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Neutral Detergent Fiber (NDF)

1. Application

This procedure is applicable for the determination of neutral detergent fiber in all types of forages and feeds.

2. Summary of Methods

A neutral detergent solution is used to dissolve the easily digested pectins and plant cell contents (proteins, sugars, and lipids); leaving a fibrous residue (aNDF) that is primarily cell wall components of plants (cellulose, hemicellulose, and lignin). Detergent is used to solubilize the proteins and sodium sulfite also helps remove some nitrogenous matter; EDTA is used to chelate calcium and remove pectins at boiling temperatures; triethylene glycol helps to remove some non-fibrous matter from concentrate feeds; and heat-stable amylase is used to remove starch. Two additions of amylase (one during refluxing and one during filtration) have been observed to aid aNDF analyses and minimize filtering difficulties. Heat-stable amylases are used in hot solutions to inactivate potential contaminating enzymes that might degrade fibrous constituents.

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 Analytical electronic balance, accurate to 0.1 mg

6.4 Sintered glass crucibles (Gooch), use tall form, coarse porosity, plate 40mm in diameter, large enough to hold 40-50 ml liquid
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 NDF solution – Using a 2 L volumetric flask, add 10 L distilled water to a 20 L glass carboy. Add the following ingredients:
- 7.1.1 334.98 g disodium dihydrogen ethylene diamine tetraacetate (also known as ethylenedinitrilo – tetraacetic acid or EDTA)
- 7.1.2 82.08 g disodium hydrogen phosphate
- 7.1.3 540 g lauryl sulfate, USP grade
- 7.1.4 122.58 g sodium borate decahydrate
- 7.1.5 180 ml ethylene glycol monoethyl ether (purified grade)
- 7.1.6 Place on magnetic stir plate. Stir while adding the remaining 8 L distilled water. Let the solution stir overnight.
- 7.1.7 Check pH of the solution after it is well mixed. It should range within 6.9-7.1. If needed, adjust with concentrated HCl or NaOH as necessary.

7.2 Acetone, regent grade

7.3 Amylase solution
- 7.3.1 1 ml amylase to 40 ml distilled water

8. Methods

Sample processing:
8.1 Sample should be oven dried at 55^{o}C to $\geq$85% dry matter, then ground to pass a 1mm forage mill.
8.2 Dry 50 ml glass crucibles overnight at 105^{o}C and hot weigh, recording weight to nearest 0.1 mg.
8.3 Thoroughly mix sample and weigh out approximately 0.5 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 45 ml NDF solution in digestion burette on fiber rack. Start solution heating while you weigh out samples. Make sure water condenser is turned on and the glass condensers are cooling.

8.5 Thoroughly mix sample and then weigh 0.5 g into plastic weigh pan. Run an in-house standard to gauge run acceptability.

8.6 Add 0.5 g of sodium sulfite to each sample in pans.

8.7 When solution is gently boiling (it takes approximately 15 minutes to reach boiling) pour sample from pan into burette, rinsing pan with a squeeze bottle of NDF solution. With rinsing, the total volume of solution in the digestion burette should be approximately 50 ml.

8.8 After solution returns to boiling (note time, needs to reflux 60 minutes), add 2 ml amylase solution and rinse down sides of burette with squeeze bottle of NDF solution.

8.9 Reflux for 60 minutes.

Filtration:

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

8.11 Put crucibles on vacuum unit below each burette. Turn on vacuum and constant hot water supply.

8.12 Open vacuum under 1-2 crucibles at a time. If too many are open at one time, power 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.

8.13 Plugging on forage samples:

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

8.14 Plugging on corn or starchy samples:

- 8.14.1 Add 2 ml amylase directly to crucible.

Rinsing:

8.15 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.

8.16 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.

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

8.17 Put samples with crucibles on small muffin tin and put into a 105^{o}C oven overnight.
8.18 Weigh samples with crucibles the following day.

9. Calculations

9.1 NDF = {((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 % NDF on a dry matter basis.

12. References

12.1 Goering, H.K. and P.J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures, and some applications). USDA Agricltural Research Service. Handbook number 379 as modified by D.R. Mertens (1992, Personal Communication).

12.2 Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J. Dairy Science 74:3583-3597.

12.3 Mertens, D.R. 1992. Critical conditions in determining detergent fiber. Proceedings of NFTA Forage Analysis Workshop. Denver, CO. p C1-C8.