Dairy One
Forage Laboratory

Analytical Procedures

October 2022

Ash, Total

Ash of Animal Feed
AOAC Official Method 942.05
16th Edition
Chapter 4
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Ash, Acid Insoluble (AIA)

Keulen, J.V. and B.A. Young. 1977. Evaluation of Acid-Insoluble Ash as a natural marker in ruminant digestibility studies. J. Anim. Sci. 44:284.

Procedure notes: 5g sample, ash 450C overnight, boil 5 minutes in 2N HCl, filter through Whatman 541, and finally ash 450C overnight.

Calibrate® Services

Ruminal starch, NDF digestibility, and/or LEAF data determined by NIRS using Calibrate Technologies® patented processes for Calibrate® program users where dietary levels of rumen digested starch (RDS), rumen undigested NDF (RUNDF), and/or Leaves Enhance Alfalfa Forage (LEAF) can be calculated.

Procedure notes: https://www.calibratetechnologies.com/

Carbohydrates, Soluble

Ethanol Soluble Carbohydrates (ESC)

Hall, M.B., W.H. Hoover, J.P. Jennings and T.K. Miller Webster. 1999. A method for partitioning neutral detergent soluble carbohydrates. J. Sci. Food Agric. 79: p.2079-2086.

Procedure notes: Samples shaken for 4 hours at 180 epm with 80% ethanol to extract ethanol soluble carbohydrates comprised of simple sugars. ESC determined using a Thermo Scientific Genesys 10S Vis Spectrophotometer after a colorimetric phenol-sulfuric acid reaction.

Water Soluble Carbohydrates (WSC)

West Virginia University Procedure by W.H. Hoover and T.K. Miller Webster. Determination of Nonstructural Carbohydrates.

Hall, M.B., W.H. Hoover, J.P. Jennings and T.K. Miller Webster. 1999. A method for partitioning neutral detergent soluble carbohydrates. J. Sci. Food Agric. 79: p.2081.

Procedure notes: Samples incubated with water in a 40ºC bath for 1 hour extracting water soluble carbohydrates comprised of simple sugars and fructan. WSC determined using a Thermo Scientific Genesys 10S Vis Spectrophotometer after acid hydrolysis with sulfuric acid and colorimetric reaction with potassium ferricyanide.

Carbon (C)

Leco Application Note – "Carbon/Nitrogen in Soil and Plant Tissue"
Form No. 203-821-442
11/14 – Rev1

Procedure notes: Dry, 1mm ground samples analyzed using a Leco CN628 or Leco CN928 Carbon/Nitrogen Determinator. Leco Corporation, 300 Lakeview Avenue, St. Joseph, MI 49085. www.leco.com

Corn Silage Processing Score (CSPS)

Mertens, D.R. 2005. Particle size, fragmentation index, and effective fiber: Tools for evaluating the physical attributes of corn silages. Pages 211-220 in 4-State Dairy Nutrition and Management Conference, Dubuque, IA. MidWest Plan Service, Iowa State Univ., Ames 50011-3080.

Procedure Notes: The percentage of starch that passes through coarse sieves (particles <4.75 mm) are the adequately processed kernels. The percentage of starch passing through the 4.75 mm sieve is determined by subtracting the amount of starch that did not pass through the 4.75 mm sieve from the total starch. The percentage of starch that passed through the 4.75 mm sieve is the CSPS. 600ml of dried corn silage test sample is shaken on a series of sieves for 10 minutes. 19, 13.2, 9.5, 6.7, 4.75, 3.35, 2.36, 1.18, 0.6 mm sieves used.

Coliform and E. coli, water

Colilert®
IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092. www.idexx.com

Procedure Notes: Colilert® is a presence/absence test that detects total coliforms and *E. coli* at 1 organism/100 ml that uses the patented Defined Substrate Technology® (DST®) to simultaneously detect total coliforms and *E. coli*. Two nutrient-indicators, ONPG and MUG, are the major sources of carbon in Colilert® and can be metabolized by the coliform enzyme *ß*-galactosidase and the *E. coli* enzyme *ß*-glucuronidase, respectively.

As coliforms grow in Colilert®, they use *ß*-galactosidase to metabolize ONPG and change it from colorless to yellow to indicate presence. *E. coli* use *ß*-glucuronidase to metabolize MUG and create fluorescence to indicate presence. Since most non-coliforms do not have these enzymes, they are unable to grow and interfere.

Colilert® is US FDA Approved for Dairy Waters.

- Milk Laboratory Evaluation Form FDA 2400m (3/01)

Colilert® is also US EPA-approved for drinking water presence/absence (P/A) and Most Probable Number (MPN) and for source water. Pertinent references:

- June 29, 1989 US EPA Federal Register Colilert® coliform approval
- June 10, 1992 US EPA Federal Register Colilert® *E. coli* approval

Corn Stalk Nitrogen Testing (CSNT)

Miller, R.O. 1998. Extractable nitrate in plant tissue: ion selective method. p85-88. Y.P. Kalra (ed.) Handbook of Reference Methods for Plant analysis. CRC Press LLC, Boca Raton, FL.

Wilhelm, W.W., S.L. Arnold, and J.S. Schepers. 2000. Using nitrate specific ion electrode to determine stalk nitrate-nitrogen concentration. Agron. J. 92:186-189.

Wilhelm, W.W., G.E. Varvel, and J.S. Schepers. 2005. Corn stalk nitrate concentration profile. Agron. J. 97:1502-1507.

Stalk Analysis. Methodology prepared by Soil Nutrient Laboratory, University of Connecticut.

Stalk Analysis. Methodology prepared by Cornell Nutrient Analysis Laboratory, Cornell University.

Density, manure

Standard Vial Method. 2002. Dairy One.

Procedure Notes: Samples weighed into fixed volume vessel. Density calculated and expressed in kg/l, lbs./ft3, and lbs./gal.

Dry Matter (DM)

Oven – 60ºC for 4 hours (forced air)

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. p15.

NFTA Method 2.2.1.1 – Partial Dry Matter using Forced-air Drying Ovens. NFTA Forage Analyses Procedures. Undersander, D., D.R. Mertens, and N. Thiex. 1993. Pages 25-26

Oven – 135ºC for 2 hours
Loss on Drying (Moisture) for Feeds
AOAC Official Method 930.15
Chapter 4
Page 2

Oven – 105ºC for 3 hours
NFTA Method 2.1.4 – Dry Matter by Oven Drying for 3hr at 105C. Shreve, B, N. Thiex, and M. Wolf. 2006.

Fat

Crude, Acid Hydrolysis
Crude Fat in Pet Food.
AOAC Official Method 954.02
16th Edition
Chapter 4
Page 25-26

Procedure Notes: Utilized for non-dairy based liquid samples.

Crude, Acid Hydrolysis
ANKOM Automated HCl Procedure
Total Fat by Acid Hydrolysis Filter Bag Technique using ANKOM^{HCl} Hydrolysis System
02/25/2014

Procedure Notes: Utilized for non-liquid samples. HCl concentration – 4N. Solvent formula – 45% Petroleum Ether, 45% Diethyl Ether, 10% Ethanol.

Samples weighed (nominal 0.75g sample + 0.75g diatomaceous earth) into XT4 filter bags and hydrolyzed with hydrochloric acid for 60 minutes in a sealed Teflon vessel in batches of 15. Final extraction performed with solvent at 90C for 60 minutes using the ANKOM XT15 Extractor. Total Fat content determined by loss of weight.

ANKOM HCl Hydrolysis System and ANKOM XT15 Extractor.
ANKOM Technology, 2052 O'Neil Road, Macedon, NY 14502. www.ankom.com

Crude, Ether Extraction
Rapid Determination of Oil/Fat Utilizing High Temperature Solvent Extraction
ANKOM Technology Method 2
01-30-2009
AOCS Standard Procedure Am 5-04

Procedure Notes: Utilized for non-liquid samples. Samples weighed into XT4 filter bags and extracted with diethyl ether at 90C for 60 minutes in batches of 15. The process uses the Soxhlet principle in a closed stainless-steel extraction vessel allowing solvent temperatures to exceed solvent boiling points. Fat content determined by loss of weight.

ANKOM XT15 Extractor

Crude, Roese-Gottlieb Method, Base Hydrolysis
Fat in Dried Milk
AOAC Official Method 932.06 A (b) and 932.06 B
16th Edition
Chapter 33
Page 56

*Procedure Note*s: Utilized for dairy based products - milk (liquid and powder), whey, and other milk-based byproducts.

Fatty Acids

Total Fatty Acids (TFA)
Direct FAME synthesis

O'Fallon, J.V., J.R. Busboom, M.L. Nelson and C.T. Gaskins. 2007. A direct method for fatty acid methyl ester synthesis: Application to wet meat tissues, oils, and feedstuffs. J. Anim. Sci. 85: p.1511-1521.

Procedure Notes: Fatty acid methyl esters (FAME) determined directly from fresh tissue, oils, or feedstuffs, without the need for prior organic solvent extraction. FAME synthesis is conducted in the presence of up to 33% water. Wet tissues or other samples are permeabilized and hydrolyzed for 1.5 hr. at 55C in 1N KOH in MeOH containing C13:0 as an internal standard. The KOH is neutralized, and the FFA are methylated by H2SO4 catalysis for 1.5 hr. at 55C. Hexane is the added to the reaction tube, vortex-mixed and centrifuged. The hexane layer pipetted into gas chromatography (GC) vials and then analyzed using a Thermo Trace 1310 Gas Chromotograph fitted with a Supelco SP-2560, 100m x 0.25mm x 0.20um capillary column and a Flame Ionization Detector (FID).

Fatty acids reported: C12:0 Lauric, C14:0 Myristic, C16:0 Palmitic, C16:1 Palmitoleic, C18:0 Stearic, C18:1 Oleic, C18:2 Linoleic, C18:3 Linolenic, C20:0 Arachidic, C20:1 Gadoleic, C20:5 Eicosapentaenoic (EPA), C22:0 Behenic, C22:6 Docosahexanoic (DHA), C24:0 Lignoceric, Other, MUFA, PUFA, RUFAL.

Thermo Trace 1310 Gas Chromotograph
Thermo Fisher Scientific Inc., 81 Wyman Street, Waltham, MA 02454. www.thermoscientific.com

Fiber

Acid Detergent Fiber (ADF)

Acid Detergent Fiber in Feeds
ANKOM Technology Method 14
Filter Bag Technique (for DELTA)
06/10/2020.

Procedure Notes: Solutions as in AOAC 973.18 – Fiber (Acid Detergent) and Lignin (H2SO4) in Animal Feed. Samples individually weighed at 0.5g into filter bags and digested for 75 minutes as a group of 24 in 2L of ADF solution in ANKOM DELTA Digestion Unit. Samples are rinsed three times with boiling water for 5 minutes in filter bags followed by a 3-minute acetone soak and drying at 105ºC for 2 hours.

ANKOM Technology, 2052 O'Neil Road, Macedon, NY 14502. www.ankom.com

Crude Fiber

Crude Fiber Analysis in Feeds
ANKOM Technology Method 1
Filter Bag Technique (FBT)
AOCS Approved Procedure Ba 6a-05 (Rev E 4-13-11)
05/19/2017

Procedure Notes: Same digestion solutions as in AOAC 962.09 – Fiber (Crude) in Animal Feed and Pet Food. Acetone used for presoak fat extraction. 0.5g weighed for forages, dried manure, wood, and wood pellets. 1.0g weighed for all other sample types.

Lignin

Method for Determining Acid Detergent Lignin in the Daisy^{II} Incubator
ANKOM Technology Method 9
06/24/2022.

Procedure Notes: Solution as in AOAC 973.18 – Fiber (Acid Detergent) and Lignin (H2SO4) in Animal Feed. ADF performed as above and residue digested as a group of 24 in 72% w/w sulfuric acid for 3 hours in ANKOM Daisy^{II} Incubator at ambient temperature.

aNDF (amylase and sodium sulfite treated Neutral Detergent Fiber)

Neutral Detergent Fiber in Feeds
ANKOM Technology Method 15
Filter Bag Technique (for DELTA)
06/10/2020

Procedure Notes: Solutions as in 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.

Samples individually weighed at 0.5g into filter bags and digested for 75 minutes as a group of 24 in 2L of NDF solution in ANKOM DELTA Digestion Unit. Four ml Alpha Amylase and 20g sodium sulfite are added at the start of digestion. Samples are rinsed three times with boiling water for 5 minutes. Four ml Alpha Amylase is added to the first two rinses. Water rinses are followed by a 3-minute acetone soak and drying at 105ºC for 2 hours.

aNDFom (aNDF on an organic matter (ash free) basis)

aNDF analyzed as above but with the addition of an ashing step to remove inorganic materials such as minerals, soil, and sand by burning the fibrous residue at 550C for 2 hours.

NDFD (Neutral Detergent Fiber Digestibility – 24, 30, 48 hr. time points)

In Vitro True Digestibility using the Daisy^{II} Incubator
ANKOM Technology Method 3
01/24/2017

Procedure Notes: 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 collected from TMR fed, high producing lactating cows. Dry, ground feed samples (0.25g; 1mm particle size) incubated in Van Soest buffer/rumen fluid mixture for specified hours under anaerobic conditions at 39ºC. After incubation, samples extracted using aNDF procedure to remove bacterial contamination. Residue is undigested fibrous material and is used to determine in-vitro true digestibility (IVTD) and neutral detergent fiber digestibility (NDFD). Lag time 4.9 hours.

NDFDom (Neutral Detergent Fiber Digestibility on an organic matter (ash free) basis – 12, 72, 120 or 30, 120, 240 hrs.)

NDF Digestibility (NDFD) analyzed as above but with the addition of an ashing step to remove inorganic materials such as minerals, soil, and sand by burning the undigested fibrous residue at 550C for 2 hours. NDFDom results expressed on an organic matter (ash free) basis as a percentage of the aNDFom.

uNDFom (undigestible Neutral Detergent Fiber on organic matter (ash free) basis – 12, 72, 120 or 30, 120, 240 hrs.)

NDF Digestibility (NDFD) analyzed as above but with the addition of an ashing step to remove inorganic materials such as minerals, soil, and sand by burning the undigested fibrous residue at 550C for 2 hours. Undigested NDF expressed on an organic matter (ash free) basis as a percentage of the dry matter.

Genetic Testing (NonGMO and Verticillium Wilt)

Subcontracted to Merieux NutriSciences
3600 Eagle Nest Drive, North Bldg, Crete , Illinois 60417

Non-GMO

Chinese National Standard GB/T 38133-2019 “Genetically modified alfalfa detection by real-time PCR” issued October 18, 2019. J101, J163, KK179 Events.

Verticillium Wilt

Entry Exit Inspection and Quarantine Industry Standard of the People’s Republic of China, SN/T 1145-2014 “Detection and identification of *Verticillium albo-atrum* Reinke & Berthold”, issued April 09, 2014.

Procedure Notes: Testing of Alfalfa Hay only. Polymerase Chain Reaction (PCR) tests for the presence of genetically modified organisms (GMO) to 0.01% detection limit (LOD) for Non-GMO testing (glyphosate (herbicide) resistance and low lignin traits) and to 0.2 Haploid Genome Equivalent (HGE; 0.2 HGE means to a level of one-fifth of one spore) detection limit (LOD) for the presence of the fungi responsible for Verticillium Wilt (Vaa).

Grain Particle Size (GPS)

Method of Determining and Expressing Fineness of Feed Materials by Sieving
ANSI/ASAE S319.4
FEB2008
(R2012)

Procedure Notes: Determines distribution of particles within a grain sample by shaking the sample through a series of 12 sieves. The weight of the sample retained on each sieve is divided by the total sample weight to determine the percentage retained on each sieve and the mean particle size in microns.

U.S. Sieve #’s used include 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, 270

Gross energy (GE)

Gross energy (gross calorific value) of solid and liquid materials
IKA C2000 *Basic* or C3000 Calorimeter System

Procedure Notes: Instrument is set to IKA's dynamic mode with an outer vessel temperature set at 25ºC and calibrated with benzoic acid. Analysis time is 7-12 minutes. Dried samples weighed into polyethylene bags. Oil type samples weighed into gelatin capsules. Samples placed in a crucible, then ignited in an oxygen rich atmosphere in a sealed decomposition vessel where the increase in temperature of the system is measured.

The specific gross calorific value of the sample is calculated from the weight of the sample, the heat capacity of the calorimeter determined from calibration standards, and the increase in temperature of the water within the inner vessel of the measuring cell. Results expressed as calories per gram (cal/g).

IKA Works, Inc., 2635 North Chase Pkwy SE, Wilmington, NC 28405-7419. www.ika.com

Microbiological Testing

Mold and Yeast Counts

Subcontracted to Microbac
3821 Buck Dr, Cortland, NY 13045

Yeast and Mold Counts in Foods and Dried Cannabis Flower
AOAC Official Method 997.02
Dry Rehydratable Film Method (Petrifilm™ Method)
Official Methods of Analysis of AOAC INTERNATIONAL (OMA) Online

Minerals

Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, Mo, Co, S, Al, B, Cr, Sr

Minerals in Feeds and Forage matrices by microwave digestion and ICP-OES
CEM-Dairy One Digestion Method
June 2016

Procedure Notes: Samples digested using CEM Microwave Accelerated Reaction System (MARS6) with MarsXpress Temperature Control using 50ml calibrated Xpress Teflon PFA vessels with Kevlar/fiberglass insulating sleeves then analyzed by ICP using a Thermo iCAP Pro XP Inductively Coupled Plasma Radial Spectrometer.

Sample weights – 0.5g for forages, ingredients, byproducts (1.0g for Co or Cr); 0.5g for grain mixes; 0.2g for mineral mixes; Manure - 0.5g dried, ground or 2-10g wet sample.

Samples first pre-digested at ambient temperature 10 minutes with 8ml nitric acid (HNO_3) and 2ml hydrochloric acid (HCl) and then an additional 10 minutes with 1ml 30% hydrogen peroxide (H_2O_2). After pre-digestion complete, samples digested in two stages: Stage one - 10-minute ramp to 135ºC and held for 3 minutes at 1500W. Stage two - 12-minute ramp to 200ºC and held for 15 minutes at 1600W. Vessels brought to 50-ml volume, aliquot used for analysis.

Water – 35ul concentrated nitric acid added to 14ml of water, mixed, then aspirated on ICP for analysis.

Manure Reference: Wolf, Ann, M. Watson, and N. Wolf. 2003. Digestion and dissolution methods for P, K, Ca, Mg and trace elements. Recommended methods of manure analysis. ed J. Peters, pp30-39. University of Wisconsin Extension Publication. A3769

CEM, 3100 Smith Farm Road, Matthews, NC 28106. www.cem.com
Thermo Fisher Scientific Inc., 81 Wyman Street, Waltham, MA 02454. www.thermoscientific.com

Chloride Ion (Cl^-)

Chloride titrations with potentiometric indication
Metrohm Application Bulletin No. 130/4 e
Metrohm Ltd., C-H-9101 Herisau, Switzerland

Procedure Notes: 0.2-0.5g dried, ground sample or 1-5g wet sample extracted for 15 minutes in 50ml 0.1N HNO_3, followed by potentiometric titration with $AgNO_3$ (0.01N or 0.10N) using a Metrohm 905 Titrando Titration Unit equipped with an Ag-ring electrode controlled by Metrohm Tiamo software. For water samples, 25ml of 0.2N HNO_3 added to 25ml of sample.

The method by Metrohm is similar to the concepts found in: Cantliffe, D.J., MacDonald, G.E. and Peck, N.H. 1970. The potentiometric determination of nitrate and chloride in plant tissue. New York's Food and Life Sciences Bulletin. No.3, September 1970. Plant Sciences. Vegetable Crops Geneva. No. 1: 5-7.

Metrohm USA, 6555 Pelican Creek Circle, Riverview FL, 33578. www.metrohmusa.com

Iodine (I)

Subcontracted to Michigan State University Veterinary Diagnostic Laboratory
4125 Beaumont Road, Lansing MI 48910-8104

Procedure Notes: Feed iodine analysis is performed by inductively-coupled plasma mass spectrometry (ICP-MS) in accordance with a modified version of the method for the determination of extractable iodine content in feed using ICP-MS found in the Verband Deutscher Landwirtschaftlicher Untersuchiungs- und Forschungstalten (VDLUFA, 2006) Method book volume 3 (11.7.1) and Volume 7 Environmental analysis (2.2.2.3). In brief, a solution of 7% ammonium hydroxide is added to 1 gram of feed to a final sample weight of 50 grams and subsequently incubated at 95°C for one hour. Upon completion of the digestion, samples are sonicated for one additional hour at room temperature, centrifuged to pellet debris, and an aliquot is diluted for analysis on an Agilent Technologies 7900 ICP-MS. The ICP-MS is tuned to yield a minimum of 7500 cps sensitivity for 1ppb yttrium (mass 89). The analysis is performed in Helium mode to reduce spectral interference and is tuned to limit to less than 1.0% oxide as determined by the 156/140 mass ratio and less than 2.0% double charged ions as determined by the 70/140 mass ratio. This method was validated in-house at the MSU Veterinary Diagnostic Laboratory (MSU-VDL) using National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 3232 Kelp Powder and SRM 3290 Dry Cat Food. Recovered concentrations of iodine from each SRM were within 2% of the certified concentration and within the associated 95% confidence interval.

References:
Verband Deutscher Landwirtschaftlicher Untersuchiungs- und Forschungstalten (VDLUFA). Methodenbuch Band III Futtermittel. 11.7.1 Bestimmung des Gehaltes von extrahierbarem Jod in Futtermitteln mittels inducktiv gekoppeltem Plasma und Massenspektrometrie (ICP-MS).

Verband Deutscher Landwirtschaftlicher Untersuchiungs- und Forschungstalten (VDLUFA). Methodenbuch Band VII Umweltanalytik. 2.2.2.3 Bestimmung des Gehaltes von extrahierbarem Iod in Futtermitteln mittels inducktiv gekoppeltem Plasma und Massenspektrometrie (ICP-MS).

Selenium (Se)

Subcontracted to Michigan State University Veterinary Diagnostic Laboratory
4125 Beaumont Road, Lansing MI 48910-8104

Wahlen R, EvansL, Turner J, Hearn R: The use of collision/reaction cell ICP-MS for the determination of elements in blood and serum samples. Spectroscopy 20 (12): 84-89, 2005

Procedure Notes: 0.5g aliquots of dried, ground feed samples are digested overnight at 95°C in 5mL of nitric acid. The digested samples are diluted with water to 100x the initial feed mass. 200uL of each diluted digest is pipetted and diluted with a solution containing 0.5% EDTA and Triton X-100, 1% ammonium hydroxide, 2% propanol and 20ppb of scandium, rhodium, indium and bismuth as internal standards. An Agilent Inductively Coupled Plasma – Mass Spectrometer (ICP/MS)[1] is used for the analysis. The ICP/MS is tuned to yield a minimum of 7500 cps sensitivity for 1ppb yttrium (mass 89), less than 1.0% oxide level as determined by the 156/140 mass ratio and less than 2.0% double charged ions as determined by the 70/140 mass ratio.
Selenium concentration is calibrated using a 6-point linear curve of the analyte-internal standard response ratio. Standards were from Inorganic Ventures[2]. A NIST[3] Typical Diet standard was used as a control

[1] Agilent Technologies Inc, Santa Clara CA 95051
[2] Inorganic Ventures, Christainsburg, VA 24073
[3] National Institute of Standards and Technology, Gaithersburg MD 20899

Mycotoxins

Subcontracted to Trilogy Lab
870 Vossbrink Dr., Washington, MO 63090

Trilogy Lab – LCMSMS – Internal SOP-15-197
Aflatoxin – B1, B2, G1, G2, Zearalenone, T2 Toxin, Vomitoxin (DON). 3-Acetyl DON 15-Acetyl DON

Procedure Notes: Samples are extracted using 84/16 Acetonitrile/water, purified with a solid phase column (TCM-160), diluted with an acidified solution and finally injected on the LCMSMS.

Trilogy Lab – LCMSMS – Internal SOP-14-168
Ochratoxin A, Fumonisin B1, B2, B3

Procedure Notes: Samples are extracted using 3/1 Methanol/water, purified with a solid phase column (MT3000), diluted with an acidified solution and injected on the LCMSMS.

Near Infrared Reflectance Spectroscopy (NIRS)

Moisture in Forage
AOAC Official Method 991.01
16th Edition
Chapter 4
Pages 2-4

Fiber (Acid Detergent) and Protein (Crude) in Forages
AOAC Official Method 989.03
16th Edition
Chapter 4
Page 20-21

Procedure Notes: Foss NIRSystems Models XDS and 6500 with ISIScan v.4.6.12. Components analyzed by NIRS include: DM, CP, SP, RDP, ADI-CP, NDI-CP, ADF, aNDF, lignin, starch, WSC, ESC, fat, ash, Ca, P, Mg, K, S, Cl, NDFD 24, NDFD 30, NDFD 48, starch digestibility, aNDFom, uNDFom 30, 120, 240, NDFDom 30, 120, 240, Total Fatty Acids, C18:1, C18:2, C18:3, RUFAL. Silages also receive lactic acid, acetic acid, and ammonia CPE.

GLOBAL NIRS calibrations (Dairy One Cooperative, Ithaca, NY) were originally developed according to the principles in AOAC methods and transitioned to LOCAL calibrations when applicable using WinISI v.4.6.11 similar in approach to Shenk et al. (1997).

Near infrared reflectance spectroscopy (NIRS) is an instrumental method for rapidly and reproducibly measuring the chemical composition of forage and feed samples. It is based on the fact that each of the major chemical components of a sample has near infrared absorption properties that can be used to differentiate one component from another and to determine nutrient concentration.

NIR is a calibration-based technology, meaning that analysis is limited to only feeds and nutrients for which calibrations have been developed. Dairy One has built broad based calibrations by incorporating samples collected over several decades. Calibrations are based on reference chemistry using traditional procedures.

Foss North America, 6509 Flying Cloud Drive, Suite 130, Eden Praire, MN 55344. www.foss.us

Nitrates (%NO_3 or ppm NO_3-N)

Reflectoquant® Nitrate Test
RQflex® Reflectometer Method

Procedure Notes: 1g of dried, ground sample or 10g of wet sample is extracted in 50ml deionized water for 20 minutes by shaking at 280 oscillations/minute. Samples are filtered through Whatman 934-AH (1.5um) filter paper, then analyzed by RQflex® Reflectometer using Reflectoquant® Nitrate test strips.

When the Nitrate test strip is immersed in the aqueous sample, a reducing agent reduces nitrate ions to nitrite ions. In the presence of an acidic buffer, the nitrite ions react with an aromatic amine to form a diazonium salt. The salt reacts with N-(1-naphthyl)-ethyelene-diamine to form a red-violet azo dye that is measured reflectometrically. Nitrate concentration is proportional to the color reaction.

Each strip contains two reaction zones generating dual replicate analyses per sample. The RQflex® Reflectometer's double optic system measures the analyte concentration based on the light reflected from the dual reaction zones. Barcode-controlled software calculates the mean of those two measurements.

Millipore Sigma 400 Summit Drive, Burlington, Massachusetts 01803. www.emdmillipore.com

pH

pH in Feed and Forage by electrode

Procedure Notes: 15g wet sample placed into 250-ml beaker. 200ml deionized water added, stirred, and allowed to stabilize five minutes. Manure -35ml liquid sample poured into 50ml beaker. 15g solid or semi-solid sample weighed into 200 ml deionized water, stirred, and allowed to stabilize five minutes.

Alternative for forage and manure – aliquot used after extraction for volatile fatty acids: 50g samples blended at 20000 rpm for 2 min. in 750ml deionized water (Manure 50g and 450ml water), filtered through cheesecloth.

pH of Water
AOAC Official Method 973.41
16th Edition
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All samples analyzed using Thermo Orion Combination Sure-Flow pH Electrode and Thermo Orion 410 A meter. Calibrated with buffers referenced to NIST SRMs. pH 4 buffer contains potassium hydrogen phthalate and pH 7 buffer contains sodium phosphate dibasic and potassium phosphate monobasic.

Thermo Fisher Scientific, 81 Wyman Street, Waltham, MA 02454. www.thermoscientific.com

Protein

Acid Detergent Insoluble Crude Protein (ADICP)

ADF residue analyzed using a Leco TruMac N Macro or CN928 Determinator to determine the protein fraction bound to the acid detergent fiber.

Crude Protein (CP) and Total Nitrogen (N)

Procedure Notes: Dry, 1mm ground samples analyzed by combustion using a CN628 or CN928 Carbon/Nitrogen Determinator. Liquid samples analyzed using a TruMac N Macro or CN928 Determinator.

Multiple References:

Protein (Crude) in Animal Feed
AOAC Official Method 990.03
16th Edition
Chapter 4
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Crude Protein in Meat and Meat Products including Pet Foods
AOAC Official Method 992.15
16th Edition
Chapter 39
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Crude Protein in Cereal Grain and Oilseeds
AOAC Official Method 992.23
16th Edition
Chapter 32
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Leco Application Notes:
"Nitrogen/Protein in Feeds, Grains, and Pet Food"
Form 20X-821-485
03/15 – Rev0.

"Nitrogen in Soil and Plant Tissue"
Form 203-821-443
11/14 – Rev2.

"Nitrogen/Protein in Feeds, Grains, and Oil Seeds"
Form No. 203-821-392
01/16 – Rev2

Manure samples – Watson, M., A. Wolf, and N. Wolf. 2003. Total nitrogen. Recommended methods of manure analysis. ed J. Peters, pp18, 23-24. University of Wisconsin Extension Publication. A3769.

Leco Corporation, 300 Lakeview Avenue, St. Joseph, MI 49085. www.leco.com

Degradable Protein (Rumen Degradable Protein - RDP)
Cornell Streptomyces griseus (SGP) enzymatic digestion.

Procedure Notes: Enzyme concentration held constant. Residues containing undegradable protein analyzed using Leco TruMac N Macro Determinator or CN928.

Concentrates incubated for 18 hrs. Cornell Nutrition Conference Proceedings, 1990. pp. 81-88.
Forage samples incubated for 2 hrs. at higher SGP concentration. J. Dairy Sci. 1999. 82: 343-354.

Neutral Detergent Insoluble Crude Protein (NDICP)
aNDF performed without sodium sulfite then residue analyzed using a Leco TruMac N Macro or CN928 Determinator to determine the protein fraction bound to the neutral detergent fiber.

Non Protein Nitrogen (NPN)

Ammonia Crude Protein Equivalent (CPE) or Ammonium-N
Diffusion Analysis
Timberline TL-2800 Analyzer

Procedure Notes: Extraction - Samples are extracted in deionized water using a single speed blender at 20,000 rpm for 2 minutes (50g/750ml) or a reciprocal shaker for 30 minutes at 280 epm (Forage - 5g/100ml wet or 1g/100ml dry; Manure - 10g/150ml). For urea, a prepared urease solution is added to a duplicate sample prior to shaking (5g/100ml wet or 1g/100ml dry). All extracts then centrifuged at 4000 rpm for 5 minutes, decanted into tubes, then analyzed.

Analysis - A peristaltic pump directs the sample, caustic, and absorbing solutions into a diffusion cell. Within the cell, the sample is mixed with the caustic solution, resulting in a pH of 11-13 which converts the ammonium ion present in the sample to dissolved ammonia gas. The sample/caustic solution flows past one side of a membrane that is permeable to gases but not to liquids or ionic species. The dissolved ammonia gas in the sample/caustic mixture diffuses across the membrane. On the other side of the membrane, a buffered solution absorbs the diffused ammonia gas then flows through a low volume heat exchanger to establish thermal equilibrium then into the conductivity detector. The conductivity cell measures the change in electrical conductance of the absorbing solution. This change is proportional to the concentration of ammonium in the original sample.

Timberline Instruments, 1880 S. Flatiron Ct. Suite I, Boulder, CO 80301. www.timberlineinstruments.com

Extraction using reciprocating shaker – Kalra, Y.P. 1998. Determination of Ammonium-Nitrogen in Plant Tissue. *Handbook of Reference Methods for Plant Analysis*. 11:90.

Principles of operation – Carlson, R.M. 1978. Automated separation and conductimetric determination of ammonia and dissolved carbon dioxide. Analytical Chemistry 50:1528-1531.

Urea Crude Protein Equivalent (CPE)
Timberline TL-2800 Analyzer. Analyzed as above in Ammonia CPE after addition of prepared urease enzyme solution.

Urea Crude Protein Equivalent (CPE)
Urea in Animal Feed, colorimetric method
AOAC Official Method 967.07
16th Edition
Chapter 4
Page 22

Procedure Notes: Alternate urea method used for samples containing high levels of minerals including mineral mixes, liquid supplements, molasses, and grain mixes with added minerals.

Organic Nitrogen, manure
Calculated by difference (Total Nitrogen minus Ammonium-Nitrogen).

Soluble Protein (SP)
Cornell Sodium Borate-Sodium Phosphate Buffer Procedure.

Procedure Notes: Soy products incubated at 39°C. All other samples incubated at ambient temperature. Residue containing insoluble protein analyzed using Leco TruMac N Macro Determinator.

Cornell Nutrition Conference Proceedings, 1990, pp. 85-86.

Standard Plate Count, water

US FDA Milk Laboratory Evaluation Form FDA 2400a (1/01)

Procedure Notes: Petrifilm Aerobic Count Method – 1ml of sample deposited onto petrifilm and covered. Sample distributed with spreader and gel allowed to solidify for 1 minute. Incubated 48 hours at 32°C. Colonies counted when incubation time is complete and reported as colonies per ml.

Starch, Total

YSI 2950D-1 or 2700 SELECT Biochemistry Analyzers

Determination of Cook in Cereal
Xylem YSI Life Sciences Application Note Number 222LS-02

YSI Incorporated Life Sciences, 1725 Brannum Lane, Yellow Springs, Ohio 45387. www.ysilifescience.com

Procedure Notes: Samples are pre-extracted for sugar by incubation in 40ºC water bath and filtration on Whatman 41 filter paper. Residues are thermally solubilized using an autoclave, then incubated with glucoamylase enzyme to hydrolyze starch to produce dextrose (glucose).

Prepared samples injected into sample chamber of YSI Analyzer where dextrose diffuses into a membrane containing glucose oxidase. The dextrose is immediately oxidized to hydrogen peroxide and D-glucono-4-lactone. The hydrogen peroxide is detected amperometrically at the platinum electrode surface. The current flow at the electrode is directly proportional to the hydrogen peroxide concentration, and hence to the dextrose concentration. Starch is determined by multiplying dextrose by 0.9.

Starch, Fecal and TTSD

Starch for fecal samples analyzed as in Starch, Total or by NIRS.

TTSD% (Total Tract Starch Digestibility) = 100.0% - (1.25 * Fecal Starch %)
J. Dairy Sci. 97:1862-1871. Fecal starch as an indicator of total-tract starch digestibility by lactating dairy cows.

Sulfates, water (SO_4 and SO_4-S)

Turbidimetric Method
AQUAfast AC2082 Tablet Chemistry
Thermo Fisher Scientific, 166 Cummings Center, Beverly, MA 01915, 1-800-225-1480. www.thermo.com/water

Procedure Notes: $BaCl_2$ tablet is added to the water sample resulting in precipitation of sulfate as $BaSO_4$. Suspension is measured photometrically at 520nm with a Thermo Scientific AQUAfast AQ4000 Colorimeter to determine the sulfate concentration in mg/l (ppm). Sulfate-sulfur (SO_4-S) calculated as sulfates (SO_4) divided by 2.996.

Total Dissolved Solids, water (TDS)

Conductivity Method
Conductivity/TDS Meter, Sper Scientific Model 850039

Procedure Notes: The total quantity of free ions is determined by ability of the sample to conduct an electrical current. Electrode immersed in water where meter measures the temperature of water and adjusts accordingly, meter is allowed to stabilize for 30 seconds and then reading recorded in ppm.

SPER Scientific, 8281 E. Evans Rd., Suite #103, Scottsdale, AZ 85260. www.sperdirect.com

Total Solids, manure

Oven – 105°C for 16 hours (gravity oven)

Procedure Notes: Used for liquid or solid samples with no bedding.

Oven – 60°C for 6-8 hours (forced air) + NIRS - AOAC 991.01 – Moisture in Forage.

Procedure Notes: Used for Liquid or solid sample with bedding.

Hoskins, B., A. Wolf, and N. Wolf. 2003. Dry matter analysis. Recommended methods of manure analysis. ed J. Peters, pp14-17. University of Wisconsin Extension Publication. A3769.

Volatile Fatty Acids (VFA) and Lactic Acid

Water Extraction Method
Analysis by Gas Chromatography and Biochemistry Analyzer

Procedure Notes: Extraction – 50g samples blended at 20000 rpm for 2 min. in 750ml deionized water (Manure 50g and 450ml water), filtered through cheesecloth, then filtered through disposable syringe filter. Adapted from Personal Communication, L.E. Chase, Ph.D., Cornell University.

Gas Chromatography – Acetic, Propionic, Butyric, Iso-butyric acids

Procedure Notes: Aliquot of extract mixed 1:1 ratio with 0.06M oxalic acid containing 100ppm trimethylacetic acid (internal standard). Samples injected into a Perkin Elmer Clarus 680 Gas Chromatograph containing a Supelco packed column with the following specifications: 2m x 2mm Tightspec ID, 4% Carbowax 20M phase on 80/120 Carbopack B-DA.

References:

- "GC Separation of VFA C2-C5" Supelco GC Bulletin 749F, 1975.
- "Analyzing Fatty Acids by Packed Column Gas Chromatography" Supelco GC Bulletin 856A, 1990.
- "Volatile Fatty Acid SOP" W.H. Miner Institute, Chazy, NY.

Sigma Aldrich (Supelco), 3050 Spruce Street, St. Louis, MO 63103. www.sigmaaldrich.com
Perkin Elmer, 940 Winter Street, Waltham, MA 02451. www.perkinelmer.com

Biochemistry Analyzer – Lactic acid

Procedure Notes: Aliquot of extract analyzed for L-Lactate using YSI 2950D-1 or 2700 SELECT Biochemistry Analyzer equipped with an L-Lactate membrane. YSI User's Manual, page 4-7.

Samples injected into sample chamber of YSI Analyzer where L-Lactate diffuses into a membrane containing L-Lactate oxidase. The L-Lactate is immediately oxidized to hydrogen peroxide and pyruvate. The hydrogen peroxide is detected amperometrically at the platinum electrode surface. The current flow at the electrode is directly proportional to the hydrogen peroxide concentration, and hence to the L-Lactate concentration. Total lactic acid is determined by multiplying L-Lactate by 2.0.

YSI Incorporated Life Sciences, 1725 Brannum Lane, Yellow Springs, Ohio 45387. www.ysilifescience.com