

## **Acid Detergent Insoluble Nitrogen (ADIN) and Acid Detergent Fiber Crude Protein (ADF-CP); also known as ADP or ICP (Insoluble CP)**

### **1. Application**

This procedure is applicable for the determination of acid detergent fiber insoluble nitrogen in all types of forages. Acid detergent insoluble nitrogen (ADIN) is the nitrogen remaining in the acid 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**

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

## 8. Methods

### Option A: Determination of ADIN using total ADF residue (filter paper)

- 8.1 Sample should be oven dried at 55°C to  $\geq 85\%$  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 ADF residues 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 a digestion burette on fiber rack. Start solution heat 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 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 weigh filter paper before filtration.
- 8.9 Put filter paper on funnel on vacuum unit below each burette. Turn on vacuum and hot water, in excess of 95°C.
- 8.10 Open vacuum under 4-6 funnels at a time. If not enough are open the filter paper may tear due to too much vacuum. 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.
- 8.11 Plugging on forage samples:
  - 8.11.1 Create more suction by slowly closing a few more vacuum ports.
  - 8.11.2 If sample refuses to unplug after 15 minutes sample will have to be re-run, cutting sample size in half (0.50g).

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 the filter paper with acetone to finish the rinsing portion.
- 8.15 Fold filter paper to retain sample, dry a minimum of 3 hours at 105° C in an oven.
- 8.16 Weigh hot the samples with filter paper, recording to nearest 0.1 mg.
- 8.17 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 ADIN using partial ADF residue (from fritted glass crucibles)**

- 8.18 Sample should be oven dried at 55°C to  $\geq 85\%$  dry matter, then ground to pass a 1mm forage mill.
- 8.19 Dry 50 ml fritted glass crucibles overnight at 100°C and hot weigh, recording weight to nearest 0.1 mg.
- 8.20 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:

- 8.21 Pour approximately 95 ml ADF solution in digestion burette on fiber rack. Start solution heat while weighing out samples. Make sure water condenser is turned on and the glass condensers are cooling.
- 8.22 When solution is gently boiling, approximately 15 minutes, pour sample from 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.
- 8.23 After solution returns to boiling note time and rinse down sides of burette with squeeze bottle of ADF solution.
- 8.24 Reflux for 60 minutes.

#### Filtration:

- 8.25 Hot weight eight glass crucibles with filter mat, or metal crucibles with Dacron and filter mat, before filtration.
- 8.26 Put crucibles on vacuum unit below each burette. Turn on vacuum and hot water, in excess of 95°C.
- 8.27 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.
- 8.28 Plugging on forage samples:
  - 8.28.1 Continue running hot water on outside of crucible.
  - 8.28.2 Use rubber policeman to break up fiber mat on bottom of crucible. Be very gentle – do not scrape filter mat too harshly.
  - 8.28.3 Add acetone to crucible until it slowly filters out.
  - 8.28.4 If sample refuses to unplug after 15 minutes sample will have to be re-run, cutting sample size in half (0.50 g).
- 8.29 Plugging on corn or starchy samples:
  - 8.29.1 Add 2 ml amylase directly to crucible.

#### Rinsing:

- 8.30 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.
- 8.31 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.
- 8.32 Suction off acetone, rinsing down sides of crucibles and the fiber mat with acetone to finish the rinsing portion.
- 8.33 Dry a minimum of 3 hours at 100° C in an oven. Weigh hot, samples with crucibles, recording to nearest 0.1 mg.
- 8.34 Sample a portion of the ADF 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.
- 8.35 Weigh partial ADF residue, recording weight to nearest 0.1 mg.

- 8.36 Determine nitrogen content of the ADF residue sub-sample using the “Kjeldahl Nitrogen and Crude Protein in Forages” procedure.

## 9. Calculations

- 9.1 Option A: Percent Acid Detergent Insoluble Nitrogen (ADIN), DM basis using total ADF residue (filter paper):
- 9.1.1  $\% \text{ ADIN (DM basis)} = \{(\text{ml titrated} - \text{blank})(.8756)/(\text{sample wt in grams})(\% \text{ lab DM})\} * 100$
  - 9.1.2 Acid Detergent Insoluble Nitrogen (as percent of total nitrogen), also called ADIN to N ratio.  $\% \text{ ADIN (of total N)} = \{[\% \text{ ADIN (DM basis)}] / [\% \text{ N (DM basis)}]\} * 100$
  - 9.1.3 Percent Acid Detergent Fiber Crude Protein (ADF-CP), DM basis.  
 $\% \text{ ADF-CP (DM basis)} = \% \text{ ADIN (DM basis)} * 6.25$
- 9.2 Option B: Percent Acid Detergent Insoluble Nitrogen (ADIN), DM basis using particle ADF residues (from fritted glass crucibles or filter paper):
- 9.2.1  $\% \text{ ADIN (DM basis)} = [\% \text{ N of ADF residue} * \% \text{ ADF (DM basis)}] / 100$
  - 9.2.2 Acid Detergent Insoluble Nitrogen (as percent of total nitrogen), also called ADIN to N ratio.  $\% \text{ ADIN (of total N)} = \{[\% \text{ ADIN (DM basis)}] / [\% \text{ N (DM basis)}]\} * 100$
  - 9.2.3 Percent Acid Detergent Fiber Crude Protein (ADF-CP), DM basis.  
 $\% \text{ ADF-CP (DM basis)} = \% \text{ ADIN (DM basis)} * 6.25$

## 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 % ADF-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. Agriculture Handbook No. 379.
- 12.2 Goering, H.K., C.H. Gordon, R.W. Hemken, D.R. Waldo, P.J. Van Soest, and L.W. Smith. 1972. Analytical estimates of nitrogen digestibility in heat damaged forages. Journal of Dairy Science. 55(9): 1275-1280.