Preamble
The Walloon Agricultural Research Centre (CRA-W) located in Gembloux, Belgium, maintains numerous contacts with farmers and horticulturists, companies, universities, and Belgian and foreign research centers. CRA-W is involved in various European, national, and regional research projects. Among many other initiatives, CRA-W has focused on promoting expertise in near-infrared spectroscopy (NIRS) since the 1980s. This chapter is mainly based on the knowledge and experience accrued by CRA-W after almost 40 years of NIRS forage analyses. The content of this section is quite technical and aims to provide clear and practical information to anyone interested in forage analysis.

27.1 INTRODUCTION

Forages are the primary self-produced feed resources of ruminants and make a significant contribution to the feeding autonomy of the farm. Forages are cropped under different forms, such as grazing or harvesting to produce silages and haylages. It is obvious that in the current economical context (e.g., price volatility of main inputs and agricultural productions), it is essential to give utmost importance to the quality of forage production to minimize feeding costs and cover animal requirements while protecting the environment.
At the nutritional level, diet must bring all the vital nutrients (e.g., energy, proteins, fibers, minerals, vitamins) in the right proportions, based on the metabolic state of the animals (e.g., pregnancy, growing, fattening). On the basis of the average values of the forages, a farmer may either overestimate the nutritive value of feed and not cover his animals’ needs or underestimate it, thus with the risk to produce manure that is too rich and could potentially pollute the environment. From the minerals that are taken from the soil by forages, it is also possible to calculate nutritional indices (based on the nitrogen content of the plant) and to put in place a reasoned system of soil fertilization.

This being the case, it is evident that the tools that will allow to determine the chemical composition and nutritive value of forage production also become essential not only to adapt the diets to the farm’s animal requirements but also to manage the overall operation of the farm.

Near-infrared spectroscopy is one of these tools and presents many advantages:

- Speed of analysis: the spectral acquisition and the prediction step usually take less than 5 min
- Costs (≈10 × cheaper than reference methods), around 30 € for the determination of the main constituents by NIR spectroscopy
- Simultaneous quantification of different parameters and criteria
- Ecologically friendly (reduced use of reagents, especially after validations of the NIR calibrations)
- Simple sample preparation, which is generally limited to a drying and a grinding step at the laboratory level and requires practically no preparation if the analysis is performed at the farm
- Analysis is easy to implement

Therefore, near-infrared reflectance spectroscopy can contribute significantly to the best qualification of forages for animal nutrition.

**27.2 TYPES OF FORAGES**

Grasslands are the largest ecosystems in the world. They cover 26% of the world land area (FAOSTAT, 2009). At the European level, permanent grasslands cover 57 million of Mha. Temporary grasslands cover smaller areas (less than 9.7 Mha). Other fodder crops cover 9 Mha where maize silage accounts for 5 Mha (Huyghes et al., 2015).

Forages comprise all the fibrous materials consumed by herbivores. The most frequently encountered forages are grass (e.g., fresh, silage, pre-wilted silage, or haylage) from temperate or tropical origin, maize (e.g., whole plant silage, corn cob mix silage), sugar beet pulp, cabbage, etc. All these types of forages can be subjected to NIRS analyses for predicting chemical composition and digestibility.

In temperate regions, forages from grasslands are usually composed of a mix of grasses (ryegrass, cocksfoot, timothy, etc.) and legumes (alfalfa, white, and/or purple clovers). These are grazed or harvested to produce haylages and silages. Many other plant species are also used in a multispecies mixture for feeding herbivores. This is the case, for instance, of mixtures of cereals, grasses, legumes, and protein crops harvested immature as silage. Whole plant maize in the form of silage or sugar beet pulp is also included in the forage resources of a farm. From a nutritional point of view, the uniqueness of these forage resources is their heterogeneity.

Some resources such as corn silages and sugar beet pulp are energy rich. Others such as grass products provide protein and/or fibers to the food rations. Moreover, within a same category of forage, there is a strong variability, so it is essential that the producer is aware of the quality of the forage. Indeed, low-quality forages may lead to an undesirable economic performance and metabolic disorders (e.g., acidosis or ketosis) resulting from the distribution of unbalanced diets.
The five classes of forage are generally described as follows:

- **Green forages (Figure 27.1)** are grazed grass, whole plant maize, immature cereals, and protein mixed crop.
- **Silage forages (Figure 27.2)** are composed of grass, whole plant maize, or beet pulp. Silage is a method of preserving wet forage through anaerobic lactic fermentation. This fermentation has the effect of stabilizing the product by inhibiting its degradation by acid production thus lowering the pH.
- **Dry fodder (Figure 27.3):**
- **Artificially dehydrated fodder (Figure 27.4)** like alfalfa pellets, etc.
- **Straws and stalks** are products like straws of cereals, peas, or corn cob.

### 27.3 FORAGE CONSTITUENTS

From a chemical point of view, forages are composed of two elements: water and dry matter (Figure 27.5). According to the type of forage, this proportion can be very different. For example, maize silage forage contains roughly less than 35% of dry matter versus hay forage with 85%.

Knowledge of water content is important for several reasons:

- It is related to the ability of the animals to ingest.
- It allows reporting the results of analyses to a fixed base and thus compares the different foods between them.
- It allows assessing the risks during conservation.
FIGURE 27.2  Maize silage from the experimental farm of CRA-W.

FIGURE 27.3  Haystack from the experimental farm of CRA-W.
Dry matter is composed of an organic fraction (carbohydrates, proteins, starch, fibers, etc.) and an inorganic matter (i.e., minerals and oligo minerals) obtained by calcination.

- **Organic matter is composed of**
  - **Crude proteins (CPs):** they play a role in the formation of tissue, enzymatic, and hormonal activities and represent an energy source through various pathways of metabolic degradation, milk, and meat production. The metabolism of the protein is very complex especially in ruminants. That’s why there are different ways of evaluating the quality of forage. Crude proteins are commonly determined by dosing total nitrogen (N) and multiplying the N value by 6.25. This represents a mixture of true protein nitrogen and nonprotein nitrogen.
  - **Fats:** they are present in small quantities in forage. Their roles are mainly energetic and act as a potential source of omega 3 fatty acids.

![FIGURE 27.4](image1) Alfalfa pellets in a small cup from a Foss DS2500 in the NIR laboratory of the CRA-W.

![FIGURE 27.5](image2) Major constituents of forage.
Carbohydrates: they are the main sources of energy for rumen microbes, but they are also used directly by the animal. There are two groups of carbohydrates according to their functions: nonstructural and structural.

- **Nonstructural carbohydrates** that include simple sugars or rapidly digestible compounds (i.e., glucose, fructose, sucrose, and maltose) and polysaccharides (i.e., starch, fructosan). Starch is the storage polysaccharide in legumes, while fructosan plays the same role in grasses.

- **Structural carbohydrates** are the substances that form the cell walls and lend rigidity to the plant: they include cellulose, hemicellulose, and lignin. The main structural carbohydrate found in plants is cellulose.
  - Cellulose is a homopolymer consisting of glucose. It has a crystalline structure that makes it difficult for rumen microbes to degrade it enzymatically
  - Hemicelluloses are heteropolymers consisting of xylose, arabinose, galactose, mannose, and glucose. They do not have a crystalline structure, so they are easily digestible by rumen microbes
  - Lignin is a complex compound that settles on cellulose, hemicellulose, pectin, and structural protein compounds as the plant ages. It binds to the constituents of cell walls making them vulnerable to biotic and abiotic stresses

Analysis of the fiber or cell wall present in forages is a major concern in ruminant nutrition because diets often contain large amounts of forage, and the fiber fraction affects both feed intake and animal performance.

The complexity of its structure explains the difficulty of digesting it. The more cellulose present in forage, the harder to digest.

The fibrous fraction that dissolves plant pectins, starch, fructans, proteins, sugars, and lipids, commonly known as NDF (neutral detergent fiber) and insoluble in a neutral detergent, contains hemicellulose, cellulose, and lignin but not pectin; it also includes fiber-bound protein and minerals, as well as heat-damaged protein (N-ADF)\(^\text{1}\).

The level of NDF in the animal ration influences the animal's intake of dry matter and the time of rumination. The concentration of NDF in forage is negatively correlated with energy concentration.

The complex of lignin and cellulose is generally grouped under the term lignocellulose or nonsoluble fibers in an acid detergent (ADF fibers, “acid detergent fiber”). This fibrous component contains cellulose and lignin, as well as fiber-bound protein and minerals. It increases with the maturity of the plant. Hemicellulose is estimated by subtracting the ADF from the NDF. The proportions of ADF and NDF of forage are indicative of its food value. ADF fiber is generally related to digestibility and the energy value of the forage: the more ADF fiber in the forage, the lower the digestibility and the energy content.

Treatment of the ADF residue with 72% sulfuric acid followed by calcination permits determining the fraction of crude lignin (ADL—acid detergent lignin). Lignin is totally resistant to enzymatic degradation in the digestive tract of ruminants; consequently, it has no nutritional value and, furthermore, adversely affects the digestion of other structural carbohydrates. With the aging of the plant, the cellulose chains surround themselves with lignin and hemicellulose, which leads to a decrease in the digestibility of cellulose and fodder. Cellulose can then be estimated by the difference between ADF and lignin plus ash.

Non-nitrogenous extracts consist mainly of sugars.

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\(^1\) N-ADF refers to a contamination of nitrogen in ADF (acid detergent fiber).
• **Inorganic matter** is contained in soluble form in mineral substances (i.e., calcium, phosphorus, sodium, magnesium, sulfur, and trace elements) essential for the vital functions of the animals and in insoluble form in the physical constituents of soil (i.e., clays, silts, and sands), which is characteristic of forage contaminated by these soils. The role of minerals is multiple and varied according to the animal species and its physiological stage (e.g., pregnancy, sowing, fattening). Minerals intervene at the level of skeleton development (major role of calcium, phosphorus, and magnesium), cell activity of organisms (role of potassium), hormonal and enzymatic reactions (role of trace elements), and ruminal digestion of feed.

Rumen microorganisms have specific requirements for major elements, such as phosphorus and sulfur and trace elements such as copper, zinc, cobalt, and manganese.

### 27.4 LABORATORY REFERENCE METHODS OF CHEMICAL ANALYSES

NIR spectroscopy is directly related to wet chemistry reference methods of analysis. Indeed, NIR calibrations are calculated from databases where spectra and the corresponding sample reference values match.

Reference methods are generally described under ISO regulations, and sometimes different official reference methods exist for the same parameters. That’s why when a database is built or a validation step is carried out, it is always important to use a single reference method.

Below, a list of wet chemistry reference methods commonly used for forage analyses is provided.

- **Water:** drying the sample generally in an oven at 60°C for 2 days and at atmospheric pressure. The drying conditions may vary according to the nature of the fodder
- **Analytical dry matter:** drying the sample in an oven at 103°C for 4 h.
- **Total ash:** obtained by calcining the dry matter in a muffle furnace at 550°C. They are then solubilized with nitric or hydrochloric acid on a sand bath. The soluble and insoluble ashes are separated by filtration.
- **Total proteins or crude proteins:** the nitrogen content is determined by the chemical destruction of the organic matter it contains (Kjeldahl method). It is then multiplied by a factor of 6.25 to obtain the total protein material.—Another method is based on the Dumas method

A distinction is made between major elements (0.2%–1%) and minor or micro-nutrients (10–500 ppm or mg kg⁻¹), but this distinction is purely quantitative as the trace elements are as essential as the major elements. The major elements routinely analyzed are potassium (K), phosphorus (P), sodium (Na), magnesium (Mg), and calcium (Ca). The most often analyzed trace elements are iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn). The identification of cations is based on atomic absorption using a spectrophotometer, while phosphorus is determined by colorimetry.

- **Total proteins or crude proteins:** the nitrogen content is determined by the chemical destruction of the organic matter it contains (Kjeldahl method). It is then multiplied by a factor of 6.25 to obtain the total protein material.—Another method is based on the Dumas method


NF EN ISO 16634-1: Determination of the total nitrogen content by combustion according to the Dumas principle and calculation of the crude protein content—Part 1: Oilseeds and animal feeding stuffs
• Soluble sugars: the reference analysis method consists of an extraction in ethanolic medium followed by a Luff–Schoorl method prior to inversion (reduction of sugars) and following inversion (total sugars). The results are expressed as percentage of glucose.
  
  Norm: REGULATION (EC) no. 152/2009—Methods of sampling and analysis for the official control of feed

• Starch: based on the difference between the rotatory polarimetric measurement of a hydrolysate (hydrochloric acid) and that of an ethanol extract of the sample.—Another method is based on an enzymatic reaction.
  
  Norm: REGULATION (EC) no. 152/2009—Methods of sampling and analysis for the official control of feed • (N): NF ISO 6493

• Fibers: the Weende method is most commonly used to determine raw cellulose content. It is based on an acid attack (sulfuric acid) followed by an alkaline attack (sodium hydroxide) on the sample.
  
  Norm: REGULATION (EC) no. 152/2009—Methods of sampling and analysis for the official control of feed • (B): NF V03–040: Determination of raw cellulose—General method • (I): NF EN ISO 6865: Determination of crude fiber content—Method with intermediate filtration

A more precise characterization of cell walls, based on the use of detergent solutions at different pHs, allows the quantification of three distinct fractions, namely:

• Insoluble fibers after treatment in neutral pH, comprising hemicellulose, cellulose, and lignin, which corresponds to the totality of the cell walls (NDF for neutral detergent fiber)
  
  • Insoluble fibers after treatment in acid pH, comprising cellulose and lignin (ADF for acid detergent fiber). Hemicellulose is the result of the difference between NDF and ADF

• Insoluble fibers after a 72% H₂SO₄ attack on lignin (ADL for acid detergent lignin). Cellulose is the result of the difference between ADF and ADL
  

• Enzymatic digestibility of organic matter: the digestibility of organic matter (dMO) is the determining parameter in the prediction of the energy value of forage. Indeed, for a given substrate, the digestive energy and dMO are closely correlated. There are a large number of methods devised to measure dMO; the most easily standardized and routinely applied methods are enzymatic techniques using pepsin and cellulase.

### 27.5 SAMPLE PREPARATION AND NIR ANALYSIS

Due to the heterogeneity of forages (Figure 27.6a and b), sampling is a critical step of their analysis.

Samples must be as representative as possible of the whole stock of forages (bales or silo). When sampling, several portions must be taken (at least 10 subsamples of 50 g each) and collected in a bag that must be hermetically closed and stored under cool conditions until their arrival at the laboratory. In NIR analyses, sample preparation is relatively easy but critical (Reddersen et al., 2013). For forages, preparation usually considers a drying and a grinding step. Forage samples have to be properly dried (until constant weight), generally for 2 days at 60°C maximum to avoid Maillard reactions in particular (Figure 27.7).

The operator will carefully arrange the samples in a thin layer (or in a micro-perforated plastic bag) and turn them regularly to obtain a homogeneous drying that will prevent mold growth. The grinding step is also very important and can influence the result of a NIR analysis. The recommendations are to grind the samples using a 1-mm sieve. Sometimes pregrinding will be necessary. In any case, it is important to always use the same grinding protocol. The grinders commonly used for forage are the following:
A hammer mill for a rough grinding (Figure 27.8)
A knife hammer used with a 1-mm sieve (Figure 27.9)

Ground samples are submitted to NIR analysis at least 30 min after grinding to allow time for the samples to reach the moisture level equilibrium.

Generally, roughly 10 g of samples is analyzed (Figure 27.10) so all the preliminary sampling steps are critical especially with heterogeneous products such as forages.
FIGURE 27.7  Oven for drying fodder (Chemistry lab at CRA-W). The recommended temperature is 60°C. The samples can be placed in a micro-perforated bag or in a tub until constant weight.

FIGURE 27.8  Retsch grinder. (CRA-W Laboratory.)
FIGURE 27.9  Cyclotec grinder. (CRA-W Laboratory.)

FIGURE 27.10  Grass sample in a quarter cup from a Foss XDS in the NIR laboratory of CRAW before NIR analysis.
The NIR spectrum (Figure 27.11) will vary especially according to the sample composition and its granulometry.

27.6 PERFORMANCE OF NIRS CALIBRATIONS FOR PREDICTING CHEMICAL COMPOSITION OF FORAGES

Studies on the potential of near-infrared spectroscopy (NIRS) for assessing feeding values of forages are numerous (Norris et al., 1976; Andueza et al., 2011; Deepa et al., 2016). A way to evaluate the accuracy of a calibration is to calculate the RPD (residual prediction deviation) value, which is the ratio of the standard deviation for a component, by the error of the corresponding NIR calibration (RPD = SD_{REF}/SEC_{NIR}). A calibration is generally considered as accurate for RPD values of 3 and higher.

In most cases, RPD ratios for forage calibrations are higher than 3 and confirm the good potential of near-infrared spectroscopy in forage analyses. More recent developments (Ward et al., 2011) used NIRS for a rapid assessment of the mineral concentration of forages. But the RPD ratio was lower, and the method could only be used for screening.

The databases for forages at CRA-W contain several thousand samples analyzed by wet chemistry. These databases present a very wide variability since the data were acquired during the last 40 years. The algorithm of calibrations commonly used is the partial least squares (PLS).

Depending on the application, the GLOBAL or the LOCAL methods will be used. In the GLOBAL system, all the spectra are used to build the calibrations. In the LOCAL approach, a new specific calibration is built automatically when a new sample is scanned. The system is set up in a way that the software can select in the database only the most similar spectra of the sample spectrum to be analyzed. Generally, the LOCAL approach gives better results (Sinnaeve et al., 1994; Tran et al., 2010).

Tables 27.1 and 27.2 give the characteristics and performance of NIR calibrations conducted by CRA-W. The parameters are expressed at 100% of dry matter (DM).
In addition to classical NIR analyses on dried and ground samples, calibrations are developed for the determination of the chemical composition and enzymatic digestibility of undried forage (Andueza et al., 2013). Indeed, it is most interesting for farmers or nutritionists to mount near-infrared devices.
on silage cutters or to analyze forage directly at the farm site using a portable spectrometer to obtain a first evaluation of the quality of the product (estimation of humidity, crude protein content). The main purpose is to secure a quick determination of the composition by skipping the drying and grinding stage thus saving a significant amount of time.

For example, the NIR fresh grass database built by CRA-W contains different types of samples harvested during the 2014 and 2015 growing seasons (Decruyenaere, 2006). Calibrations were developed for determining the dry matter content (DM 60°C) and the chemical composition: total ash, crude protein, crude cellulose, and enzymatic digestibility of organic matter (cellulase method).

In practice, before the NIR analysis, grass samples were cut into 8–10 cm long strands. Samples were then homogenized and analyzed on a Foss XDS equipped with a rectangular full cup (Figure 27.12). Every sample was scanned five times, and the absorbance of the samples was recorded as log (1/R).

At the time of creation of the database and in an effort to save time and money, fresh samples were not analyzed through wet chemistry but dried in a ventilated oven at 60°C for 48 h for dry matter determination and then ground through a 1-mm sieve using a cyclotec grinder. The next step consisted of subjecting the ground and dried sample to the same NIR analyzer (XDS). The measurements were made in duplicate in this case, and the classical chemical composition and enzymatic digestibility of organic matter were then predicted from existing and approved NIR calibrations. Since the NIR predictions are checked on a regular basis as part of the activities undertaken by interlaboratory studies (BIPEA, Germ services, REQUASUD), the level of confidence on dried grass forage is very high.

Compared to NIR calibrations developed with dried samples, the performances (Table 27.3) obtained from fresh samples are slightly lower.

![Figure 27.12](image-url) Analyze of wet forage with a Foss XDS spectrometer in the NIR laboratory of CRA-W. (The Foss XDS spectrometer is a monochromator NIR instrument working in reflectance and covering the range of 400–2498 nm.)
The results are similar to those obtained by other research teams. Previous work (Sinneave et al., 1994; Park et al., 1998) has shown the potential of NIR analyses of fresh materials to determine the chemical composition and digestibility of grass silages. According to Cozzolino and Labandera (2002), the NIR fresh analyses can be used to predict the dry matter and crude protein of forages with errors of calibration of 1.2% and 1.9%, respectively. This potential has been confirmed by more recent studies. Thus, Park et al. (2014) have shown that moisture, proteins, and ash can be estimated with errors of calibration of 1.89%, 1.14%, and 1.47% DM, respectively.

NIR fresh analyses allow estimating the dry matter content and the chemical composition and digestibility of grass with precision. The range of variation of the samples used to develop the calibrations suggests that this precision is sufficient to differentiate the samples qualitatively. The estimation of dry matter content, a parameter of importance for the calculation of livestock diets, seems sufficiently accurate. The database should, however, be supplemented with new samples to consolidate the calibrations.

### 27.8 HAND-HELD NIR SPECTROMETERS

In addition to classical analyses of dried fodder, nutritionists and farmers are also interested in the analyses of fresh forage directly at the farm. Indeed, changes in nutrient in the silages can be important according to the season, the place where the samples are taken or in the way of how the silage has been done. Having a real-time analysis tool, even with a more limited accuracy can be very interesting to help the farmers feed their cattle the most efficient way. Miniaturized and mobile NIR instruments allow very frequent forage analysis with immediate results and for a lower cost.

There are various devices on the market that are more or less adapted to the measurement of fresh fodder. Each of them has its specificity and needs to be optimized. In 2018, CRA-W had the opportunity to test the FieldSpec of ASD (Figure 27.13), the microNIR 1700 of VIAVI (Figure 27.14), and the NIR Flame of Ocean Optics (Figure 27.15) on the forages of its experimental farm but also from 12 other farms in Wallonia. The materials tested were mainly maize and grass silage (Figure 27.16).

In this mini-study, the variability is of course very limited by the number of farms and because all the silages analyzed were produced in 2017 and 2018. That’s why presenting RPD and SECV values at this stage of the project were not very significant. What emerges from this study is that analyses of wet samples at the farm are much more complicated than on dried samples in a laboratory, especially for maize.

The main reason is certainly the sample presentation (Figure 27.17) but also the restricted spectral ranges of most of the miniaturized spectrometers, the influence of the external environmental conditions (temperature, rain, and relative moisture), and the unconscious human behavior which

### TABLE 27.3
Performance of NIR Calibrations for Wet Grass Forages (CRA-W Database)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
<th>$R^2$</th>
<th>SEC</th>
<th>RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residual dry matter %</td>
<td>192</td>
<td>13.32</td>
<td>69.39</td>
<td>28.67</td>
<td>13.54</td>
<td>0.99</td>
<td>1.48</td>
<td>9.1</td>
</tr>
<tr>
<td>Protein % DM</td>
<td>222</td>
<td>2.76</td>
<td>29.64</td>
<td>14.79</td>
<td>5.43</td>
<td>0.93</td>
<td>1.85</td>
<td>2.9</td>
</tr>
<tr>
<td>Ash % DM</td>
<td>219</td>
<td>6.00</td>
<td>31.66</td>
<td>11.11</td>
<td>4.24</td>
<td>0.93</td>
<td>1.19</td>
<td>3.6</td>
</tr>
<tr>
<td>Cellulose % DM</td>
<td>218</td>
<td>18.50</td>
<td>34.69</td>
<td>24.82</td>
<td>3.48</td>
<td>0.90</td>
<td>1.38</td>
<td>2.5</td>
</tr>
<tr>
<td>Digestibility of organic matter % DM</td>
<td>218</td>
<td>44.80</td>
<td>93.17</td>
<td>72.96</td>
<td>9.26</td>
<td>0.93</td>
<td>3.08</td>
<td>3.0</td>
</tr>
</tbody>
</table>

*Max, maximum value; Min, minimum value; N, number of spectra; $R^2$, coefficient of determination; SD, standard deviation; SEC, standard error of calibration.*

$$RPD = \frac{SD}{SEC}.$$
FIGURE 27.13  ASD spectrometer. (NIR Laboratory of CRA-W.)

FIGURE 27.14  VIAVI spectrometer. (NIR Laboratory of CRA-W.)

FIGURE 27.15  Flame NIR. (NIR Laboratory of CRA-W.)
unintentionally tends to place the spectrometers at the best place in the silage. Nevertheless, the first results seem to confirm that a dry matter determination is possible with a good accuracy in silage with such instruments.

For the other main parameters such as fibers, digestibility, ash, or protein, the level of accuracy seems to be more limited but acceptable in grass. In fresh maize silage, the sample presentation (unground grain, leaves, etc.) was probably the main limiting factor for a good NIR analysis, resulting in less accurate models as on grass silage.

### 27.9 REQUASUD: A LABORATORY NETWORK FOR NIR ANALYSIS IN BELGIUM

The Walloon Agricultural Research Center manages a network of laboratories called REQUASUD (www.requasud.be). Each of these laboratories owns a Foss XDS NIR spectrometer. The CRA-W is responsible for calibration updates, generating standardization procedures on an annual basis, and checking the results obtained by the organizations of Inter-Laboratories Studies (ILS) for different products (forage, cereals, food, etc.).
The network is driven by the MOSAIC (Foss) system, while the LOCAL methods are used for forage analysis. The principle consists of selecting from a vast database hundreds of spectra similar to the spectrum of the sample being predicted. Specific calibrations for each component are built automatically. The minimal and maximal number of samples selected in the database and the number of PLS factors used have been previously optimized.

These ring tests take place twice a year. The first test is usually conducted in March and focuses on grass (hay and silage), while the second test is usually run in September and targets maize (whole plant and silage). Two blind duplicate samples of each type (fermented and not fermented) are sent to each laboratory. Before being sent, homogeneity tests are conducted to ensure that each laboratory has received identical samples.

The samples are analyzed through wet chemistry procedures and NIR spectroscopy. The requested components are dry matter, crude proteins, cellulose, ash, and digestibility (including starch for maize only). The results are compiled, and a value is assigned to every parameter of each sample. This assigned value corresponds to the average of the results of all laboratories minus outliers. The outliers are identified by statistical tests:

- **Grubbs**: makes possible the detection of outliers in terms of average dispersion. The principle of this test is to compare absolute values vs. reduced deviations
- **Cochran**: makes possible the detection of outliers in terms of dispersion and is applied to the standard deviations of the measurements
- **Z-score analysis**: reflects the consistency between the analytical result and the assigned value. It is a measure of how many standard deviations below or above the population mean a raw score is.

## 27.10 NIR APPLIED TO FECES TO ASSESS THE QUALITY OF FORAGES

Methods for assessing the quantitative and qualitative characteristics of grass are numerous but difficult to apply in the case of grazing ruminants. Several studies have shown that NIRS spectra of feces can be correlated with the chemical or biological composition of samples in order to develop calibrations (Decruyenaere, 2015, Dixon and Coates, 2009, Boval et al., 2004).

Major studies show that NIRS has great potential for estimating in vivo digestibility and voluntary intake by grazing ruminants and that feces are a good indicator of ingested diets. Based on both large or small and varied databases, results suggest that NIRS spectral libraries could be developed for characterizing ruminant feed intake. The accuracy of the NIRS models in estimating in vivo digestibility and voluntary intake is similar to or better than that of other methods generally used to assess these parameters. NIRS could also be used to predict ruminants’ diet composition in terms of plant species. However, these predictions should only be used for ranking, because of the current lack of accurate procedures for determining diet selection individually.

The main difficulty associated with this method is generating diet–fecal pairs as reliably as possible. NIRS calibrations for predicting in vivo diet characteristics are derivative calibrations. The samples analyzed for reference values (diet samples) differ from the samples submitted for NIRS analyses (feces). In connection with research on forages, in vivo trials with animals confined in pens or digestibility crates appear to be the best reference method for generating NIRS calibrations.

Future work will involve developing NIRS calibrations for predicting independent datasets and using them to create decision-support tools for improving diverse grazing management schemes. The focus should be on comparing different feeding strategies rather than obtaining an exact estimate of feed intake values. As a low-cost and rapid prediction technique, NIRS could contribute significantly to the development of a methodology that would help improve our knowledge of forage and animal variability.
27.11 CONCLUSION

In a world where everything needs to be optimized, NIR spectroscopy is a wonderful tool that can replace traditional physicochemical methods to determine the composition of forage and to evaluate its quality. The classical NIR analyses of dry and ground samples are well in place now. The future is in miniaturization and portability. That’s why we are seeing on the market a new generation of hand-held spectrometers. This is only the beginning, and a lot of work has still to be done, but for sure in the future, at least a preliminary analysis of forage will occur at the farm level.

Forage analysis in the field is one of the elements of what we can call “smart farming” where the farms will be equipped with different types of sensors to control and optimize all its processes.

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