, standardized analyses)
- Diode array instruments provide farm level analytical capability for real-time decision-making (real, near real time measurements) This architecture facilitates

Tab. 1: Technical background and comparison of different systems (NIR spectroscopy) Only examples, not complete lists (Williams and Norris, 2001; Burns and Ciurczak, 2007; Workman and Weyer, 2007; Griffiths and de Haseth, 2006; Fearn, 2001; Feudale *et al.*, 2002; Roberts *et al*., 2004; FAO, 2011)

Feature	FT-NIR	Diode Array	Monochromator
Role in network	Master/reference lab	Field/on-farm	Routine lab
Spectral resolution	High	Medium–low	Medium
Moving parts	Yes (interferometer)	No	Yes
Field use	Limited	Excellent	Moderate
Lab accuracy	Excellent	Moderate	Good
Measurement speed	Medium	Very fast	Medium
Robustness	Medium	High	Medium
Calibration transfer	Excellent	Moderate	Good
Typical users	Eurofins central labs	Dairy One, Rock River field tools	CVAS, regional labs
Main advantage	Accuracy and stability	Speed and portability	Reliability and balance
Cost	High	Low–medium	Medium
Main limitation	Cost, complexity	Lower precision	Moving parts

distribution of calibration models and guarantees analytical consistency at several sites (Shenk and Westerhaus, 1991; Williams and Norris, 2001).

Standardized protocols for sample preparation and spectral acquisition also help ensure comparability, as local laboratories are guided by procedures established by central labs (Martens and Næs, 1989; Roberts *et al*., 2004).

MEMS based NIR spectrometers (Micro Electro Mechanical Systems) are based on different principles than monochromators or diode array spectrometers but do include other aspects of both in a miniature-sized, integrated optical system (Crocombe, 2018; Beć *et al*., 2020a). The MEMS NIR instruments obtain spectra in some processes with a moving micro mirror, micro grating diffraction system, or micro-interferometer (Pasquini, 2018; Beć *et al*., 2020a). These small mechanical features (mirrors, gratings and lamellae) are actuated electrostatically or thermally depending on the structure of the system (Crocombe, 2018). The direction dictates what wavelength range the detector receives–so rather than a rotating or prism-based monochromator, the measurement is carried out by a vibrating MEMS element that dynamically determines wavelengths (Beć *et al*., 2020a; Crocombe, 2018). Main types:

1. **MEMS micro mirror** (DMD, Digital Micromirror Device concept): use the principle of determining wavelengths by tilting thousands of microscopic mirrors (e.g., those using TI DLP technology in multiple devices) in a differential manner.
2. **MEMS diffraction grating principle:** a micro grating pivoting mechanism tunes the wavelength of the reflected or transmitted light.
3. **MEMS Fabry–Perot interferometric principle:** an oscillating mirror pair alters the optical path length to selectively allow different wavelengths to reach the detector. Examples include Si Ware NeoSpectra System and Texas Instruments DLP NIRscan System.

New MEMS enabled portable NIR devices provide more analyses for on-farm decision making (Crocombe, 2018; Beć *et al*., 2020b). Most conventional inline NIR systems are now MEMS-based or contain MEMS elements, which are critical for industrial or agricultural machinery that need to be compact, vibration-resistant, and dust-proof (Pasquini, 2018; Crocombe, 2018). Feed mixer, unloading facers, and forage harvester mounted sensors enable continuous feedback during processing and feeding (Sørensen *et al*., 2021; Müller *et al*., 2019).

Inline NIR technology is specifically designed for continuous measurement between individual steps for solids, slurries, or feeds on conveyors, augers, or mixers (Workman & Weyer, 2012). Although a majority of modern sensors are MEMS based, their best analytical precision is mainly reserved by monochromator and diode array systems, especially in laboratories or industrial reference systems (Williams & Norris, 2001; Roberts *et al*., 2004).

- Classical inline NIR systems – Many of the classical inline NIR solutions like FOSS ProFoss, Bruker Matrix, Unity SpectraStar, etc., are monochromator or diode array optics. They are high-reliability, laboratory-grade systems (Williams & Norris, 2001; Workman & Weyer, 2012).
- Contemporary "compact inline" systems: MEMS-based inline sensors are developed recently, making these smaller, cheaper, and easier to incorporate into feed mixers, silage unloaders, and forage harvesters (Beć *et al*., 2020b; Crocombe, 2018). NeoSpectra Scanner / NeoSpectra Puck can be applied in silage facers and mixer wagons, as well as AgriNIR Inline systems that utilize MEMS or compact diode-array spectrometers (Crocombe, 2018; Sørensen *et al*., 2021).

The variety of NIR sensors determines spectral range (the range for MEMS instruments is typically from 1350 to 2500 nm), desired analytical accuracy, and environmental conditions (vibration, temperature, dust, moisture or feed residues) (Pasquini, 2018; Beć *et al*., 2020b).

The NIR market is progressing rapidly from laboratory to inline, and then to handheld "smart sensor" devices (Table 2).

- The AgriNIR and X NIR devices based on the diode array model are better suited with rapid acquisition, moderate resolution and robustness in the field; though lower precision than monochromator based devices, they are much smaller and applicable for use on farms. However, diode array systems, such as the AgriNIR are a compromise: they have higher spectral stability and wavelength range than MEMS devices, but are smaller and faster than larger monochromator based laboratory spectrometers.
- The HarvestLab 3000 is closest to a laboratory grade NIR instrument (as for example in FOSS or Bruker systems). It provides both a wider spectral range and very fine resolution: that is why it is frequently used as a reference grade inline analyzer.

Tab. 2: NIR instrumentation strategies in the case of some portable NIR devices. A limited list here, mainly examples (based on manufacturer specifications and scientific publications – Williams and Norris 2001; Burns and Ciurczak 2007; Workman and Weyer 2007; Roberts *et al*. 2004; FAO 2011; Dinamica Generale; ITPhotonics; John Deere)

Device	Manufacturer / Origin	Optical Principle / Technology	Notes / Typical Application
AgriNIR	Dinamica Generale (Italy)	Diode array spectrometer with 19 optical filters (SW NIR, 1150–2500 nm)	Used either as a portable or inline analyzer on farms and feed mixers; not MEMS based.
X NIR	Dinamica Generale (Italy)	Same diode-array principle as AgriNIR (identical optical platform) but in a handheld version	Portable field device for rapid forage analysis.
PoliSPEC NIR	ITPhotonics (Italy) / Caltech Instruments	MEMS-based spectrometer, likely Fabry–Perot interferometer type	Compact, IP67-rated instrument for on site TMR and plant sample analysis.
John Deere HarvestLab 3000	John Deere / Zeiss	Monochromator type spectrograph (optical grating based), covering ~1100–2500 nm	High-accuracy inline system mounted on forage harvesters, tankers, and tractors. Not MEMS but diffraction grating based.

- PoliSPEC NIR, the new MEMS based generation highlighting miniaturization, low energy consumption, and field mobility — albeit usually with a narrower spectral range and also a lower signal to noise ratio than full scale laboratory instruments.

Spectral Pre-Processing

To minimize the influence of light scattering, baseline drift, and particle size effects in near-infrared spectroscopy (NIRS), several standardized spectral transformations are routinely applied prior to chemometric modeling (Burns and Ciurczak, 2007). The most widely used methods include Standard Normal Variate (SNV) correction (Williams and Norris, 2001), which normalizes each spectrum to its own mean and variance to reduce multiplicative scatter effects; and Multiplicative Scatter Correction (MSC), which aligns individual spectra to a reference spectrum to correct additive and multiplicative differences across samples (Williams and Norris, 2001). Savitzky–Golay derivative filtering (Savitzky and Golay, 1964) is commonly used to enhance spectral resolution and remove baseline offsets by calculating first or second derivatives of the spectra. Detrending further eliminates slope variations and low-frequency baseline curvature, improving model robustness. Finally, wavelength selection algorithms—including interval Partial Least Squares (iPLS) (Nørgaard *et al*., 2000) and heuristic optimization techniques such as genetic algorithms—help identify the most informative wavelength regions, thereby reducing noise and computational load while retaining analytical sensitivity (Workman, and Weyer, 2007; Brereton, 2023; Rinnan *et al*, 2009).

Chemometric Modeling

The predominant modeling technique in forage and feed NIR analysis is Partial Least Squares (PLS) regression, which effectively reduces spectral dimensionality while establishing correlations between latent spectral variables and reference chemical data. Owing to its robustness against multicollinearity and high interpretability, PLS has long remained the benchmark method for routine calibration in spectroscopic applications (Martens and Næs, 1989; ge K. Smilde *et al*., 2004; Cozzolino, 2014).

In recent years, however, there has been a marked increase in the application of machine learning approaches capable of modeling nonlinear relationships between spectral features and compositional parameters. These include Artificial Neural Networks (ANN), Support Vector Regression (SVR), Random Forest Regression, and Gaussian Process Regression (GPR), which have demonstrated improved predictive performance, particularly for heterogeneous sample sets and complex feed matrices (Zhang *et al*., 2022; Roger *et al*., 2016). Such methods often outperform traditional PLS models in nonlinear contexts, including regions characterized by strong interactions between constituents (e.g., moisture–protein interactions) (Balabin and Smirnov, 2011; Bevilacqua and Marini, 2014).

Forage and Feed Samples Examined

A wide spectrum of forage and feed types has been evaluated in NIRS calibration studies.

- Typical materials include corn silage, grass silage, and alfalfa silage, whole crop cereal silages cut in different growing stages (boot stage, in heading, milky-dough stage), whole crop cereal-legume blends, as well as alfalfa and meadow hays derived from temperate climates. In addition, fresh forages such as ryegrass, clover, early cut whole crop cereals are frequently analyzed.
- Industrial by-products—such as distillers dried grains with solubles (DDGS), sugar beet pulp, brewery grains, corn gluten feed, soybean hulls—are also included to expand calibration range and enhance model generalizability across diverse feed matrices.
- Cereal grains (corn, wheat, barley, oat, rye), full fat seeds (soya, rapeseed, sunflower), extracted meals (soybean meal, sunflower meal, rapeseed meal) and mixed concentrates are frequently analyzed.
- Complex blends like total mixed rations (TMRs). However, the accuracy and reliability of the mixture results raise some concerns. In this case, the size of the calibration database plays a critical role.

These global NIR-networks generally -besides forage samples- have databases for water, soil, milk and manure samples, also.

Chemical and In Vitro Reference Methods

Reference laboratory analyses underpin all NIR calibration models and are typically conducted using standardized wet chemistry protocols:

- Gravimetric oven drying at 70°C and 105 °C to determine dry matter content.
- Kjeldahl or Dumas methods for crude protein determination; On average the Dumas method provided results that were relatively higher by about 1.4% than the Kjeldahl method, but the difference between the methods depended on the type of feedstuff.
- Soxhlet ether extraction for crude fat quantification;
- Van Soest procedures for neutral detergent fibre (NDF and aNDFom), acid detergent fibre (ADF), and acid detergent lignin (ADL). Original publication for NDF, ADF and ADL: Van Soest and Wine, (1967)
- Assays include enzymatic starch analysis (fotometric method).

However, **reference methods are not standardized**. When NIR results are questioned, it is often the case that differences in the reference methods are responsible for discrepancies between chemical analytical results and NIR outcomes, as well as for variations among results obtained by different NIR laboratories. The **worldwide harmonization of reference methods would significantly enhance comparability**, transparency, and the reliability of results.

For crude protein determination, both the Kjeldahl and the Dumas methods are in use (While *et al*.,1998; Thompson *et al*., 2002). Even within the Kjeldahl method, procedural variations exist.

- Crude protein without NH_3-N: This is the result of the Kjeldahl method where all the NH_3-N has been substracted. This crude protein is used in the Dutch calculation of VEM (Net Energy for Dairy Cows).
- Crude protein with NH_3-N after pre-drying: This is crude protein + the amount of N which remains in the material, after the first drying process (at 60 degrees). This value is used in the German and Danish energy and protein calculation.
- Crude protein total: First the NH_3-N is measured in the sample before it is dried. Afterwards this NH_3-N can be re-calculated and added as protein to the crude protein value. This value is used in the Dutch calculation of DVE (Digestible Protein in the Small Intestine) and OEB (Degraded Protein Balance - rumen nitrogen balance).

Furthermore, there are new protein parameters required by the modern softwares using CNCPS-model: e.g. adjusted protein (Crude Protein – ADICP), ADICP (Acid Detergent Insoluble Crude Protein), NDICP (Neutral Detergent Insoluble Crude Protein), NDRCP (Neutral Detergent Residue Crude Protein).

The crude fiber value is no longer reported in modern analytical reports. Moreover, further harmonization is required for fiber analysis, especially in forage/TMRs/PMRs containing starch. The aNDFom (amylase-treated, organic matter corrected neutral detergent fiber) would be preferable by the AOAC (AOAC Official Method 2002.04; Mertens, 2002) compared to the original NDF method (Van Soest and Wine, 1967). Some laboratories, such as Cumberland Valley Analytical Services (CVAS) report aNDF and NDFom values for corn silage and use a new parameter: the NDR (neutral detergent residue). Dairyland Laboratories Inc. provide both aNDF and aNDFom values for corn silage and TMR/PMR samples. Moreover, there are other technical alternatives (ANKOM or Fibertec methods) for fiber fraction analyses (Hall and Mertens, 2023). Modern softwares using CNCPS-model requires soluble fiber content, also. The physial structure of the NDF (peNDF) can be determind by the Ro-Tap Sieve Shaker and method (Mertens, 1997) applied both classic chemistry or NIR-technology. The concept of physically effective undigested NDF (peuNDF), combining uNDF and physical effectiveness, has been discussed in applied dairy nutrition, particularly in the work of Grant and colleagues, as a new parameter developed by the Miner Institute (Grant *et al.* 2020).

The determination of starch is often still carried out polarimetrically in many European countries, whereas the AOAC method now recommends enzymatic-photometric starch quantification (AOAC Official Method 2014.10 in AOAC INTER NATIONAL, 22nd Edition, 2023). This enzymatic-photometric method is recognized as an AOAC and AACC harmonized approach for starch measurement in feeds and foods. Especially in wet by-products, total mixed rations (TMRs) and partial mixed rations (PMRs) containing viscous, gel-forming substances, part of the sugar fraction is mistakenly measured as starch in polarimetric assays. In contrast, such interference is eliminated in photometric methods.

The determination of total sugars is also critical, as multiple analytical protocols are in use. The WSC (water-soluble carbohydrates) method employs water for extraction, whereas the ESC (ethanol-soluble carbohydrates) method. Both Dairyland Laboratories Inc. and CVAS report results derived from each method. There are other differences, also. The Luff–Schoorl titrimetric method, uses an alcohol-based extraction. It is a cheap, robust but slow method and give total sugar content. The DNS method (3,5-dinitrosalicil acid) quick and has lower cost giving total sugar concentration. The HPLC method advantages: high specificity, simultaneous determination of multiple components and high accuracy, but expensive instrumentation needed, sample preparation required and requires technical expertise. (AOAC Official Method 923.09 titrimetric method; AOAC Official Method 945.66 titrimetric method; AOAC Official Method 982.14 HPLC method; AOAC Official Method 977.20 HPLC method; AOAC Official Method 986.25 enzymatic method)

When measuring fat content, the question arises as to whether hydrolytic pre-treatment is applied universally to all samples or solely to those containing protected fat sources (TMRs/PMRs), as practices differ among laboratories. For example, Eurofins Agro provide total fat content, independently of the sample type. Hydrolytic pretreatment is discussed in AOAC 2003.05, indicating it's required when samples contain protected or bound fats. AOAC Official Metho d 920.39 / 922.06 / 2003.05:

- 920.39: *Crude Fat in Feed – Ether Extraction (Soxhlet).*
- 2003.05: *Total Fat in Foods – Hydrolytic Pre Extraction and Solvent Extraction.*

Wet chemical methods are not capable of determining ruminal degradability, degradation rates, or the proportion of undegradable fractions of protein, starch, or fiber fractions. For this purpose, NIR laboratories require *in vitro* reference databases. As one of the key determinants of competitiveness for NIR laboratories is their ability to provide input data for software based on the CNCPS model, such *in vitro* databases have been extensively developed over the past decade. However, methodological inconsistencies remain. In the United States, 30-hour NDF degradability has been widely used, whereas in Europe the 48-hour value has become standard. The National Academies of Sciences, Engineering, and Medicine recommended in 2021 that the 48-hour *in vitro* degradability should be adopted as the standard, as it shows a closer correlation with *in vivo* data. Unfortunately, this implies that many NIR laboratories in the United States still report 30-hour values, while robust background databases for 48-hour values have not yet been fully established. This creates challenges for European users.

Parameters and parameter packages

As the large number of parameters may be confusing for users, the common international practice is for laboratories to provide pre-defined **data packages tailored to specific forage types**. This user-friendly approach supports non-expert livestock producers in their daily decision-making. However, these data packages are not always sufficiently flexible; therefore, many laboratories also offer optional parameters that can be selected for specific or specialized applications.

The large number of parameters reported in NIR result sheets can be confusing, and even experts often express negative opinions about them. However, it is important to recognize that these data can be categorized into distinct groups (Table 3).

Certain groups of parameters support or evaluate **crop production practices**. Other parameter-groups provide information on the **technical aspects of harvesting**, enabling improved machine settings in subsequent seasons. A third major group is relevant for **livestock managers**, who need to assess the quality, feed value, and potential risks associated with a given forage. A fourth group, which is often less intuitive for professionals with a classical background, consists of **input data for software applications**. Many of these programs are now based on dynamic models, which account not only for quantitative balances but also for temporal synchronization. Finally, there is a group of parameters that can assist **veterinarians** in determining whether observed production depression or herd-level health issues are related to nutritional factors or are caused by other problems, such as management deficiencies, bacterial or viral infections, or parasitic infestations.

We present several examples illustrating which parameters provide relevant information for different divisions within a specialized large-scale farm, and from which perspective this information is important. In smaller farms, such a level of differentiation is not feasible, as the farmer typically performs crop production, harvesting, preservation, and dairy production tasks alone. Nevertheless, even in such cases, it can be informative to distinguish which parameters are primarily relevant for the nutritionist and which are of greater importance to the veterinarian.

Tab. 3: The data presented in the NIR-report are categorized according to the target division and their intended use. Not a complete list, only examples (own compilation)

Crop manager	Crude protein (fertilization, growth stage), nitrate content (fertilization technology: dose, application, timing), NDF, ADF, ADL (growth stage at harvest), Fiber digestibility (NDFd30 or 48: growth stage at harvest), Starch content and digestibility (growth stage at harvest), RFV (relative feed value), RFQ (relative forage quality), Milk kg per ton silage, Milk kg per ha, Beef kg per ton silage
Mechanical specialist	DM (wilting technology), Ash (soil contamination), peNDF (chop size), CSPS (seed cracking efficiency of the harvestor), Fermentation profile: lactic acid, acetic acid, LA/AA ratio, propionic acid, butyric acid, Ammonia and pH (compaction and sealing technology).
Livestock managers	DM (dry matter intake, effluent, face management), Crude protein (milk production), nitrate content (reproductive performance, animal health risk), NDF (fiber supply, milk fat and acidosis risk), peNDF (acidosis risk), Starch (energy content, ketosis and acidosis risk), Total sugar (rumen supply and silage aerobic stability, face management), NFC, NSC (ketosis and acidosis risk), Fermentation profile: lactic acid, acetic acid, LA/AA ratio, propionic acid, butyric acid, ammonia and pH (as silage anaerobic stability and animal health risk)
Diet formulation (input data)	NDFd $_{12,30,48,120,240}$ (*kd*, dry matter intake, CNCPS), dNDF$_{48}$ (milk fat content, CNCPS), uNDF$_{240}$ (dry matter intake, CNCPS), ADICP, NDICP, adjusted protein (available protein for milk production, CNCPS), starch content and digestibility (rumen supply and CNCPS), total sugar (rumen supply and CNCPS), NFC, NSC (energy supply, ketosis and acidosis risk), NDF dig. rate (CNCPS), Starch dig. rate (CNCPS), Crude fat, fatty acids, unsaturated fatty acids (rumen health, milk fat, reproductive performance), Amino acids (nitrogen utilisation efficiency)
Vet. aspects	NDF (acidosis risk), peNDF (acidosis risk), Starch, NFC, NSC (ketosis and acidosis risk), Fermentation profile: lactic acid, acetic acid, LA/AA ratio, propionic acid, butyric acid, ammonia and pH (rumen and animal health risk)

Examples are presented of parameters that are currently reported in NIR reports for forages by some of the major NIR laboratory networks (Table 4).

Tab. 4: Parameters reported in NIR reports for forages by some of the major NIR laboratory networks (parameters listed were compiled from technical documentation of CVAS, Dairyland Laboratories, Eurofins Agro, Rock River Laboratory, Dairy One).

Parameter	CVAS	Dairyland Labs	Eurofins Agro	Rock River Lab	Dairy One
Dry Matter (DM)	✓	✓	✓	✓	✓
Crude Protein (CP)	✓	✓	✓	✓	✓
ADICP, NDICP	✓	✓	✓	✓	✓
NDRCP	✓	✓	–	✓	–
RDP / RUP	✓	✓	✓	✓	✓
Soluble Protein	✓	✓	✓	✓	✓
Amino Acid, total	✓	✓	–	✓	✓
Neutral Detergent Fiber (NDF)	✓	✓	✓	✓	✓
aNDFom (ash-corrected NDF)	✓	✓	✓	✓	✓
NDF Digestibility (NDFd 12,24,30,72,120,240)	✓	✓	✓	✓	✓
NDF Digestibility (NDFd48)	–	–	✓	–	–
uNDF240 (undigestible NDF)	✓	✓	✓	✓	✓
Acid Detergent Fiber (ADF)	✓	✓	✓	✓	✓
Acid Detergent Lignin (ADL)	✓	✓	✓	✓	✓

Parameter	CVAS	Dairyland Labs	Eurofins Agro	Rock River Lab	Dairy One
Starch	✓	✓	✓	✓	✓
Sugar (WSC)	✓	✓	–		✓
Sugar (ESC)	✓	✓	✓	✓	
Crude Fat (EE)	✓	✓	✓	✓	✓
Fatty acids	✓	✓	–	✓	optional
Ash	✓	✓	✓	✓	✓
NEL (Net Energy for Lactation)	✓	✓	✓	✓	✓
ME (Metabolizable Energy)	✓	✓	✓	✓	✓
TDN (Total Digestible Nutrients)	✓	✓	✓	✓	✓
RFV (Relative Feed Value)	✓	✓	✓	✓	✓
RFQ (Relative Forage Quality)	✓	✓	✓	✓	✓
DCAD	✓	✓	✓	✓	✓
NFC (Non-Fiber Carbohydrates)	✓	✓	✓	✓	✓
pH (silage)	✓	✓	✓	✓	✓
Fermentation acids (lactic, acetic, butyric)	✓	✓	✓	✓	✓
Ammonia-N (% of CP)	✓	✓	✓	✓	✓
CNCPS carbohydrate pool	–	–	✓	–	–
CNCPS protein pool	–	–	✓	–	–
French dataset (PDIA, PDIN. PDIE, UFL)	–	–	✓	–	–
German dataset (nXP, RNB)	–	–	✓	–	–
Dutch dataset (DVE, VEM, VEVI, OEB, NELVC)	–	–	✓	–	–

A key limitation of U.S.-based laboratory networks in Europe is that they primarily provide data aligned with American feeding systems, most notably the parameters required for the CNCPS model (e.g., AMTS profiles). As a result, values corresponding to European feed evaluation systems—such as the French, German, Dutch, or Nordic models—are typically not available. Consequently, users relying on French, German, or other region-specific ration formulation software cannot fully utilize the outputs of these laboratories.

From this perspective, the Dutch central laboratory of Eurofins Agro is more competitive, as it incorporates equations from local feed evaluation systems and provides results adapted to regional requirements, including the use of local languages.

Table 5. presents the correlation of parameters measured by different NIR-laboratory networks, compared to reference datasets (obtained from 'wet' chemical analytical methods) and NIR prediction accuracy.

Tab. 5: NIR prediction accuracy for forage analysis of different NIR laboratories. Based on technical reports of Dairy One, Rock River Laboratory, Eurofins Agro, CVAS, Dairyland Laboratories and scientific references include Shenk and Westerhaus (1991), Williams (2001), Deaville and Flinn (2000), Roberts *et al.* (2004), Williams and Norris (2001)

Parameter	CVAS	Dairyland Labs	Eurofins Agro	Rock River Lab	Dairy One	Typical Range
Dry Matter	R^2: 0.90–0.98 RMSE: 1.0–2.5 %	0.92–0.98 / 1.0–2.0	0.90–0.97 / 1.2–2.5	0.92–0.98 / 1.0–2.0	0.90–0.97 / 1.2–2.5	0.90–0.99 / 0.8–3.0
Crude Protein	R^2: 0.92–0.99 RMSE: 0.5–1.5 %	0.94–0.99 / 0.4–1.2	0.92–0.98 / 0.5–1.5	0.94–0.99 / 0.4–1.2	0.92–0.98 / 0.5–1.5	0.93–0.99 / 0.3–1.5
NDF	R^2: 0.88–0.97 RMSE: 1.5–3.5 %	0.90–0.97 / 1.2–3.0	0.88–0.96 / 1.5–3.5	0.90–0.97 / 1.2–3.0	0.88–0.96 / 1.5–3.5	0.88–0.98 / 1.0–4.0
ADF	R^2: 0.85–0.95 RMSE: 1.5–3.0 %	0.88–0.96 / 1.2–2.5	0.85–0.94 / 1.5–3.0	0.88–0.96 / 1.2–2.5	0.85–0.94 / 1.5–3.0	0.85–0.97 / 1.0–3.5
NDFd	R^2: 0.70–0.90 RMSE: 2.5–5.0 %	0.75–0.92 / 2.0–4.5	0.70–0.88 / 3.0–5.0	0.75–0.92 / 2.0–4.5	0.70–0.88 / 3.0–5.0	0.70–0.93 / 2.0–6.0

Calibration and Validation: Model Performance Metrics

Model quality in chemometric applications is typically evaluated using standard statistical indicators, most notably the coefficient of determination (R^2) and several root mean square error metrics, including RMSEC (calibration), RMSECV (cross-validation), and RMSEP (prediction). These parameters are widely used to assess both model fit and predictive performance in spectroscopic calibration studies (Rocha *et al*., 2020; Sadergaski *et al*., 2022).

Lower values of RMSEC, RMSECV, and RMSEP indicate improved model accuracy and robustness, while their comparison enables the detection of overfitting and generalization capability (De Bleye *et al*., 2012).

In addition to these metrics, the Residual Predictive Deviation (RPD), defined as the ratio between the standard deviation of reference data and the prediction error, serves as an integrative indicator of model applicability and predictive usefulness (Kamruzzaman *et al*., 2022). High RPD values are generally associated with models suitable for screening, quality control, or precise quantitative prediction, depending on threshold ranges reported in the literature.

Interpretative thresholds for RPD are widely accepted as follows:

- → RPD > 3.0 – Excellent analytical accuracy suitable for quantitative use.
- → RPD = 2.0 – 3.0 – Good screening ability with acceptable precision.
- → RPD < 2.0 – Limited practical utility.

A highly reliable and industry-grade calibration model typically achieves an RPD ≥ 3.0, indicating strong predictive performance and robustness across varying forage types and measurement conditions.

COMPARISON OF NIR SPECTROSCOPY AND CONVENTIONAL CHEMICAL ANALYSIS FOR FORAGE EVALUATION

Forage quality is a cornerstone of ruminant nutrition, determining the energy, protein and structural carbohydrate supply available to dairy cattle, beef herds and small ruminants. Classical analytical methods such as Kjeldahl, Dumas, Soxhlet extraction and the van Soest fiber system are accurate and widely validated, yet they are labor intensive, time consuming and expensive. Their turnaround time limits their widespread use in daily feeding management.

Near-infrared spectroscopy (NIR) has become an important analytical tool in forage quality assessment due to its speed, non-destructive nature, and ability to support real-time decision-making in livestock nutrition. In contrast, conventional chemical analyses—such as Kjeldahl nitrogen determination, Van Soest fibre fractionation, and solvent-based fat extraction—offer high accuracy but at the cost of time, labor, and chemical use. Recent systematic review highlights that NIR can achieve comparable predictive performance for key nutritive traits, especially when robust calibration models are available.

The quantification of forage quality is fundamental for ruminant nutrition, feed preparation and accurate animal management. Chemical analysis methods have traditionally been the gold standard in nutritional laboratories that are applied to crude protein (Kjeldahl), acid and neutral detergent fibre (ADF/NDF) analysis by the Van Soest system, and Soxhlet extraction for ether extract. But, they are time-consuming and labor-intensive methods that need hazardous reagents regularly. Over the last 30 years, near-infrared spectroscopy (NIR) has been developed as a fast non-destructive approach for determining nutritive value in silages, hays, and TMR. Among the advantages of NIR over classical chemical assays is its non-destructive characteristic and lack of chemical reactants, thus significantly decreasing its operational cost and environmental impact (Fodor *et al*., 2024), which is the primary reason for its implementation. In addition to that, high-throughput analysis of NIR also exist and can be performed in both lab and field applications with low-cost portable spectrometer portable measurements (Alomar *et al*., 2021). Such flexibility is particularly relevant for precision feeding programs that need real-time detection on forage quality to dynamically adapt rations.

Although much slower than pure NIR, chemical methods remain essential as reference procedures. However, their accuracy for the measurement of distinct compounds (e.g. crude protein or lignin) continues to be much greater than that of NIR for poorly absorbing or trace compounds (Chen *et al*., 2013). For instance, mineral elements or secondary metabolites produce weak NIR signals, so measurement is only possible by wet chemistry. In addition for formal feed assessment systems and regulatory laboratories, standard systems adopted by AOAC or ISO for laboratory verification and inter-lab reproducibility are also required.

The data of the NIR spectrum have many limitations including the reliance on calibrated database. Sample diversity, species composition, growing conditions and storage history are all important variables that can affect the robustness of predictive models (Hossain *et al*., 2024). In the absence of representative calibration sets, the outlier samples exhibit drastically lower predictive accuracy. Furthermore, instrument variability—variation in detectors, light sources, and path lengths—requires model transfer procedures or recalibration (Maduro Dias *et al*., 2024). Therefore, NIR, despite its statistical capabilities, is not quantitative-based in principle, its value comes from pre-known relationships to chemical reference data.

Practically, the incorporation of NIR spectroscopy into intelligent farming systems is a quantum leap of progress. Studies in feed laboratories and devices, estimate well dry matter/ crude protein composition of whole crop corn, corn silage and TMR mixtures, which can make immediate management decisions in the field. These on-farm adoption provide economic benefits via immediate forage allocation, decreased waste disposal and enhanced herd nutrition.

Chemical analysis, in contrast, has specialist workers and lab facilities. In addition, sample prep consists of digestion, filtration and reactions in multiple analytical steps, each with individual human errors and cumulative uncertainty. The average batch analysis time for one sample of forage might be 24-72 hours, depending on the parameters. By comparison, with sufficient calibration, NIR can work at times of tens of applications/hour to monitor feed inventories (De Boever *et al*., 2018). Thus, many recently developed food industry research programs are a combination of the techniques. (1) the primary reference information, based on scientific chemical analyses, and, (2) feed (continuous) operational feedback from NIR, such that operational activities are more or less continuous along production chains.

Note that contemporary ration formulation software needs specific quantities of inputs that cannot be easily acquired using common chemical analytical techniques. Some of such systems (among other applications) require the observation of fiber degradability parameters at multiple time points (degradability of NDF at 12, 24, 30, 48, 120, and 240 h *in vitro* incubation, degradation rate (kd in %/h), concentrations of degradable and un-degradable fiber fractions (dNDF48

and uNDF240, g/kg DM), starch degradability (7 h *in vitro* degradability) and soluble/rumen-degradable protein (RDP). Competitiveness of privately owned NIR laboratories generally rely on their customers for obtaining compatible data with their clients' software. Through the proliferation of dynamic rumen systems, this competition has prompted swift advancements in *in vitro* parameters following the development of dynamic rumen models and this pace cannot be achieved by methods based on classical analytical methods. Due to this, the current chemical analytical laboratories in the field are losing competitiveness as a result.

Despite development in technologies, NIR should not be treated as an absolute substitution for wet chemistry. It works rather as a complementary method for standard, nondestructive, statistically derived quality control to act. (Ferré and Garrido, 2022), the reliability of NIR assessment increases significantly where appropriate sampling design, recalibration on a periodic basis, and chemometric verification techniques, including partial least squares regression (PLSR), and cross-validation, are applied. Under these requirements, NIR allows the rapid translation of laboratory data to real-time nutritional insights. It should rely on analytic objective whether they satisfy regulatory targets or are efficient in operations. Continuous improvement of machine learning, spectral and sensor standardization and small-sensor miniaturisation is anticipated to bridge performance disparity further and NIR spectroscopy will be the predominant tool in sustainable forage quality of management and precise feeding to livestock in the next 10 years.

A summary Table 6 illustrates the main differences between the two systems in terms of their respective strengths and weaknesses, summary Table 7 presents the differences between the NIR spectroscopy and 'wet' chemical analysis based on various criteria.

Tab. 6: The key differences between NIR spectroscopy and conventional chemical Analysis in terms of their advantages and disadvantages (based on Shenk and Westerhaus, 1991; Givens *et al.*, 1997; Deaville and Flinn, 2000; Williams and Norris, 2001; Roberts *et al*., 2004; Van Soest *et al.*, 1991; AOAC, 2019; Sniffen *et al*., 1992)

NIR Spectroscopy	
Advantages	**Limitations**
1. Rapid and Non-Destructive Measurement NIR allows for the rapid assessment of dry matter, pro-tein, fiber, and other forage constituents without sample destruction, reducing time from hours to seconds	**1. Dependence on Calibration Quality** NIR accuracy de-pends heavily on representative calibration databases; for-age diversity in plant species, harvest maturity, and ensiling conditions can reduce predictive accuracy
2. No Hazardous Chemicals Unlike traditional methods (e.g., Kjeldahl or Soxhlet), NIR avoids aggressive reagents such as sulfuric acid or organic solvents, reducing cost, environmental impact, and safety risks	**2. Lower Sensitivity to Minor Components** Trace elements, minerals, and compounds with weak NIR absorption often cannot be detected with sufficient precision, necessitating traditional chemical methods
3. Suitability for On-Farm and Real-Time Applications Portable instruments enable in-situ forage eval-uation, supporting precision feeding strategies and enhancing reactivity to variability in silage and TMR quality	**3. Instrument and Model Transfer Issues** Differences be-tween devices, optical setups, or measurement geometries require careful standardization or transfer algorithms for consistent results
4. High Throughput and Low Operating Cost Once calibration models are established, NIR provides near-zero marginal cost per sample, enabling large dataset generation.	
5. Digestibility data for fiber fractions, starch, and protein (based on an *in vitro* reference dataset) can be used as input for software implementing dynamic rumen models. CNCPS data are available.	
Conventional Chemical Analysis	
Advantages	**Limitations**
1. High Analytical Accuracy and Sensitivity Wet chemistry provides reference-level quantification for protein, fibre fractions, minerals, and secondary metabolites. These measurements form the founda-tion for NIR calibration itself.	**1. Time- and Labor-Intensive Processes** Protein, fibre, and lipid assays may require hours to days, limiting throughput.
2. Universality Across Sample Types Unlike NIR, chemical methods require no calibration models and remain robust across highly diverse forage matrices.	**2. Use of Hazardous Chemicals** Procedures rely on strong acids, bases, and solvents, creating waste and environmen-tal burdens
3. Regulatory and Standardised Methods Many wet chemistry methods (e.g., AOAC) are legally required for feed evaluation and food safety assays.	**3. Higher Cost per Sample** Reagents, labor, and waste management lead to significantly higher unit costs relative to NIR.
	4.Digestibility and CNCPS can not be given. It is not capable of supporting modern feed ration formulation software based on dynamic rumen models and is therefore not com-petitive.

Tab. 7: Differences between the NIR spectroscopy and 'wet' chemical analysis based on various criteria. (based on Shenk and Westerhaus, 1991; Givens *et al.*, 1997; Deaville and Flinn, 2000; Williams and Norris, 2001; Roberts *et al*., 2004; Van Soest *et al*., 1991; AOAC, 2019; Sniffen *et al*., 1992)

Criterion	NIR Spectroscopy	Chemical Analysis
Speed	Seconds	Hours–days
Sample preparation	Minimal	Extensive
Chemical use	None	High
Accuracy	High (with calibration)	Very high
Trace components	Limited	Strong
Cost per sample	Very low	High
On-farm usability	Excellent	Poor
Digestibility data (fiber fractions, starch, and protein)	Yes	No
Supporting modern formulation softwares based on CNCPS model	Yes	No
Flexibility	Strengths	Limitations
Market sensitivity and competitiveness	Strengths	Limitations

Corn silage samples comparison

It aims to analyze the variability between conventional chemical analysis and NIR-based predictions for different laboratory networks. The results of the analysis are presented in Table 8. Ten corn silage samples were collected for comparison purposes. The samples were obtained through standard forage extraction and preparation protocols. The samples were dried at 70 °C to constant weight and then ground to pass through a 1 mm sieve. The samples were also homogenized to reflect uniformity following the grinding process. Every such homogenized sample had four representative subsamples. One subsample was subjected to standard wet chemical analysis and the rest of the subsamples were sent to three NIR laboratory networks for laboratory analyses – Cumberland Valley Analytical Services (CVAS), Dairyland Laboratories (Dairyland Laboratories), and Eurofins Agro (Eurofins Agro). All laboratories performed analyses of the samples based on their normal models of calibration with near-infrared spectroscopy (NIR) and as per standard operating procedures. Standard chemical analysis was also performed using reference methods compatible with the forage study.

Data analysis (statistical methods and laboratories) analyzed differences in analysis across laboratories and agreement between laboratories was obtained. We calculated descriptive statistics (mean, standard deviation) for all parameters. Differences among experimental settings were analyzed through one-way analysis of variance (ANOVA). Post-hoc comparisons were conducted to determine the pairwise difference between laboratories when significant ($p < 0.05$) effects were observed. The prediction accuracy of NIR results was assessed in relation to traditional chemical analysis based on coefficient of determination and root mean square error.

$$R^2 = 1 - \frac{\sum (y_i - \hat{y}_i)^2}{\sum (y_i - \bar{y})^2} \quad (1)$$

$$RMSE = \sqrt{\frac{1}{n} \sum (y_i - \hat{y}_i)^2} \quad (2)$$

where y_i represents the reference (wet chemistry) values, $\hat{y}_i$ the NIR predicted values, and $\bar{y}$ the mean of reference values.

All statistical studies were performed at $\alpha = 0.05$. The results of the systematic differences among the laboratories and the predictive performance of NIR spectroscopy over traditional analytical analyses were examined for statistical methods (Table 9).

Starch: One-way ANOVA group differences were significant with each other ($F = 6.05$, $p = 0.0019$). Post hoc Tukey analysis showed that NIR2 was significantly different from all other groups, but no difference was found among wet chemical data, NIR1, and NIR3. R^2 values proved the good consensus between wet chemical and NIR1 ($R^2 = 0.66$), between wet chemical and NIR3 ($R^2 = 0.68$), but poor consensus for NIR2 ($R^2 = -0.73$),

Tab. 8: Comparative analysis of starch- and ADF content in corn silage samples (n = 10)

	Starch			ADF		
	Mean	**Std**	**CV%**	**Mean**	**Std**	**CV%**
Wet chemistry	345,3	46,9	13,6	220,6	24,3	11,0
NIR1	331,7	34,4	10,4	236,2	21,0	8,9
NIR2	401,8	38,9	9,7	218,2	22,3	10,2
NIR3	355,4	35,0	9,9	218,1	25,4	11,6
Mean	**358,6**	**–**	**–**	**223,3**	**–**	**–**
Std	**30,4**	**–**	**–**	**8,7**	**–**	**–**
CV%	**8,5**	**–**	**–**	**3,9**	**–**	**–**

Tab. 9: Statistical analysis of corn silage samples (n = 10) and complete validation table (R^2, RMSE, bias, RPD) compared to wet chemical data

	Starch				ADF			
Modell	**R^2**	**RMSE**	**Bias**	**RPD**	**R^2**	**RMSE**	**Bias**	**RPD**
NIR1	0.662	25.87	−13.6	1.81	0.468	16.80	+15.6	1.45
NIR2	−0.735	58.60	+56.5	0.80	0.913	6.79	-2.3	3.58
NIR3	0.685	24.99	+10.1	1.88	0.927	6.20	-2.5	3.91

which indicated a systematic difference. Root mean square error (RMSE) analysis clearly confirmed the good agreement observed within wet chemistry and NIR1 (RMSE = 25.9) and between wet chemistry and NIR3 (RMSE = 25.0), while NIR2 showed significantly greater deviation (RMSE = 58.6), showing poor agreement and systematic differences. The residual predictive deviation (RPD) values revealed limited predictive performance of the NIR models, (NIR3 (RPD = 1.88) and NIR1 (RPD = 1.81) were relatively moderate in their suitability for screening purposes; NIR2 (RPD = 0.80) exhibited a poor prediction capability). Systematic biases indicated systematic underestimation and overestimation, with NIR1 (–13.6) vs NIR3 (+10.1), but NIR2 displayed strong positive bias (+56.5), which suggested systematic deviation from the models.

ADF: No significant differences were found for ADF values across laboratories (ANOVA: F = 1.39, p = 0.262). While mean scores of NIR1 were slightly higher, the variation within groups was comparable, revealing comparable performance from laboratory to laboratory and with even distribution across laboratories. Validation Results: The validation showed great prediction performance for NIR2 (R^2 = 0.91, RMSE = 6.79, RPD = 3.58) and NIR3 (R^2 = 0.93, RMSE = 6.20, RPD = 3.91) demonstrating the highest accuracy and high stability. The models presented less of a bias which proved appropriate for quantitative applications. In contrast, NIR1 exhibited only limited predictive power (R^2 = 0.47, RMSE = 16.8, RPD = 1.45) and a strong positive bias, limiting its application to the rough screening for all features.

GLOBAL NIR LABORATORY NETWORKS

Modern global NIR systems operate as multi tiered analytical infrastructures integrating central, regional, and local facilities into a unified data ecosystem. At the core of such systems are central “master” laboratories, which maintain reference instruments and serve as custodians of calibration integrity and spectral standardization. These laboratories generate and validate the master calibration models used for routine forage and feed analysis worldwide. Surrounding this center, regional NIR laboratories function as intermediate nodes, applying and adapting the master calibrations to the specific feed materials and environmental conditions of distinct geographic regions.

At the operational level, on farm and portable NIR instruments extend analytical capacity directly to the production environment. These field deployable spectrometers collect real time spectra of silages, hay, or total mixed rations, and their data are synchronized with the central network through cloud based calibration databases. Within these databases, global spectral libraries are continuously updated, incorporating new sample data and verified reference analyses.

The integration of automated calibration updates and bias correction algorithms ensures that instruments remain harmonized despite differences in hardware, optical configuration, or climate induced sample variability. It is also a common approach that the infrastructure—from grinding to spectral acquisition—and the sample preparation methods are fully standardized, meaning that the local (satellite) laboratories follow a uniform protocol defined and prescribed by the central laboratory. Through these mechanisms, networks such as INGOT, EVEREST, or the International Forage Evaluation Consortium provide globally standardized calibrations, enabling reliable cross laboratory comparability. In the field of forage analysis, global networks are being established by the companies below:

Eurofins Agro network (BLGG 2013): reference forage laboratory is in Wageningen (The Netherlands). Eurofins Agro is presented in Belgium, Bulgaria, Chile, Canada, China, Colombia, Denmark, Ecuador, Finland, France, Germany, Hungary, Mexico, Norway, Portugal, Romania, Tudkey, UK, Vietnam.

Dairy One network: reference laboratory is in Ithaca, New York, US. Satellite labs are in 7 different locations in the US. DL presented in Argentina, Brasil, Chile, Canada, Republic of South Africa, Marocco, Australia, New Zealand, Spain, The Netherlands, Belgium, Luxemburg, France, Italy, Hungary, Poland, Ukraine, Belarus, Turkey, China, Russia

Dairyland Laboratories Inc. network: reference laboratory is in Arcadia, Wi, US. Satellite labs are in 7 different locations in the US. DL presented in Argentina, Brasil, Chile, Canada, Republic of South Africa, Marocco, Australia, New Zealand, Spain, The Netherlands, Belgium, Luxemburg, France, Italy, Hungary, Poland, Ukraine, Belarus, Turkey, China, Russia.

Rock River Laboratory Inc. network: reference laboratory is in Watertown, WI, USA. 5 satellite Rock River Laboratories in the US, 4 satellite laboratories supported by he RRL in the US. International laboratories: 3 laboratories in Brasil, 2 laboratories in Mexico, and RRL are presented in Australia, Ukraine, Chile, Colombia, Germany, Spain, Turkey.

CVAS (Cumberland Valley Laboratories) network: reference laboratory is in Waynesboro, PA, US. Satellite labs are in 6 different locations in the US. There are 3 CVAS supported labs in Canada. CVAS presented in Argentina, Uruguay, Mexico, Canada, Republic of South Africa, Australia, Japan, Spain, the UK, Italy, Hungary, Croatia, Poland, Ukraine, Belarus, Thailand, Chine, Russia.

At the same time, they retain the capacity for localized fine tuning, allowing each participating laboratory or farm to adjust models to regional feed characteristics while maintaining traceability and analytical coherence across the global NIRS community. This architecture exemplifies the convergence of centralized reference control and decentralized operational flexibility, a defining feature of next generation agricultural spectroscopy.

Global NIR laboratory networks provide a rapid catch-up opportunity for countries where laboratory systems for forage analysis are not yet modernized or the opportunities for NIR-calibration development is limited. In such countries, the availability of fast, comprehensive laboratory results—covering a wide range of new analytical parameters—may have a significant impact on the efficiency of forage crop production and harvesting, as well as on the productivity of

milk and beef production systems. Beyond improving production performance, these advancements also may contribute to better rumen- and animal health and may accelerate the overall development of the livestock sector.

These global NIR-networks, in addition to analyzing forage samples, *are expanding the range of sample types to provide broader services*. Thus, besides forage samples, they typically also maintain calibration databases for *water, soil, milk and manure samples*. Generally the farm technology support is also involved in the services.

Global NIR Laboratory Networks and Local Calibration Development in Forage Analysis: Benefits and Risks

The effectiveness of NIR systems is fundamentally linked to calibration strategy. Two primary approaches can be distinguished: global (centralized) calibration models and local (site-specific) calibration models.

Global calibration systems rely on large, multi-regional spectral databases, often maintained by international laboratory networks or instrument providers. These databases incorporate extensive variability in feed composition, environmental conditions, and processing methods, resulting in robust and broadly applicable models. Studies such as Hossain *et al.* (2024) have demonstrated that multi-regional datasets significantly enhance predictive accuracy by capturing diverse sources of variation. Furthermore, continuous updates and algorithm refinement—highlighted by Maduro Dias *et al.* (2024)—improve model stability and reduce systematic bias. However, global calibration systems may reduce local calibration autonomy, as laboratories become dependent on externally maintained models. This can limit adaptability to region-specific conditions, such as novel crop varieties or unique silage practices, and may introduce long-term reliance on proprietary systems.

Local calibration models are developed using region-specific reference samples, allowing for greater contextual accuracy and flexibility. Research by Chen *et al.* (2013) indicates that locally calibrated models can outperform global ones when applied to narrowly defined sample sets. Additionally, local systems provide independence from centralized infrastructure, which is particularly advantageous in regions with limited technical resources. Nevertheless, local calibration approaches require substantial investment in sample collection, laboratory analysis, and continuous model validation. Limited dataset diversity may also lead to overfitting, reducing model generalizability (Cozzolino and Morón, 2014).

Towards a Hybrid Calibration System: given the strengths and limitations of both approaches, modern forage analysis increasingly adopts a hybrid calibration strategy, combining global data resources with local model refinement. In this framework: the global databases provide a robust baseline calibration, while local datasets are used for fine-tuning and validation and instrument-specific standardization techniques ensure compatibility across platforms. Emerging developments in cloud-based data sharing and machine learning further support this integration, enabling continuous model improvement while preserving local adaptability.

The performance of NIR spectroscopy in forage analysis is determined by the combined effects of instrument technology and calibration strategy. Global laboratory networks leverage FT-NIR, monochromator, and diode array systems in a complementary manner to achieve both analytical precision and operational efficiency. At the same time, the balance between global standardization and local adaptability remains a critical consideration.

A sustainable and effective NIR system will therefore depend on maintaining transparency, supporting calibration flexibility, and fostering collaboration between instrument manufacturers, laboratories, and end users. Over the past decades, global collaborative initiatives, centralized calibration centers and large spectral databases have elevated NIRS to a key technology in modern forage laboratories and on farm decision making.

The recent expansion of global near-infrared spectroscopy (NIR) laboratory networks has dramatically reshaped how analytical calibration models are developed, shared, and maintained within the feed and forage industry. Large international organizations—such as the global NIRS consortiums established by commercial instrument providers and public–private research alliances—now maintain centralized databases containing tens of thousands of spectra from diverse feed and forage materials. This approach has improved model robustness and promoted cross-instrument standardization, particularly for common nutritional parameters such as dry matter, crude protein, neutral detergent fibre (NDF), and acid detergent fibre (ADF).

By pooling global data, these NIR networks reduce the need for each laboratory to collect extensive local reference samples before achieving acceptable model performance. For instance, Hossain et al. (2024) demonstrated that multi-regional calibration datasets significantly increased prediction accuracy in livestock diet quality assessment by capturing variability in growing conditions, cultivar, and processing. Similarly, the review by Maduro Dias, Nunes, and Borba (2024) emphasized that global collaboration allows continuous updates of algorithms and model parameters across heterogeneous environments, thereby minimizing systematic bias linked to specific optical setups or geographic differences.

Beyond accuracy, the global-network approach promotes inter-laboratory harmonization. Calibration transfer between instruments from the same manufacturer can be readily achieved through standardized reference spectra and mathematical treatments such as piecewise direct standardization (PDS) or external parameter orthogonalization (EPO). Consequently, NIR operators can achieve consistent performance across multiple sites within a corporate or research consortium (Fodor et al., 2024). Such harmonization is essential for international feed trading systems, where analytical uniformity directly impacts nutritional labeling and regulatory compliance.

However, the same integration that offers technical advantages may also introduce structural weaknesses and dependency. The most evident risk is the erosion of calibration sovereignty—that is, the ability of a local laboratory or farm to adapt models to its own regional feed materials and climatic conditions. When calibration databases and prediction equations are proprietary assets held by multinational instrument providers or centralized consortia, local users often lose the capacity to independently refine or recalibrate models. This dependency may hinder adaptability to new crop varieties, novel silage types, or changing preservation technologies, and it can drive long-term vendor lock-in.

Local calibration development, in contrast, provides flexibility and contextual relevance. Locally built models reflect the specific feedstuff composition, soil mineral profile, and processing practices characteristic of a region. Studies such as Chen et al. (2013) showed that site-specific models often outperform global ones when applied to new or regionally limited feed materials, because local reference samples better capture subtle spectral–chemical relationships

Furthermore, in developing regions where infrastructure and internet access may be limited, localized calibration systems offer greater autonomy and resilience. They avoid

dependence on external updates or cloud-based databases, which can be costly or unavailable.

Nevertheless, local calibration strategies also entail substantial drawbacks. They demand large, well-curated reference datasets, continuous validation, and frequent model re-training. Maintaining such datasets can be financially and logistically challenging for small laboratories or farm-scale facilities. Limited sample diversity also risks overfitting, where the calibration performs well only for local materials but fails for imported or cross-seasonal samples. As highlighted by the comprehensive review of Cozzolino and Morón (2014), model transfer and robustness remain primary barriers for the widespread decentralization of NIR forage analysis.

From an operational perspective, NIR networks integrate automated quality control, version control of calibration files, and instrument diagnostics, enhancing analytical traceability and reproducibility.

Yet this centralized model may compromise transparency: users often cannot view the internal structure of multivariate models, making the analytical process a "black box." Such opacity can reduce scientific confidence when NIR results are used in regulatory or litigation contexts. Data-sharing agreements also raise questions about intellectual property and sample privacy, especially when agricultural data include sensitive production information.

A sustainable calibration ecosystem likely requires a hybrid approach combining global knowledge bases with local adaptability. Open-access initiatives and federated calibration frameworks are emerging to allow laboratories to contribute anonymized spectral data while preserving local customization capacity. Decentralized network architectures, leveraging cloud and AI-assisted learning, could help maintain calibration quality without surrendering ownership. As Maduro Dias et al. (2024) suggested, continuous collaboration between instrument manufacturers, research institutions, and end users is vital to prevent dependency asymmetry and ensure that both global and local knowledge are exploited for scientific and economic benefit.

Ultimately, the choice between global NIR network participation and local calibration development is not binary. Instead, the optimal strategy balances global standardization, which ensures comparability and efficiency, with local calibration autonomy, which ensures contextual accuracy and resilience. For the near future, maintaining transparency in model governance and fostering open calibration platforms appear essential to prevent concentration of analytical control in a few global entities while still harnessing the collaborative strength of large-scale spectral data infrastructures.

A summary Table 10 highlights the key differences between the two systems in terms of their advantages and disadvantages, summary Table 11 presents the differences between the two systems based on various criteria.

Near infrared spectroscopy has established itself as a leading technique in forage analysis. When supported by high quality calibration databases, modern preprocessing and robust chemometric models, NIRS achieves accuracy close to classical reference methods for most nutrients. Its rapid, non destructive measurement capability makes it indispensable for real time feeding management and quality control.

Tab. 10: The key differences between the global NIR databases and local NIR databases in terms of their advantages and disadvantages (compiled from literature on NIR calibration systems and database structures – Shenk and Westerhaus, 1991; Williams and Norris, 2001; Roberts *et al*., 2004; Fearn, 2001; Feudale *et al.*, 2002; FAO, 2011; ICAR, 2012; ISO, 2016)

Aspect	Global NIR Databases	Local NIR Databases
Advantages	Large, diverse datasets improve model robustness and predictive accuracy	Models are tailored to local feed composition and environmental conditions
	Enable cross-instrument standardization and calibration transfer	Greater flexibility and adaptability to new crops, silage types, and practices
	Reduce need for extensive local reference sample collection	Better representation of region-specific spectral–chemical relationships
	Continuous updates through global col-laboration improve model performance	Increased autonomy, no dependence on external providers or cloud systems
	Promote inter-laboratory harmonization and comparability	More resilient in regions with limited infrastruc-ture or internet access
	Support large-scale monitoring and standardized feed evaluation	Greater transparency and control over calibration models
	They are more competitive and can re-spond more quickly to market dynamics.	
Disadvantages	Reduced calibration sovereignty for local users	Require large, well-curated local reference da-tasets
	Dependency on centralized systems and instrument providers (vendor lock-in)	High cost and effort for maintenance, validation, and recalibration
	Limited flexibility to adapt to local varia-bility	Limited sample diversity may lead to overfitting
	Models may not fully capture region-specific characteristics	Poor transferability to non-local or imported feed materials
	Lack of transparency ("black box" mod-els)	Slower model development compared to global systems
	Data privacy and intellectual property concerns	Lack of standardization across laboratories
	Potential bias from centralized optical setups or geographic differences	No automatic updates or shared improvements
		Less compatitive, can not respond quickly to market dynamics.

Tab. 11: Differences between the global NIR databases and local NIR databases based on various criteria (compiled from recent literature and international guidelines on NIR calibration systems Shenk and Westerhaus, 1991; Hossain *et al*., 2024; Williams and Norris, 2001; Chen *et al*., 2013; Roberts *et al*., 2004; Fearn, 2001; Feudale *et al*., 2002; Fodor *et al*., 2024; Cozzolino and Morón, 2014; FAO, 2011; ICAR, 2012; ISO, 2016).

Criterion	Global NIR Databases	Local NIR Databases
Data scope	Large, multi-regional spectral datasets improve robustness and variability coverage (Hossain *et al*., 2024)	Limited to region-specific samples reflecting local feed composition and conditions (Chen *et al*., 2013)
Predictive performance	High accuracy for common parameters due to diverse calibration sets (Maduro Dias *et al*., 2024)	Often superior for niche or region-specific materials due to contextual relevance (Chen *et al*., 2013)
Calibration effort	Reduced need for local reference sample collection	Requires extensive local sampling, wet chem analyses, validation, and model development. Limited opportunities
Standardization	Enables cross-instrument calibration transfer (PDS, EPO) and inter-laboratory harmonization (Fodor *et al*., 2024)	Limited standardization; models often instrument- and site-specific
Adaptability	Less flexible to local variability, new cultivars, or novel processing conditions	Highly adaptable to local agricultural practices and environmental conditions
Model maintenance	Continuously updated via centralized networks and collaborations (Maduro Dias *et al*., 2024)	Requires ongoing local recalibration and maintenance
Infrastructure dependency	Strong reliance on centralized databases, cloud systems, and providers	Independent from external systems; suitable for low-infrastructure environments
Transparency	Often limited ("black box" models), restricted access to model structure	Full transparency and control over model development and parameters
Economic aspects	Efficient for large-scale operations; involve subscription or licensing costs	Higher initial and maintenance costs for small laboratories
Risk factors	Vendor lock-in, reduced calibration sovereignty, data privacy concerns	Overfitting risk, limited transferability, smaller datasets (Cozzolino and Morón, 2014)
Operational impact	Facilitates large-scale monitoring and standardized feed evaluation	Best suited for targeted, site-specific decision support
Service development, competitiveness	They possess stronger infrastructural, human, and financial resource capacity to support the development of new sample types and the expansion of measurable parameters. As a result, they are more competitive and can respond more quickly to market dynamics.	They have more limited opportunities for development and are less competitive.

PORTABLE NIR SPECTROMETERS

Recent technological developments have introduced handheld near-infrared (NIR) spectroscopy devices. Interest in compact, portable spectrometers is steadily increasing (Crocombe, 2018; Pasquini, 2018; Beć *et al*., 2020a,b; Digman *et al*, 2022; Feng *et al*, 2022¸ Rego *et al*., 2020; Rukundo *et al*, 2021; Yan *et al*, 2023). These devices have demonstrated good performance when applied to pre-treated samples, such as dried and ground forage (Cozzolino, 2014). Moreover, portable NIR spectrometers are increasingly integrated into forage harvesting machinery and have been adapted for use in other agricultural equipment, such as liquid manure applicators (Griffiths *et al*., 2007; Sørensen *et al*., 2021).

Understanding and effectively managing the variability in forage nutritional composition is essential for improving dairy farm performance and supporting animal health. The nutritive value of forages—such as alfalfa–grass haylage and corn silage—can vary substantially, with direct implications for milk production efficiency and environmental sustainability (Van Soest, 1994). By enabling frequent, data-driven adjustments of animal diets based on real-time forage analysis, handheld NIR devices can improve feed efficiency, reduce environmental burdens, and enhance the economic and ecological sustainability of dairy operations (Batten, 1998; Roberts *et al*., 2004; Decruyenaere *et al*., 2009).

Existing studies highlight both the capabilities and limitations of handheld NIRS systems in real-world applications. For example, Beć *et al*. (2020a) report on predictive model development for different forage types and emphasize the critical role of scanning methodology in determining prediction accuracy. Similarly, Crocombe (2018) evaluates multiple handheld NIR devices, focusing on their precision and accuracy in on-farm applications, particularly in comparison with conventional moisture measurement methods and in relation to the robustness of calibration models for nutritive parameters.

Type of portable NIR spectrometers

NIR devices used in forage analysis can be broadly categorized into three groups based on their functionality, calibration approach, and application context.

"Research-grade" instruments, such as those developed by NeoSpectra, TrinamiX, and AgroCares, are characterized by their strong validation in scientific studies and their ability to predict a wide range of forage quality parameters. These include not only primary chemical constituents—such as crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) (Norris *et al*., 1976; Coleman *et al*., 1995; Andrés *et al*., 2005, Cozzolino and Morón, 2004; Decruyenaere *et al*., 2009; Roberts *et al*., 2004 Givens *et al*., 1997)—but also more complex biological

traits, including neutral detergent fiber digestibility (NDFD) (Decruyenaere *et al*., 2009; Coleman *et al*., 1995; Andrés *et al*., 2005) and *in vitro* total digestibility (IVTD) (Decruyenaere *et al*., 2009; Cozzolino and Morón, 2004; Andrés *et al*., 2005; Roberts *et al*., 2004).

A key advantage of these systems lies in their flexibility, as they allow the development and implementation of custom calibration models. Consequently, they typically provide superior predictive performance. However, their accuracy is highly dependent on the quality and robustness of the applied calibrations (Martens and Næs, 1989; Shenk and Westerhaus, 1991; Williams and Norris, 2001; Decruyenaere *et al*., 2009).

"Plug-and-play" agricultural devices, such as X-NIR and AgriNIR, are designed for immediate use under field conditions. These systems come with pre-installed calibrations for commonly required parameters, including dry matter (DM), crude protein (CP), starch, NDF, ADF, fat, and ash. Their primary advantage is ease of use and rapid deployment without the need for advanced calibration development. However, this convenience is often associated with limitations in the range of measurable parameters, particularly with respect to more complex digestibility-related traits such as NDFD and IVTD, which are less frequently available or less reliably predicted.

Industrial or on-line NIR systems are the third category, exemplified by Polispec NIR and HarvestLab 3000. Polispec NIR instruments are designed for continuous, real-time monitoring of forage materials during processing or handling (e.g., in mixer wagons or along conveyor systems) and represent typical industrial process-NIR applications (Burns and Ciurczak, 2007; Pasquini, 2018). They can cover a broad spectrum of parameters depending on the applied calibration models and are particularly valuable for dynamic process control. Their main strength lies in enabling real-time decision-making and continuous quality monitoring in operational environments (Workman and Weyer, 2012).

HarvestLab™ represents an on-machine near-infrared (NIR) sensing system designed for integration into agricultural equipment, such as forage harvesters and slurry tankers. Previous research has shown that HarvestLab™ systems are capable of estimating the quality of mixed, undried forage, although systematic errors may occur and require calibration adjustments (Griffiths *et al*., 2006; Sørensen *et al*., 2021). Its primary advantage lies in its ability to perform continuous, real-time measurements during field operations, thereby providing immediate feedback that supports precision agriculture practices, including variable rate application (Müller *et al*., 2019; Sørensen *et al*., 2021).

The system is well established for the estimation of key compositional parameters, such as dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and starch (Decruyenaere *et al*., 2009; Roberts *et al*., 2004). In addition, it can be used to predict more complex variables, such as neutral detergent fiber digestibility (NDFD) and *in vitro* digestibility (IVTD), although the reliability of these predictions is highly dependent on the applied calibration models (Coleman *et al*., 1995; Andrés *et al*., 2005). The success of such applications relies heavily on the development of robust and reliable calibration models (Martens and Næs, 1989; Shenk and Westerhaus, 1991).

Despite its strengths, HarvestLab™ has certain limitations. Its performance is strongly influenced by crop type and calibration quality, and it offers less flexibility compared to research-grade handheld NIR instruments, which allow for more extensive customization of predictive models (Crocombe, 2018; Beć *et al*., 2020b). With the inclusion of HarvestLab™, the category of on-line NIR systems can be more clearly defined. Systems such as Polispec NIR function as flexible, industrial-grade solutions suitable for a wide range of environments and applications, whereas HarvestLab™ is specifically designed for field integration and optimized for use during forage harvesting operations. This distinction highlights the different application domains: Polispec systems are more adaptable for multi-environment, process-oriented monitoring, while HarvestLab™ is tailored to real-time data acquisition directly during harvesting (Pasquini, 2018; Workman and Weyer, 2012).

Comparison of handheld and on-line NIR devices: parameters

Across all device categories, most handheld NIR instruments are capable of reliably measuring core compositional parameters, including DM, CP, NDF, and ADF (Table 12). The primary differences between systems arise in their ability to accurately predict digestibility-related variables, the quality and adaptability of their calibration models, and their intended operational context (handheld versus on-line applications). In general, chemical constituents are more robustly and consistently predicted by NIR spectroscopy, whereas biological parameters—such as digestibility—are more sensitive to variability and typically exhibit lower predictive accuracy.

Conclusion: NeoSpectra, TrinamiX, and AgroCares are most appropriate for research-based and high-level modeling projects; X-NIR and AgriNIR offer convenient solutions for regular on-farm decision-making; while Polispec and HarvestLab™ are most suitable for continuous and real-time observation. Within this latter range, HarvestLab™ serves as particularly advantageous for harvesting and Polispec is more suitable for monitoring during the processing of feeds and feeding operations.

The continuous progress in NIRS technology allows for miniaturized, portable instruments to reach the predictive efficiencies of classic benchtop systems. As adoption expands, attention will need to be paid to the effect of device type and scanning approach on reliability and accuracy of forage quality prediction.

Forage evaluation with handheld NIRS technology is aimed at a relatively straightforward approach, where samples can be analyzed directly without prior preparation. This is done through calibration models customized for wet, unprocessed forage (Cozzolino, 2014; Crocombe, 2018). Nevertheless, some issues still exist, namely moisture absorption bands and sample heterogeneity (Pasquini, 2018; Beć *et al*., 2020). Other pragmatic limitations are maintaining appropriate signal-to-noise ratio and a dependable electrical supply on portable systems, and ensuring operational dependability in different and sometimes hostile environmental scenarios (Crocombe, 2018; Beć *et al*., 2020).

Comparison between handheld and on-line NIR systems: the type of forage

Performance characteristics of handheld and on-line near-infrared (NIR) spectroscopy systems are determined by the characteristics of the physical structure and compositional variability of the forage matrix.

Performance variation is observed among forages (Table 13). Corn silage is generally predicted with high accuracy in all categories of devices due to its relatively homogeneous composition. Whereas grass silage and haylage display a more diverse behaviour, requiring more rigorous calibration models. Since TMR is highly heterogeneous and best suited to on-line measurement systems, it should be used accordingly, whereas the more accurate predictions made on dry hay are more reliable, regardless of the device type.

Tab. 12: Comparison of handheld and on-line NIR devices for parameters (own compilation based on published studies and manufacturer specifications – Alomar *et al*, 2021; Cozzolino, 2014; Rinnan e al, 2009; De Boever, Goyvaerts and Van Waes, 2018; Pasquini, 2018; Crocombe, 2018; Rego *et al*., 2020; Rukundo *et al*., 2021; Digman *et al*., 2022; Feng *et al*., 2023; Fodor *et al*., 2024; John Deere, 2024; TrinamiX, 2024; AgroCares, 2024; Dinamica Generale, 2024; PoliSPEC, 2024)

Parameter / Device	NeoSpectra	TrinamiX	AgroCares Scanner	X-NIR	AgriNIR	Polispec NIR	HarvestLab™
Dry Matter	✓	✓	✓	✓	✓	✓	✓
Crude Protein	✓	✓	✓	✓	✓	✓	✓
NDF / aNDF	✓	✓	✓	✓	✓	✓	✓
ADF	✓	✓	✓	✓	✓	✓	✓
ADL	✓	✓	✓	✓ (limited)	✓	✓	(limited)
NDF Digestibility	✓	✓	✓	(limited)	(limited)	✓	✓ (model-dependent)
DM or OM Digestibility	✓	✓	✓	(limited)	(limited)	✓	✓ (model-dependent)
Starch	✓	✓	✓	✓	✓	✓	✓
Crude Fat	(possible)	(possible)	✓	✓	✓	✓	✓
Ash	✓	✓	✓	✓	✓	✓	✓
Energy values (NEL, ME)	(derived)	(derived)	(derived)	✓	✓	✓	✓
Real-time / on-line meas.	×	×	×	×	×	✓	✓

Tab. 13: Comparison of handheld and on-line NIR devices by forage type (own compilation based on published validation studies and manufacturer documentation of Alomar *et al*., 2021; Rego *et al*., 2020; Rukundo *et al*., 2021; Digman *et al*., 2022; Feng *et al*., 2023; John Deere, 2026; TrinamiX, 2025; AgroCares, 2026; Dinamica Generale, 2026; Polispec, 2026).

Parameter / Device	NeoSpectra	TrinamiX	AgroCares	X-NIR	AgriNIR	Polispec NIR	HarvestLab™
Grass silage / haylage	✓✓ (high validation)	✓✓	✓✓	✓	✓	✓✓	✓✓
Corn silage	✓✓ (well validated)	✓✓	✓✓	✓	✓	✓✓	✓✓✓
Fresh forage (green chop)	✓ (moisture sensitive)	✓	✓	✓	✓	✓✓	✓✓✓
Dry hay	✓✓✓	✓✓✓	✓✓✓	✓✓	✓✓	✓✓	✓
Total Mixed Ration (TMR)	✓ (heterogeneity challenge)	✓	✓	✓✓	✓✓	✓✓✓	✓✓
Forage mixtures (multi-species)	✓✓ (with calibration)	✓✓	✓✓	✓	✓	✓✓✓	✓ (model-dependent)
Processed / flowing forage (on-line)	×	×	×	×	×	✓✓✓	✓✓✓

Notes: ✓ acceptable , ✓✓ good performance/reliable applicability, ✓✓✓ excellent performance / highly suitable for the given task.

The ratings (Table 11) are derived from the joint analysis of data which involves: predictive accuracy (R^2, RMSE derived from literature data), sample inclusiveness, moisture effect robustness, measurement conditions (static and dynamic environment), practical application (on-line as an example). Availability of the parameters or extent their performance are determined by literature-derived calibration capabilities and specifications of the manufacturer and are influenced by the quality of calibration, the kind of sample and the conditions concerning measurements.

Sample heterogeneity is one of the leading determinants of predictive performance. NeoSpectra, TrinamiX, AgroCares and other well-known research-grade handheld devices exhibit robust performance over a wide range of forage types, if well-developed calibration models are employed to calibrate it. They are especially robust for relatively homogeneous materials such as dried hay, but they also work well for heterogeneous matrices such as silages and mixtures, provided that calibration strategies are well developed. Farm-oriented "plug-and-play" systems (e.g., X-NIR and AgriNIR) are more efficient at generalizing to standard applications across the forage range to a low scale, but provide limited flexibility to more variable or mixed samples.

Moisture content is another critical parameter governing NIR performance. Fresh and wet forages, especially those used in green chop and silage, can introduce highly damaging spectral noise with the formation of strong water absorption bands, and their high frequency and size can also affect the accuracy of predictions especially as it pertains to more involved or biologically inferred parameters (Pasquini, 2018; Cozzolino, 2014). Research-grade instruments tackle this shortcoming through advanced, moisture-aware calibration models (Martens and Næs, 1989; Shenk and Westerhaus, 1991), while platforms such as Polispec NIR and HarvestLab 3000 adapt to this challenge by making use of continuous data acquisition and signal averaging, increasing robustness under high-moisture conditions (Sørensen *et al*., 2021).

Environmental aspect of measurement also has a major impact. Compared with handheld devices for real-time applications in forage collection, total mixed ration (TMR) preparation and conveyor based processing, on-line NIR systems surpass the capabilities of handheld systems (Pasquini, 2018). Their capacity to perform continuous monitoring increases the representativeness of measurement compared to handheld devices limited by point-based sampling particularly in heterogeneous materials (Crocombe, 2018; Beć *et al*., 2020b; Sørensen *et al*., 2021).

In general, the overall performance of NIR systems is dominated by their inability to estimate the core composition parameters for key forage types when subjected to sample heterogeneity, moisture, and measurement conditions. Research-grade handheld devices are the ideal solution for a wide range of forage types, plug-and-play systems for routine on-farm use, and on-line systems like Polispec, HarvestLab™ offer a clear advantage for the handling of heterogeneous materials, for continuous process processing and for real-time decision support. The choice of a particular NIR apparatus for forage analysis does not depend purely on the measurable variables, but to a great extent on the type and physical state of the forage. Conventional handheld equipment is more flexible and convenient, while on-line tools offer much better quality in the treatment of variability and for measurement of true representatives against reality.

CONCLUSIONS

The use of near infrared (NIR) spectroscopy has been essential to modern forage analysis and it does so in a non-destructive and rapid manner and can predict a multiplicity of nutrition parameters. The use of the device to support decision-making within and outside of the lab allows enhanced feed management efficacy, as well as the development of precision livestock farming. In particular, the NIR approach is well established for the determination of major chemical constituents such as dry matter, crude protein and fiber fractions, with high spectral correlations.

Nonetheless, numerous limitations should be highlighted. Precision in NIR predictions hinges on the reliability, generalizability and representativeness of calibration models used in inference which need constant updating and validation. Additionally, biological-process parameters, including digestibility, are still more complex to evaluate due to their indirect connection to spectral data. Variability in sample types, moisture content and measurement conditions may also compromise prediction accuracy, particularly in fresh or unprocessed forages.

Furthermore, regional differences in feed assessment systems and reporting requirements may restrict the generalization of NIR-based information to the various nutritional models. This points to the potential need for calibration consistency or for flexible calibration schemes.

The investigation of improved calibration robustness in the presence of larger and more diverse datasets, advanced chemometric and machine learning techniques and the performance of portable and on-line devices under volatile field conditions should be the focus of future work. We need to also better characterize complex biological parameters, as well as harmonize NIR outputs with systems across multiple international feed evaluation systems. *A potentially broader approach could be to use an integrated set of parameters supported by the harmonized analytical methodology, a corresponding software framework, for instance, the CNCPS dynamic rumen model (and similar software in the form of AMTS or NDS) adopted across Europe. Such measures would help to overcome fragmentation on the continent, streamline investment-intensive developments through more focused, coordinated action, and strengthen global competitiveness for future developments*. Further technological and methodological development will reinforce the significance of NIR spectroscopy to sustainable harvest-, preservation and feeding technology of diverse forages.

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