

## Neutral Detergent Fiber

### Materials:

600 ml Berzelius beakers  
Fiber digestion apparatus  
Aluminum weighing pans  
Whatman filter paper (#541, 5.5 cm)  
Spatula  
Filtering Flasks  
Buchner Funnels  
Desiccator

### Reagents:

#### **Neutral Detergent Solution:**

1. To 500 ml dd water, add 8 g Sodium hydroxide (Mallinckrodt Cat # SX059-1, FW 40.00, CAS # 1310-73-2 or Macron Cat # 770810, FW 40.00, CAS # 1310-73-2), 29.22 g ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich Cat # ED-1KG, FW 292.24, CAS # 60-00-4) and 13.62 g Sodium borate (Sigma Cat #S-9640, FW 381.37, CAS # 1303-96-4).
2. With heat, dissolve 9.12 g Sodium phosphate dibasic (anhydrous) J.T. Baker Cat #3828-01, FW 141.90, CAS # 7558-79-4) in 50 ml dd water.
3. Dissolve 63.33 g Sodium dodecyl sulfate (Sodium laurel sulfate) (MP Cat # 102918, FW 288.4, CAS # 151-21-3) in 1,000 ml dd water.
4. Pour 1, 2, and 3 above into 2,000 ml volumetric.
5. Bring up to volume with dd water and allow to stand overnight.
6. Check pH. It should be 6.8-7.2. Adjust with NaOH.

#### **Amylase solution:**

We use a heat stable alpha-amylase (DSM Maxamyl™ HT). Brand name: Validase HT340L, Valley Enzymes, South Bend, IN.

**Acetone** (Fisher Cat # A18-20, FW 58.08, CAS # 67-64-1)

### Procedure:

1. Transfer approximately 0.25 g duplicates of sample (air dry) to Berzelius beakers.
2. Label an aluminum weighing pan with sample ID for each duplicate. Place a filter paper in each pan. Dry for 20 minutes at 100 °C, cool in desiccator, and record weight.
3. To each beaker, add 100 ml neutral detergent solution. Then, add 2 ml amylase mixture. Place on reflux apparatus and turn on condensers.
4. Heat to boil (5 to 10 minutes) and reflux gently exactly 60 minutes. If sample boils up on sides of flasks, wash down with NDF solution.

5. Filter with light suction onto filter paper. If filtration is very slow, try adding 1-2 ml amylase solution to sample in funnel.
6. Rinse beaker well with hot dd H<sub>2</sub>O (use rubber policeman if necessary).
7. Wash residue with hot dd H<sub>2</sub>O 2 to 3 times (total of approximately 250 ml), watch for foaming, rinse until clear.
8. Wash residue twice with acetone (around inside walls of funnel and across paper).
9. Carefully transfer paper and residue from funnel weighed pans. Place in drying oven at 100 °C for 8 hours or overnight.
10. Remove from drying oven and cool in desiccator. Weigh and record weight.

**Calculation:**

Sample	A Sample Weight	B DM	C Dry Paper + Pan	D NDF + Paper + Pan	E NDF % dmb
#15	0.2173	0.9136	1.5057	1.5640	29.367
	0.2338	0.9136	1.4917	1.5535	28.933

$$E = \left[ \frac{D - C}{A \times B} \right] \times 100$$

$$29.367 = \left[ \frac{1.5640 - 1.5057}{0.2173 \times 0.9136} \right] \times 100$$

To calculate mean, sd, and % CV, see section on statistical analysis.

**References**

- Goering, H. K. and P. J. Van Soest. 1970. Forage fiber analyses. Agr. Handbook No 379, ARS, USDA.
- J. C. Jeraci, T. Hernandez, I. B. Robertson, and P. J. Van Soest, 1988, JAS 66(Suppl):351