



**AGRIFOOD  
LABORATORIES**

## **METHOD REFERENCES FOR FEED AND FORAGES**

### **DRY MATTER/MOISTURE**

- a. Forages** – 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.
- b. Grains, mixed feeds, concentrates and by-products** - AOAC Official Method 930.15 - Animal feeds and Pet foods - Drying of feeds at 135°C for 2 hr.
- c. Wet pet foods, meat, dry milk products and molasses** – Dry samples at 103°C +/- 2°C for 16 h (overnight).

### **CRUDE PROTEIN (CP)**

AOAC – Official Method 990.03. Protein in animal feed. Combustion method. Protein factor of 6.25 is used for calculation.

### **SOLUBLE PROTEIN AND DEGRADABLE PROTEIN (*Streptococcus griseus*)**

Roe, M.B. and C.J. Sniffen. Techniques for measuring protein fractions in feedstuffs. Department of Animal Science, Michigan State University. – Borate-Phosphate procedure.

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

### **CRUDE FIBRE**

Grain samples containing greater than 10% fat must be defatted before determination.

- a.** AOCS Ba 6a-05. Crude fibre analysis in feeds by filter bag technique (ANKOM Technology)
- b.** AOAC – Official Method 978.10. Crude fibre in animal feed and pet food.

### **ACID DETERGENT FIBRE (ADF)**

ANKOM Technology Method 12. Acid Detergent Fiber in Feeds – Filter Bag Technique. AOAC 973.18 – Fiber (Acid Detergent) and Lignin in Animal Feed.

### **ADF NITROGEN (ADF-CP)**

Residue from ADF procedure in filter bag is analyzed for nitrogen: AOAC - Official Method 990.03. Protein in animal feed. Combustion method.

### **ACID DETERGENT LIGNIN (ADL)**

ADF residue digested in 72% w/w sulfuric acid for 3 hours at ambient temperature: AOAC 973.18D.

### **NEUTRAL DETERGENT FIBRE (aNDF)**

ANKOM Technology Method 13. Neutral Detergent Fiber in Feeds – Filter Bag Technique.

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.

Amylase and sodium sulphite is used.

### **NDF NITROGEN (NDF-CP)**

Residue from NDF procedure (without sodium sulfite) in filter bag is analyzed for nitrogen: AOAC – Official Method 990.03. Protein in animal feed. Combustion method.

### **IN VITRO NDF DIGESTIBILITY (NDFd)**

ANKOM Technology Method 3. In Vitro True Digestibility using the DAISY<sup>II</sup> Incubator – ANKOM Technology – 08/05.

Goering, H.K. and P.J. Van Soest. 1970. Forage Fiber Analyses (apparatus, reagents, procedures, and some applications). ARS/USDA Handbook No. 379, Superintendent of Documents, US Government Printing Office, Washington, D.C. 20402. p13-14.

Rumen fluid is collected from two lactating cows fed a TMR diet. Sample is ground to 1 mm and weighed (0.25-0.3 g) in filter bags. Sample plus inocula are incubated at 39°C at different time points. NDF analysis (Ankom Fibre Analyzer) is performed in the residue after incubation.

### **CRUDE FAT**

ANKOM Technology, Method 2 (01-30-09). - AOCS Official Procedure Am 5-04.

Sample is extracted using petroleum ether.

### **FAT, ACID HYDROLISIS**

AOAC Official Method 954.02 – Crude fat in pet food.

Method applicable to baked and extruded semi-moist pet foods, high temperature pelletized feeds and dried milk products. Sample is hydrolyzed in HCl solution to release bound fat and then extracted with diethyl ether and petroleum ether.

### **STARCH**

AACC International Method 76-13.01 – Total Starch Assay Procedure (Megazyme: Amyloglucosidase/∞-Amylase method). Determination of total starch in cereal products using a kit from Megazyme International Ireland Ltd. Adopted by AOAC – Official Method 996.11

### **IN VITRO STARCH DIGESTIBILITY (SCHd) (High Moisture Corn and Corn silage)**

Richards, C.J., J.F. Pedersen, R.A. Britton, R.A. Stock, C.R. Krehbiel. 1995. In vitro starch disappearance procedure modifications. *Animal Feed Science and Technology*: 55 (35-45).

Reagents and solutions as in: Goering, H.K. and P.J. Van Soest. 1970. Forage Fiber Analyses (apparatus, reagents, procedures, and some applications). ARS/USDA Handbook No. 379, Superintendent of Documents, US Government Printing Office, Washington, D.C. 20402. p13-14.

Rumen fluid is collected from two lactating cows fed a TMR diet. Sample is ground to 4 mm and weighed (0.5 g) in centrifuge tubes fitted with Bunsen valves. Samples and inocula are incubated at 39°C for 3 or 7 h. Starch procedure for residual starch determination.

### **SUGARS**

a. Glucose and Sucrose in feeds and forages - Megazyme K-SUCGL. Sucrose/D-Glucose Assay procedure.

Outlaw, W.H. Jr. And Tarczynski, M.C. (1988). In: Methods of enzymatic analysis (Bergmeyer, H.U. Ed.). 3<sup>rd</sup> ed. Vol 6, pp 96-103. VCH Verlagsgesellschaft mbH, Weinheim, Germany.

Kunst, A., Draeger, B. And Ziegenhorn, J. (1988). In: Methods of enzymatic analysis (Bergmeyer, H.U. Ed.). 3<sup>rd</sup> ed. Vol 6, pp 163-172. VCH Verlagsgesellschaft mbH, Weinheim, Germany.

**b.** Total sugars (Dextrose Equivalent) in Feeds and Mills - AOAC – Official Method 923.09. Invert sugar in sugars and syrups.

**c.** Ethanol soluble carbohydrates or Simple sugars and Water soluble carbohydrates or Total sugars in forages.

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.

Modification: Sample extraction is made by sonicator

### **ASH**

AOAC - Official Method 942.05 - Ash in animal feed. Sample is subject to 600°C for 2 h.

ACID INSOLUBLE ASH – Official Method Ba 5b-68

### **MINERALS AND METALS**

**a.** AOAC - Official Method 985.01- Metals and other elements in plant and pet foods by ICP – Sample is dry ashed, followed by acid digestion with HCl. Minerals are then analyzed by inductively couple plasma after appropriate dilution.

**b.** Mineral supplements – AOAC Official Method 965.09.

### **SULPHUR**

**a.** AOAC Official Method 923.01 – Sulfur in plants. Magnesium nitrate method.

**b.** Sulphur by Combustion. LECO Sulphur Analyzer.

### **SALT (AS SODIUM CHLORIDE)**

AOAC - Official Method 969.10 – Soluble chlorine in animal feed. Potentiometric titration.

### **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. – Percentage of total starch that passes through a 4.75 mm screen.

### **PARTICLE SIZE IN FORAGES**

Heinrichs, J. 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.

### **PHYSICALLY EFFECTIVE NDF (PeNDF)**

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

### **PEPSIN DIGESTIBILITY OF ANIMAL PROTEIN (PD)**

AOAC – Official Method 971.09 – 0.2% pepsin as per AOAC.

**NITRATE**

AOAC Official Method 968.07 – Nitrate and nitrite in animal feed. Colorimetric method.

**AMMONIA**

**Recommended Methods of Manure Analysis.** Ammonium-N determination by electrode (adapted from *Standard Methods for the Examination of Water and Wastewater; Method 4500-NH<sub>3</sub>F*). Ch 4 Sec 2: 26 (2003). Five ml extract mixed with 45 ml deionized water are transferred to an extraction cup and 2 ml of NaOH (10 M) are added. Ammonia-N concentration is read with Ion Electrode.

**UREA**

AOAC - Official Method 967.07 – Urea in animal feed. Colorimetric method.

**UREASE ACTIVITY**

AOCS - Recommended Practice Ba9-58 – Sampling and analysis of oilseed by-products.

**FREE FATTY ACIDS**

AOCS – Official Method Ca 5a-40.

AOAC - Official Method 940.28. Free fatty acids in crude and refined oils.

**PEROXIDE VALUE**

AOAC – Official Method 965.33. Peroxide value of oils and fats.

**INSOLUBLE IMPURITIES**

AOCS - Method Ca 3a-46. Insoluble impurities in oils and fats.

**UNSAAPONIFICABLE**

AOAC - Official Method 933.08. Unsaponifiable residue of oils and fats.

**IODINE VALUE**

AOAC - Official Method 41.1.13 – Iodine value in oils and fats.

**QUANTITATIVE ANALYSIS OF TOXINS**

Toxins present in feed and cereals are determined using specific enzyme immunoassay kits of RIDASCREEN<sup>®</sup>FAST:

Zearalenon – Art N° R5502

Aflatoxin – Art N° R5202

T-2 Toxin – Art N° R5302

EZ-Tox<sup>™</sup>DON Test in grain and feed are determined by the enzyme immunoassay kit from Diagnostix (Cat # 600120-DON)