

Committee on Feeds, Fertilizers, and Related Agricultural Topics

Feeds

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Summary

Eight topics of study in the feed area are discussed within this report. Topics with potential collaborative study protocols pending upon completion of data collection are "Loss on Drying for 3 Hours at 105°C," and an SPE/LC method for determining vitamin A. A topic being reinvestigated is AOAC Official Method **942.05**, Ash of Animal Feed. Five collaborative studies, which are underway or in some stage of report review, are discussed in the *Selected Topics Section*.

Loss on Drying for 3 Hours at 105°C—New Method

The National Forage Testing Association's (NFTA) Moisture Task Force investigated the reproducibility, accuracy, and applicability of various oven moisture methods for estimating moisture in forages (personal communication, report to NFTA Board of Directors). A round robin test was conducted to gather data. Two alfalfa hay, 2 grass hay, and 2 corn silage materials were sent to each of 8 laboratories. All materials came from previous NFTA Proficiency Testing materials. Each laboratories ran moisture by 3 oven methods: 105°C for 3 h, 105°C for 6 h, and 135°C for 2 h. The Karl Fischer moisture technique used is now AOAC **2001.12**.

Comparison of oven methods.—Analysis of variance for each sample revealed that laboratories and methods all resulted in significantly different oven moisture values. All laboratories results were within ± 3 standard deviations of the mean for each sample. The AOAC ranking test for laboratories, however, did identify 2 laboratories as outliers. Removing these 2 laboratories from the data pool did not change the outcome of the statistical analysis for 5 of the 6 samples. For one of the grass hay samples, trimming the outlying laboratory eliminated the significant effect of laboratories on oven moisture results. In every case, the current NFTA method (135°C for 2 h) produced significantly higher moisture than the other methods. Little difference was observed in reproducibility among the various methods.

Comparison to Karl Fischer moisture technique.—Paired t-tests comparing each oven method to The Karl Fischer technique were conducted. There was no significant difference (at the 0.01 level) between 105°C for 3 h and Karl Fischer values for the materials studied. Both the 105°C for 6 h and 135°C for 2 h methods resulted in significantly higher moisture results than The Karl Fischer method using homogenization extraction.

Conclusions.—Based upon agreement with the Karl Fischer technique, the 105°C for 3 h oven method best represents the true moisture content of alfalfa and grass hays. Even for corn silage, this method appears better than other oven methods currently available. The NFTA will adopt this method as the reference method for moisture, and will coordinate an AOAC collaborative study on the 105°C for 3 h method.

SPE/LC Method for Determining Vitamin A in Feed—New Method

Large variances are observed in the results of analysis of vitamin A in the AAFCO Feed Check Sample Program. Poor performance of vitamin A methods is due to many problems, some of which occur during the extraction of vitamin A from the hydrolysate, and subsequent evaporation of hexane. Problems that occur at this step include degradation of retinol upon exposure to heat and light, problems associated with certain lots of hexane, and incomplete extraction.

SDB-XC Poly(styrenedivinylbenzene) extraction disks have been used successfully to quantitatively extract retinol from the hydrolysis mixture. Vitamin A was determined on 20 samples by both the AOAC **974.29** colorimetric method and by the SPE/LC method. Vitamin A concentrations obtained by SPE/LC ranged from 81 to 125%, and averaged 102% of those obtained by AOAC **974.29**. Five 10 g portions of soybean meal blank material were spiked with vitamin A acetate standard solutions (0.109, 0.547, 1.09, 11.02, 110.2 IU vitamin A acetate), and recoveries were 94, 94, 93, 101, and 97%, respectively. Five samples of feed containing vitamin A were spiked with vitamin A acetate standard solution, and recoveries from 87 to 100% were obtained.

The use of solid-phase extraction (SPE) disks can facilitate the extraction process, reduce labor input, improve recoveries, and eliminate the use of hexane and the evaporation step. Detection limits for the SPE disks can be as low as 100 IU/lb, depending on the volume of hydrolysate applied to the disk and the volume of methanol used to elute the vitamin A. An AOAC collaborative study on the method is planned.

AOAC Official Method 942.05, Ash of Animal Feed—Existing Method

Problems with AOAC Official Method **942.05**, Ash of Animal Feed, have been reported by the pet food industry; 2 h at 600°C is not always sufficient for carbon-free ash of feed materials, such as corn gluten meal. The problems reported have been reproduced in the GR's laboratory. The Corn Refiners Association confirmed that 2 h is not a sufficient ash time for corn gluten meal. Materials other than corn gluten meal have been reported as problematic.

The method specifies placing the crucible and test portion directly into a 600°C furnace. Frequently this will cause the test portion to ignite and blow out of the crucible. Today's furnaces can be ramped a slow rate from room temperature to 600°C, eliminating this concern and eliminating the need for the precharring step specified in some other AOAC official ash methods. The original collaborative study was completed before the days of electronic analytical balances. Using modern electronic balances, recommendations should be made to weigh to the nearest 0.1 mg. In addition, a statement providing for preashing of the crucibles is needed. A working group of the AAFCO Laboratory Methods and Services Committee has been established to make recommendations for changes to the method.

Selected Study Director Topics

Amylase-Treated Neutral Detergent Fiber

Study Director David Mertens, U.S. Dairy Forage Research Center, Madison, WI, Tel: +1-608-264-5228, Fax: +1-608-264-5275, E-mail: davem@dfrc.wisc.edu, is nearing completion of the collaborative study report manuscript.

Crude Protein in Animal Feeds, Forages, Cereal Grains, and Oilseeds—Block Digestion, Steam Distillation Method, 1-3

Study Directors Nancy Thiex and Harold Manson, Oscar E. Olson Biochemistry Labs, South Dakota State University, Brookings, SD, Tel: +1-605-688-5466, Fax: +1-605-688-6295, E-mail: nancy_thiex@sdstate.edu and harold_manson@sdstate.edu; Shirley Anderson, Foss North America, 7682 Executive Dr, Eden Prairie, MN 55344, Tel: +1-952-974-9892, ext. 161, Fax: +1-952-974-9823, E-mail: sanderson@fossnorthamerica.com; and Jan-Ake Persson, Foss Tecator, Tecator AB, Box 70, SE-263 21 Höganäs, Sweden, Tel: +46 42 361500 or +46 42 361638, Fax: +46 42 340349, E-mail: jan-ake.persson@foss.tecator.se. A collaborative study was conducted to evaluate the repeatability and reproducibility of an extension of AOAC Official Method **991.20**, Nitrogen (Crude) in Milk, to animal feed, forage (plant tissue), grain, and oilseed materials. Test portions are digested in an aluminum block at 420°C in sulfuric acid with potassium sulfate and a copper catalyst. Digests are cooled and diluted, and concentrated sodium hydroxide is added to neutralize the acid and make the digest basic. The liberated ammonia is distilled into a weak boric acid solution using steam distillation. The trapped ammonia is titrated with a stronger standardized acid, hydrochloric acid, using colorimetric endpoint detection. Fourteen blind samples were sent to 13 collaborators in the United States, Denmark, Sweden, Germany, and the United Kingdom. Recoveries of nitrogen from lysine, tryptophan, and acetanilide were 86.8, 98.8, and 100.1%, respectively. The within-laboratory relative standard deviation (RSD_r, repeatability) ranged from 0.4 to 2.38% for crude protein. Among- and within-laboratory RSD_R, (reproducibility) ranged from 0.44 to 2.38%. The Study Directors recommend the method for First Action status. A lower concentration (1% H₃BO₃) trapping solution was com-

pared to the concentration (4% H₃BO₃) specified in the original protocol, and found comparable for use in an automatic titration system, where titration begins automatically as soon as distillation starts. The Study Directors recommend this optional alternative to 4% boric acid trapping solution be allowed for automatic titrators that titrate throughout distillation.

Crude Fat, Diethyl Ether Extraction, in Feeds, Cereal Grains, and Forages by Randall/Soxtec/Submersion Method, 1c-017 and Crude Fat, Hexanes Extraction, in Feeds, Cereal Grains, and Forages by Randall/Soxtec/Submersion Method, 1c-028

Study Directors Nancy Thiex and Bryan Gildemeister, South Dakota State University, Brookings, SD, Tel: +1-605-688-5466, Fax: +1-605-688-6295, E-mail: nancy_thiex@sdstate.edu and bryan_gildemeister@sdstate.edu; Shirley Anderson, Foss North America, 7682 Executive Dr, Eden Prairie, MN, Tel: +1-952-974-9892, ext. 161, Fax: +1-952-974-9823, E-mail: sanderson@fossnorthamerica.com. The submersion method for fat extraction is an AOAC Official Method for Meats and Meat Products. Its use is also widespread in feed laboratories. Approximately 1/3 of the laboratories reporting crude fat results on animal feed to the AAFCO Check Sample Program are reporting fat values obtained using this method (personal communication, George Latimer, Office of the Texas State Chemist). It therefore seems appropriate that this method should be collaborated for animal feeds and forages, and if results are acceptable, the method should be made an AOAC Official Method for Animal Feed, Cereal Grain, and Forage (Plants).

Comparisons of the various fat methods reported in the AAFCO Check Sample Program were made by George Latimer, Chair of the AAFCO Check Sample Committee. On data from 90 check samples, the mean percent recovered by the proposed method was 96.71% of AOAC Official Method **920.39** (diethyl ether, traditional Soxhlet extraction), and the median percent recovered was 98.21%. Regression analysis of the proposed method on AOAC Official Method **920.39** gave a correlation coefficient of 0.9973, R-squared of 99.46%, slope of 1.00062, and an intercept of -0.137367. On this basis, the methods appeared comparable.

The proposed submersion method considerably decreases the extraction time required to complete a batch of samples. The increase in throughput is very desirable in the quest for faster turnaround times and the greater efficiency in the use of labor. In addition, this method provides for the reclamation of the solvent as a step of the method.

While seeking collaborators for this study, it became apparent that few laboratories are using diethyl ether because of safety considerations with the potential for peroxide formation in ether. A second important need for the study is to provide an official alternative solvent to diethyl ether for laboratories performing fat extractions. Petroleum ether, which is not an official solvent for animal feed, was in wide use for fat extractions (personal communication and AAFCO reports). Therefore, comparability tests for petroleum ether and other potential solvents were completed.

Ruggedness tests (1) were performed as part of the method validation process. Variables studied were predry time (2 h vs 4 h); boil time (20 vs 40 min); solvent (diethyl ether vs petroleum ether); rinse time (30 vs 60 min); test portion weight (1 vs 3 g); extraction cup dry time (2 vs 4 h); and solvent drip rate (2 vs 4 drops/s). Ruggedness tests were performed on 3 feed materials. The method was found to be rugged in all variables tested, with one exception: the use of petroleum ether as a solvent produced a consistently low bias compared to diethyl ether.

Petroleum ether, pentanes, and hexanes were tested for comparability to diethyl ether in an effort to find an alternative to diethyl ether for laboratories desiring to avoid use of a solvent with the potential for peroxide formation. The solvents chosen were petroleum ether, pentanes, and hexanes. Solvents were chosen for comparison based upon boiling point, boiling point range, polarity, price, and (in the case of petroleum ether) current usage. Ten materials were chosen for the comparability tests to represent a wide range of feed and forage materials and included a fat supplement, alfalfa hay, soybean meal, corn silage beet pulp, pig starter, meat and bone meal, extruded supplement and cattle protein supplement, and a corn based feed. Correlation coefficients, slope, and bias for petroleum ether, pentanes, and hexanes (compared to diethyl ether and omitting the high fat supplement) are 0.9949, 0.9645, and 0.4662; 0.9938, 1.0216, and 0.3941; and 0.9970, 0.9858, and 0.1661, respectively. Hexanes appear to be the best match to diethyl ether.

Comparability tests described above were repeated using 3 laboratories and 6 feed and forage materials. Correlation coefficients, slope, and bias for petroleum ether, hexanes, and pentanes (compared to diethyl ether and omitting the high fat supplement) are 0.9878, 1.0077, -0.3193; 0.9925, 1.0201, -0.1672; and 0.9880, 1.0274, -0.4141, respectively.

Based upon these results and discussions of them at AAFCO Laboratory Methods and Services Committee Meetings the past year (2), it was decided that the most suitable alternative to diethyl ether is hexanes. Petroleum ether is already in common use as an alternative, but it shows a low bias for some materials, including forages. There are also problems with consistency of the solvent from manufacturer to manufacturer, lot to lot, and country to country, as a fat solvent. Some of this may be due to the wide range of the components of the solvent. The wide boiling point range also makes it a poor choice for recovery and re-use of the solvent.

Water (Moisture)/Dry Matter in Animal Feed, Grain, and Forage (Plant Tissue) by Karl Fischer Titration, I-28

Study Directors Nancy Thiex and Terri Van Erem, South Dakota State University, Oscar E. Olson Biochemistry Labs, PO Box 2170, Brookings, SD 57007-1217, Tel: +1-605-688-5466, Fax: +1-605-688-6295, E-mail: nancy_thiex@sdstate.edu and terri_vanerem@sdstate.edu. A Karl Fischer method for determining water (dry matter) in animal feed and forages was collaboratively studied. Water is extracted from the animal feed or forage material into methanol-formamide (50 + 50) directly in the Karl Fischer titration vessel by high-speed ho-

mogenization. The water was then titrated at 50°C with one component Karl Fischer reagent based on imidazole. Ten blind samples were sent to 9 collaborators in the United States, Canada, and Germany. The within-laboratory RSD_f ranged from 1.14 to 6.99% for water or from 0.09 to 0.56% for dry matter. Among- and within-laboratory RSD_R ranged from 5.35 to 10.73%, or from 0.44 to 0.77% for dry matter. The Study Directors recommend the method for First Action status. A comparable alternative extraction procedure using boiling methanol is also recommended for First Action status.

Recommendations

(1) *Amylase-Treated Neutral Detergent Fiber*: Study Director David Mertens, U.S. Dairy Forage Research Center, Madison, WI, Tel: +1-608-264-5228, Fax: +1-608-264-5275, E-mail: davem@dfrc.wisc.edu. Study Director is finalizing the study report and plans to submit it for review in the next month. Continue study.

(2) *Chelated (Organic) Minerals*: Study Director Grahame A. Leach, Chelated Minerals Corp., PO Box 27872, Salt Lake City, UT 84127, Tel: +1-801-973-4500, Fax: +1-801-972-4068, E-mail: keylated@biokeylate.com. No activity during the past year. Discontinue study.

(3) *Crude Protein*: Topic Advisor Peter F. Kane, Office of the Indiana State Chemist, Purdue University, 1154 Biochemistry Bldg, West Lafayette, IN 47907-1154, Tel: +1-765-494-1560, Fax: +1-765-494-4331, E-mail: kanep@purdue.edu. No pending workload and no activity during the past year. Discontinue topic. Recommend Methods **990.02** and **990.03** for Final Action status.

(4) *Crude Protein in Animal Feeds, Forages, Cereal Grains, and Oilseeds—Block Digestion, Steam Distillation Method, I-3*: Study Directors Nancy Thiex and Harold Manson, Oscar E. Olson Biochemistry Labs, South Dakota State University, Brookings, SD 57007, Tel: +1-605-688-5466, Fax: +1-605-688-6295, E-mail: nancy_thiex@sdstate.edu and harold_manson@sdstate.edu; Shirley Anderson, Foss North America, 7682 Executive Dr, Eden Prairie, MN 55344, Tel: +1-952-974-9892, ext. 161, Fax: +1-952-974-9823, E-mail: sanderson@fossnorthamerica.com; and Jan-Ake Persson, Foss Tecator, Tecator AB, Box 70, SE-263 21 Höganäs, Sweden, Tel: +46 42 361500 or +46 42 361638, Fax: +46 42 340349, E-mail: jan-ake.persson@foss.tecator.se. Collaborative study report has been submitted to AOAC INTERNATIONAL and is in the review process. Continue study.

(5) *Crude Fat, Diethyl Ether Extraction, in Feeds, Cereal Grains, and Forages by Randall/Soxtec/Submersion Method, Ic-017*: Study Directors Nancy Thiex and Bryan Gildemeister, South Dakota State University, Brookings, SD, Tel: +1-605-688-5466, Fax: +1-605-688-6295, E-mail: nancy_thiex@sdstate.edu and bryan_gildemeister@sdstate.edu; Shirley Anderson, Foss North America, 7682 Executive Drive, Eden Prairie, MN 55344, Tel: +1-952-974-9892, ext. 161, Fax: +1-952-974-9823, E-mail: sanderson@fossnorthamerica.com. Collaborative study protocol has been submitted to AOAC INTERNATIONAL and is in the review process. Continue study.

(6) *Crude Fat, Hexanes Extraction, in Feeds, Cereal Grains, and Forages by Randall/Soxtec/Submersion Method, Ic-028*: Study Directors Nancy Thiex and Bryan Gildemeister, South Dakota State University, Brookings, SD, Tel: +1-605-688-5466, Fax: +1-605-688-6295, E-mail: nancy_thiex@sdstate.edu and bryan_gildemeister@sdstate.edu; Shirley Anderson, Foss North America, 7682 Executive Dr, Eden Prairie, MN 55344, Tel: +1-952-974-9892, ext. 161, Fax: +1-952-974-9823, E-mail: sanderson@fossnorthamerica.com. Collaborative study protocol has been submitted to AOAC INTERNATIONAL and is in the review process. Continue study.

(7) *Inorganic Elemental Constituents of Plant Samples, Microwave Digestion*: Topic Advisors Robert Miller, Colorado State University, Fort Collins, CO, Tel: +1-970-493-4382, Fax: +1-970-416-5820, E-mail: rmiller@lamar.colostate.edu and Nancy Thiex, South Dakota State University, Brookings, SD, Tel: +1-605-688-5466, Fax: +1-605-688-6295, E-mail: nancy_thiex@sdstae.edu. No activity during the past year. Continue topic.

(8) **999.13** *Lysine, Methionine, and Threonine in Pure Amino Acids (Feed Grade) and Premixes*: Study Directors Johannes Fontaine, Degussa AG, Hanau, Germany, Tel: +49-6181 593259, Fax: +49-6181 593908, E-mail: Johannes.Fontaine@degussa-huels.de and Marcelle Eudaimon, Customer's Laboratory, Zone Industrielle Nord, Rue de Vaux, F-80884 AMIENS CEDEX 2 France, Tel: +(33)225470-11 or 17, Fax: +(33)22547038. Method has Official First Action Status. Continue study.

(9) *Microscopy*: Topic Advisor Mike Buckner, Division of Consolidated Lab Services, 1 N 14th St, Room 127, Richmond, VA 23219, Tel: +1-804-225-4070, Fax: +1-804-796-7795, E-mail: mbucker@dgs.state.va.us. No activity during the past year. Continue topic.

(10) *Water (Moisture)/Dry Matter in Animal Feed, Grain, and Forage (Plant Tissue) by Karl Fischer Titration, I-28*: Study Directors Nancy Thiex and Terri Van Erem, South Dakota State University, Oscar E. Olson Biochemistry Labs, PO Box 2170, Brookings, SD 57007-1217, Tel: +1-605-688-5466, Fax: +1-605-688-6295, E-mail: nancy_thiex@sdstate.edu and terri_vanerem@sdstate.edu. Collaborative study report has

been submitted to AOAC INTERNATIONAL and is in the review process. Continue study.

(11) *Neutral Detergent Fiber, Acid Detergent Fiber, and Lignin Using Filter Bag Technology*: Study Director Andrew Komarek, ANKOM Technology Corp., Fairport, NY 14450, Tel: +1-716-425-3940, Fax: +1-716-425-3941, E-mail: akomarek@ankom.com. Continue study.

(12) **2000.12** *Phytase Activity in Feeds*: Study Director Peter H.G. Randsdorp, SDM Food Specialties, Department ISL/SP pp 010-0585, PO Box 1, Delft, 2613-MA, The Netherlands, Tel: +31-15-2793543, Fax: +31-15-2792357, E-mail: Peter.Randsdorp@dsm-group.com. Method has Official First Action status. Continue study.

(13) *Sample Preparation*: Topic Advisor George W. Latimer, Office of the Texas State Chemist, College Station, TX 77841-3160, Tel: +1-979-845-1121, Fax: +1-979-845-1389, E-mail: GWL@otsc.tamu.edu. Continue topic.

(14) **999.12** *Taurine in Pet Foods, Liquid Chromatographic Method*: Study Director Claudia Hischenhuber, NESTEC Ltd. Research Centre, Quality & Safety Assurance Department, Vers-Chez-Les-Blanc, Lausanne 26, 1000-CH, Switzerland, Tel: +41-21-7858352, Fax: +41-21-7858553, E-mail: claudia.hischenhuber@rdls.nestle.com. Method has Official First Action status. Continue study.

(15) *Vitamin A, LC Method*: Topic Advisor John L. MacDonald, Ralston Purina Co., St. Louis, MO. Retire Topic Advisor. Continue topic.

(16) A number of methods were never recommended for Final Action. The following are recommend for Final Action at this time: AOAC Official Method **990.03** Protein (Crude) in Animal Feed, Combustion Method; AOAC Official Method **990.02** Protein (Crude) in Animal Feed, Semiautomated Method-Alternative System; AOAC Official Method **941.04** Urea and Ammoniacal Nitrogen in Animal Feed, Urease Method.

References

- (1) Youden, W.J., & Steiner, E.H. (1975) *Statistical Manual of the AOAC*, Association of Official Analytical Chemists, Arlington, VA
- (2) *Minutes* (2000) AAFCO Laboratory Methods and Services Committee Meetings, August 11, Charletson, WV, and September 11, Philadelphia, PA