DATE: FEBRUARY 2007

Neutral Detergent Insoluble Nitrogen (NDIN) and Neutral Detergent Fiber Crude Protein (NDF-CP)

1. Application

This procedure is applicable for the determination of neutral detergent fiber insoluble nitrogen in all types of forages. Neutral detergent insoluble nitrogen (NDIN) is the nitrogen remaining in the neutral detergent fiber residue and, while some occurs naturally in all plant material, is generally considered to be an estimate of heat damage occurring during storage or processing. Nitrogen in excessively heated samples is usually unavailable to animals.

2. Summary of Methods

NDIN is determined as the nitrogen in NDF residue. The two options used to determine NDIN differ in the amount of the NDF residue that is analyzed for nitrogen. If the total NDF residue is collected on filter paper and analyzed for nitrogen, NDIN (% DM basis) is determined by measuring the nitrogen (corrected for a filter paper blank) in the total NDF residue and dividing by the original dry sample weight. The other option involves NDF residues from fritted glass (Gooch) crucibles. It is difficult, if not impossible to collect all NDF residues from fritted glass (Gooch) crucibles, therefore only a sub-sample of the total NDF residue is analyzed for nitrogen. When only a part of the NDF residue is analyzed, by sampling the NDF residue from a fritted glass crucible (or from filter paper), the nitrogen content of the NDF residue must be determined by dividing the nitrogen in the NDF sample by the NDF sample weight. Then NDIN (% DM basis) is calculated by multiplying the nitrogen content of NDF by the NDF content in the dry matter. When sampling NDF residues from fritted glass crucibles, be careful not to scrape glass particles into the partial NDF residue that is analyzed for nitrogen. Neutral detergent fiber crude protein (NDF-CP) is NDIN expressed as crude protein on a dry matter basis.

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°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.

6. Apparatus and Materials

6.1 See procedure "Kjeldahl Nitrogen and Crude Protein in Forages" for nitrogen determination method.
6.2 See procedure "Neutral Detergent Fiber (NDF)" for neutral detergent fiber method.
6.3 Filter paper, acid hardened, #4 Whatman or equivalent.

7. Reagents

7.1 See procedure "Kjeldahl Nitrogen and Crude Protein in Forages" for nitrogen determination method.
7.2 See procedure "Neutral Detergent Fiber (NDF)" for neutral detergent fiber method.

8. Methods

Option A: Determination of NDIN using total NDF residue (filter paper)

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 at least 6 filter papers overnight at 100°C to determine average filter paper DM content. Weigh filter papers to be used to collect NDF residues to nearest 0.1 mg.
8.3 Thoroughly mix sample and weigh out 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 45 ml NDF solution in digestion burette on fiber rack. Start solution heating while weighing out the 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 weigh filter paper before filtration.
8.11 Put filter paper on the funnel on the vacuum unit below each burette. Turn on vacuum and hot water.
8.12 Open vacuum under 4-6 funnels at a time. If not enough are open the filter paper may tear. Open stop cock on burette to drain into paper, 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 Create more suction by slowly closing a few more vacuum ports.
  8.13.2 If sample refuses to unplug after 15 minutes sample will have to be re-run, cutting sample size in half (0.50 g).

Rinsing:
8.14 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.
8.17 Suction off acetone, rinsing down the side of the filter paper with acetone to finish the rinsing portion.
8.18 Fold filter paper to retain sample, dry a minimum of 3 hours at 105^{o} C in an oven.
8.19 Weigh hot, samples with filter paper, recording to nearest 0.1 mg.
8.20 Insert filter paper and sample into Kjeldahl flasks, add 5 ml additional acid to digest the filter paper and determine nitrogen by "Kjeldahl Nitrogen and Crude Protein in Forages" procedure.

Option B: Determination of NDIN using partial NDF residue (from fritted glass crucibles)

8.21 Sample should be oven dried at 55^{o}C to $\geq$85% dry matter, then ground to pass a 1mm forage mill.
8.22 Dry 50 ml fritted glass crucibles overnight at 100^{o}C and hot weigh, recording weight to nearest 0.1 mg.
8.23 Thoroughly mix sample and weigh out 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:

8.24 Pour approximately 45 ml NDF solution in digestion burette on fiber rack. Start solution heating while weighing out the samples. Make sure water condenser is turned on and the glass condensers are cooling.

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

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

8.27 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.

8.28 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.

8.29 Reflux for 60 minutes.

Filtration:

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

8.31 Put crucibles on vacuum unit below each burette. Turn on vacuum and hot water.

8.32 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.

8.33 Plugging on forage samples:

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

8.34 Plugging on corn or starchy samples:

- 8.34.1 Add 2 ml amylase directly to crucible.

Rinsing:

8.35 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.

8.36 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.
8.37 Suction off acetone, rinsing down sides of crucibles and the fiber mat with acetone to finish the rinsing portion.
8.38 Dry a minimum of 3 hours at 100^{o} C in an oven. Weigh hot, samples with crucibles, recording to nearest 0.1 mg.
8.39 Sample a portion of the NDF residue from fritted glass crucible using a Teflon or plastic policeman into Kjeldahl flasks. Do not scrape so hard as to dislodge glass from the fritted disk.
8.40 Weigh partial NDF residue, recording weight to nearest 0.1 mg.
8.41 Determine nitrogen content of the NDF residue sub-sample using the "Kjeldahl Nitrogen and Crude Protein in Forages" procedure.

9. Calculations

9.1 Option A: Percent Neutral Detergent Insoluble Nitrogen (NDIN), DM basis using total NDF residue (filter paper):
- 9.1.1 % NDIN (DM basis) = {(ml titrated– blank)(.8756)/(sample wt in grams)(% lab DM)}*100
- 9.1.2 Neutral Detergent Insoluble Nitrogen (as percent of total nitrogen), also called NDIN to N ratio. % NDIN (of total N) = {[% NDIN (DM basis)] / [% N (DM basis)]} * 100
- 9.1.3 Percent Neutral Detergent Fiber Crude Protein (NDF-CP), DM basis. %NDF-CP (DM basis) = % NDIN (DM basis) * 6.25

9.2 Option B: Percent Neutral Detergent Insoluble Nitrogen (NDIN), DM basis using particle NDF residues (from fritted glass crucibles or filter paper):
- 9.2.1 % NDIN (DM basis) = [% N of NDF residue * % NDF (DM basis)] / 100
- 9.2.2 Neutral Detergent Insoluble Nitrogen (as percent of total nitrogen), also called NDIN to N ratio. % NDIN (of total N) = {[% NDIN (DM basis)] / [% N (DM basis)]} * 100
- 9.2.3 Percent Neutral Detergent Fiber Crude Protein (NDF-CP), DM basis. %NDF-CP (DM basis) = % NDIN (DM basis) * 6.25

10. Quality Control

Samples are typically run in duplicate due to increased risk of filter paper tearing when using option A.

11. Reporting

Results are reported as % NDF-CP 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 Agricultural 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.