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Acid Detergent Fiber Procedure (ADF)

1. Application

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

2. Summary of Methods

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.

3. Safety

All chemicals should be considered a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.

4. Interferences

5. Sample Collection, Preservation, and Handling

All samples are dried at 55^{o}C in a cabinet-type forced air dryer for 12-16 hours. After drying the sample is ground to pass through a 1 mm forage mill. A subsample is then dried at 105^{o}C for 3 hours to determine laboratory DM content.

6. Apparatus and Materials

6.1 Refluxing apparatus, condenser connections should be made from neoprene rubber or ground glass.
6.2 600 ml Berzelius beakers
6.3 Sintered glass crucibles (Gooch), use tall form, coarse porosity, plate 40mm in diameter, large enough to hold 40-50 ml liquid
6.4 Analytical electronic balance, accurate to 0.1 mg
6.5 Suction manifold of 6 crucible capacity with trap in line and valve to break vacuum
6.6 Drying ovens set at 105^{o} C

7. Reagents

7.1 ADF Solution
- 7.1.1 10.0 L Distilled Water
- 7.1.2 360 g Hexadecyltrimethylammonium Bromide
- 7.1.3 500 ml sulfuric acid technical
- 7.1.4 Bring to 18.0 L with 6.0 L Distilled Water
- 7.1.5 Standardize ADF Solution:
  a. Pipette 10 ml ADF Solution into 150 ml beaker.
  b. Mix 1.0 g of Phenolphthaline to 100 ml of 95% ethanol. Mix thoroughly and add 5 drops of indicator to beaker of ADF solution.
  c. Titrate using 1 N sodium hydroxide. Solution should begin "clear" and end of the reaction should be "very light pink."
  d. Adjust solution to desired titration of 10.0 ml If NaOH is below 10.0 ml add 10 ml sulfuric acid technical for every 0.1 ml below or 100 ml distilled water for every 0.1 ml above.

7.2 Acetone, reagent grade - Use grade of acetone that is free of color and will leave no residue upon evaporation.

8. Methods

Sample processing:

8.1 Sample should be oven dried at 55°C to ≥85% dry matter, then ground to pass a 1mm forage mill.

8.2 Dry 50 ml glass crucibles overnight at 100°C and hot weigh, recording weight to nearest 0.1 mg.

8.3 Thoroughly mix sample and weigh out approximately 1.0 g of sample into 600 ml Berzelius beaker or comparable refluxing container.

NOTE: The UW Soil and Forage Analysis Laboratory uses a modified method for fiber analysis using modified burettes for refluxing instead of the 600 ml Berzelius beakers. The procedure that follows assumes that these modified burettes are being used in the assay. Please contact the lab if you have questions about this modification.

Digestion:

8.4 Pour approximately 95 ml ADF solution in digestion burette on Fiber rack. Start heating the solution while weighing out the samples. Make sure water condenser is turned on and the glass condensers are cooling.

8.5 When solution is gently boiling, approximately 15 minutes, pour sample from weigh pan into burette, rinse pan with a squeeze bottle of ADF solution. With rinsing, the total volume of solution in the digestion burette should be approximately 100 ml.

8.6 After solution returns to boiling note time and rinse down sides of burette with squeeze bottle of ADF solution.

8.7 Reflux for 60 minutes.

Filtration:

8.8 Hot weight glass crucibles with filter mat, or metal crucibles with Dacron and filter mat, before filtration.

8.9 Put crucibles on vacuum unit below each burette. Turn on vacuum and constant hot water supply, in excess of 95°C.

8.10 Open vacuum under 1-2 crucibles at a time. If too many are open at one time, capacity will be lost on vacuum. Open stop cock on burette to drain into crucible, turn off burner on burette. Rinse burette thoroughly with hot water. Make sure all fiber is out of burette then keep approximately 40-45 ml hot water in burette for later rinsing.

8.11 Plugging on forage samples:

- 8.11.1 Continue running hot water on outside of crucible.
- 8.11.2 Use rubber policeman to break up fiber mat on bottom of crucible. Be very gentle – do not scrape filter mat too harshly.
- 8.11.3 Add acetone to crucible until it slowly filters out.
- 8.11.4 If sample refuses to unplug after 15 minutes sample will have to be re-run, cutting sample size in half (0.50 g).

Rinsing:

8.12 After all samples are evacuated from burettes and filtered turn vacuum off. Open stop cocks on burettes and evacuate hot water. Let water soak in sample for 1 minute then suction off water with vacuum.

8.13 After water is filtered off, turn off vacuum and add 20-30 ml acetone to samples. Rinse down sides of crucible while adding acetone. Let soak approximately 1 minute.

8.14 Suction off acetone, rinsing down sides of crucibles and the fiber mat with acetone to finish the rinsing portion.

8.15 Put samples with crucibles on small muffin tin and put into 105° C oven overnight.

8.16 Weigh hot samples with crucibles the following day, recording to nearest 0.1 mg.

9. Calculations

9.1 %ADF = {((Crucible Weight + Fiber) –Crucible Weight w/o Fiber) / (Sample Weight x lab DM as decimal)} x 100

10. Quality Control

An in-house standard is run to gauge run acceptability.

11. Reporting

Results are reported as % ADF on a dry matter basis.

12. References

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