National Forage Testing Association Reference Method

NFTA Method 4.1 Determination of Acid Detergent Fiber by Refluxing

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Introduction

This procedure is intended as a preparatory step for lignin analysis. If done sequentially following NDF, the difference between the NDF and ADF will be an estimate of hemicellulose. While wide used in energy calculations, ADF is not recommended for this purpose.

Scope

This procedure is applicable for the determination of acid detergent fiber (ADF) in all types of forages.

Basic Principle

An acidified quaternary detergent solution is used to dissolve cell solubles, hemicellulose and soluble minerals leaving a residue of cellulose, lignin, and heat damaged protein and a portion of cell wall protein and minerals (ash). ADF is determined gravimetrically as the residue remaining after extraction.

Equipment

Refluxing apparatus
Berzelius beakers (600 mL)
Fritted glass (Gooch) crucibles (coarse porosity, 50 mL)
Analytical electronic balance, accurate to 0.1 mg
Suction filtering device with trap in line and valve to break vacuum
Forced-air drying oven set at 100^{o}C

Reagents

Acid detergent solution:

To prepare mix:

1 liter 1.00N Sulfuric acid ±0.005N. Normality must be verified by titration with a primary base standard (method 3.1.2) before adding CTAB. A solution approximately 1.0 N sulfuric acid can be made by adding 51.04 g (27.7 mL) of concentrated reagent grade sulfuric acid (95-98% purity) to 972.3 mL water (AOAC 935.70). Titrate by method 3.1.2 and add water (if normality too high) or sulfuric acid (if normality too low) to adjust normality to 1.00N ±0.005N.

20 g Cetyl trimethylammonium bromide (CTAB), technical grade

Acetone, reagent grade

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Safety Precautions

- Always add sulfuric acid to water. Wear face shield and heavy rubber gloves. If acid is splashed on skin, wash immediately with copius amounts of water.
- CTAB powder will irritate mucous membranes, eyes and skin. Wear gloves and dust mask while handling.
- Acetone is highly flammable. Do not let vapors accumulate in work area. Use effective fume removal device. Also avoid inhaling or contact with skin. Make sure all traces of acetone have evaporated from the crucibles containing fiber residue before placing in the drying oven.

Procedure

1. Samples should be microwave dried or oven dried at 55°C to more than 85% dry matter, then ground to pass a 1 mm screen.
2. Dry 50 mL fritted glass crucibles overnight at 100°C and hot weigh (W1), recording weight to nearest 0.1 mg. (Hot weigh techniques described in method 2.2.2.2.)
3. Thoroughly mix and weigh sample (W2) (approximately 0.9 to 1.1 g, record weight accurate to 0.1 mg) into Berzelius beaker. Scan remaining sample on NIR or weigh a second subsample for laboratory dry matter determination.
4. Add 100 mL acid-detergent solution at room temperature. Place beaker on heater under the cold water condenser.
5. Heat to boiling in 5-10 min; reduce heat to avoid foaming as boiling begins. Reflux 60 min from onset of boil, adjusting heat so boiling remains at slow, even level.
6. Half way through (after about 30 min), wash down sides of beaker with minimal amount of acid detergent solution. A wash bottle is convenient for dispensing solution.
7. At end of 60 min, remove beaker, swirl, and filter through tared (step 2) fritted glass crucible, using minimal vacuum. Police and rinse the Berzelius beaker with boiling water while inverted over the crucible to insure quantitative transfer of all fiber particles into the crucible.
8. Soak residue in crucible twice with boiling (95-100°C) water. Use water to break up residue mat, filling crucible each time with vacuum off and allowing to soak a minimum of 15 to 30 sec (2 min recommended) after each wash. While filling the crucible with hot water or acetone, rinse the top edge and sides to remove residual acid detergent.
9. Rinse twice with 30-40 mL acetone by filling crucible, each time with vacuum off, allowing a minimum of 15 to 30 sec (2 min recommended) before vacuuming dry.
10. Dry 3 hr or overnight in forced-air oven (100°C) and weigh hot, recording weight (W3) to nearest 0.1 mg.

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Comments:

- Sulfuric acid for acid detergent fiber solution must be standardized to be between 0.995 and 1.005 N. Variation in normality outside of this range can result in low or high ADF values.
- Timing of refluxing is critical and should not vary more than 5 min from the 60 min described by the method.
- Acid must be thoroughly washed from the sample because it will become concentrated when water is removed during drying. The combination of strong sulfuric acid and high temperature can char the sample and result in low ADF values. If black discoloration occurs during drying, repeat the analysis with increased rinsing.
- Difficult filtration may result from plugging of the fritted glass crucibles. Crucibles should be cleaned regularly with acid or alkaline cleaning solution. (Alkali cleaning will tend to deteriorate fritted disk faster.) The filtration rate of crucibles should be as uniform as possible for a given set of samples. To check the filtration rate of crucibles, fill them with 50 mL of distilled water and record the time required to drain completely without vacuum. This should be about 180 sec. If filtration takes more than 240 sec, crucibles need cleaning. If cleaning does not improve the filtration rate, the crucible should be discarded. If filtering takes less than 120 sec, check crucible for cracks or holes in the fritted disk. If filtering takes less than 100 sec, the crucible should be discarded.
- The proper vacuum is critical to good filtering. It should be sufficient to remove the solutions rapidly but not so great that fiber particles plug the fritted disk.
- Rinse water must be in excess of 95^{o}C. This is particularly true of samples containing pectic substances, mucilages or glycoproteins.

Calculation

Percent Acid Detergent Fiber (ADF)

$$\% \; ADF = \frac{W_3 - W_1}{W_2 \; X \; Lab \; DM} \; X \; 100$$

Where W1 = tare weight of crucible in grams
W2 = initial sample weight in grams
W3 = dry weight of crucible and dry fiber in grams after refluxing
Lab DM = dry matter of sample W2

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Example Calculation

W1 = Weight of crucible = 15.5238 grams
W2 = Initial Weight of sample = 1.0223 grams
W3 = Weight of sample + crucible after drying = 15.8256 grams
Laboratory dry matter = 0.95%

$$\% \; ADF = \frac{15.8256 - 15.5238}{1.0223 \; X \; 0.95} \; X \; 100 = 31.07544\%$$

Reporting Rules

Report Acid Detergent Fiber (ADF) as a percent to one place past the decimal.

Example:
The above example calculation should be reported as 31.1%

Quality Control:

Include one or more quality control (QC) samples in each run, choosing QC samples by matching analyte levels and matrices of QC samples to the samples in the run. Include at least one set of duplicates in each run if single determinations are being made.

An acceptable average standard deviation among replicated analyses for acid detergent fiber ranges from about ±0.20 for samples with 20% ADF to ±0.35 for samples with 40% ADF, which results in warning limits (2s) ranging from ±0.40 to 0.70 and control limits (3s) ranging from ±0.60 to 1.05. Plot the results of the control sample(s) on an X-control chart and examine the chart for trends. Results outside of upper or lower warning limits, ±2s (95 percent confidence limits), are evidence of possible problems with the analytical system. Results outside of upper or lower control limits, ±3s (99 percent confidence limits), indicate loss of control and results of the run should be discarded. Two consecutive analyses falling on one side of the mean between the warning limits and the control limits also indicate loss of control

See the NFTA's Quality Assurance/ Quality Control Guidelines document for additional guidance.

Reference

Fiber (Acid Detergent) and Lignin in Animal Feed. (973.18) Official Methods of Analysis. 1990. Association of Official Analytical Chemists. 15th Edition.