

# NIRS: analytical tools in discernment in animal nutrition research and allied Field

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## Abstract

Near-infrared spectroscopy (NIRS) is rapid technique, environmental friendly, non-destructive and achieved with maximum accuracy analytical method to predict the chemical composition of feeds for easy animal feed evaluation as compare to traditional methods of chemical analysis of feed samples and having time consuming and expensive. The majority of the time, no sample preparation is necessary. If samples contain C-H, N-H, or O-H bonds, it is also incredibly flexible. Because of the various types of electromagnetic radiation, spectroscopy and principle are separated into distinct categories for usage in various industries. NIRS accuracy prediction is strongly reliant on acquiring a calibration set that accurately represents variation in the primary population, reliable laboratory analysis, and use of the finest statistical algorithms.

**Keywords:** Animal feed evaluation; chemical composition; chemical analysis; Near-infrared spectroscopy.

## Introduction

Near infra-red (NIR) spectroscopy is a technology that has vast applications in agriculture, veterinary science and allied fields. Near Infrared Spectroscopy (NIRS) was first discovered around 1800 by Frederick William Herschel; the true fathers of NIRS for use in agriculture were Phil Williams and Karl Norris. NIRS is the study of the interaction between matter and electromagnetic radiation (Huck, 2015). The first section of the electromagnetic spectrum to be visible to the naked eye was NIR electromagnetic radiation. The measurement of spectral data as well as interpretation of spectrum data read by computers depends on a variety of feedstuffs. To evaluate sample composition or properties, near-IR spectrometry uses the absorption, emission, or scattering of light in the near-infrared section of the electromagnetic spectrum (700-3000 nm) by atoms or molecules. Animal nutritionists seek a method for measuring the nutritional value of pastures and feeds that is accurate, precise, quick, easy to use, and cost-effective (Smith and Flinn, 1991). The chemical composition of feedstuffs can be determined using near-infrared spectroscopy (NIRS) (Swart et al., 2012). Aside from its speed, NIRS is a physical non-destructive approach with great accuracy that requires minimal sample preparation. In comparison to traditional chemical analysis, NIRS uses no reagents and produces no waste. It is a multi-analytical approach that may provide qualitative and quantitative information about complex sample (Lohumi et al., 2015; Kumar et al., 2016; Abasi et al., 2018; Pasquini, 2018), and it is straightforward to use and operate once calibrated (Givens et al., 1997). The purpose of forage or feed analysis is to forecast animal performance based on the forage and feed used in a ration designed to meet nutrient requirements (Corson et al., 1999). NIR absorption spectra of compounds having functional groups made up of hydrogen bound to carbon, nitrogen, and oxygen are less useful for identification and more valuable for quantitative study. Quantitative applications of NIR spectroscopy are most common, with measurements taken directly from solid meals using either transmission or diffuser reflection. Statistical techniques for multivariate data- Based on the amount of IR radiation absorbed at distinct wavelengths, NIR devices may be calibrated to quantify the levels of various ingredients in a feed sample.

## Principle

Part of the radiation that strikes a solid or granular substance is reflected from the sample surface. Specular reflection is a mirror-like reflection that provides little information about the sample. The majority of specularly reflected radiation returns to the energy source. Before exiting the sample, a portion of the radiation will pass through the sample's surface and be reflected off numerous sample particles. This is known as diffuse reflection, and the diffusely reflected energy emerges from the surface at random angles throughout the year of 180°. The chemical ingredients in the sample can absorb a fraction of the radiation each time it interacts with a sample particle. As a result, the quantity of energy absorbed at certain wavelengths in diffusely reflected radiation contains information about the chemical makeup of the sample. The size and form of the sample particles influence the amount of radiation that penetrates and exits the sample surface. This impact can be compensated either by grinding solid or granular materials to a tiny, uniform particle size with a sample preparation mill, or by applying mathematical corrections when the instrument is calibrated (Givens et al., 1997; Turker-Kaya & Huck, 2017).

## The NIR instruments

Basic NIR spectrometer contains light source, monochromator and detector to get the spectrum of sample

### *Light source*

The light source will produce a beam that will irradiate the samples that will be examined. Halogen light with tungsten filament and quartz window, capable of providing a continuous spectrum in the ranges of 320 nm to 2500 nm, is a commonly used light source. LEDs (Light Emitting Diodes), which may emit up to 1600 nm depending on their composition, can be employed. LEDs do not require a wavelength selection mechanism, unlike halogen lamps must.

### *Monochromator*

The radiation is spilled by monochromators before it reaches the sample. Monochromators include FT, tunable filter, and diffraction grating spectrometers. A diffraction grating is made up of a large number of parallel lines or slits that are separated by a distance equal to the wavelength of light. When a polychromatic beam of light strikes the grating, it is distributed in various directions, with the angle of diffraction being proportional to the wavelength of light. It is suitable for NIRS because of its high detector sensitivity and high source intensity in the NIR band.

### Detector

After wavelength separation, detectors are used to record the signal. In NIRS, photon detectors are the most common. Silicon, lead sulphide (PbS), and indium gallium arsenide are examples of detector types (InGaAs). From the visible area to 1100 nm, silicon detectors are fast, low noise, compact, and very sensitive. PbS detectors are slower, but they're quite common because they're sensitive from 1100 to 2500 nm and have good signal-to-noise ratios. The most expensive InGaAs detector combines the speed and size of a silicon detector with the wavelength range of a PbS detector to create a hybrid detector. NIR spectroscopy employs devices made of semiconductors such as PbS or InGaAs, such as single-channel detectors, multi-channel detectors, and charged coupled devices (CCD) arranged in rows or planes to record multiple wavelengths at once, increasing the speed with which spectral information about the sample can be acquired. Typically, three modes are available to take an NIR spectrum: reflectance, transmittance, and transreflectance (Marcel, 2013).

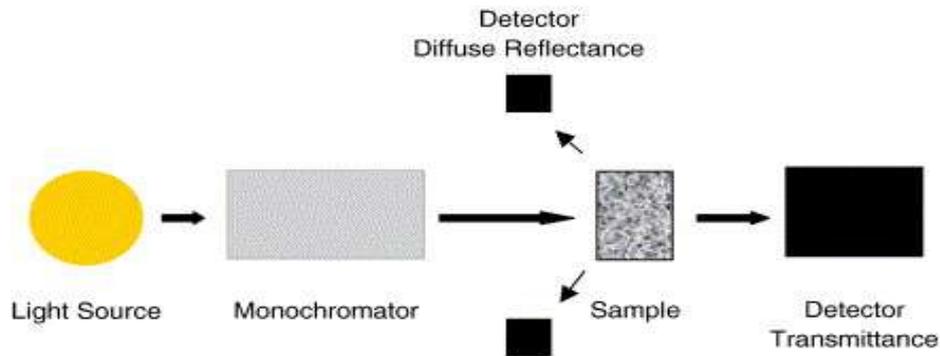


Fig. 1 NIR Instrumentation

### Sample presentation

Slurries and powders with larger sample quantities (>10 gram) are better suited to NIRS. Working on dried and ground food has traditionally been required for sample presentation, but more and more applications are now appearing on intact fresh products (cereals, forages, fruits, meat, etc.). The spectrum must be verified as representative of the sample, and the samples must be dried, crushed, and homogenised depending on the physical form of the product. Wheat flour can be measured as such, whereas fresh coarse fodder must be dried, *ground*, and thoroughly combined. NIR equipment can scan samples ranging from micrograms (NIR microscopes) to several kilos, depending on the sample container.

### Necessities for calibration

For use of NIRS in routine mode, the instrument must be calibrated. For calibration NIRS requires define the product and the area (a village, a province, a country, etc.). Samples with the most diverse origin (in term of soils, climate, growing stages, fertilizers, etc.) and samples preservation for future analyses. Required the prepared and homogenized the samples and spectra of the samples (500 samples). Based on the spectral variation, 50 representative samples which cover the total variance. Wet chemistry analysis must for the selected samples. For accurate analysis, it is to be merge wet chemistry analyses with the spectra and compute a model. Reselect required for few samples (20 from another location or growing season) for analysis the wet chemistry. The results to be sure that the model performance is as per expectation. If it is satisfactory, it helps for routine analyses using the developed equations and if it is unsatisfactory, repetition required until it works satisfactorily.

Because of the high cost of wet chemistry tests, the calibration process is a significant investment that might cost a lot of money. Another option is to look for or purchase models that have been developed over time by specialised companies or research centres. Although purchasing models is a faster way to begin routine analyses than constructing particular and local equations, performance monitoring is still necessary for correct and usable analyses. If the equations don't work, the user should provide some spectra and wet chemistry data to the vendor, who will recalibrate and offer new models when the 'local' samples are integrated (Marcel, 2012).

### Calibration equation

After analysis of calibration sample set by primary reference method (wet chemistry). Instrument calibration can be done by generation of regression equations. Best equation which is able to relate results of wet analysis most appropriately to predicted results of NIRS is selected as final equation. Validation of final equation is done by using new set of samples or similar set used already

Protocols for equation generation are not too specific. A mathematical relationship is required to convert spectral data into quantifiable properties of sample. Basic principle behind spectroscopy is Beer Lambert's law. A variety of methods like classical least squares, Multiple linear regression, Partial least square regression and Artificial neural networks can be used to develop a regression line.

Multiple linear regression used for this purpose is as follows:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_Z X_Z$$

Where, Y is the constituent of interest,  $\beta$  values are regression constants and X values are log (1/ reflectance) measurements or their derivatives at wavelengths 1 to Z.

After this selection and validation of final equation is done with the help of standard error of calibration (SEC) and standard error of performance (SEP) respectively.

$$SEC = [\sum(X_i - Y_i)/(N-p-1)]^{0.5}$$

Where,  $X_i$  is determined by conventional analytical method,  $Y_i$  is the value determined by NIRS, N is the number of samples & p is the number of terms in calibration equation. SEC is equal to standard error of Multiple linear regression.

Validation of final equation can be done either by new sample set or by using the same set of samples. For validation standard error of performance (SEP) can be calculated as under:

$$SEP = [\sum(X_i - Y_i)^2/(N-1)]^{0.5}$$

Where,  $X_i$  and  $Y_i$  are described as above except that  $X_i$  and  $Y_i$  are from different population.

### *Absorption Bands in the NIR Region*

Overtones and combinations are the most common absorption bands seen in the NIR region. C—H, N—H, and O—H are the most common bonds involved. The size and form of the sample particles influence the amount of radiation that penetrates and exits the sample surface. This effect can be compensated for by grinding solid or granular materials to a fine, uniform particle size with a sample preparation mill, or by adding mathematical corrections when the instrument is calibrated. Functional groups with a hydrogen atom connected to a carbon, nitrogen, or oxygen, which are prevalent groups in main food ingredients such as water, proteins, lipids, and carbohydrates, contribute to the NIR area. It's worth noting that the strong absorption bands associated with water OH groups are focused around 1450 and 1940 nm. These bands are the most conspicuous in the spectrum of cheese, which has 30–40 percent moisture, and they are still visible in wheat and egg white samples with lower moisture. Bands formed by -NH groups in proteins (Franklin, 2002).

## **Uses**

Now a day, NIRS routinely used in most of Veterinary Research Institute, National Dairy Research Institute and various Veterinary colleges for feed and fodder analysis in Department of Animal Nutrition and allied departments. Protein, moisture, starch, lipids, ash, oil, and salts are all measured using near infrared spectroscopy in feed (Givens & Deaville, 1999). NIRS for predicting diet quality of animal via fecal scans. NIRs are also employed in grain storage quality, soil quantities such as organic carbon, total nitrogen, and micro elements, protein denaturation, biogenic amines, amino acid composition, lysine availability, and mammalian meat and bone meal contamination in feed (Murray et al., 2019). NIRS in agricultural products, agro-forestry label verification, cereal product, dairy product analysis, and beverage analysis chemicals, petrochemicals, dietary supplement, fish and feed industries, gases, wool, textiles, polymers, pharmaceutical applications, chemical, petrochemical, dietary supplement, fish and feed industries. In each industry, technology is used in a different way, and many applications are tailored to specific organisations and their products and demands. Cheese, meat and meat products, fish (Prieto et al., 2017), egg products, milk, butter, beer, wine, fruit juices, baked goods, cereal, produce, jams, jellies, nutritional supplements, and more have all benefited from NIR spectroscopy (Bhanuse and Patil, 2017; Hazarika *et al.*, 2019).

### *Other applications*

To determine the content and quality of red meat, processed meats, poultry, and fish (Murray et al., 2019). It assesses the chemical elements of a food that have an impact on its end-of-life quality. NIR can be used to inspect incoming raw materials to ensure that vendors are shipping high-quality commodities. NIR can be used to check the product mix to ensure that all ingredients were introduced appropriately. NIR analysis of the finished product can be used to ensure product quality and verify label claims. Before harvest, raw fruit can be analysed using NIR to identify the optimal oil and moisture levels. For sectors that require quick process analysis, real-time results are possible. Qualitative study of raw materials in intermediate and completed products based on their significance in the moisture control, fat or oil control, and control of fat added to the product in the form of spray oil processes.

## Limitations

Wet chemistry must still be used to measure a limited number of samples. Calibration is less accurate than wet chemistry. Measurement outside of the calibration sample range is invalid. Small calibration sample sizes can lead to a false sense of security. It is best for large feed components, not minor ones. Calibrations must be developed with great care because they are matrix specific. For the newbie, the complexity of data handling options can be perplexing. Calibration techniques take time and are only worthwhile when large sample numbers are analysed afterwards. It is expensive instruments. The development of calibrations is the most significant restriction of the technology; different calibrations apply to different products, such as maize leaf vs. sugarcane leaf. NIRS is currently unsuitable for small sample groups. NIRS equipment is cost effective and cost around 20 to 25 lac which attached with computer.

## Conclusion

NIR is wonderful diagnostic tool for feed and fodder samples and allied field's sample analysis in various field. NIR spectroscopy technologies are efficient and provide feed and forage composition predictions. In comparison to traditional method, fast screening techniques for feed and fodder analysis have become increasingly significant in Animal Nutrition labs.

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## Declaration of interest

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