

# Cumberland Valley Analytical Services, Inc.

## Procedure References

### **Acid Insoluble Ash**

Sand and Silica in Plants, Gravimetric method (920.08). Official Methods of Analysis, 17<sup>th</sup> edition. 2000. Association of Official Analytical Chemists.

### **ADF**

Fiber (Acid Detergent) and Lignin in Animal Feed (973.18). Official Methods of Analysis, 17<sup>th</sup> edition. 2000. Association of Official Analytical Chemists. (Modifications: Whatman 934-AH glass micro-fiber filters with 1.5µm particle retention used in place of fritted glass crucible.) ADF method only.

### **ADF – ash free**

ADF procedure as listed under ADF. Final glass fiber filter and sample ashed in 535°C furnace for 2 hours

### **ADF Nitrogen (ADIN, ADIP)**

Total residue from ADF procedure pressed and wrapped in foil. Analyzed for nitrogen: Leco FP-528 Nitrogen Combustion Analyzer. Leco, 3000 Lakeview Avenue, St. Joseph, MI 49085.

### **Ash**

Ash of Animal Feed (942.05). Official Methods of Analysis, 17<sup>th</sup> edition. 2000. Association of Official Analytical Chemists. (Modifications: 1.5g sample weight, 4 hour ash time, hot weigh)

### **Chloride**

Sample is extracted with 0.5% nitric acid and analyzed by potentiometric titration with silver nitrate using Brinkman Metrohm 848 Titrino Plus. Brinkmann Instruments Inc., One Cantiague Rd P.O. Box 1019, Westbury NY 11590.

### **Corn Silage Processing Score (CSPS)**

Mertens, D.R. Determination of Starch in Large Particles, Ro-tap Shaker Method. U.S. Dairy Forage Research Center. 2002. Particle size analysis of greater than 4.75mm, 4.75 – 1.18 , and less than 1.18. Starch analysis on particles greater than 4.75mm.

### **Crude Fiber**

Fiber (Crude) in Animal Feed and Pet Food (978.10). Official Methods of Analysis, 17<sup>th</sup> edition. 2000. Association of Official Analytical Chemists.

### **Degradable Protein (Strep. griseus)**

Krishnamoorthy, U., C.J. Sniffen, M.D. Stern, and P.J. VanSoest. 1983. Evaluation of a mathematical model of rumen digestion and an in vitro simulation of rumen proteolysis to estimate rumen-undegraded nitrogen content of feedstuffs. Br. J. Nutr. 50:555.

### **Dry Matter**

**Forages:** Two step process: First step: Partial dry matter adapted from Goering, H.K. and P.J. Van Soest. 1970. Forage Fiber Analysis. USDA Agricultural Research Service. Handbook number 379. U.S. Dept. of Agriculture. Superintendent of Documents, US Government Printing Office, Washington, D.C. 20402. Second step: Modified to 105°C for 3 hrs per National Forage Testing Association recommendations, 2002.

**Grains, mixed feeds, concentrates and by-products:** Moisture in Animal Feed, Drying at 135°C (930.15). Official Methods of Analysis, 17<sup>th</sup> edition. 2000. Association of Official Analytical Chemists.

### **Fat**

Crude Fat in Feeds, Cereal Grains, and Forages (2003.05). Official Methods of Analysis, 18<sup>th</sup> edition. 2006. Association of Official Analytical Chemists. Tecator Soxtec System HT 1043 Extraction unit. Tecator, Foss NA 7682 Executive Drive, Eden Prairie, MN 55344.

### **Fat, Acid Hydrolysis**

Fat (Crude) or Ether Extract in Pet Food, Gravimetric Method. (954.02). Official Methods of Analysis, 17<sup>th</sup> edition. 2000. Association of Official Analytical Chemists.

### **Fatty Acid**

Sukhija, P.S.; Palmquist, D.L. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 1988, 36, 1202-1206.

### **Fermentation Analysis**

Extraction: Fermented feed sample is mixed and a 25g wet sample is taken and diluted with 200ml deionized water. Sample mixture sits overnight, then is blended for 2 min and filtered through coarse (20-25 µm particle retention) filter paper. Extract is used in the following procedures:

#### **pH and Titratable Acidity**

30ml extract is introduced to a Mettler DL12 Titrator. pH is read and sample is titrated with 0.1N NaOH to a pH of 6.5. Mettler-Toledo, Inc. 1900 Polaris Parkway, Columbus, Ohio, 43240.

#### **Ammonia**

25ml extract is mixed with 75ml deionized water and introduced to a Labconco Rapidstill II model 65200 analyzer. Sample is titrated with 0.1 N HCl to determine ammonia. Labconco. 8811 Prospect Ave, Kansas City, Missouri 64132

#### **Lactic Acid**

1:1 ratio of extract to deionized water is introduced to a YSI 2700 Select Biochemistry Analyzer to determine L-lactic acid. Result is multiplied by four to obtain total lactic acid. YSI, Inc. Yellow Springs, Ohio, 45387.

#### **Acetic, Propionic, Butyric, & Iso-butyric acids**

3ml of extract is filtered through a 0.2 micron filter membrane and a 1.0µl sub-sample is injected into a Perkin Elmer AutoSystem gas chromatograph using a Restek column packed with Stabilwax-DA. Perkin Elmer, 710 Bridgeport Avenue, Shelton, CT 06484.

### **Lignin**

Goering, H.K. and P.J. Van Soest. 1970. Forage Fiber Analysis. USDA Agricultural Research Service. Handbook number 379. U.S. Dept. of Agriculture. Superintendent of Documents, US Government Printing Office, Washington, D.C. 20402.

#### **Modifications:**

Fiber residue from the ADF step is recovered on a 1.5µm particle retention 7cm Whatman Glass Fiber Filter in a California Buchner Funnel instead of using a Gooch crucible. Greater surface area allows for better filtration. Fiber residue and filter is transfer to a capped tube and approx. 45 ml of 72% Sulfuric Acid is added. Tubes are gently agitated for 2 hours to insure that all fiber material is continually washed with acid. The contents of the tube after incubation in acid is filtered onto a second filter (same type as above) which is then rinsed, dried and weighed. The glass fiber filters and lignin residue are then ashed for 2 hours in a furnace to remove lignin organic matter. Finally, the filter and ash residue is weighed back and subtracted from the original weight to determine grams of lignin.

### **Metals / Minerals**

Metals and Other Elements in Plants (985.01). Official Methods of Analysis, 17<sup>th</sup> edition. 2000. Association of Official Analytical Chemists. Perkin Elmer 5300 DV ICP. Perkin Elmer, 710 Bridgeport Avenue, Shelton, CT 06484. (Modifications include: Ash 0.35g sample for 1 hr at 535°C. Digest in open crucibles for 20 min in 15% nitric acid on hotplate. Samples diluted to 50ml and analyzed on ICP.)

## **Molybdenum**

2g sample ashed at 480°C for 4 hours. Digest in open crucible for 20min in 15% nitric acid on hotplate. Sample diluted to 50ml and analyzed on axial view ICP.

## **Mycotoxins**

**Aflatoxin** - HPLC - Aflatoxins in Corn, Almonds, Brazil Nuts, Peanuts and Pistachio Nuts. Multifunctional Column (Vicam) Method. Ofttserova M Et Al, Journal of AOAC International, Vol. 92, No. 1, 2009 (with modifications).

### **Vomitoxin (DON)**

HPLC - AOAC method (2002) 986.18 (with modifications).

**3 & 15 Acetyl DON** - GC - Tackel and Casper, Journal of AOAC International, Vol. 79, No. 2, 1996.

### **T2 Toxin**

screening by TLC - Romer, T., Journal of AOAC, Vol. 69, No. 4, 1986  
confirmation by GC - Krska R, Baumgartner S, Josephs R. 2001. The state-of-the-art in the analysis of type-A and-B trichothecene mycotoxins in cereals. Fresenius J Anal Chem 371(3):285-99.

### **Zearalenone**

screening by TLC - AOAC 976.22 with modifications  
confirmation by HPLC – Ofttserova M Et Al, Journal of AOAC International, Vol. 92, No. 1, 2009 (with modifications).

**Fumonisin** - HPLC - Fumonisin B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub> in Corn. Ofttserova M Et Al, Journal of AOAC International, Vol. 92, No. 1, 2009 (with modifications).

**Ochratoxin** by HPLC – Ofttserova M Et Al, Journal of AOAC International, Vol. 92, No. 1, 2009 (with modifications).

**Mycotoxin LCMSMS** – By LCMSMS – Trilogy internal procedure SOP-15-197

## **NDF**

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. J.Dairy Science 74:3583-3597. (Modification: Whatman 934-AH glass micro-fiber filters with 1.5um particle retention)

## **NDR**

“Neutral Detergent Residue” is the NDF procedure outlined above but without the use of sodium sulfite. Sodium sulfite facilitates removal of some nitrogen from the fiber complex and theoretically opens the fiber matrix up to greater solubilization of neutral detergent fiber.

## **NDF – ash free**

NDF procedure as listed under NDF. Final glass fiber filter and sample ashed in 535°C furnace for 2 hours

## **NDFD**

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Agric. Handbook 379. ARS, USDA, Washington, DC.

## **NDR Nitrogen (NDIN, NDIP)**

Total residue from NDF procedure pressed and wrapped in foil. Analyzed for nitrogen: Leco FP-528 Nitrogen Combustion Analyzer. Leco, 3000 Lakeview Avenue, St. Joseph, MI 49085.

**Nitrate**

Nitrate in Forages, Potentiometric Method (986.31). Official Methods of Analysis, 15<sup>th</sup> edition. 1990. Association of Official Analytical Chemists. (Modifications: 25ml DI, 1g sample and 1 ionic strength adjustor packet shaken together for 1 hour, then filtered. Nitrate-Nitrogen standards used to calibrate meter.)

**Nitrogen**

Protein (Crude) in Animal Feed (990.03). Official Methods of Analysis, 17<sup>th</sup> edition. 2000. Association of Official Analytical Chemists. Leco FP-528 Nitrogen Combustion Analyzer. Leco, 3000 Lakeview Avenue, St. Joseph, MI 49085.

**Non-Protein Nitrogen (NPN)**

Urea and Ammoniacal Nitrogen in Animal Feed (941.04). Official Methods of Analysis, 17<sup>th</sup> edition. 2000. Association of Official Analytical Chemists. Modified calculation and reporting unit.

**Particle Size**

Heinrichs, J. and P. Kononoff. Evaluating Particle Size of Forages and TMRs using the New Penn State Forage Particle Separator. The Pennsylvania State University Department of Dairy and Animal Science. DAS 02-42 2002.

**PE NDF**

Mertens, D.R. Determination of Starch in Large Particles, Ro-tap Shaker Method. U.S. Dairy Forage Research Center. 2002. NDF analysis on particles less than 1.18mm

**Protein Dispersibility Index (PDI)**

A.O.C.S. Official Method Ba 10-65. Modified by U.S. Dairy Forage Research Center: Jih-Tay Hsu.

**Selenium**

Selenium in Feeds and Premixes (996.16). Official Methods of Analysis, 17<sup>th</sup> edition. 2000. Association of Official Analytical Chemists.

**Soluble Fiber (analytical)**

Neutral detergent soluble fiber including ethanol insoluble residue organic matter, ethanol insoluble residue crude protein, neutral detergent residue organic matter, neutral detergent residue crude protein, ethanol insoluble residue starch. Hall, M. B., J.P. Jennings, and T.K. Miller Webster. 1999. A method for partitioning neutral detergent-soluble carbohydrates. *J. Sci. Food Agric.* 79:2079.

**Soluble Protein**

Borate-Phosphate procedure as detailed in Nitrogen Fractions in Selected Feedstuffs. U. Krishnamoorthy, T.V. Muscato, C.J. Sniffen, and P.J. Van Soest. 1982. *J. Dairy Science* 65:217-225.

**Starch**

Hall, M. B. 2009. Analysis of starch, including maltooligosaccharides, in animal feeds: a comparison of methods and a recommended method for AOAC collaborative study. *JAOACI* 92: 42-49. Correction for free glucose as described in Hall (2009).

**Sugar**

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28:350. Referenced in: Hall, Mary Beth. February 2000. Neutral Detergent-Soluble Carbohydrates - Nutritional Relevance and Analysis. University of Florida, bulletin number 339.

**Sulfur**

Leco Organic Application Note "Sulfur and Carbon in Plant, Feed, Grain, and Flour" Form 203-821-321, 5/08-REV1. Approx. 0.15g sample is introduced to a Leco S632 Sulfur Combustion Analyzer with the use of tungsten oxide as a combustion aid. Leco Corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085.

**Urea**

Urea and Ammoniacal Nitrogen in Animal Feed (941.04). Official Methods of Analysis, 17<sup>th</sup> edition. 2000. Association of Official Analytical Chemists.

**Urease Activity**

A.O.C.S. Official Recommended Practice Ba 9-58