Guidelines for Optimal Use of Near Infrared Spectroscopy (NIRS) with Sample Preparation and Presentation Across Instrument Platforms

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Abstract Guidelines for optimal use of near infrared spectroscopy (NIRS) with sample preparation and presentation across instrument platforms will cover methods and resources specifically for NIRS analysis. Recommendations for sample preprocessing and scanning required to ensure compatibility across calibrations will be highlighted. Proper calibration determinations, monitoring, and performance will be demonstrated to create a baseline for correct use. Additional materials about the use and reporting of nutritive content predictions for publication will be discussed. Goals of this topic session are to develop an understanding of the basics of NIRS technology and provide the basics to what a laboratory is required to have for this analytical technique.

Background

Utilization of Near Infrared Spectroscopy Instrumentation (NIRS) is a resource for laboratories around the world to provide rapid and non-destructive cost savings forage nutritive analysis. This topic session should provide an understanding of the basics of NIRS technology and provide the basics to what a laboratory is required to have for this analytical technique. The Near Infrared Spectroscopy Forage and Feed Consortium (NIRSC, Berea, KY) shares in the effort and costs to produce calibrations for use with continued work to strengthen forage and feed testing and to encompass new technology as it comes available. The NIRSC works toward a vision to lead collaboration and applications for agriculture through educational efforts, promotion of best sample handling and preparation practices, and proper NIR instrument and calibration use. The goal of the NIRSC is to create uniformity in all aspects of NIRS analysis of feeds and forages for the membership and beyond. Our mission is to promote and standardize the use of NIRS through the development of robust, accurate prediction calibrations.
An accompanying resource *Guidelines for Optimal Use of NIRSC Forage and Feed Calibrations in Membership Laboratories* (McIntosh et al. 2022) will be used to guide though some basic principles necessary for a laboratory to perform using best practices and provide an overview of what is necessary to have a NIRS laboratory.

NIRS Methods Demonstrated

Although NIRS analysis of feed and forage is a rapid and effective method for determining sample composition there are limits to its application and these limits must be observed. Failure to observe these limits, can lead to inaccurate or misinterpreted results. The usefulness and applicability of any analytical results are limited first and foremost by the quality of the sample submitted to the laboratory. The best practices in any lab cannot compensate for an improperly collected sample that ultimately does not scan well in the laboratory. Though we have no control over the sampling procedures employed by a lab's clientele, we must make every effort to emphasize the importance of proper sampling techniques to customers. In addition, many NIRSC members are researchers who must be proficient in collecting primary samples for analysis.

Proper Sampling

Laboratories and researchers must be proactive in their approach to proper sampling. The NIRSC offers several different calibrations for a wide variety of feedstuffs. Each calibration was developed to analyze a specific group of materials such as legume hay, grass hay, mixed hay, haylage, and both fermented and un-fermented corn silage. Each product calibration includes multiple constituents, or parameters. For

NIRS results to be reliable, it is imperative to limit calibration application to the range of sample types known to be included in the calibration set. The NIRSC cannot stress the importance enough about proper sampling and the techniques that facilitate representative sample collection. Protocols must be observed before the sample meant for analysis reaches the laboratory. Laboratories cannot fix an unrepresentative sample; the laboratories can only analyze it. For ways producers misuse forage analysis refer to *Common Abuses of Hay Testing Results* (Putnam, 2019). When improperly collected samples arrive to the lab (e.g., extremely small samples, grab samples, or cores from a single bale) potential discrepancies among laboratories can be minimized using these proactive measures rather than dealing with sampling issues in a reactive manner.

Sample Handling

Sample handling is easily the most significant contributor to the accuracy and precision of analytical results. Errors can be introduced to the system in the laboratory at any stage from splitting and sub-sampling to contamination and induced physical alterations such as using grinders and equipment not for forage and feed preprocessing. These errors add to the errors introduced in obtaining the primary sample. Heterogeneity of forage material makes the processes of splitting and sub-sampling prone to errors. Not all particles within a sample exhibit the same composition (e.g., a sample may contain particles of leaf and particles of stem), thus it is vital that a sample and associated sub-sample accurately reflect the makeup of the entire lot. Achieving this accurate reflection is further complicated by the non-random distribution of particles within the given sample at any stage of collection or analysis. Sufficient sample size and proper mixing are the best mechanisms to mitigate these effects. Samples are also vulnerable to physical or chemical alterations induced by improper sample handling, chemical treatments, mold, and moisture. Contamination from either foreign sources or separate samples, electrostatic separation, or 'loss of fines', and the effects from grinding or excessive heat exposure (e.g., Maillard Reaction) are known to alter the chemical makeup of forage material and can alter analytical results.

When a sample is received by the laboratory the NIRSC recommends that the entire laboratory sample submitted be dried and ground for analysis. Any laboratory sample must be handled and documented for evidentiary integrity so that the test result(s) can be traced back to the lot. This includes controlling error during processes that select a portion of the laboratory sample and during processes that prepare the sample and includes reception of the laboratory sample. As follows, any handling or manipulation of a sample in any stage must preserve the primary sample integrity (AAFCO 2018; Thiex and Ramsey 2016). Therefore, sample preparation that converts the sample received at the laboratory into a homogeneous material suitable for analysis (Undersander et al. 1993) must attempt to keep sampling and analytical errors as low as possible.

Sample Grinding

Grinding is the most physically intensive process to which samples are exposed prior to analysis. Particle size is a very important factor in NIR analysis because finer milled particles are more reflective to NIR light, provide adequate surface area, and create a homogeneous sample (Murray and Cowe 2004). Due to the complex processes occurring during grinding (e.g., leaves and stems may require different amounts of time to pass through the screen it is vital that all material which enters the mill be collected into the final sample container. These processes may also introduce striations into the sample. Additionally, improper grinding protocols may lead to heating of the materials, which may alter DM or other analysis procedures for NIR or wet chemistry (Undersander et al. 1993; Williams 2008; Williams et al. 2017). The publication ISO (2012) provides performance tests for particle size reduction (grinding) to determine grind quality and recovery. Regardless of grinding equipment, all material for NIRS analysis must have a final pass through a 1 mm screen of a cyclonic mill. Abrams (1989) showed that the finer and more uniform particle size produced with 1 mm cyclonic milling reduced Standard Error of Calibration (SEC), and ISO (2012) reports that expected coefficient of variation goes down with smaller particle sizes. It is important to

make sure the same grinding method which was used in developing the calibration is used for routine analysis.

Sample Preparation

It is the premise of NIRSC that all samples that are received will be analyzed by NIRS and subsequent wet chemistry if required. Even though there are portable NIR devices and other NIR technologies that can analyze samples of standing forage, harvested piles, and stored feed; the standard presentation for laboratory forage NIR analysis is a dried and ground sample. Samples that are dried and ground remove variables including analysis interference from water and heterogeneity and stabilizes the material (Murray and Cowe 2004).

Drying samples in the laboratory has two purposes. First, the laboratory sample (AAFCO 2018) must be dried enough to process it further for subsequent analysis; and a test portion must be used to determine the dry matter of the forage material submitted to the laboratory. All samples, regardless of the judged dryness of the laboratory sample, should be dried further before pre-processing or scanning. Samples should be dried in a 55°C forced-air oven (Undersander et al. 1993). Oven drying is generally preferred over microwave protocols (Murray and Cowe 2004) and is the only acceptable method for subsequent NIRS analysis using NIRSC calibrations. Drying at higher temperatures (>55°C) may cause chemical changes in the sample that could alter the subsequent constituent analysis. All samples analyzed by NIRSC calibrations must be oven dried to obtain reliable results. Please note that there are different types of drying for use in reporting lab analysis (as-received/as-is) and the dryness level needed to process a sample to scan it properly using NIRS instrumentation. Predicted dry matter or moisture in a sample is the presented moisture content of a sample to the NIR instrument at time of scanning.

Moisture in samples can cause issues in correctly predicting constituents and data fluctuation across a project or subsampled material. The optimal range of water in a ground sample for NIR analysis is roughly between 93.5-97% Dry Matter (DM) (McIntosh et al. 2019). McIntosh et al. (2019) showed that samples scanned at between 84-93.5% DM underestimated, in most samples (unless noted below with a different scanning range) crude protein, overestimated fibers and ash, and underestimated sugars, carbohydrates, and digestibility.

Optimum Scanning Ranges (McIntosh et al. 2019):

- Grass Hay (GH) 95 to 97% DM
- Mixed Hay (MH) 94 to 97% DM
- Legume Hay (LH) 93.5 to 95% DM
- Haylage (HL) 93.5 to 95.5% DM
- Corn Silage (CS & UC) 94 to 95.5% DM.

Sample Presentation

Determining if the sample(s) about to be scanned is within the recommended scanning range by product it is always good practice to randomly select a few samples and scan them for current dry matter (DM). Proper technique for loading dried and ground material into a sample cup for NIR scanning is vital to obtaining accurate spectral data. If the dried and ground sample is not handled properly while packing a sample cup, the resulting analysis will not be representative of the sample. For example, a non-homogeneous ground sample will result in a distorted sample prediction, while a dusty sample cup or instrument window can produce a faulty artifact in the sample spectra, therefore altering predicted value. Consistency and thoroughness in packing a sample cup for scanning will help produce NIR analyses representative of the original sample. In addition, consistency among NIRSC labs in preparing samples for NIR scanning will help reduce discrepancies between laboratories.

Outcomes

The materials and methods demonstrated in this session are to provide basic understanding of NIRS technology and calibration use across instrumentation; not only specific to member laboratories but also relates to any laboratory using this technology. Recommendations for sample preprocessing and scanning required to ensure compatibility across calibrations will be highlighted. Proper calibration determinations, monitoring, and performance will be demonstrated to create a baseline for correct use. Additional materials about the use and reporting of nutritive content predictions for publication will be discussed.

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