ANIMAL AND PLANT NUTRITION, HEALTH, AND SAFETY

Critical Factors in Determining Fiber Content of Feeds and Foods and Their Ingredients

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***Background*: Because of its chemical complexity, the estimation of dietary fiber content of feed and food materials is a difficult analytical challenge. Three major fiber analyses are conducted routinely in the United States including crude fiber (CF), detergent fiber, and total dietary fiber (TDF). *Objective*: Factors crucial to the successful measurement of dietary fibers are described and suggestions provided as to how to overcome potential analytical problems within assays. *Methods*: An accounting of methodological details that result in variation in fiber concentration values is presented along with suggestions as to how to decrease the variation. *Results*: CF analysis remains in use in the livestock feed and pet food industries for nutrition labeling purposes in spite of the fact that the analysis does not separate mammalian enzyme-digestible from indigestible carbohydrate components, and values obtained are usually 30–50% of the actual dietary fiber concentration. Detergent fiber methods quantify the insoluble dietary fibers (IDF) accurately, but not the soluble dietary fiber (SDF) components. TDF methods account for intrinsic and intact fibers, isolated and extracted fibers, and synthetic fibers found in feed and food ingredients and complete diet matrixes. *Conclusions*: The CF procedure should be abandoned as it fails to quantify fiber properly. Detergent analyses quantify IDF. TDF methods quantify both IDF and SDF. *Highlights*: Accurate dietary fiber quantification is essential given the role of fiber in health and well-being of animals and humans.**

From the human nutrition perspective, fiber is the carbohydrate and lignin fractions of food and food ingredients that is indigestible by mammalian enzymes, although the carbohydrates may be fermentable in the large bowel (1). Chemically, this fraction includes cellulose, hemicelluloses, lignin (a complex polyphenolic closely associated with cellulose and hemicelluloses), and soluble fibers such as pectins, beta-glucans, gums, mucilages, and oligosaccharides. For ruminants, in which fermentation is a major digestive process that occurs anterior to gastric digestion, fiber is defined typically as lignin and insoluble carbohydrate that cannot be fermented or is slowly fermented by anaerobic bacteria and that occupies space in the gastrointestinal tract.

Practical and routine methods of measuring different fiber concentrations are desirable. These methods are a compromise between the nutritional concept of fiber and use of chemical solubility methods to isolate and measure the nutritionally relevant fiber fractions (2). Because there is no guarantee of direct correspondence between chemical solubility and nutritional availability, fiber is defined by the method used to measure it (Codex Type 1 methods or empirical methods). The Codex definition, "a method which determines a value that can only be arrived at in terms of the method per se and serves as the only method for establishing the accepted value of the item measured," suggests that different fiber methods must always have different test names, and none can be termed "fiber" without a modifier. Crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), amylase-treated NDF (aNDF), total dietary fiber (TDF), insoluble dietary fiber (IDF), soluble dietary fiber (SDF), and nonstarch polysaccharides (NSP) are analyses used today to quantify fibers in food and feeds.

The association of fiber values with a specific method suggests that fiber methods must be followed exactly to be reproducible. Fiber results often are variable because of modifications commonly introduced by various labs (2) with the justification that modifications are done (*1*) to meet the specific needs of a particular application or research project, (*2*) for convenience, or (*3*) to increase the speed of fiber analysis. Without careful evaluation, method modifications have the potential to result in fiber concentrations that are not comparable with those obtained using the parent method; thus, values should not be reported using the same method name to avoid confusion. Modifications to original procedures should be described in detail in the Materials and Methods section of manuscripts. Any modification to a fiber method must be evaluated thoroughly with a variety of substrates in order to evaluate its applicability or inapplicability to diverse matrixes (2, 3).

The objective of this paper is to discuss the critical steps and conditions for select fiber analyses and describe the potential

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problems inherent in the methods themselves. The following three major fiber assays will be considered: CF, detergent fibers, and TDF. In addition, within laboratory repeatability and among laboratory total reproducibility data are presented for detergent fibers and TDF in Tables 1 and 2, respectively.

Fiber Methods

CF

CF is the dried residue lost on ignition after extraction of a fat-extracted test portion with hot 0.255 N sulfuric acid and 0.313 N sodium hydroxide. This method was designed originally to separate plant carbohydrates into digestible and indigestible fractions. It was developed by Henneberg and Stohmann in the 1860s at the Weende Agricultural Experiment Station in Germany as a part of the Weende System of Proximate Analysis (7). Although CF was a standard for measuring fibrous components, it was observed early on that some CF was partially fermentable by microorganisms (8) in the animal fermentative compartments (e.g., reticulorumen, cecum, large bowel). The alkali in the CF method is used to extract protein, but it also solubilizes some lignin and phenolic compounds. The acid in the method dissolves many of the hemicelluloses. Thus, some of the lignin, phenolic complexes, and hemicelluloses are included in the nitrogen-free extract that is calculated by difference. Because CF includes most of the cellulose but little of the hemicelluloses and variable portions of the lignin in a material, it underestimates the true fiber content by 30–50%. Historically, CF has been useful because it is negatively related to digestibility and energy value, especially within a feed type. Unfortunately, and despite its gross inaccuracies, it remains today the legal measure of fiber in plant tissues, manufactured livestock feeds, and pet foods in the United States. Three AOAC methods for CF exist; AOAC **930.10** relates to CF in plant tissues, and AOAC **962.09** and **978.10** relate to CF in animal feed and pet food. The American Oil Chemists Society (AOCS) established the filter bag method as an official method (Ba6a.O5) for CF analysis.

NDF, ADF, and ADL

Animal nutritionists have largely replaced the CF assay with the detergent system of fiber analysis. Detergents are used to remove protein from fiber residues. The technique of using detergents to separate mammalian enzyme-digestible and indigestible parts of plants was originally proposed by Van Soest in the 1960s. ADF (9) was designed to remove hemicelluloses and obtain a low-nitrogen residue for the measurement of lignin (ADF is primarily cellulose and lignin, with some ash). Both protein and hemicellulose complexes can contaminate lignin determinations. The concept behind NDF (10) analysis is that plant cells can be divided into the less digestible cell walls (NDF is primarily cellulose, hemicelluloses, and lignin with some ash, silica, and cutin) and the highly digestible cell contents (containing starch and sugars).

The ADF is the residue remaining after boiling a test portion in acid detergent solution; it was developed as a preparatory step for lignin determination (9). The sulfuric acid in the ADF solution hydrolyzes the hemicelluloses into their component sugars, thus resulting in their elimination in the filtrate. Substrates with higher ADF values are lower in digestible energy than those with lower ADF values, and they often are used in nutritional equations to estimate digestibility, total digestible nutrients (TDN), and/or net energy for lactation (NE_L).

There are two methods for quantifying ADL. Acid detergent sulfuric acid lignin (ADSL) is obtained by treating the ADF residue with 72% sulfuric acid for 3 h with occasional stirring at room temperature, resulting in the dissolution of cellulose

Table 1. Within-laboratory repeatability and among-laboratory total reproducibility for acid detergent fiber (ADF), acid detergent sulfuric acid lignin (ADSL), amylase-treated neutral detergent fiber (aNDF), and amylase-treated neutral detergent fiber expressed on an organic matter basis (aNDFom)

Fiber method	No. of labs	No. of tested materials	Concn value/range, %	SD_r avg. or range[a]	SD_R avg. or range[b]	RSD_r avg. or range[c]	RSD_R avg. or range[d]
ADF[e]	10	6	39.5	0.38	1.13	1.06	3.16
ADSL[e]	10	6	6.7	0.29	0.62	0.81	1.74
ADF[f]	22	6	3.5–72.7	0.3–0.9	0.6–2.4	1.3–8.6	3.4–17.8
ADSL[f]	22	6	0.9–20.3	0.1–0.6	0.2–0.7	2.7–39.0	3.7–52.6
aNDF[g]	11	11	–0.1 to 90.4	0.5–1.7	0.8–2.2	neg–8.2	neg–16.4
aNDFom[g]	11	11	0.4–89.3	0.3–1.8	0.3–2.2	1.4–68.4	2.0–78.9
aNDFom[h]	11	11	37.7	1.02	1.28	2.85	3.58

[a] SD of within-laboratory repeatability.

[b] SD of total reproducibility among laboratories.

[c] RSD of within-laboratory repeatability ($RSD_r = SD_r$ / Mean).

[d] RSD of total reproducibility among laboratories ($RSD_R = SD_R$ / Mean).

[e] Using crucibles (4).

[f] Using FiberTec (5).

[g] Using crucibles and FiberTec (6).

[h] aNDFom = aNDF organic matter (6).

Table 2. Within-laboratory repeatability and among-laboratory total reproducibility for total dietary fiber (TDF), soluble dietary fiber (SDF), and insoluble dietary fiber (IDF)

Fiber method	No. of labs	No. of tested materials	Concn range, %	SD_r range[a]	SD_R range[b]	RSD_r range[c]	RSD_R range[d]
TDF[e]	9	9	1.0–86.9	NR[f]	NR	0.56–66.25	1.56–66.25
TDF[g]	8–10	8	1.2–47.3	0.18–1.07	0.22–3.70	1.48–22.93	4.13–29.23
SDF[g]	8–10	7	0.4–9.6	0.10–0.90	0.24–1.59	5.70–58.35	10.40–84.64
IDF[g]	8–10	8	0.9–40.9	0.09–0.80	0.30–2.76	1.59–17.07	2.68–35.33
TDF[h]	13–16	8	11.6–47.8	0.41–1.43	1.18–5.44	1.65–12.34	4.70–17.97
SDF[i]	13–15	8	10.5–29.9	0.47–1.41	0.95–3.14	2.43–8.60	6.85–19.48
IDF[i]	13–15	8	3.8–17.3	0.28–1.03	0.85–1.66	3.86–18.10	7.53–26.20
IDF[i]	13–15	8	1.1–25.7	0.13–0.71	0.42–2.24	2.03–12.25	4.76–39.64

[a] SD_r range = SD of within-laboratory repeatability.

[b] SD_R range = SD of total reproducibility among laboratories.

[c] RSD_r range = RSD of within-laboratory repeatability ($RSD_r = SD_r$ / Mean).

[d] RSD_R range = RSD of total reproducibility among laboratories ($RSD_R = SD_R$ / Mean).

[e] AOAC **985.29**.

[f] NR = Not reported.

[g] AOAC **991.43**.

[h] AOAC **2009.01**.

[i] AOAC **2011.25**.

and leaving behind the lignin fraction that is measured on an ash-free basis (9). Acid detergent permanganate lignin (ADPL) is measured by oxidizing lignin in the ADF residue, which leaves a cellulose fraction that is measured on an ash-free basis (11). The two lignins are highly correlated, and, typically, the ADPL value is higher than the ADSL value. Lignin is considered the greatest impediment to digestion of cell wall carbohydrates. Accurate quantification of this component aids in understanding the overall use of fibrous carbohydrates by the animal.

NDF is the insoluble residue left after boiling a material in neutral detergent solution. The original NDF method used sodium sulfite to improve the solubilization of proteins (10). However, this method did not remove starches adequately, and the neutral detergent residue method (12) was developed that used a heat-stable alpha-amylase to enhance the removal of starch from NDF residues, but this method removed sodium sulfite. Hintz et al. (13) observed that sodium sulfite was needed to remove protein-carbohydrate complexes formed when materials were heated. Mertens (6) included heat-stable amylase with the original NDF method that used sodium sulfite to obtain the aNDF (AOAC **2002.04**; 14) that also can be used to measure ash-free aNDF or aNDF organic matter after ashing.

The cellulose, hemicelluloses, and lignins measured by the various NDF methods represent the insoluble fibrous content of materials. Because they give the plant rigidity and enable it to support itself as it grows, cellulose and hemicelluloses are classified as structural carbohydrates. Although lignin is indigestible, the structural carbohydrates can be (to varying degrees) fermented by microorganisms in animals with a reticulorumen (e.g., cow, sheep, goat), a cecum (e.g., horse, rabbit, pig, guinea pig), or a large bowel (most species). Concentrations of NDF often are used in nutritional equations to estimate intake, digestibility, TDN, and/or NE_L.

Hemicelluloses often are calculated as the difference between NDF and ADF. There can be instances in which this difference is zero or negative; however, this result may not be related to errors in analysis. Because the neutral detergent solution contains EDTA, which chelates Ca and helps to solubilize pectins in boiling solutions, NDF can contain less pectin than ADF. Bailey and Ulyatt (15) reported that ADF may contain up to 50% of the pectins in clover in addition to some hemicelluloses (12, 15, 16). Neutral detergent solution also extracts tannins more effectively than acid detergent solution, as indicated by a reduced ADF concentration when pre-extracted by neutral detergent solution for materials with 10 g/kg condensed tannins (17). When the ADF concentration is larger than the NDF concentration, the result should be discarded, and a new test portion reanalyzed sequentially after NDF and reported as sequential ADF.

TDF

The impetus for establishment of the TDF methodologies was passage of the Nutrition Labeling and Education Act of 1990 by the U.S. Congress that required the concentration of "dietary fiber" to be listed on the Nutrition Facts Panel of human foods. Development of these procedures began in 1981 when human nutrition experts in the field of dietary fiber gathered at the AOAC INTERNATIONAL Spring Workshop in Ottawa, Canada, and concluded that two methods for the determination of TDF in foods should be developed, including (*1*) a rapid enzymatic-gravimetric method based on procedures developed previously by Asp et al. (18), Furda (19), and Schweizer and Wursch (20), and (*2*) an enzymatic-gravimetric-liquid chromatographic method based on modifications of the enzymatic-chemical methods of Southgate (21) and Theander and Aman (22) that quantified the individual dietary fiber components (23).

TDF constituted the actual "definition" of dietary fiber in human nutrition prior to May 27, 2016, when the U.S. Food and Drug Administration (FDA) published a final ruling amending

their Nutrition and Supplement Facts label regulation (i.e., residue recovered as TDF from test portion analysis was the fiber value presented on the Nutrition Facts panel of human foods). The FDA final rule defined dietary fiber as nondigestible soluble and insoluble carbohydrates (with three or more monomeric units) and lignin that are intrinsic and intact in plants as well as isolated or synthetic nondigestible carbohydrates (with three or more monomeric units) determined by FDA to have physiological effects that are beneficial to human health (24).

In the enzymatic-gravimetric method, the sum of insoluble and soluble polysaccharides and lignin was quantified and reported as a unit (AOAC **985.29**; 25–27). With slightly modified methodology, IDF and SDF could be measured separately (AOAC **991.43**; 28). These methods were the "standards" for dietary fiber until the early 1990s. It then was recognized that nondigestible oligosaccharides, which do not precipitate in the 78% ethanol used in the original TDF procedures, and resistant starch (first recognized by Englyst et al. as a result of their research on measurement of NSP) possess many of the characteristics commonly associated with dietary fiber (i.e., not enzymatically digestible but potentially fermentable; 29). This led to the development of specific dietary fiber methods for the measurement of fructooligosaccharides (AOAC **997.08** and **999.03**; 30, 31), galactooligosaccharides (AOAC **2001.02**; 32), resistant maltodextrins (AOAC **2001.03**; 33), and resistant starch (AOAC **2002.02**; 34). However, a portion of some components that were measured by the specific methods also was measured by the TDF method, leading to double counting. This problem was solved by following procedures outlined in AOAC **2009.01** and **2011.25**, both enzymatic-gravimetric-liquid chromatographic methods and termed the "integrated TDF method" (35, 36). AOAC **2009.01** quantifies TDF including resistant starch and dietary fiber that is not precipitated in 4 parts alcohol:1 part water (nonprecipitable SDF) of degree of polymerization >3 (35). AOAC **2009.01** combines the key attributes of AOAC **985.29**, **991.43**, **2001.03**, and **2002.02** (35). AOAC **2011.25** quantifies IDF, SDF, and TDF inclusive of the resistant starch and the water:alcohol-soluble nondigestible oligosaccharides and polysaccharides of degree of polymerization >3 (36). AOAC **2011.25** combines the key attributes of AOAC **985.29** (and its extensions, **991.42** and **993.19**), **991.43**, **2001.03**, and **2002.02** (36). These methods are applicable to plant materials, foods, and food ingredients consistent with the Codex Alimentarius Commission definition of dietary fiber adopted in 2009 and modified slightly in 2010 and including naturally occurring, isolated, extracted, modified, and synthetic fibers. As a result of these advancements in analytical technology, dietary fibers, whether intrinsic and intact, extracted/isolated, or synthetic, can be quantified accurately.

The main steps in enzymatic-gravimetric dietary fiber methods (e.g., AOAC **985.29** and **991.43**) include enzymatic treatments for starch and protein removal, precipitation of SDF components by aqueous ethanol, isolation and weighing of the dietary fiber residue, and correction for protein and ash in the residue (37). Gravimetric methods tend to have higher coefficients of variation at low dietary fiber concentrations. This is because corrections for protein and ash become proportionately larger at low dietary fiber concentrations. For routine analysis of dietary fiber, these methods are more convenient and less expensive. In addition, they measure the components of fiber in most natural ingredients that are composed mostly of cell wall polysaccharides and lignin.

In the enzymatic-gravimetric-liquid chromatographic methods (e.g., AOAC **2009.01** and **2011.25**), the enzymatic removal of starch is the first key step of the procedure and is accomplished by use of a pancreatic alpha-amylase + amyloglucosidase in maleate buffer while mixing to maintain a continuous suspension. In the present method, this is done for 16 h, but in a modification to that method that currently is being evaluated, incubation time has been reduced to 4 h so as to be more physiological. Nonresistant starch is solubilized and hydrolyzed to glucose and maltose. The reaction is terminated by pH adjustment and heating. Protein in the test material is digested with protease. For the measurement of IDF, the digestate is filtered and the IDF determined gravimetrically after correction for protein and ash in the residue. For the measurement of water-soluble but water:alcohol-IDF [SDF precipitate (SDFP)], ethanol is added to the filtrate of the IDF. The precipitated SDFP is captured by filtration and determined gravimetrically after correction for protein and ash in the precipitate. Nonprecipitable water:alcohol-soluble dietary fiber (SDF solubles) in the filtrate is recovered by concentrating the filtrate, deionizing through ion exchange resins, concentrating, and then quantifying by LC or, alternatively, by concentrating the filtrate and simultaneously deionizing and quantifying by LC.

These methods enable detailed analysis of sugars, starches, nondigestible oligosaccharides, noncellulosic polysaccharides, cellulose, and lignin and often are used for the analysis of complex food matrixes that include isolated/extracted fibers (e.g., inulin) and/or synthetic fibers (e.g., resistant maltodextrins) in addition to natural ingredient fiber sources. They quantify, for all practical purposes, all components of dietary fiber present in a material. Although these methods were initially applied to human foods and their ingredients, animal nutritionists now are using these methods to quantify fibers in complete feeds, feed ingredients, and byproduct feeds used for nonruminant livestock, poultry, pets, and exotic animals.

Survey of Fiber Methods in Use Today

At its January 2016 meeting, the Association of American Feed Control Officials' (AAFCO) Laboratory Methods and Services Committee created a fiber best practices working group. This working group was tasked with reviewing fiber methods in use and creating a document detailing critical factors that the labs need to observe to achieve consistent reliable fiber values.

The working group sent a six-page survey to the members of these organizations: AgLabs (listserv of state and federal regulatory labs), AAFCO's Laboratory Methods and Services Committee members, National Forage Testing Association, and fiber equipment manufacturers customer lists. Fifty surveys were returned that represented 11 government labs, 11 university research labs, and 28 private labs. The survey revealed that the filter bag technique was used by many labs (Table 3). This paper is an outcome of the work of the fiber best practices working group.

A review of AAFCO's feed proficiency testing program monthly method and summary reports (38) showed that the ANKOM filter bag (39) was used the most compared with the hot plate/Berzelius beaker/sintered glass crucible method and the Fibertec extractor method.

Generally, analytical methods used by regulatory bodies must have demonstrated repeatability and reproducibility to obtain recognition of a third-party standards body, such as AOAC INTERNATIONAL. Regulatory laboratories may use methods

Table 3. Number of responses to survey questions regarding fiber methods being used by laboratories

	Fiber method		
Technology	CF	ADF	NDF
Hot plate/Berzelius beaker/sintered glass crucible	9	9	8
Fibertec extractor (boiling column/sintered glass crucible)	1	3	2
ANKOM filter bag in pressurized kettle	15	33	28
Fibertherm bag in reflux kettle	1	1	1

that have not been established as standards for screening purposes, but generally do not use them to take regulatory action. The filter bag detergent fiber techniques fall in this category, because reproducibility of the methods has not been formally established, and they are not standard (or "official") methods. Because of their widespread use, the authors believed it was important to include a discussion of the filter bag technologies in this paper. The authors recommend that reproducibility of these methods be formally established in the near future so that they can become standards for the determination of detergent fibers.

Factors Resulting in Variation in Crude and Detergent Fiber Analyses

Prior to publication of the AOAC document entitled "Method for the Analysis of Cattle Food" published in 1887 (40) and the methods of the International Congress of Applied Chemistry (41), there were numerous variations in methods used to measure CF. Earlier methods varied in the ratio of reagent to test portion mass, types and strengths of acids and bases, time of boiling, temperature of acid and base when added, condensing of reagents during boiling, volume and temperature of water used to wash residues between and after extractions, filtration apparatus, and pre-extraction with ether. Normality of acids and bases must not vary by more than ±0.005 N units. Care must be taken when preheating the CF acid and base solutions prior to adding them to the reflux vessel. Boiling of the solutions or prolonged simmering before addition will result in the loss of water and an increase in the concentration of the acid and base. To minimize evaporation, a large quantity of solution should be preheated in a vessel equipped with a reflux condenser or heated using the heat exchanger described in AOAC Official Method **962.09C(f)** (39).

Other factors in CF analysis that affect applicability or accuracy of results are fiber concentration and matrix composition of the test portion. For example, a CF guarantee on milk replacers is required, likely as a means of detecting soy or other vegetable protein sources. However, the concentration of CF in milk replacer (0.2–0.6%) does not allow for its accurate measurement as the limit of quantitation of this test is 0.3–0.5%. In addition, an ammonium hydroxide fat hydrolysis step is required for milk replacers that is different than the fat pre-extraction step used traditionally in CF analysis. Fat is difficult to extract from milk replacers because it is homogenized and encapsulated in a dairy protein matrix; thus, a more robust hydrolysis step is needed to avoid interference from lipids.

The analytical techniques for measurement of crude and detergent fibers (7, 10) have stayed basically the same, although slight changes to the NDF methods (6, 12) have been made over the years. The only modification of the ADF method has been that of extracting the test portion using acetone for materials with >5% fat. This modification was needed to solve a problem observed by a regulatory lab who found that feed mixtures containing added fat generated ADF values higher than those calculated from ingredient composition data. An in-house study was conducted before editorial changes to the ADF method were made to confirm that pre-extraction of fat (>5%) was needed for ADF as is the case for NDF as fats complex with detergents and reduce their extraction capacity. Although the guiding principles have remained the same, the methods and equipment have been modified for greater throughput and reduction in tedious steps. Originally, crude and detergent fibers were first determined by boiling test materials in a beaker and filtering residues through Gooch crucibles, and many labs still use this method. Tecator introduced the Fibertec system in 1976 that allowed simultaneous extraction and sequential filtration of six test portions in heated crucibles, thereby eliminating the need to transfer the solution to a filtering crucible. The ANKOM^{200} Fiber Analyzer was introduced in 1992 that allowed the determination of up to 24 test portions placed in filter bags in a pressurized extraction vessel. Gerhardt followed this with its Fibretherm System that allowed simultaneous determination of 12 test portions placed in filter bags in a reflux kettle. Although there are different extraction systems and methods, all must be standardized to obtain acceptable reproducibility among laboratories and agreement with AOAC Official Methods.

Segregation, Comminution, and Selection of the Test Portion

In general, the fiber content of large particles is greater than that of small particles, and the fiber content of less dense particles is greater than that of more dense particles for most feed ingredients. Thus, any process that promotes segregation because of a range in particle size, shape, or density (such as vibration during shipment or storage, and comminution) will increase heterogeneity that will make selection of a representative test portion more challenging. One of the most problematic processes is comminution (e.g., grinding). The tough, large particles that are retained in the grinding mill and are most slowly comminuted are higher in fiber. If these particles are brushed or vacuumed from the mill or otherwise excluded from the analytical sample, a systematic error (error bias) will result. Even when all of the material has passed through the screen, the material in the collection container is segregated by the comminution process, with the initial material being lowest in fiber and the material that passes through the screens last being highest in fiber. Comminution equipment always should be evaluated for producing a uniform particle size, shape, and density to control segregation issues.

Although comminution to a finer particle size improves the uniformity of the analytical sample, it may also promote segregation of particles. For materials that do not readily segregate, appropriate mixing can reduce heterogeneity. The mixing container must not be more than two-thirds full to achieve effective mixing; mixing in a container that is full or almost full is always ineffective. One mixing technique that can be effective for many materials is a three-dimensional motion combining a figure eight movement with rotation. Two mixing

techniques that are always ineffective are stirring and shaking the container back and forth.

For materials that readily segregate, mixing is ineffective because segregation returns as soon as the mixing motion is stopped. Therefore, the act of "mixing" should never be assumed to produce an analytical sample of uniform composition. In fact, mixing can be a second insidious segregation process (especially the common technique of "stirring"), leading to false confidence and taking of short cuts in selection of the test portion. Refer to AAFCO's *Guidance on Obtaining Defensible (GOOD) Test Portions* (42) for more information on proper selection of a test portion.

Drying of High-Moisture Materials Before Comminution and Analysis (2)

Proteins and carbohydrates can form insoluble compounds (Maillard or browning products) when exposed to high temperatures in the presence of moisture. These Maillard products are measured as artifact fiber and lignin. Thus, high-moisture materials should never be exposed to temperatures above 60°C during drying in a forced air oven, and a maximum of 50°C is preferred to avoid a significant bias in fiber results.

Particle Size (2)

Fiber reagents function by extracting and solubilizing nonfibrous compounds from feed particles. Extraction efficiency increases as the size of particles decreases (and surface area increases) because reagents and washing solvents have less matrix to penetrate. Furthermore, fibrous residues are filtered on coarse-porosity filter membranes, suggesting that fine fiber particles may be washed out of the residue or plug the filter membrane. These factors explain why materials of smaller particle size result in lower fiber values. However, a compromise is necessary between a fine particle size to increase extraction efficiency and coarse particle size to prevent loss of fiber particles and plugging of the filtration vessel. Grinding through a 1 mm screen using a cutting mill (e.g., Wiley) is recommended. A 2 mm screen with a cyclone or centrifugal mill is roughly equivalent. The centrifugal mills (e.g., Retsch ZM200 or Fritsch P-14) or cyclone mills (e.g., Foss Cyclotec or Udy) generate smaller average particle size than a cutting mill with a similar size screen because centrifugal mills force the particles through the screen openings at an acute angle instead of allowing the particles to drop vertically (at a right angle) through the screen as in the cutting mill. As the angle of incidence to the screen decreases from 90°, the effective screen opening decreases. Using the same size screen, cyclone and centrifugal mills will produce an average particle size that is one-half that of cutting mills, resulting in slightly lower fiber values and greater filtering difficulties during fiber analyses (Mertens, D.R., Mertens Innovation and Research LLC, unpublished data). For Gooch crucibles with coarse fritted disks, which have been used traditionally for detergent fiber analysis, the optimum particle size is when the largest particles are <1 mm in dimension, the range in particle size is narrow, and there is a minimum small proportion of small particles (fines) that can pass through filter vessels. However, Van Soest (11) recognized that small lignin particles could be lost after ADF was treated with 72% sulfuric acid and used asbestos in crucibles as a filter mat to retain small particles. Asbestos is no longer used and Mertens (Mertens, D.R., Mertens Innovation and Research LLC, unpublished data) observed that glass micro fiber mats with porosity of 2.7 μm (Whatman GF/D) were better than ceramic fibers for ADSL determination. Raffrenato et al. (43) observed that glass micro fiber mats with porosity of 1.5 (Whatman GF/C or 934-AH) resulted in larger ADSL recoveries compared with Gooch crucibles with coarse fritted disks (porosity of 40–60 μm). Alternative filtration membranes for materials or residues that have particles that are smaller than recommended are discussed in the section *Determining Fiber in Difficult-to-Filter Materials*, such as feces, digesta, and in vitro residues.

Standardizing Reagents (2)

To provide consistent and accurate fiber values, reagents and solutions must be standardized. Commercially purchased fiber assay solutions must be periodically checked to see that they are within normality and/or pH specifications.

Neutral detergent solution must be standardized to a pH of 6.9–7.1. If pH differs by more than 0.2 units from 7.0, reagents should be checked to determine if the wrong chemicals were used, and, if so, the neutral detergent solution should be discarded. If pH is between 6.8 and 7.2, an adjustment of pH must be performed by adding either HCl or NaOH to obtain a pH of 7.00. Chemicals in neutral detergent solutions can precipitate if the solution gets cold, and the solution should not be used until all components are completely solubilized.

The amylase solution used in the NDF analysis must be standardized so that the amount of enzyme solution added at boiling and during the first or second filtration steps removes all traces of starch from the fritted disk of Gooch crucibles. *See* AOAC *Official Method* **2002.04C(e)** for the standardization of alpha-amylase (14). The alpha-amylase used needs to be heat-stable.

Sodium sulfite must be added to each test portion before refluxing in the NDF procedure. It is important for the removal of protein from NDF and is especially critical in the removal of nitrogenous contamination from cooked or heated feeds, animal byproduct feeds, and feces or digesta. Hintz et al. (13) varied sodium sulfite from 0.25 to 1 g and observed that 0.5 g or more was adequate and did not have to be weighed precisely; therefore, this amount can be added using a calibrated scoop.

Measurement of ADF depends on the use of 1N sulfuric acid, which must not vary by more than ±0.004 N. The CF 0.255 N H_2SO_4 and 0.313 N NaOH must not vary by more than ±0.005 N. Normality values are verified by titrating an aliquot of the solution against a standardized base or acid. If the solution is not within its specified range, the normality should be adjusted by adding water or concentrated acid or base and then rechecked by titration.

Test Portion Mass (2)

The ratio of test portion mass to extraction solution volume can have a small, but significant, effect on fiber analyses. The standard ratio for detergent and crude fiber analyses is 1.0 g test portion per 100 mL solution (ANKOM detergent test portion size is 0.45–0.50 g). Selection of the test portion mass is a compromise among several factors including sufficient mass to be representative, extraction efficiency, reagent cost, weighing errors, and sampling errors (e.g., selecting a higher ratio of

coarse particles than fines). Larger test portion masses improve representivity but also increase reagent costs when maintaining the same test portion:solution ratio. Smaller test portion masses magnify all sampling, analytical, and weighing errors. For example, if the residue weighs 0.01 g with a weighing error of 0.0002 g, the error is 2%; however, if the residue weighs only 0.002 g, the error is 10%. The lower the fiber content, the larger the test portion needed to maintain confidence (or reduce relative error) in the residue weight.

Varying Reflux Times and Temperatures

Extraction of fiber is both time- and temperature-dependent. As the time and temperature increase, the amount of fibrous residue recovered decreases. This is especially true in the case of acid and base reagents. It is crucial to closely adhere to the time of refluxing from the onset of boiling. Refluxing should be at a temperature that causes a rolling agitation of feed particles. Heating units for individual beakers or reflux columns should be calibrated to bring 100 mL water at room temperature to a boil in 3–4 min. When refluxing in Berzelius beakers, the beakers must be placed on the hot plates with a staggered time between each beaker placement to ensure that the reflux time is consistent for each beaker. This time is determined by the amount of time it takes to filter a test solution (2). Because the ANKOM system performs digestion under pressure, boiling does not occur at 100°C, so agitation must be accomplished mechanically by the instrument. The pressure inside the ANKOM^{200} Fiber Analyzer should be verified by tapping a hole in the lid and using a certified pressure gauge. After extensive use of the ANKOM system, the seals for the lid and mechanical agitator rod can leak, reducing pressure. Pressure in the analyzer is a function of head space, reagent volume, and temperature change during heating. The temperature of the extraction unit should be confirmed to be 100°C with the top open and using a certified reference thermometer. Too often it is observed that the temperature of water in baths and extractors does not match that of the indicator on the unit (Mertens, D.R., Mertens Innovation and Research LLC, personal communication).

Incomplete Transfer of Residues into the Filtration Vessel (2)

A major source of error is the loss or incomplete transfer of all fibrous residues from the Berzelius beaker into the crucible or other filtration vessel. Sometimes residues adhere to the sides or bottom of the beaker. These residues must be freed before they can be transferred. At other times, the last drop from the beaker is allowed to flow down the outside of the beaker when it is turned upright after pouring its contents into the crucible. The beaker should be kept inverted over the crucible and be rinsed with a fine stream of hot water to transfer all particles. If the beaker must be turned upright during transfer, it is critical to wipe the last drop from the lip of the beaker onto the lip of the filtration device. Often, this last drop contains significant fiber because particles have settled in the beaker during transfer. Transfer should be sufficiently complete that beakers do not need to be washed between uses. Beakers should be checked routinely for cleanliness to ensure that previous transfers were complete.

Filtration Using Crucibles (2)

Several factors are important in making filtration of fiber residues effective and efficient. Normally, minimum filtration vacuum should be used to prevent plugging the filter membrane with fiber residues and loss of fine particles. The vacuum source should be constant and have reserve capacity. It is also important that the vacuum manifold and vacuum lines be constructed to minimize the trapping of foam that will greatly reduce the effective vacuum at the crucible.

AOAC *Official Method* **2002.04B(c)** describes a manifold that minimizes vacuum leaks and foam in the system yet is durable and economical to construct (14). The manifold is designed for Gooch crucibles but can easily be modified for use with Buchner funnels or paper funnels. The basic design fits crucibles tightly and allows back flushing of problem crucibles by removing and reinserting them into the holder.

The choice of filtration vessel is a compromise between filtration ease and fiber recovery. Coarse membranes will allow some fine fiber particles to be lost, but fine membranes often plug, making filtration difficult. Crucibles recommended for NDF, ADF, and CF analyses of feed and food materials are the FiberTec P2 (Foss Manufacturing Co.) standard crucible (retention size 40–90 μm) and the 50 mL Gooch crucible with a coarse fritted disk (retention size 40–60 μm). For purposes of comparison, Whatman 40 filter paper has a retention size of 8 μm and Whatman 41/54/541 filter paper has a retention size of 20–25 μm. Fecal, digesta, and in vitro residues require filtration membranes with smaller porosity, as described in a following section.

The filtration rate of crucibles is checked by measuring the time it takes for 50 mL water to pass through each crucible without vacuum [refer to AOAC **2002.04B(b)**] (14). It should take approximately 180 s. If it takes less than 120 s, the crucible should be checked to ensure it is not cracked and leaking. If it takes longer than 240 s, the crucible should be cleaned with acid and measured again. If it still takes 240 s, the crucible should be cleaned with alkali. If cleaned crucibles take longer than 240 s to filter, they should be discarded, as filtration problems will result. The filtration rate should be checked on all new crucibles before use, and should be checked at least annually.

Filtration difficulties also can be caused by gradual plugging of the fritted disks of crucibles with fine particles or ash after repeated use. Crucibles can be easily cleaned by pulling hot water through the fritted disk in reverse of normal filtration flow. Crucibles also can be cleaned by ashing for 5 h at 500–525°C and then back flushing with hot water.

Occasionally, crucibles can be cleaned with 6N HCl and/or an alkaline cleaning solution containing 5 g disodium EDTA, 50 g trisodium phosphate, and 200 g potassium hydroxide per liter of water. The crucibles should be allowed to soak in either solution for 30 min, and the alkaline solution should be used with heat at 70–80°C. The alkaline treatment can weaken the glass, so it should be used only on crucibles that do not filter normally [refer to AOAC **2002.04C(h)**] (14).

Filtration Using Filter Bags

Filter Bag Technology (ANKOM Technology, Macedon, NY) is designed to allow for filtration to take place continuously during solubilization in both crude fiber and detergent solutions.

The F57 filter bag (ANKOM Technology) is designed to retain fine particles milled according to AOAC Official Method **962.09** (1 mm screen with a cutting mill or 2 mm screen with a cyclone or centrifugal mill). The F58 filter bag (ANKOM Technology) is designed to retain finer particles produced by finer milling. The use of a single replicate blank bag during the analysis may alert the user to potential bias. Corrections of empty bag weights for F57 blank bags should have values from 0.9940 to 0.9980 depending upon the method involved. Blank bag correction factors greater than 1.0000 indicate either inadequate washing or gain of particles that have been lost from other bags during extraction. If fiber loss contaminates blank bags or a smaller particle size of test portions is desired, the F58 bag should be used. Sufficient pressure is critical for proper liquid flow through the bags as both extraction and rinsing efficiency are impacted.

Washing Residues with Hot Water and Acetone (2)

A common error made by fiber analysts is incomplete washing of fiber residues to remove the fiber solutions and soluble feed components. All too often, residues are rinsed, rather than soaked, during the washing steps. Feed particles are filled with voids that can trap solutions and components. These voids cannot be washed free of contaminants by simply rinsing the outside of the particle. The laws of mass action must be used to equilibrate the liquids within the void with clean wash water outside of the particle. This is a time-dependent process. Thus, fibrous residues must be soaked in 30–40 mL clean hot water (95–100°C) for at least 2 min (preferably 5 min) in each wash to remove the fiber solution and soluble compounds trapped in the voids of particles. The larger the volume of water and the longer the time of soaking, the more complete the extraction of soluble contaminants of fiber will be.

The same principles are true for acetone washes used to remove residual lipids (fats) from the fiber residue. Simply washing the outside of particles with acetone will not extract all the lipid. Both the time and volume of clean acetone are important. A minimum of 20 mL acetone for 2 min is needed (30 mL for 5 min preferred). Acetone should not be added before all rinse water has been removed. Although this will occasionally improve filtering, it does not remove detergent or detergent solubles from residues. Adding acetone before water washing is complete typically results in inflated fiber values.

It is especially important that all traces of acid be washed from ADF residues and filtration vessels. With crucibles, it is desirable to rinse the underside of the crucible, and with filter paper, it is wise to rinse the edges of the paper. If residual acid remains, it will migrate to the edges of particles and become concentrated during drying. The concentrated acid will char the fiber or filter paper during drying. Charring signifies oxidation and loss of organic matter resulting in low residue weights.

The removal of acid or base from filter bags is also important. The use of four hot fresh water washes of 5 min each with the lid closed, heat on, and pressure maintained at approximately 12 psi should sufficiently remove the acid or base. A pressure gauge is needed to determine if the desired pressure is achieved. However, the water of the fourth rinse may be checked by the use of litmus or pH paper during the final minute of the rinse to ensure the removal of the acid or base.

Drying and Weighing Fiber Residues (2)

Filtration vessels with fiber residue should be placed in the oven all at one time at the end of the day. This prevents moisture from wet vessels placed in a forced air oven from contaminating vessels that have been drying in the oven. Vessels should remain in the oven (100–105°C) until they achieve a constant dry weight. This normally takes 8 h or overnight drying.

Residual acetone from the NDF and ADF filtration vessels should be removed as completely as possible either by vacuum or allowing vessels with residues to stand in an operating hood before placing them in an oven to avoid an explosion.

Weighing technique is critical for obtaining dry weights of very hygroscopic fiber residues. If too many filtration vessels are placed in the desiccator at one time, the desiccator lid is held open during transfer from the oven or weighing, or the desiccant is the wrong type or is not changed often, dry weights obtained using a desiccator are incorrect regardless of the oven temperature or drying time, as they will progressively take on more water from the atmosphere. Weighing residues in vessels directly out of the hot oven used for drying is faster, minimizes handling during transfers, and eliminates the errors associated with desiccators. The balance should be warmed with three hot vessels before residues are weighed, and a heat-resistant pad should be used to minimize heat transfer to the balance pan. The balance should be tared to 0 (the tare weight will change when the balance is warming) and the vessel with residue added. Within seconds, the balance will arrive at a minimum weight, and then the weight will increase as moisture is absorbed by the vessel and hygroscopic residue. The minimum weight should be recorded and the weighed vessel removed. Time between successive weighings should be consistent (44).

With filter bags, generally a large number of substrates are extracted at the same time. If the bags are placed in a desiccator after drying, each time the lid or door is opened to remove a bag, moist, ambient air is introduced. Because the desiccator is opened up to 24 times, the moisture can more readily affect the remaining bags. This occurs for crucibles and filter papers, too, and, in most cases, the desiccant is maintained poorly and thus rendered ineffective. When a collapsible ANKOM desiccant pouch is used, the air can be pushed out of the pouch each time a filter bag is removed. This will reduce the introduction of moist air that will affect weight of the remaining filter bags. Alternatively, sample bags may be hot weighed in beakers as described previously.

Calculation and Dry Matter Errors (2)

Laboratories have been known to have errors in the equations used to calculate results. The most common source of discrepancies in fiber results among laboratories is because of differences in dry matter estimates and the variation associated with adjusting fiber values to a dry matter basis.

Determining Fiber in Difficult-to-Filter Materials (2)

Any test portion that takes more than 10 min of filtration time under vacuum should be discarded because the results will be inaccurate. Instead, the test material needs to be rerun using one of the following modifications: (*1*) Reduce the test portion mass.

This will increase the sampling and analytical errors, but it often is the best approach to use with difficult materials. (*2*) Use filter aids such as glass wool (about 0.25 g), or glass microfiber filter mats (Whatman GF/D, 4.25 cm) that will keep gelatinous materials and ash or fine residues from plugging the fritted disk of the crucible, or use sea sand (CAS-No. 14808-60-7) placed on top of microfiber filter mats. Celite (diatomaceous earth, acid washed - Celite 545 AW, or equivalent) is no longer recommended because its small particles (10–200 μm) plug fritted disks. The sea sand, particle size >90% between 100 and 315 μm, should be washed with acid and ashed at 525 ± 15°C before use; otherwise, there can be some weight loss when using it. (*3*) Back-flush the crucible by removing then reinserting it into the crucible holder to force air back through the fritted disk to dislodge the residue plugging the frit. This also can be done with the Fibertec system.

For detergent fibers, materials containing >5% fat should be extracted with a suitable solvent such as acetone to remove some of the lipids before fiber analysis. Fats bind to detergents and reduce their extraction effectiveness. If there is a presence of fat globules floating on the surface of the solutions or the wash water, the analysis should be repeated by first pre-extracting the fat. With CF, all samples, regardless of fat content, should be pre-extracted with the solvent specified in the method.

Starch is a major cause of filtration problems during NDF analysis. A milky or opaque appearance of the neutral detergent solution indicates high starch. If filtration is difficult, add additional amylase solution to the crucible. Many times, this will unplug the fritted disk and allow filtration. Shorten soaking times to a minimum to keep soaking solutions as hot as possible (>85°C).

Soluble fiber (usually pectin) interference is suspected if the fiber residue has a glossy, translucent sheen and filtration becomes more difficult with each water soak. Fiber residues from these materials must be kept hot to filter readily. Decrease soaking time to a minimum and keep rinse water at boiling temperature. Preheat the crucible by filling it with hot water before beginning to transfer the residue. Do not let residues settle in the beaker before transferring to the crucible; instead, transfer as quickly as possible. Adding glass wool or sea sand on top of glass microfiber filter mats to the crucible helps to keep the gelatinous residue from plugging the filter. Adding acetone before the last water wash has been completely removed (less than 5 mL water remaining in the crucible) can salvage some samples, but acetone will precipitate any residual detergent in the residue.

Feces, intestinal digesta, and in vitro fermentation residues can be especially difficult to filter. When ground through the same screen, feces and digesta have smaller particle size than the material fed (Mertens, D.R., Mertens Innovation and Research LLC, unpublished data). In vitro residues also have smaller particle size than the original ground material because of fermentative digestion. Not only do these fine particles plug the pores of filtering vessels and slow or prevent evacuation, but they also can be lost by passing through filtration membranes. Using microfiber filter mats (Whatman GF/D, porosity = 2.7 μm or Whatman GF/C, porosity = 1.6 μm) with sea sand in crucibles is essential for the recovery of NDF in these materials. Filtration also can be enhanced by allowing the residue to settle in the beaker for 1–2 min after it has been removed from the refluxing apparatus and carefully decanting the liquid from the beaker with minimal transfer of particles to the crucible. It helps to slowly transfer the liquid under vacuum in a way that does not cover the entire surface of the filter mat. If the crucible begins to plug during the washing step, carefully scrape the surface of the mat to provide a new surface for filtration. Patience and use of minimum vacuum during the transfer step are important in obtaining accurate results with these materials.

Factors Resulting in Variation in TDF Analyses

In addition to factors discussed above (specifically, segregation, comminution, test portion selection, particle size, and test portion mass) associated with crude and detergent fiber analyses that may cause variation in fiber results, there are specific issues related to the TDF methods.

Starch Removal

Starch removal is dependent on solubilization and enzyme systems used to degrade nonresistant starch. But with the discovery of the physiological importance of resistant starch (starch not digested or absorbed in the small intestine but that passes to the large bowel where it is partially fermented), separation of enzymatically digestible starch from resistant starch is critical and is perhaps the most difficult step in the entire process. As indicated previously, the incubation time allowed for this process to occur is under discussion. Materials that contain high starch concentrations and low dietary fiber concentrations (e.g., rice, white bread) are subject to the greatest within- and among-laboratory variation for AOAC Methods **2009.01** and **2011.25**.

High-Fat Materials

Substrates should be pre-extracted with the solvent specified in the method to remove the lipids in products with a fat content >10% before fiber analysis.

Difficult Filtration

Filtration times of more than 1 h are not uncommon. Techniques to reduce long filtration times include reducing the test portion weight or scraping the top surface of the diatomaceous earth bed. Higher filtration times tend to produce inconsistent and artificially high fiber values.

Agitation During Hydrolysis

Most water baths do not provide sufficient agitation during enzymatic hydrolysis to keep the solution and test portion adequately mixed to allow their interaction. This can result in the lack of solubilization of nonfibrous components that can create artificially high and inconsistent fiber values.

Waterbath Temperature Control

The dietary fiber procedures have very specific temperature requirements. Water baths that can maintain the higher temperatures required by the procedures must be used. Temperature should be routinely checked using a certified thermometer. Higher temperatures are necessary to denature the protein in the test material.

Single Test Portion Process (or Splitting Fiber Residues)

Dietary fiber procedures call for the duplication of each test material because dietary fiber residues require both an ash and protein correction. To increase throughput, it is not unusual for laboratories to generate only a single residue and then split it in half with one half for ash correction and the other half for protein correction. This process introduces weighing, analytical, and sampling errors that lead to higher variability in results and thus greater error. Subsampling assumes homogeneity of the residue.

Technician Variability

As indicated previously, TDF analysis is much more labor-intensive and has many more steps (>40) in the method than do crude or detergent fiber procedures. Each additional step provides opportunity for variation from technician to technician. Variation will occur in each step of this multistep process. However, many laboratories worldwide have participated in the interlaboratory evaluation of the various TDF methods, and results were sufficiently robust for AOAC Official Method status to be conferred.

Quality Assurance/Quality Control for All Fiber Analyses

It is imperative that laboratories validate all fiber methods used by each analyst in their laboratory. Section 5.4 of the *AAFCO 2014 Quality Assurance Quality Control Guidelines for Feed Laboratories* (45) may be referred to for greater detail on selection and in-house verification and validation of methods. The verification or validation should be repeated periodically, especially when training new personnel or installing new equipment. In addition to analytical methods, laboratory sampling procedures should be validated before use and revalidated or verified on a periodic basis (42).

The inclusion of quality control checks is vital to monitor systematic and random errors in fiber methods. Section 5.9 and Table 3 of the *AAFCO 2014 Quality Assurance Quality Control Guidelines for Feed Laboratories* (45) provides a listing of quality control checks frequently used in feed laboratories. For fiber methods, routine quality control checks should include a laboratory reagent blank, laboratory control sample(s), and use of multiple replicates. The laboratory should have procedures in place for evaluating quality control results and dealing with results that are unacceptable or nonconforming. In addition to the analytical methods, laboratories should incorporate quality control to monitor laboratory sampling procedures used to generate test portions for various fiber methods.

Sources of laboratory control materials that have consensus values include the AAFCO Animal Feed with Values for CF, NDF, and ADF (46); The National Forage Testing Association Forage with Values for NDF and ADF (47); AAFCO Proficiency Testing Program, Pet Food with Values for CF (48); The American Oil Chemists Society Laboratory Proficiency Program Soybean and Distillers Dried Grains with Values for CF (49); and The American Association of Cereal Chemists Check Sample Program for total dietary fiber (50).

Other quality controls include maintaining a log of reagent preparation and amylase standardization. The normality and pH of each batch or lot of solutions must be checked and adjusted as needed. The activity of amylase stock solutions should be determined every 6 months during storage and amylase working solutions adjusted accordingly.

Summary and Conclusions

Fiber is a complicated chemical and physical entity that today takes many forms (e.g., intrinsic and intact, extracted/isolated, synthetic). Several analytical methods are available for its measurement, all subject to random variation that occurs with all assays. Although random variation cannot be eliminated, systematic variation because of errors or inadequate technique must be minimized. There are crucial steps in each method that should be followed exactly to obtain reproducible results because, at some level, all fiber methods essentially define the fiber that is measured. As a result of its inability to accurately quantify dietary fibers, the crude fiber method should no longer be used for research, labeling, or regulatory purposes. AAFCO is strongly encouraged to consider replacement of crude fiber with the appropriate detergent fiber(s) or dietary fiber(s) as new feed labeling guides and model bills are developed. Detergent fibers, if assayed properly, provide accurate information on IDF components (cellulose, hemicelluloses, and lignin), and values can be used with confidence to predict select physiological responses by animals to the ingestion of fibrous feeds. However, detergent procedures do not quantify the SDFs (pectins, beta-glucans, gums, mucilages, nondigestible oligosaccharides, resistant starches, isolated/synthetic dietary fibers) found to be important in nonruminant animal and human nutrition and health. Total dietary fiber methods have been intensely studied in recent years as a result of the renewed interest in dietary fiber in human nutrition. Indeed, a formal definition of dietary fiber was recently approved by the FDA. Near continuous evaluation and improvement of these methods occurs in an attempt to perfect their measurement and to make them as relevant as possible to the physiology of the animal and human whose diets contain a variety of fibrous constituents. Laboratories should participate in ring tests and collaborative studies to evaluate new methods or method modifications.

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