Fat and Neutral Detergent Fiber (NDF) Analysis

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Fat:

Supplies Needed:

-Lab Equipment

Heating Block
Tube Drying Manifold
Centrifuge
Green or Red Pipette Pump
Size 16 Teflon Lined Screw Caps

-Disposables

16 x 100 Screw Top Culture Tubes
2mL Glass Pasteur Pipettes

-Gas

Compressed air from fume hood air line (if doing gravimetric fat analysis)
Bottled nitrogen if analyzing samples for fatty acid profile

I. Reagents:
   A. 50:50 ratio of Hexanes and Diethyl Ether
   B. Dilute HCl (1 drop HCl/ 40mL ddH_20)

II. Personal Protective Equipment:
   a. Lab Coat
   b. Safety glasses/goggles
   c. Latex gloves
   d. Conduct procedure in designated fume hood

III. Procedure:
   1. Weigh up 0.5g of sample into tared 16 x 150 mm screw-top glass tube. Obtain the weight of a second empty 16 x 150 mm screw-top glass tube.

   2. Add 4 mL of 50:50 Hexane and Diethyl Ether solvent mixture to the tubes containing samples and cap tightly (once tight slightly relieve pressure) with a Teflon lined cap (**For forages add 5 to 6 mL**). Vortex the tubes and place in a 50°C heating block for 9 hours. **NOTE: Make sure hot plate is on low setting.**

   3. After the 9 hours, remove the heated tubes from the heating block and place in a test tube rack to cool for about 10 minutes. Add 3 mL of dilute HCl mixture and vortex at a moderate speed (**For forages add 4 mL**). Centrifuge at 1000 x g for 6 minutes at 25°C.

   4. Carefully pipette off the top solvent layer with a glass Pasteur pipette and dispense this into the pre-weighed empty screw-top glass tube. Be careful not to disturb the meniscus. Use a new pipette for each sample.

5. Add 2 mL of 50:50 Hexane and Diethyl Ether mixture to the original tube and vortex moderately again. Centrifuge again at 1000 x g for 6 minutes at 25°C.

6. Pipette off the top solvent layer again with the same Pasteur pipette that was used previously for that sample and add this to the same corresponding tube previously used.

7. In a fume hood, dry off the solvent in heating block set at 50ºC. Set the manifold fingers into the tubes to blow air/nitrogen in to aid in evaporation. (Use air if doing gravimetric analysis, nitrogen if continuing on with a FA profile).

8. Check on tubes after about 10 minutes. Then, roll the tubes in your hands to coat the walls of the tubes with lipid a couple times to release any trapped solvent as the solvent evaporates.

9. Remove the tubes from the heating block when no remaining solvent is left.

10. Allow tubes to cool for 10 min then weigh the tubes.

11. Place the original sample containing tube under the manifold fingers to evaporate off any remaining solvent, rotate if needed. Vortex sample periodically. Sample will **NOT** dry completely due to water remaining in tube. Be careful not to over dry the sample (may create artifact fiber). Leave in a fume hood overnight. These are the tubes to be used for conducting fiber analysis.

NDF

I. References:

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 Sci. 74:3583.

Van Soest, P.J. and W.C. Marcus. 1964. Methods for the determination of cell wall constituents in forages using detergents and the relationship between this fraction and voluntary intake and digestibility. J. Dairy Sci. 47:704. (Abstract of 1964 Amer. Dairy Sci. Assoc., Univ. of Ariz. Tucson).

II. Reagents:

A. Neutral Detergent Fiber Solution
B. Sodium Sulfite
C. Alpha-Amylase
D. Acetone

III. Personal Protective Equipment:

a. Lab Coat
b. Safety glasses/goggles
c. Latex gloves

IV. Procedure:

1. Weigh up 0.5 g sodium sulfite into empty 600 mL tall-form NDF beakers. Rinse the fat tubes containing the fat-extracted sample into the beakers with NDF solution. Be sure to remove the entire sample from the tubes. Bring the volume up to 100 mL of NDF solution.

2. Place the beakers on the hot plates to begin boiling. It is recommended to set these to boil in sets of duplicates for timing purposes. Once these have begun to boil, reduce the heat to maintain a constant and steady reflux for 60 minutes. Be sure to rinse down the sides of beakers to ensure all the sample remains in the NDF solution. After boiling initiation, add 0.5mL alpha-amylase to each beaker.

3. Once boiling is completed, filter the mixture in the beakers with dried down, pre-weighed Whatman 541 filters. Clean out the beakers and filter using hot dH_20. Be sure to remove the entire sample from the beaker. Rinse with acetone followed by hot dH_20 again. Rinse until the sample stops producing soapy foam.

4. Carefully remove the filters and place in a filter rack. Dry in a 100°C oven overnight.

5. Wash all beakers and any other dishes remaining.

V. Calculations:

$$\% \text{ Fat} = \frac{(\text{Fat Residue + Tube Wt}) - (\text{Empty Tube Wt})}{(\text{Sample Wt}) * (\%\text{DM})}$$

$$\%\text{NDF} = \frac{(\text{NDF Residue + Filter}) - (\text{Filter Wt})}{(\text{Sample Wt into glass tube}) * (\%\text{DM})}$$