NIRS Basics: Principles and Practical Uses in Forage Testing

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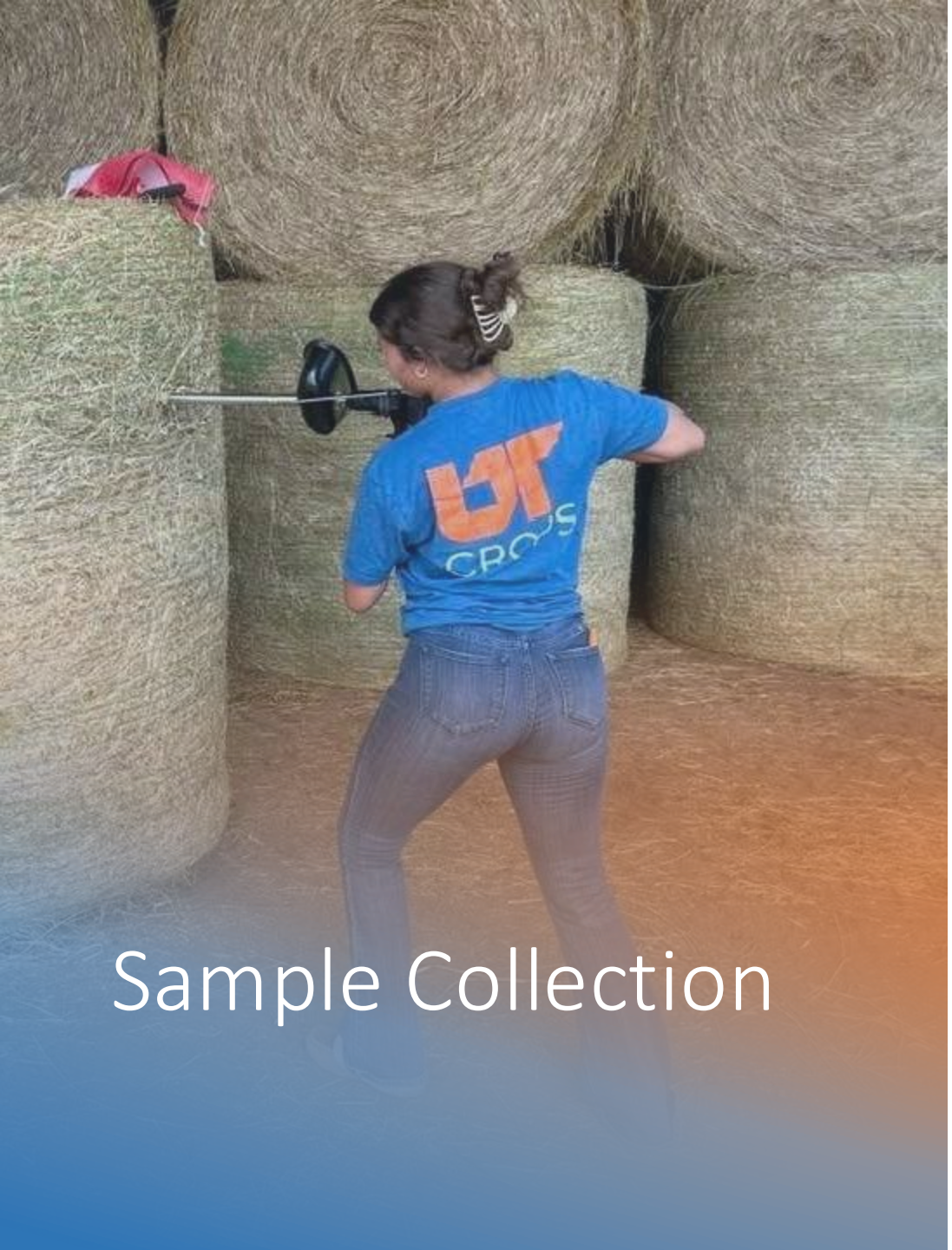
Using NIRS for Forage and Feed Analysis

- 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.
- Proper sampling, handling, grinding, preparation, and presentation.

Preprocessing Theory

- 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 to be sent out.
- Calibrations contain spectra from samples with a wide moisture content.
- Scanning occurs if dry enough to grind, can this be trusted?
- The spectral scattering effect from water molecules was first detected in the early 1990s, with no specific recommendation or range determined.
- Typical forage calibration models use adjusted wet chemistry results converted to 100% DM.



Sample Collection

- 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 researchers and customers.
- Laboratories and researchers must be proactive in their approach to proper sampling.
- Laboratories cannot fix an unrepresentative sample; the laboratories can only analyze it.
- When improperly collected samples arrive to the lab (e.g., extremely small samples, grab samples, or cores from a single bale) potential discrepancies occur.
- "Undesirable Results" can be minimized using these proactive measures rather than dealing with sampling issues in a reactive manner.

Proper Sampling

- Laboratories and researchers must be proactive in their approach to proper sampling.
- Most laboratories offer several different calibrations for a wide variety of feedstuffs.
- Each calibration is developed to analyze a specific group of species or grouped together by type including multiple constituents, or parameters.
- It is imperative to limit calibration application to the range of sample types known to be included in the calibration set.
- Protocols must be observed before the sample meant for analysis reaches the laboratory.
- An unrepresentative sample cannot be fixed; only analyzed.

Sample Handling and Composition

- 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.
- 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.
- 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.

Sample Preparation

- Samples that are dried and ground remove variables including analysis interference from water and heterogeneity and stabilizes the material.
- Samples should be dried in a 55°C forced-air oven.
- Drying at higher temperatures (>55°C) may cause chemical changes in the sample that could alter the subsequent constituent analysis.
- Predicted dry matter (DM) or moisture in a sample is the presented moisture content of a sample to the NIR instrument at time of scanning.
- Please note that there are different types of DM for use in reporting lab analysis (as-received/as-is)

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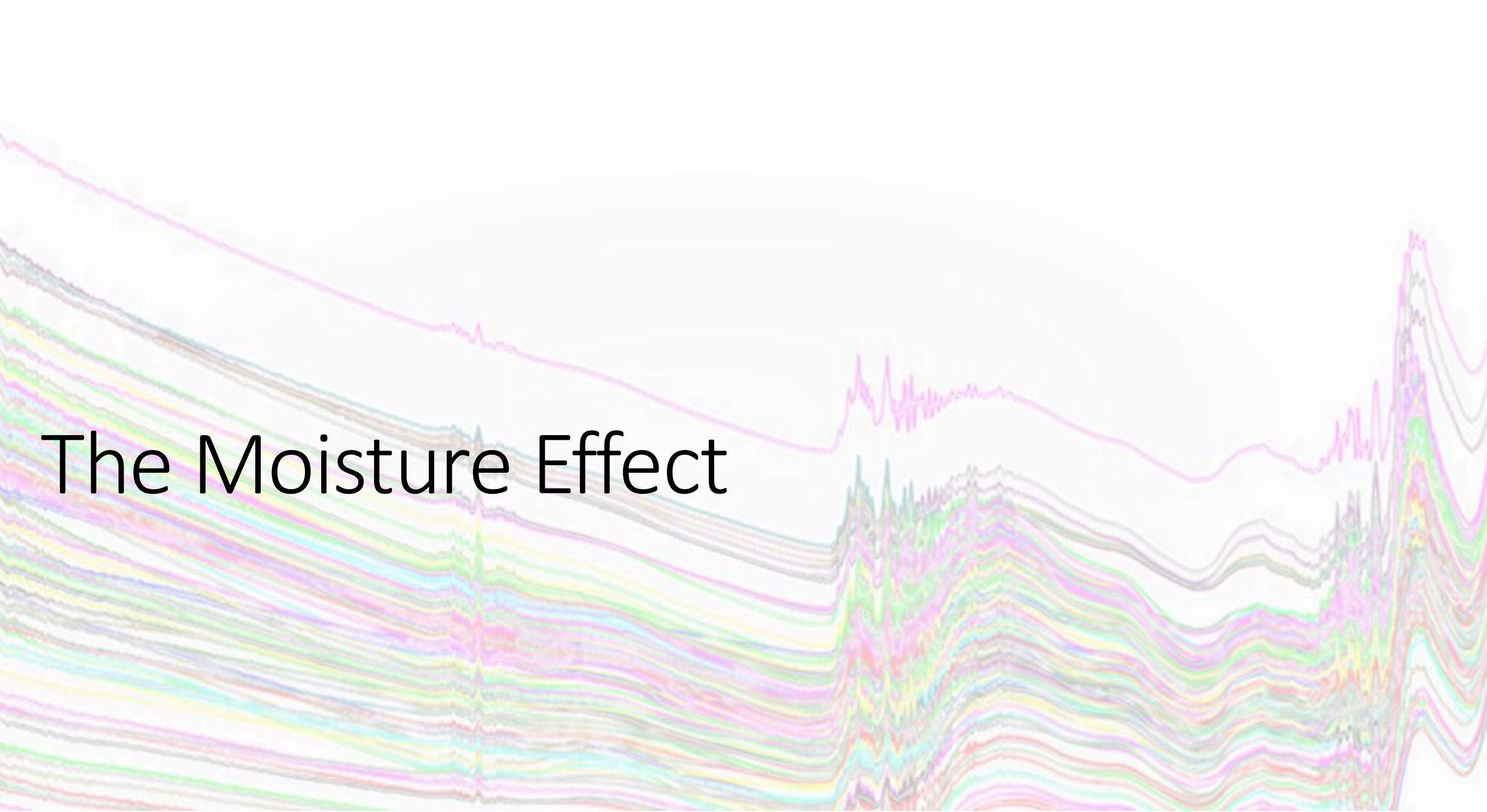
- Errors can be introduced to the system in the laboratory at any stage from splitting and sub-sampling to contamination and induced physical alterations such as using grinders and equipment not for forage and feed preprocessing.
- Heterogeneity of forage material makes the processes of splitting and sub-sampling prone to errors.
- 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.
- Excessive heat exposure mechanical or induced are known to alter the chemical makeup of forage material and can alter analytical results

Sample Grinding

- The same grinding or preparation method used in developing the calibration is used for routine analysis.
- Sample preparation that converts the sample received at the laboratory into a homogeneous material suitable keeping analytical errors as low as possible.
- Improper grinding protocols may lead to heating of the materials, which may alter DM or other analysis procedures for NIRS or chemistry.
- Regardless of grinding equipment, all material for NIRS analysis have followed grinding with a final pass through a 1 mm screen of a cyclonic mill.
- The finer and more uniform particle size reduced Standard Error of Calibration (SEC) and expected coefficient of variation goes down with smaller particle sizes.

Sample Presentation and Final Product

- Instrumentation must be maintained, checked, and user must know how to detect errors.
- 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.
- Sample cup or instrument window contamination can produce a faulty artifact in the sample spectra.
- Packing a sample cup with a representative sample from the ground material is extremely important.
- Scanning on the appropriate model and knowing the limitations.
- Reporting the data and making decisions.



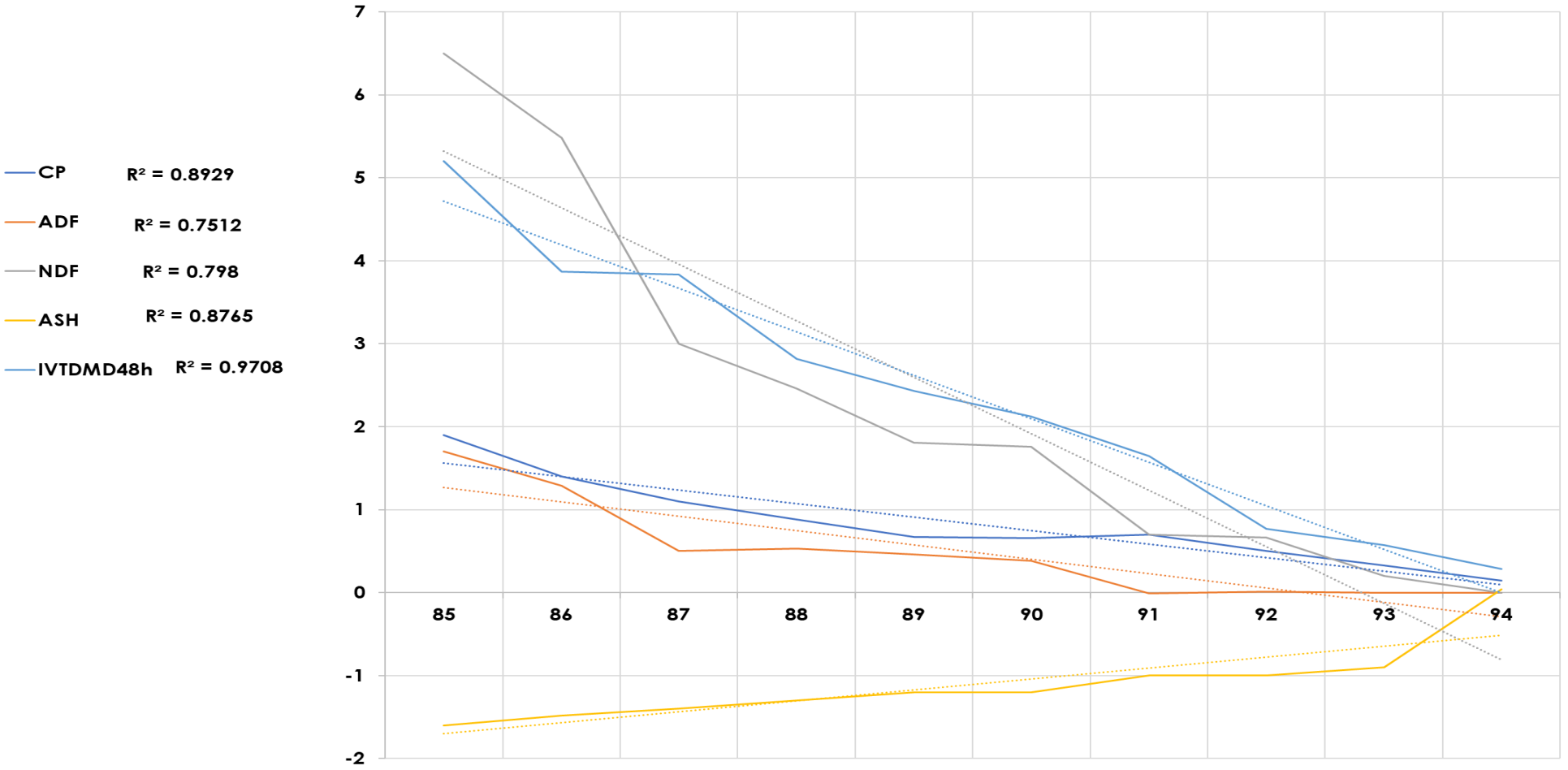
The Moisture Effect

Actual Sample

Sample	Species	DM	CP	ADF	NDF	Ash	IVTDMD48h
108677	Corn Silage	82.38	7.24	26.11	36.14	6.29	97.57
108677	Corn Silage	85.54	8.48	23.97	33.98	5.58	92.96
108677	Corn Silage	88.83	9.18	22.84	33.68	5.05	88.73
108677	Corn Silage	94.59	9.88	21.06	32.27	4.17	85.74

Experiment Representation

Percent change in nutritive value by predicted dry matter (DM) of forage samples



X-axis represents DM 83.7-93.8%. Y-axis represents percent change in nutritive analysis results as DM increases, across all samples, further divided by type not represented

Resolved:

“Russian Roulette”

DAIRYLAND Laboratories, Inc.

Dairyland Laboratories
217 E. Main St.
Arcadia, WI 54612
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Fax: 608-323-2184
Email: info@dairylandlabs.com

To: UT Beef & Forage Center
David McIntosh-CoordinatorIII
2505 EJ Chapman Dr., PBB 112
Knoxville, TN 37996

Product: 113050

Moisture	5.86%
Dry Matter	94.14%

		Dry Basis	90% Range*
Crude Protein	%DM	13.21	13.7 - 23.9
ADF	%DM	43.62	25.8 - 41.6
aNDF	%DM	58.89	32.3 - 55.6
aNDFom	%DM	56.76	29.9 - 53.5
Ash	%DM	6.54	8.88 - 13.8

*Mixed hay statistics provided for comparison.

Calculations

NFC	%DM	22.37
RFV		86.85

		ADF
TDN	%DM	54.92
Nel 3x	Mcal/cwt	55.78
Neg	Mcal/cwt	24.56
Nem	Mcal/cwt	49.79

Results at Purchase of Hay

Analy
GF22-

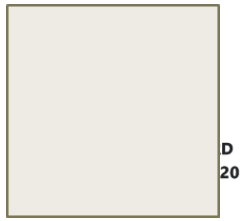
1st Cut Hay
Client Sample ID: 1

Analysis	Dry Basis	As Is
Dry matter (%)	--	90.14
Moisture (%)	--	9.86
PROTEIN		
Protein {N x 6.25} (%)	18.09	16.31
Soluble Protein (%)	5.90	5.32
Soluble Protein as %CP	32.61	--
ADF-CP [ADP] (%)	2.50	2.25
ADP as %CP	13.82	--
Adjusted Crude Protein	18.09	16.31
NDF-CP [NDP] (%)	3.93	3.54
NDP as %CP	21.72	--
FIBRES		
Acid Detergent Fibre (%)	30.56	27.55
aNeutral Detergent Fibre (%)	45.09	40.64
Lignin (%)	7.59	6.84
NON-FIBRES		
Fat (%)	2.37	2.14
Ethanol Soluble CHO (%)	7.86	7.08
Water Soluble CHO (%)	9.09	8.19
Starch (%)	0.57	0.51
Non-Structural Carbohydrates (%)	9.66	8.71

Results from Received Hay

Soil, Plant & Pest Center
5201 Marchant Dr. | Nashville, TN 37211
615.832.5850 | soillab@tennessee.edu
soillab.tennessee.edu

2023 Certified NIRS by NFTA



Forage Anal

County: J
Email: RE

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Near-Infared Spectrosc

Water Content		as received
DM	Dry Matter	90 %
Moisture	Moisture	10 %
Protein		*100% DM basis*
CP	Crude Protein	12.95 %
ADICP	Acid Detergent Insoluble CP	0.75 %
NDICP	Neutral Detergent Insoluble CP	1.50 %
InsolCP	Insoluble Crude Protein	6.40 %
Lysine	Lysine	0.45 %
Fiber		*100% DM basis*
ADF	Acid Detergent Fiber	37.55 %
NDF	Neutral Detergent Fiber	60.10 %
Lignin	Lignin	6.55 %

Analysis (100%DM)	Sample	DM	CP	ADF	aNDF	Notes
5/13/2023 8:03:38 PM	113050	95.95	13.02	39.90	56.82	Dried to GH Optimum Range for Scanning
5/13/2023 8:02:32 PM	113050	95.94	13.31	40.11	57.37	Dried to GH Optimum Range for Scanning- 2nd sampling
5/22/2023 4:01:59 PM	113050	91.78	19.61	30.83	65.59	Moisture added for demonstration purposes
Reference Lab Wet Chemistry	113050	94.14	13.10	43.62	58.89	Wet Chemistry Analysis

Importance of Dry Matter at Scanning

- Moisture in samples can cause issues in correctly predicting constituents and data fluctuation across a project or subsampled material.
- Optimal range of water in a ground sample for NIRS analysis is roughly between 93.5-97% Dry Matter (DM).
- 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.
- 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).

Optimum Scanning Ranges:

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

Consistency in preparing samples for NIR analysis will help reduce discrepancies between laboratories, across entire projects, and ultimately in the final analysis data.

“Take it to the barn”

- Although NIRS calibrations have been reliable with samples scanned at higher moisture levels, it is imperative that a target DM should be presented to achieve the best possible prediction, whether a one-time sample or an entire project.
- Consistency in preparing samples for NIRS analysis will help reduce discrepancies between laboratories, across entire projects, and ultimately in the final analyses of that data.
- NIRS analysis as an analytical technique has a long and credible history.
- NIRS is a secondary method that never can be more accurate than the reference method upon which it is based. It will be more precise and repeatable!
- Statistically robust prediction models allow for a rapid and repeatable assay procedure for nutritional values that help the livestock industry detect and manage variability in composition among and within feedstuffs.
- The cost-effectiveness of NIRS analysis allows the total analytical error (sampling and laboratory) to be reduced because a larger number of sub-samples or sequential samples can be assayed with a limited analytical budget than is possible using the more expensive wet chemistry approaches.
- To enhance trust, nutritionists, producers and laboratories are encouraged to communicate more fully and openly so that NIRS prediction model and wet chemistry statistics are understood more clearly.

NIRSConsortium

Forage and Feed

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Appreciation to the co-authors and contributors to the efforts put forward by the NIRS Forage and Feed Consortium's *Guidelines for Optimal Use of NIRSC Forage and Feed Calibrations in Membership Laboratories (Second Edition, 2022).*

IMPORTANT NIRS EVENTS COMING UP

NIRSC Hub Event- Hands on Training and Problem Solving, April 15 & 16, 2026, Knoxville, TN

International Diffuse Reflectance Conference (IDRC 2026), July 26-30, 2026, Knoxville, TN

“Call me, I like to know you are human” David McIntosh