



Guidelines for Optimizing Accuracy and Consistency in the NIRSC Laboratory

INTRODUCTION

1. NIRSC Organizational Goals

The NIRSC Forage and Feed Testing Consortium (NIRSC) was formed in 1992 through the efforts of several universities and commercial entities because of their interest in promoting consistent and quality forage testing results for the agricultural public. The group took on official form by electing a Board of Directors, adopting by-laws, and incorporating in June of 1998 and by becoming a federal non profit in 2002 as a 501(c)(6). The mission of the NIRSC is to improve the accuracy and understanding of NIRS (near-infrared reflectance spectroscopy) testing of forages and feeds.

NIRSC carries out its mission by supporting the overall proficiency of our members. The organization does this through several activities: standardizing instruments, managing an instrument monitoring program, supporting member operator skills through workshops, monitoring and updating equations, developing new equations, participating in forage quality testing research, exchanging information between members, and promoting optimal practices. These activities improve business conditions for all of the forage and feed testing industry by promoting accuracy and reliability of the NIRS instrument through uniformity, standardization, and good practices. The last activity, promoting optimal practices, is the focus of this document and collection of resources.

2. Goals of this Collection/Document

The NIRSC includes laboratories that process a tremendous volume of feed and forage samples. As such the consortium possesses the potential to significantly

influence the quality and direction of NIRSC analysis throughout the country. This potential may be limited by the homogeneity and unity of member organizations in their processing of samples and application of NIRSC equations. The quality of internal operations at each member lab can likewise influence the consortium's ability to speak and act (or be perceived to do so) with a single, reliable, and authoritative voice.

The NIRSC has assembled this document to help guide members toward optimum performance. There are two parts to this document. Part I describes NIRSC's recommended internal monitoring methods and external monitoring methods. Internal monitoring includes those actions taken within an NIRSC lab at that lab's discretion to monitor equation and/or instrument performance. External monitoring are methods set up and carried out by NIRSC to monitor equation performance and to monitor and maintain NIRSC member instruments. Part II of this document outlines specific steps and procedures recommended when handling samples for subsequent NIR analysis.

There are several specific goals we plan to achieve through this document.

- 1) Reduce as much as possible the variation in results from member labs by optimizing our collective accuracy in sample analysis. This can be achieved by promoting proper sample handling and processing and by discouraging practices that may reduce the accuracy and applicability of NIRSC equations.
- 2) Improve each member's ability to monitor internal performance by providing resources to assist with quality assurance and quality control (QA/QC). A corollary to this goal is to encourage members to utilize such resources.
- 3) Provide clear definitions of NIRSC equations and proper report notation to properly represent NIRSC member participation to clients. Please see Appendix I—Integrity of Equation Use for the official NIRSC Statement of Use and Definitions.

3. Need for this Collection of Sample Handling Methods for Quality Assurance

The NIRSC has historically supported the overall proficiency of its members, but a comprehensive description of sample handling from sample collection through sample processing for the specific purpose of NIRSC analysis has been lacking. In order for there to be confidence in results generated from NIRSC equations, uniform sample handling methods must be used based on authoritative resources. The following document aims to help the NIRSC lab identify and describe sample handling steps and provide useful authoritative resources on protocol for each step.

PART I: DESCRIPTION OF NIRSC'S INTERNAL AND EXTERNAL MONITORING MEASURES

A. INTERNAL MONITORING MEASURES

Many laboratories have internal monitoring programs. These suggestions are not meant to replace but rather to augment existing programs. They are also directed toward new member labs to provide them with the tools necessary to maintain the high quality results that are expected of NIRSC member laboratories. Finally, members new to NIR analysis may be unfamiliar with the variety of methods available to monitor and insure quality results. The reputation of the NIRSC as a whole can be no better than the reputation of any single member.

NIRSC members are not required to implement internal monitoring programs. However, any laboratory with a commitment to providing accurate and reliable results should also commit to tracking internal performance of their instrument(s) and equations.

First and foremost, it should be stated that all sample introduction material and ground sample should be maintained at the same temperature as the instrument to limit variation in spectra caused by temperature fluctuation.

There are three measures the NIRSC advocates for maintaining optimum internal monitoring of NIR results beyond those recommended by the instrument manufacturer.

- 1) Use of NFTA samples for both instrument and equation monitoring.
- 2) Use of NIR spectra available to members through the NIRSC website for equation monitoring.
- 3) Use of NFTA bulk samples as internal standards or check cells for both instrument and equation monitoring.

Each of these measures is explained in detail below.

1. NFTA Samples for Quality Monitoring

The NFTA results from each participating NIRSC member are recorded, compiled, and analyzed. All labs' submissions are confidential. Summaries of results are coded so that lab identity and anonymity is preserved.

The goals of this project are:

- a. To determine how consistent NIRSC labs are in their predictions. We need to include a protocol here for labs to work with Paolo when having trouble with NFTA samples and to make a log or history of NFTA sample failures.

- b. To determine whether an equation could perform better on certain samples and/or DM, CP, ADF or NDF prediction.

2. Spectra for Member Internal Checks

It is a possibility that some type of corruption could occur, though rare, in an NIRSC equation. NIRSC has spectra available on its website so that members can test that NIRSC equations are functioning properly and are not corrupted and that instruments are functioning properly, and to highlight possible standardization problems. Spectra files of several products with acceptable ranges of prediction values are posted on NIRSC's website. Instructions on how members may download the files and test the equations are posted with the spectra. NIRSC offers as much support as possible to facilitate the process, but labs must perform checks on their own schedule. This creates the opportunity for users to have ownership of the process and responsibility to check that results reported to their customers are accurate.

3. Use of Bulk Samples as Internal Quality Control Standards

Rationale

Labs that participate in the National Forage Testing Association (NFTA) Proficiency Program have a supplemental quality control method available to them. The use of NFTA bulk samples as quality control standards can help monitor the performance of their NIR calibrations and/or instrument.

Recommended Procedures

When using NFTA samples as quality control standards, it is prudent to find a sample that is representative of samples typically received by your lab. The NFTA samples all have Reference Method Averages (RMAs) for Dry Matter, Crude Protein, Acid Detergent Fiber (ADF), and Neutral Detergent Fiber (NDF), against which participating labs are statistically graded. The RMAs are determined from the results of all labs using an NFTA approved reference method. This is indicated by each yearly questionnaire.

When using NFTA samples as standards, a portion of the NFTA bulk sample is packed and sealed in a NIR Sample Disk. This sample should be mixed with, and repacked from an NFTA sample on a weekly basis, so as to eliminate sample deterioration possibilities. Each time the sample is then scanned, the results are measured against the corresponding RMAs. It is recommended to scan the sample at least weekly; however, a daily scan would be better for detecting drift. If you are in need of sample, NFTA will send duplicate samples bags of specifically requested certification samples for a fee of \$25.00 per bag. RMAs are also available for Phosphorus, Calcium, Potassium, Magnesium, Sulfur, ADF-CP, and Lignin for labs that measure these constituents by NIR.

NIRSC commercial laboratory membership requires participation in the NFTA Proficiency Program. If you are interested in finding out more information, this can be found on the NFTA website at <http://www.foragetesting.org>.

Resources

NFTA Website

<http://www.foragetesting.org>

B. EXTERNAL MONITORING MEASURES

Here we will describe NIRSC's instrument monitoring program. Although it is limited to FOSS instruments at this time, the theory of instrument monitoring may be described. We have some documents from Paolo from early 2000's on that. Here we will also describe instrument standardization. Again limited to FOSS instruments, we can describe the theory of checking instrument integrity over several instruments and over time and how this is important in achieving accurate predictions from NIRS equations.

PART II: SAMPLE HANDLING STEPS AND RECOMMENDED PROCEDURES

Sample Collection

Rationale

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.

Though we ultimately have no control over the sampling procedures employed by our clientele, we must make every effort to emphasize the importance of proper sampling techniques to our customers.

The following resources may help educate customers about both the importance of proper sampling and the techniques that facilitate representative sample collection.

Recommended Procedures

The NFTA website (www.foragetesting.org) provides a 10-step protocol for proper hay sampling. There is also an online test to become a certified hay sampler that

requires the applicant to correctly answer a series of pertinent questions about the hay sampling process.

For silage and mixed rations, the University of Wisconsin Extension service offers a publication titled “Sampling hay, silage, and total mixed rations for analysis.” This document is available online (<http://learningstore.uwex.edu/pdf%5CA2309.pdf>) at no charge. Copies can also be ordered at a cost of \$1 each. Search for publication A2309 at <http://cecommerce.uwex.edu/OrderPubLookup.asp>.

Labs must be proactive in their approach to informing clients about proper sampling. The NFTA offers instructional posters that can be displayed at your place of business. When clients bring improperly collected samples to the lab (e.g. extremely small samples, grab samples, or cores from a single bale), take advantage of the opportunity to provide guidance and instruction. Direct the client to relevant posters, publications, and the NFTA website. Potential discrepancies between laboratories can be minimized by the use of these proactive measures rather than dealing with sampling issues in a reactive manner.

Resources

Sampling hay, silage, and total mixed rations for analysis
<http://www.uwex.edu/ces/crops/uwforage/Feeding.htm>

NFTA website
<http://www.foragetesting.org>

Subsampling Undried Forages in the Lab

Rationale

The National Forage Testing Association (NFTA) website in the Laboratory Procedures/Sample Preparation section, describes sample preparation as the following: “Laboratory sample preparation is the process of converting the sample received at the laboratory into a homogeneous material suitable for analysis. This process generally involves drying and/or grinding.” In a perfect laboratory world, all samples received would be of the perfect size to allow drying and grinding of the entire sample. However, we know that reality forces most labs to subsample large samples on a daily basis.

Recommended Procedures

The NFTA website states that most forage samples fall into one of three categories:

1. Those dry enough to grind and analyze immediately, >90% Dry Matter(DM).
2. Those dry enough to be coarsely ground but too wet to be finely ground, 85% - 90% DM.
3. Those samples <85% DM which need to be partially dried before the sample can be coarsely ground.

It is the premise of NIRSC that all of the samples that are received will be analyzed by NIRS. Following that premise, NIRSC will follow the recommended procedure for samples <85% dry matter. The samples will be partially dried enough to be finely ground and packed into a NIR disk for analysis. The procedure for this method is as follows:

- Remove sample from shipping container and discard any roots from plants and brush off dirt. Note and report removed material and any other sample manipulation.
- Chop samples of whole plants into about half-inch pieces using either hand clippers or a laboratory forage chopper. Cut open stalk or corn cob pieces to facilitate drying. Include any ears attached to the plants. When using the laboratory chopper, be sure to brush any sample adhering to the sides of the chopper into the receiving tray. Silages and haylages generally have particle lengths less than 1 inch and do not require chopping.
- Place the chopped sample into a clean dishpan or on a clean plastic sheet. Mix thoroughly.
- The NFTA recommendation for subsampling includes coarse grinding all material if the entire sample submitted is greater than 75 grams.
- If the entire sample cannot be dried, reduce the sample size by making a cone of sample and quartering. Save opposite quarters. Repeat mixing, coning and quartering until the volume is reduced to an appropriate size. Make certain that representative ratios of leaf and stem occur in each pile.
- Transfer reduced sample to a tared container for drying.
- Dry the reduced sample using either forced-air oven or microwave oven.
- Grind the partially dried sample to fineness desired for analysis in appropriate grinder.
- Thoroughly mix the ground sample. Transfer to an airtight container and label immediately.

A more detailed description of this procedure can also be found on the NFTA website in the Laboratory Procedures/Sample Preparation section. Also included is a list of needed equipment and safety precautions.

AAFCO also discusses mass reduction in *Guidelines for Preparing Laboratory Samples*, noting that sample mass may be reduced by splitting or subsampling. AAFCO recommends a selecting a minimum of 30 increments of a sample for

preparation. Heterogeneity errors are reduced with more increments, and fewer than 10 increments is never recommended. Grinding less than ¼ of the submitted sample prior to mass reduction is not acceptable.

Resources

National Forage Testing Association (NFTA) Website
<http://www.foragetesting.org>

Association of American Feed Control Officials Incorporated. 2000. *Guidelines for Preparing Laboratory Samples*.

Drying

Background

Sample integrity must be maintained throughout all aspects of laboratory testing. Sample drying is important for all samples that come into the laboratory. No matter how dry samples come into the laboratory, they will need to be dried to meet the NIRSC sample prep method used in calibration development.

Recommended Procedure

For all forages, it is necessary to partially dry them prior to fine grinding. Drying can be done by either a 55-60 degree C forced-air oven or a microwave oven. Drying at higher temperatures (>60 degrees C) can cause chemical changes in the sample that will adversely affect the subsequent fiber, lignin or acid detergent insoluble nitrogen analysis. The drying method for a sample to be analyzed by NIRS should be consistent with the drying method used for samples in the NIRS calibration.

NIRSC Equation	Acceptable Drying Method(s)	
	Equation Variant I	Equation Variant II
Alfalfa Hay	oven/microwave	oven
Grass Hay	oven/microwave	oven
Mixed Hay	oven/microwave	oven
Haylage	oven/microwave	oven
Corn Silage	oven/microwave	oven
Alfalfa Breeders Equation	oven	

The Alfalfa Hay, Grass Hay, Mixed Hay, Haylage, and Corn Silage equations all have variants of the equation that contain a digestible fiber parameter. Please note that oven drying is always the acceptable method and it is the mandatory method for fiber digestibility.

NFTA Forage Analysis Procedures, July 1993 offers an excellent reference to this procedure.

Section 2.2.1.1 Partial Dry Matter Using Forced Air Ovens

Section 2.2.1.2 Partial Dry Matter Using Microwave Ovens

These procedures are summarized below.

Partial Dry Matter Using Forced Air Drying Ovens-Part 1

Step 1: Dry empty pans at 55-60 degrees C for 2 hours.

Step 2: Weigh empty pans on a top loading balance and record the weight (W1) to the nearest 0.01g.

Step 3: Place a representative amount of forage into the pan. Fill pan to a maximum sample depth of 1.5 inches. Record weight of pan and wet forage to the nearest 0.01g (W2).

Step 4: Dry in a forced air drying oven at 55-60 degrees C for 16-24 hours. Be sure to allow air flow between the pans.

Step 5: Remove pans from oven and allow to air equilibrate for about 15 minutes, then weigh pan and dry forage to the nearest 0.01g (W3).

Partial Dry Matter Using a Microwave Oven-Part 1

Step 1: Dry paper boats in microwave oven for 3 minutes on full power.

Step 2: Weigh empty boat to the nearest 0.01g (W1).

Step 3: Place a representative sample in the boat and record the weight of the boat and the sample to the nearest 0.01g (W2).

Step 4: Dry in the microwave carefully to avoid hot spots, charring and fire. For the average hay, initially dry for 2 ½ minutes at 30% power. Remove from microwave and stir to allow moisture to escape. When cool, weigh the boat and forage. Place back in microwave and dry for 2 ½ minutes at 20% power. Repeat until successive weighings are less than 0.7 grams.

Step 5: After drying is complete, equilibrate to room temperature a final time until cool. Record the final dry weight (W3) of the boat and dry forage to the nearest 0.01g.

Dry matter of partially dried forages should be between 85-95%.

Laboratory Dry Matter of Partially-Dried (Oven) Forages-Step 2

Step 1: Dry aluminum dish with cover at 104 degrees C for at least 1 hour.

Step 2: Remove dishes and covers from oven and place in a dessicator and allow to cool. Weigh dishes with cover (W4) to nearest 0.1mg.

Step 3: Add approximately 2 grams ground sample to each dish and record the weight of dish with cover and sample (W5) to the nearest 0.1mg. Gently distribute the sample uniformly in the dish to expose the maximum area for drying.

Step 4: Dry in a preheated oven to 104 +/- 1 degree C for 3 hours +/- 5 minutes. Place dishes in oven so that air can circulate freely.

Step 5: Remove dishes from oven and place cover back on each dish when transferred to the dessicator. Allow to cool.

Step 6: Weigh dish with cover and dried sample (W6) to the nearest 0.1mg.

Determination of Total Dry matter using the Two-Step Procedure

$$\text{Partial DM} = \frac{(W3 - W1)}{(W2 - W1)} \times 100$$

$$\text{Lab DM} = \frac{(W6 - W4)}{(W5 - W4)} \times 100$$

$$\% \text{ Total DM} = \text{Partial DM} \times \text{Lab DM}$$

Where:

- W1= empty weight of container in grams
- W2= wet weight of forage and container in grams
- W3= dry weight of forage and container in grams
- W4= empty weight of dish with cover in grams
- W5= initial weight of sample & dish with cover in grams
- W6= dry weight of sample & dish with cover in grams

Calculation: Percent Total Moisture

$$\% \text{ Total Moisture} = 100 - \% \text{ Total DM}$$

Resources

NFTA Forage Analysis Procedures, July 1993.

Association of American Feed Control Officials Incorporated. 2000. *Guidelines for Preparing Laboratory Samples*.

Intrasoft International. 1995. NIRS 1 Version 3.10 Routine Operation Software for Near Infrared Instruments.

Grinding

Background

Good analytical data requires that samples be representative of the whole lot and that their integrity has been ensured during transport to the lab and during their preparation.

Recommended Procedure

Regardless of grinding method, all material for NIRS analysis must pass through a 1 mm screen of a cyclone mill (UDY, Cyclotec or equivalent). It is important to make sure the same grinding method is used in developing the calibration as well as for routine analysis. It maybe necessary to reduce sample bulk by grinding through a cutting mill first, then to regrind through a cyclone mill.

NFTA Forage Analysis Procedures, July 1993 offers an excellent reference to this procedure.

Section 1.1 Grinding with a Cutting Mill

Make sure the mill is clean, then insert appropriate screen into the mill. If a fine grind is desired, most samples are ground with a 1 mm screen. If large samples are to be ground, you may grind them using a 4 or 6 mm screen and then regrind using the 1 mm screen. Following your mill's operating instructions, place the entire sample into the grinder and allow it to completely pass through. A higher pitched "empty" sound, may be noted. Remove the sample container and hold it beneath the grinding chamber as you open the mill. Sweep any incompletely ground residue from the mill into the container. Remove the screen and transfer residue from it into the sample container. Mix thoroughly before analysis. Clean entire mill using air and/or a brush.

Section 1.2 Grinding with a Cyclone Mill

Make sure the mill is clean, then insert 1 mm screen to be used to properly grind all NIR samples. Insert a clean sample bottle beneath the clear plastic cyclone and turn the mill on. Following your mill's operating instructions, present the sample to the mill and allow it to grind the entire sample. Shut off the mill. Mix thoroughly before analysis. Clean entire mill using air and/or a brush.

Resources

NFTA Forage Analysis Procedures, July 1993.

Intrasoft International, 1995. NIRS 1 Version 3.10 Routine Operation Software for Near Infrared Instruments.

Mixing a Dried & Ground Sample

Rationale

Mixing a dried and ground sample is an important step before NIRS analysis or wet chemical analysis. Grinding stratifies a sample and mixing is necessary afterwards. If the dried and ground sample is not mixed properly, the resulting analyses will not be representative of the sample.

Recommended Procedures

The NIRSC recommends the following methodologies. Two different methods are listed.

Method One

US Dairy Forage Research Center: Dave Mertens, 2006:

Place a sheet of paper approximately 18" square on a flat surface and pour the entire ground sample near the center but offset slightly towards one corner. Mix the sample by pulling the corner nearest the sample pile towards the diagonally opposite corner, so that the sample mixes as it rolls. You will observe the sample color becoming more uniform as you mix. Usually 12 rolls are sufficient, but it is impossible to 'over-mix' the sample.

If the entire sample cannot be used, take at least 3 subsamples from different parts of the mixed pile using a putty knife. Ensure that the knife runs along the paper surface so that fines are included in the subsample. One advantage of this technique is that fines are rolled throughout the sample and tend not to be lost at the bottom.

Place the sample in a cup or bag. Cups are preferred because they allow the user to re-mix the sample before packing NIR cups. The body of the cup, and not just the lid, should be labeled so that accidental lid-switching cannot cause problems.

Method Two

University of Minnesota Forage Quality NIRS Laboratory, 2001

Sample becomes stratified due to fine grinding (cyclone) and needs remixing. A 15-gallon plastic drum is used as a sample tumbler. Place 70 - 100 samples (in 4 oz. plastic cups with sealed lids) into tumbler for 15 minutes. The drum rotates at 15 rpm and contains a rod that lifts and drops the bottles to provide a random tumbling.

In tests using NIR, subsamples from the same bottle were nearly identical. Standard errors for laboratory chemical procedures also decreased due to the more uniform subsampling from the tumbler.

Whirlpaks can also be tumbled by sealing open end with tape and providing an air space for sample movement.

If samples sit longer than 4 weeks before analysis, tumbling is repeated due to possible moisture stratification within the sample.

Resources

Packing and Scanning a Sample for NIRS

Rationale

Loading a dried and ground sample into a sample cell (ring cup) for NIRS scanning is an important last process in obtaining analysis scan results. If the dried and ground sample is not handled properly while packing a sample cell, 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 cell window or instrument window will produce a faulty artifact in the sample prediction.

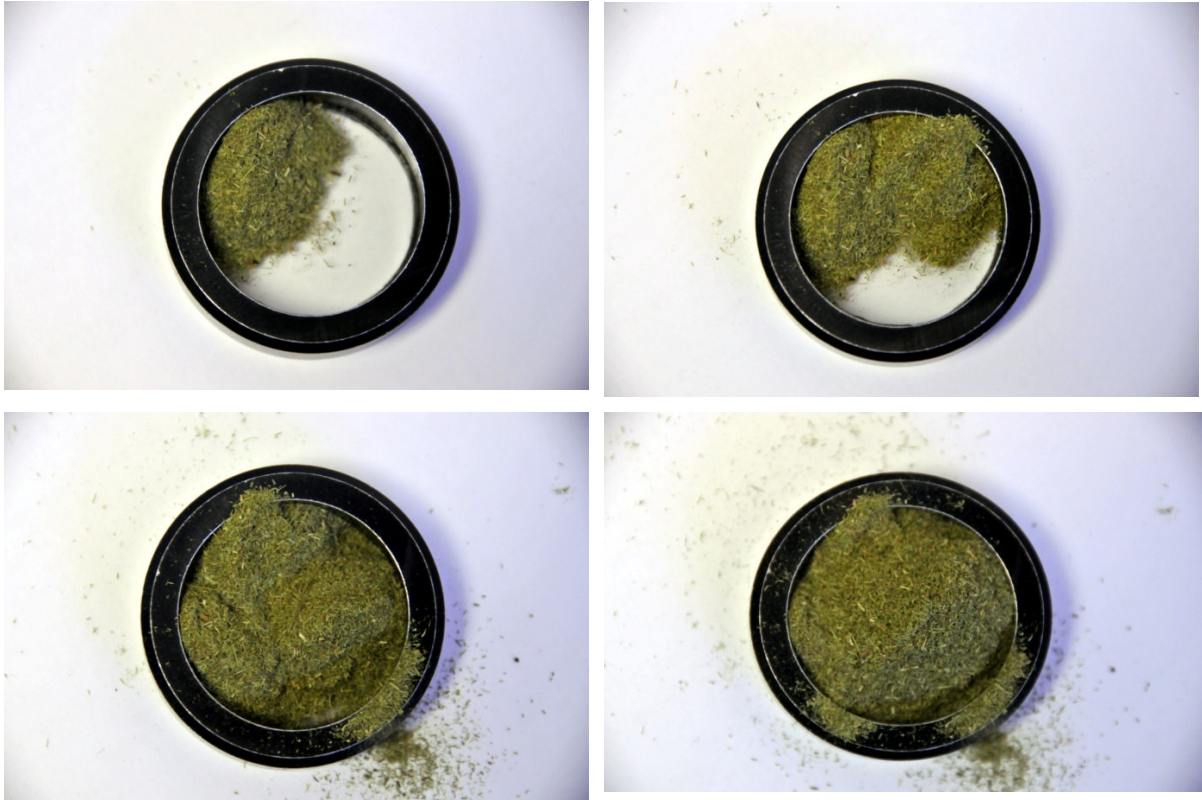
Consistency and thoroughness in packing a sample cell for scanning will help produce NIRS analyses representative of the original sample. In addition, consistency among NIRSC labs in preparing samples for NIRS scan will help reduce discrepancies between laboratories.

Recommended Procedures

Packing a dried and ground sample is described by NFTA under laboratory procedures information (www.foragetesting.org) beginning with section 2.2.2.4 “Dry Matter by Near Infrared Reflectance Spectroscopy.” Here general drying to 90-95% dry matter and grinding to pass a 1mm cyclone screen are covered, as well as subsampling the ground sample and filling a sample cup for NIRS scan. But see the sections on drying, grinding, and mixing in this document for recommendations on these methods specifically related to NIRSC equation use.

The NIRSC recommends the following methodology, which is the protocol used when scanning samples on the NIRSC Master Instrument.

1. **Mix Sample:** for samples in bags, stir with a spatula; for samples in cups, tumble specimen cups 20 half-turns. Mixing of dried and ground samples is important in order to alleviate any settling and stratification that might have occurred from grinding. Stratified samples produce varying size fractions in the ground sample and in turn produce different analyses results.
2. **Subsample:** Using a spatula, take three subsamples by scooping from separate areas of a sample bag or specimen cup. Scoop from three locations vertically as well as horizontally. If samples are in cups, rotate cups as subsampling occurs. Fill a ring cup in three quadrants with these subsamples. Overfill the remainder of the ring cup with additional subsamples.



3. Prepare the Ring Cup: Strike off excess sample from the ring cup with a straight spatula edge. Press the cardboard back onto the ring cup and tap the cup gently on its edge twice to dislodge dust. (Tapping more than twice may cause stratification.) Brush all cup surfaces with a soft brush to avoid dust transfer to the inside of the instrument. Brushing dust off the external cup surfaces is extremely important when using an autosampler (see recommendation #4). Load the cup into the instrument and scan.
4. Cleaning the Instrument scanning Window: Clean weekly. High T-values from check cell predictions are an indicator of dust on the window and that cleaning is overdue.
 - a. Autosampler: Remove the autosampler first, then rest autosampler on a soft surface, remove window, and wipe both sides of the window with a Kimwipe moistened with glass cleaner. **WARNING!! The white ceramic is usually positioned under the window. Don't touch the white ceramic!! Use a very soft brush and a vacuum cleaner to remove all the dust that may be deposited inside the autosampler.** Be sure to also apply vacuum/compressed air alternately two or three times through the apertures for entrance and exit of ring cups.

Dust may have spread around and gotten trapped in some dead corner.

- b. Spinning drawer: Remove the white ceramic (two screws) and if it is dusty, gently brush it off with a soft brush. Vacuum the inside of the drawer.
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5. Duplicate Scans: This is a more advanced and time consuming procedure than single scans. It is used by NIRSC for critical spectra collection such as master scans for calibrations. Pack two cups of the same sample at the same time and scan consecutively. After scanning, label the duplicate spectra records with the same number or name. Move the duplicates into two spectra files (such as Rep_1 file and Rep_2 file) and use the Contrast Spectra function in WinISI to look for spectra that differ considerably. NIRSC recommends re-running samples in which the two replicates exhibit RMSC >3500.

Resources

NFTA website

<http://www.foragetesting.org>

Equation Use

Rationale

NIRS analysis of feed and forages is a rapid and effective method of determining sample composition. There are limits to its application, and these limits must be observed. Failure to observe these limits can lead to inaccurate results.

The NIRSC offers nine different equations to analyze a wide variety of feeds and forages. Each equation was developed to analyze a particular group of materials, such as alfalfa hay, grass hay, legume and grass haylages, or fermented corn silage. For NIRS results to be reliable, it is imperative to limit equation application to appropriate materials.

Recommended Procedures

NIRSC equations are supplied with appropriate files to report Global H (GH) and Neighborhood H (NH) values for all analytes. It is important to monitor these

values and deal appropriately with samples yielding GH and NH values beyond acceptable thresholds.

For Global H, the limit of reliable results is 3.00. Any sample with a GH higher than 3.00 should be acknowledged as having a poor NIRS match. Wet chemistry analysis may be necessary to confirm analyte values.

Different sources cite different values for the acceptable limit for Neighborhood H values, though they are typically between 0.6 and 1.2.

Samples yielding high values for GH and/or NH are excellent samples to add to the equation calibration. Spectra from such samples should be saved and sent to the NIRSC for possible inclusion in the calibration file update.

Reporting results for samples that yield high GH and/or NH values without discussing the situation with the client or checking the values with wet chemistry analysis is a disservice to the client. This practice may also lower the quality and reputation of the laboratory, and by extension, the NIRSC.

NIRSC equations are developed using sample sets that have been dried and ground. These sets include samples that have been dried and ground according to multiple methods (e.g. microwave and oven drying, single- and multi-step grinding). Thus NIRSC equations are robust and multiple methods of sample drying and grinding may be successfully employed for use with NIRSC equations. However, all samples should be dried and ground in a manner consistent with the methods used to prepare samples for the NIRSC equation development.

Note: Oven drying must be used for fiber digestibility and RUP analysis on corn silage, hay, and haylage samples.

Please refer to Appendix I for details regarding equation definitions, spectra ownership, and NIRSC logo usage.

Resources

Foss International, NIR Instrument Maintenance and Diagnostics Workshop Manual.

Infrasoft International. 1995. NIRS 1 Version 3.10 Routine Operation Software for Near Infrared Instruments.

NIRSC. 2002. NIRS Consortium Annual Meeting. NIRS—Getting Started.

Sample Storage

Rationale

Most labs retain feed and forage samples. It is important to prevent sample deterioration during this storage interval so that additional analyses may be performed or previous results confirmed.

Recommended Procedures

Effective storage of feed and forage samples is simple. Because samples prepared for NIRS analysis have been dried to below 10 percent moisture, sample degradation is of relatively small concern. Basic principles of effective storage for dried and ground feed and forage samples include storage in airtight containers out of direct light at room temperature. Many laboratories use cups and/or sealed plastic bags for archiving samples. Such containers are typically adequate to maintain the integrity of samples. Where possible, it is advisable to further restrict exposure to air and moisture by storing samples cups/bags in larger airtight containers. Even with such measures in place, it is possible for samples to absorb measurable amounts of moisture from the atmosphere. However, other analyte values remain largely consistent, and samples can often be stored effectively in this manner for several years.

Resources

Association of American Feed Control Officials Incorporated. 2000. *Guidelines for Preparing Laboratory Samples*.

Nelson, C. J., and D. Smith. 1972. Changes in carbohydrate and nitrogen concentrations during storage of heat and freeze-dried alfalfa root tissue. *J. Agric. Food Chem.* 20:125.

Appendix I: Intellectual Property Standards Listed in NIRSC Bylaws

1. Definition of an “NIRSC Equation”

An NIRSC equation is defined as- an NIRS equation (calibration) derived from NIRSC aggregate spectra and chemical data submitted to the NIRSC. The equation (calibration) can not be biased or modified in any way.

2. Use of NIRSC Aggregate Materials

Use aggregate spectra (SPECTRA), chemistry data (CHEMISTRY), and/or forage and feed samples (SAMPLES) submitted to NIRSC:

1. Spectra shall be defined in this case as being the absorbance or reflectance data collected by a Near Infrared Reflectance instrument, used to describe chemical, biological, or physical properties of a sample. Chemistry data shall be defined in this case as being the data collected from chemical analysis to describe chemical, biological, or physical properties of a sample. Samples shall be defined in this case as being the physical samples from any physical material used in predicting spectra or determining chemical data.
2. NIRSC Aggregate spectra shall be defined as spectra pooled by NIRSC from Spectra submitted by NIRSC Members and/or collaborators to be used as defined in paragraph 5 and 6 below.
3. NIRSC Aggregate chemistry shall be defined as chemistry pooled by NIRSC from chemistry data submitted by NIRSC Members and/or collaborators to be used as defined in paragraph 5 and 6 below.
4. NIRSC Aggregate samples shall be defined as samples pooled by NIRSC from samples submitted by NIRSC Members and/or collaborators to be used as defined in paragraph 5 and 6 below.
5. NIRSC shall not sell, lease, or donate any aggregate spectra, chemistry, or samples to any non-member of the NIRSC without a vote of the membership. Approval of sale, lease or donation of aggregate spectra, chemistry, or samples will require at least a 75% approval of NIRSC voting members. NIRSC aggregate spectra, chemistry, and samples can be used for the formulation of NIRSC global calibrations, expandable calibrations, validation sets, etc.
6. NIRSC aggregate spectra, chemistry, or samples may be used by instrument manufacturers for the purpose of evaluation of their instruments, or spectral transfer to their particular format. This will require an approval from the NIRSC Board of Directors. Any research group or instrument manufacturer receiving approval for use of the NIRSC spectra, chemistry, or samples will be required to complete a Research Agreement.

3. NIRSC Logo Usage

An important goal for NIRSC membership is to maintain performance, and any logo use should reflect this. Two logo concepts have been implemented by NIRSC that would both represent support for the forage/feed and agriculture industry, but differentiate level of NIRSC participation.

1. The first logo represents that an entity is a participating NIRSC member. Under the logo is stated, "Working for better forage analysis."
2. The second logo represents that an entity uses NIRSC equations. Under the logo is stated, "Proudly using NIRSC equations." With this second logo option, the member's analysis or results sheet would need to footnote which equations are being used and sign an agreement to this effect. "Using" NIRSC equations means doing work for a 2nd party or doing some kind of reporting for a 2nd party. Method of footnoting is flexible as long as members report on which NIRSC equations are being used. The intent is to keep misrepresentation of NIRSC equations from happening while also benefiting the NIRSC.

Appendix II: Creation of a Permanent Check Cell

If creating a permanent check cell is something your lab wishes to do, it is very important to remember that it can be a very difficult process to perform correctly. Be aware of the following factors:

- The sample must be packed tightly enough to not allow sample particle shifting. This must be done without packing too tightly and breaking glass in the cell.
- The cell must be sealed well enough to prevent moisture migration, and must hold up to handling and temperature changes.

With these factors in mind, the committee feels it would be most prudent to contract InfraSoft International (ISI) to perform this task for you. It may save a large amount of time and glass.