

**NEUTRAL/ACID DETERGENT INSOLUBLE NITROGEN**  
**Up-dated November 2013**

I. Reference:

Goering, H.K., C.H. Gordon, R.W. Hemken, P.J. Van Soest and L.W. Smith. 1970. Analytical measures of heat-damaged forage and nitrogen digestibility. Paper 136 presented at the annual meeting of the American Dairy Science Association, Gainesville, Florida, June, 1970.

I. Reagents:

- A. Neutral Detergent Solution
- B. Acid Detergent Solution:
  - 1. 1 liter of 1 N sulfuric acid (H<sub>2</sub>SO<sub>4</sub>)
  - 2. 20 g of cetyltrimethylammonium bromide, technical grade (CTAB) (C<sub>19</sub>H<sub>42</sub>BrN)

II. Personal Protective Equipment:

- A. Lab coat
- B. Safety glasses/goggles
- C. Latex gloves
- D. Thermal pad for removing beakers from hot plates

III. Procedure:

- A. Dry sample at no more than 70° C and grind through a 20 to 30 mesh or 1 mm screen. Weigh 1 g into a beaker suitable for refluxing.
- B. Add 100 mL room temperature neutral or acid detergent solution. Heat to boiling in 5 to 10 minutes. Reduce heat as boiling begins in order to avoid foaming. Reflux 60 minutes from the onset of boiling; adjust boiling to a slow, even level.
- C. Filter with suction on 15.0 cm Whatman #541 filter paper.
- D. Wash paper several times with hot water. **Note: This acid detergent is an ammonium compound. Thorough washing is essential for good nitrogen analysis.**
- E. Transfer paper and the NDF/ADF residue into a Kjeldahl flask or scrape and mix sample in filter paper then weigh for N analysis on LECO.

III. Calculations:

- A. Determine g N in residue:

$$\text{g N} = \%N * \text{residue wt.}$$

- B. Determine N/ADIN as a percent of dry matter:

$$\text{N/ADIN} = \frac{\text{g N}}{\text{ADIN}} \times 100$$

- C. Determine nitrogen content of the feed using normal Kjeldahl procedures:

$$\% \text{ N in feed dry matter} = \frac{\text{g N}}{\text{DM}} \times 100$$

- D. Express ADIN as a percent of total N:

$$\frac{\text{g N}}{\text{(Sample wt.) (DM)}}$$

$$\frac{\text{g N in residue}}{\text{(Sample wt.)(DM)}}$$

$$\frac{\%N/ADIN}{\%N}$$

$$\text{N/ADIN, \% of Total Nitrogen} = \frac{\text{g N}}{\text{DM}} \times 100$$