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USDA LTAR Common Experiment measurement: Obtaining quality metrics in forage aboveground biomass

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Abstract

Harvesting crops for potential use as livestock forage must include nutritional value measurements to understand how these feedstuffs will meet the consumptive needs of animals. Nutritional value is often called “forage quality.” Nutritional value can change rapidly over hours to days as plants grow, mature, and respond to their environment. Measuring nutritive constituents in forage follows standardized methodologies across commercial and research laboratories. Crude protein (CP) estimates the true protein concentration based on tissue nitrogen analysis and the assumption that most plant protein is 16% nitrogen. Ash measures the total mineral elements present in forage, such as calcium and phosphorus, which do not contribute to its nutritional energy value. Cell wall constituents in forages are quantified using the Van Soest system (Goering and van Soest, 1970), where neutral detergent fiber (NDF) includes cellulose, hemicellulose, and lignin, while acid detergent fiber (ADF) contains cellulose and lignin, and acid detergent lignin (ADL) contains only lignin. This protocol outlines the methodology for analyzing ash, CP, ADF, NDF, and lignin in a forage sample.

Materials

1. Garden shears, manual or electric (Keep them sharp! Application of WD-40 to blades after use removes plant residue and prevents rust)
2. Quadrats with rigid PVC or metal frames (not necessary for all forage types)
3. Rulers for monitoring the correct clip height
4. Buckets
5. Large plastic trash cans
6. Small portable scale for weighing samples
7. Paper, cloth, or mesh bags big enough for samples (do not skimp on bag size; samples in packed bags dry slowly and may mold)
8. 18-oz Whirl-Pak bags for short-term ground sample storage
9. Bag labels (stickers or tags)
10. Barcode labeler
11. Forced air sample dryer
12. Wiley mill or equivalent
13. Udy or other cyclonic mills
14. NIRS instrument and software
15. Riffle splitter
16. 4-oz glass or hard-plastic airtight archival storage containers

Sample collection

1 Sampling for forage quality requires the collection of biomass samples in addition to those samples outlined in the LTAR aboveground biomass protocol. These additional samples are necessary to capture a sample that reflects the cutting height of forage harvesting equipment or animal grazing. To best represent the forage intended for removal, **do not** clip forage to ground level; instead, clip samples at a height similar to that of forage harvesting or grazing. Ensure that all personnel clip uniformly at the designated stubble height. Use a height guide or ruler because the eye can easily deceive on this measurement. Grab samples for quality analyses should not be taken directly from fresh material harvested by a machine because the contamination of these samples by soil is possible during harvest, inflating the ash value.

2 Forage quality samples should be collected at each scheduled machine harvest or managed grazing event. Continuously stocked pastures should be sampled at least monthly during the grazing season.

Sample processing

3 When measuring total biomass from quadrats or small plot harvesters:

3.1 Within 48 h before biomass harvest, hand clip forage quality subsamples with garden shears from a location within 10 ft of each quadrat area used in the LTAR aboveground biomass sampling protocol. The representative composited forage quality sample should be between 200 g to 400 g of fresh forage.

3.2 The ground area clipped at each location depends on forage type: For fine-stemmed monoculture forages, clipping a hand-sized ground area or a 6-in section of the row may suffice, whereas coarse-grained cover crop mixtures may require a quadrat for effective sampling. When forages are planted in rows, quadrat width should be a multiple of the inter-row spacing to accurately capture row and inter-row biomass. Getting a representative sample of all forage present is key. Clipping quality samples from within the biomass sampling area requires adding the quality sample biomass weight back to the total for the unit.

3.3 Three composited sub-samples per experimental unit suffice for small plots (Grimsbo-Jewitt et al., 2001), while 10-20 composited subsamples suffice on field scale units.

3.4 Place composited samples in clean paper, cloth, or mesh bags and record fresh weights. Use bags large enough to allow air circulation within the forage mass. Paper bags should have perforations to help speed drying (use a drill press to make uniform holes through a stack of bags).

4 When measuring total harvested biomass as weighed bales of hay:

- Taking forage quality samples is possible with a commercial hay core sampler, with at least one core from each hay bale composited across the experimental unit.

5 Fresh forage samples should be transported promptly from the field to the dryer—do not pile the bags in the sun for hours or leave them in a truck overnight because the forage will quickly begin to heat, and this heating can change nutritional values. Place the bags in a forced air dryer at 55 °C until the weight is constant (equilibrium) and record the dry weight. A low temperature of 55 °C is required to avoid heat damage and inaccurate crude protein results.

Sample analysis

15m

6 Near-infrared reflectance spectroscopy (NIRS) is a rapid, nondestructive technique that uses light reflectance from reference samples of known chemical composition to build prediction equations for chemical constituents in unknown samples (Abrams et al., 1987; Shenk and Westerhaus, 1993). NIRS has emerged as the dominant method of forage quality analysis which ensures its future use in the LTAR network.

7 Forage samples will be analyzed using NIRS for the primary metrics crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin, and ash.

8 The NIRS Forage and Feed Consortium (NIRSC, Berea, KY) provides validated prediction equations for forage analysis and a detailed handbook of sample preparation and scanning protocols (McIntosh et al., 2022) to research and commercial laboratories.

9 Ensure analysis consistency across locations by analyzing all samples using these NIRSC equations by:
1. analyzing samples in a research laboratory with access to NIRSC equations or
2. analyzing samples at a commercial laboratory that is using NIRSC equations.

10 Forage analysis by NIRS has extreme sensitivity to sample preparation and spectrum collection methods. Therefore, follow the protocols described in Guidelines for Optimal Use of NIRSC Forage and Feed Calibrations in Membership Laboratories (McIntosh et al., 2022) for sample drying, subsampling, grinding, and spectra collection. These protocols are consistent with National Forage Testing Association (Undersander et al., 2093) and North American Forage Improvement Assoc. (Sheaffer et al., 1995) guidelines for standardized forage testing. An abbreviated overview of key points is listed below, but the reference contains specific details.

10.1 Ideally, the entire sample collected from the field will be dried because representative subsamples from whole mixed plants are extremely difficult to obtain. However, if the sample size makes drying the entire sample unrealistic, chop the collected plants into pieces a few inches in size and mix thoroughly in a large pail/can. Collect a representative subsample of approximately one (1) gallon and place it in a dryer.

10.2 Grind the entire dry sample. Samples less than 100 g in total weight can go directly to Step 3 (12.3). Larger samples can either be subsampled prior to Step 3 (12.3) or the entire sample can be processed if preferred.

- To subsample, grind the entire dry sample coarsely to pass a 2-8-mm screen in a Wiley mill or equivalent, mix thoroughly, and then split to 100-g size using a riffle splitter or the quadrant subsampling technique (Bohnert et al., 2011).

10.3 Final preparation for NIRS analysis using the NIRSC protocol requires grinding to pass a 2-mm screen in a Wiley mill or equivalent grinder, followed by grinding to pass a 1-mm screen in a cyclone mill. Do not omit steps or use different screen sizes because this deviation will change particle size distribution, which affects analysis results. Keep grinders in good condition with effective grinding surfaces and screens.

10.4 The final ground sample size should be 50-100 g for analysis and archiving. NIRS scanning is non-destructive and requires 15 g - 25 g of dry sample, depending on the instrument model.

10.5 If samples are not ground immediately upon removal from the dryer, hygroscopic constituents will quickly absorb atmospheric moisture. To facilitate grinding, use samples containing at least 93% dry matter (7% moisture). Attempting to grind wetter samples will likely cause fine material to stick to inside surfaces of grinders, skewing the proportions of nutritive constituents.

Note

If samples stick in the grinder, place them back in the low-temperature 55 °C dryer for 1 to 2 hours.

10.6 Thorough mixing of samples, as detailed in McIntosh et al. (2022), before scanning is essential for good results. Particles will segregate during grinding and even in storage containers because of the normal movement of containers from place to place. Shaking containers or stirring with scoopulas does not provide adequate mixing. Mixing methods are described in McIntosh et al. (2022).

10.7 When scanned in the NIRS instrument, ground samples must be 95-97% dry matter (DM) for grass and grass/legume mixtures and 93.5-95% DM for legume hay and haylage. Samples outside these ranges will give erroneous results. Monitor DM results when running the first few samples.

- If samples are too wet upon initial analysis, transfer them to food-grade tin containers and place them in a dryer at 55 °C in 15-minute increments until they reach the target DM.

- If samples are too dry, leave opened containers on the benchtop for a few hours until they acquire enough atmospheric water to reach target analysis moisture.

10.8 NIRS results generated from NIRSC equations should be validated in an NIRSC wet chemistry reference laboratory. Validation methods are outlined in McIntosh et al. (2022).

Reporting results

11 Metadata required for reporting NIRS results include:

- NIRS instrument brand, model number, and software
- NIRSC calibration name, release year, and performance statistics, including SEC, r^2, and 1-VL for each constituent.
- Date scanned
- Worker name
- GH and NH statistics for the prediction
- Name of NIRSC wet chemistry reference laboratory used for validation, and validation statistics including correlation coefficient (R^2), standard error of prediction (SEP), standard error of cross validation (SECV), bias, and the number of samples used in the validation test.

Concurrently sampled covariate metrics

12
- Management data
- Biomass removed from the field
- Biomass left in the field
- Elemental concentrations in ground tissue samples
- Treatment name and crop species and variety
- Collection date
- Sampling implement (type of garden shear)
- Exact number of subsamples within a composite
- Worker name
- NIRS instrument brand, model number, and software
- NIRSC calibration name, release year, and performance statistics

Calculations

13 All forage constituents will be reported on a 100% dry matter basis (e.g., 0% moisture).

Quality assurance and quality control

14 A set of sample standards will be developed and distributed among locations studying forage quality. Standards will include a grass, a legume, a 50:50 grass:legume mix, and corn silage.

Include duplicate standard samples of the appropriate calibration class in every sample set submitted for forage-quality analysis.

15 NIRSC prediction equations will flag samples that fall outside the expected numeric bounds of the calibration (H value > 3). Carefully examine samples flagged as outliers for recording, sampling, sample preparation, or scanning errors, and if no errors exist, analyze the samples by wet chemistry at a NIRSC reference laboratory.

Archiving

16 Keep subsamples (50 g - 100 g) at Room temperature in controlled areas that are as dry and dark as possible. Glass containers are preferable because insects and rodents can chew into plastic.

Recommendations for data collection

17 Table 1. Summary of recommendations for the measurement of forage quality indicators in aboveground biomass leaving the field.

	A	B	C	D
	Attribute	**Preferred**	**Minimum**	**Comments**
	Spatial scale	Field and plot	Field	Depends on the specific objective. In addition, the temporal scale is important in perennial forages (multiple years of data collection from the same plot or field).
	Frequency	Every harvest or grazing cycle (1-6 per year, typically)	One harvest at physiological maturity for infrequently harvested forage or continuously stocked pasture; the 1st and 3rd cut for hay crops	
	Covariate metrics	Biomass leaving the field; biomass remaining in the field; C, P, K, and N contents	Biomass leaving the field; biomass remaining in the field	

Illustrative Media

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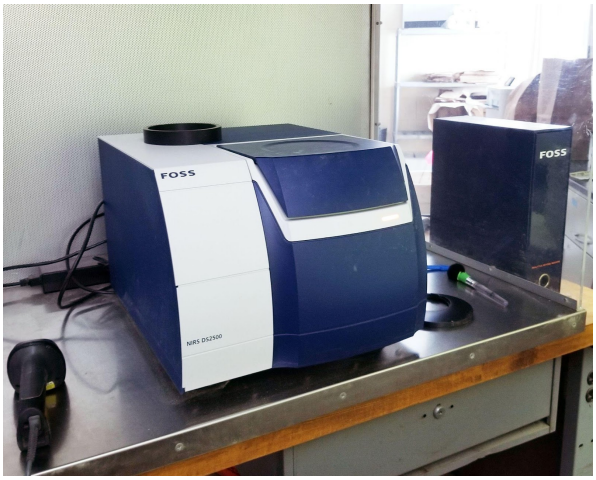
Example of garden shears appropriate for hand-clipped quality sampling.



Example of an NIRS Instrument with the lid closed.

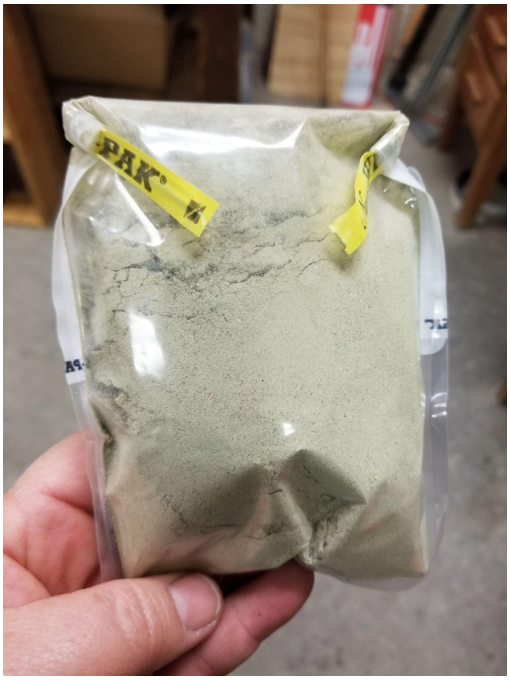
Example of an NIRS Instrument with the lid open and showing the loaded sample cup.

Examples of sample cups used with Foss NIRS instruments. Size and shape differ among instrument brands. The sample size must be adequate to completely cover the glass to a depth of at least 4 mm.

Wiley mill.

Udy cyclone mill.

Riffle splitter for subdividing samples.



Forage sample after grinding through a 1-mm cyclone mill screen.

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