Analysis of Forage Fiber and Cell Walls in Ruminant Nutrition

Hans-Joachim G. Jung

USDA-Agricultural Research Service Plant Science Research Unit and U.S. Dairy Forage Research Center Cluster; and Department of Animal Science and Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108

ABSTRACT Analysis of the fiber or cell wall present in forages is of major concern in ruminant nutrition because diets often contain large amounts of forage, and the fiber fraction affects both feed intake and animal performance. Traditional extractive, gravimetric methods such as crude fiber and neutral detergent fiber recover variable amounts of the plant cell wall, but they remain popular because of their ease of use and the large feed data bases available for these methods. More intensive chemical methods utilizing chromatography and spectrometric analysis provide greater detail on cell wall composition and structure, but they have been used little in ruminant nutrition. Lignin analysis has remained problematic because no definitive reference method exists. Recently attention has focused on the measurement of lignin composition and cell wall phenolic acids; however, these methods have yet to be widely adopted in ruminant nutrition. The detergent fiber methods have been semi-automated to increase sample handling capacity. Near-infrared spectroscopy is routinely used for prediction of fiber concentration in forages and has greatly increased the ease of obtaining fiber analysis of forage samples. Widespread adoption in ruminant nutrition of the more sophisticated methods of cell wall analysis is unlikely to occur until these methods can be demonstrated to improve diet formulation and prediction of animal performance. J. Nutr. 127: 810S–813S, 1997.

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In nutrition, the term fiber refers to the components of plant-derived foods and feedstuffs that are not digestible by mammalian enzyme systems (Moore and Hatfield 1994). In forages commonly fed to livestock, fiber refers to the plant cell wall. Mammals do not possess the enzymes to hydrolyze the predominant b1-4 linked polysaccharides that occur in cell walls and depend on microorganisms in the gastrointestinal tract to ferment these polysaccharides to absorbable nutrients. Ruminants are among the most specialized herbivores for utilizing this symbiotic relationship to exploit plant cell walls as a source of nutrients (Van Soest 1994). Obviously plant cell walls did not evolve to serve as a feed for ruminant animals. The biological functions of the cell wall have resulted in a structure that is of variable and often low digestibility by ruminants. Also, the physical volume occupied by cell walls in the rumen affects feed intake and animal performance. My objective in this review is to provide an overview and assessment of the methodologies available for the measurement of concentration, composition and structure of fiber or plant cell walls.

PHYSICAL AND CHEMICAL STRUCTURE OF PLANT CELL WALLS

Recent reviews summarize cell wall development and structure (Iiyama et al. 1993; Terashima et al. 1993). My objective here is to briefly describe the development of the plant cell wall to acquaint the reader with how the wall is organized and the relationships among its components. This information should provide a basis from which to examine the various methods used to analyze fiber and better understand the strengths and weaknesses of these procedures.

During early development a plant cell must be able to grow in size (Iiyama et al. 1993). At this stage the cell wall is referred to as a primary wall and is capable of elongating because the wall polymers are not cross-linked. The middle lamella is the region between adjoining cells and is composed primarily of pectic substances, which are a group of galacturonan polymers with neutral sugars substitutions. Legumes contain large amounts of pectic substances, whereas grasses have relatively low concentrations. The primary wall consists of several polysaccharides, including cellulose, mixed linkage b-glucans, hemiceluloses, glucuronarabinoxylans and heteroxylans (Moore and Hatfield 1994). The xylans are much more abundant in grass walls than in legumes. Pectic polysaccharides of legumes...
are substituted with small amounts of ferulate and p-coumarate esters. In contrast, primary wall in grasses has high concentrations of ferulate esterified to arabinofuranosyls and lesser concentrations of p-coumarate esters. Structural and other proteins are also deposited in the primary wall of plants.

When the plant cell stops growing and initiates the maturation process, secondary wall deposition and lignification begin. Cellulose is the major polysaccharide deposited in the secondary wall. Grasses continue to deposit xylans; however, the degree of substitution of the xylans declines compared with these polysaccharides in the primary wall. 4-O-Methyl-
neuronic and glucuronic acids, are measured colorimetrically in the hydrolysate. Klason lignin is the residue remaining after acid hydrolysis of the feed. Unlike NDF and CF, the DF method retains all the cell wall components. Starch and protein removal can be a problem in concentrate and processed feeds. Incomplete removal of these substances will result in DF overestimating cell wall concentration. The Prosky method has been recognized as an official AOAC method.

Fiber concentration. The Prosky method of fiber analysis is similar to the Prosky DF method but has the added advantage of providing compositional data (Theander et al. 1995). After pretreatment to remove non-wall components, the cell wall polysaccharides are hydrolyzed with sulfuric acid, and the neutral sugar components are identified and quantified by chromatography.

Methods of Fiber Analysis

Some of the major methods used in forage fiber analysis are listed in Table 1. Each method has its own strengths and weaknesses. Choice of analytical method must depend on the objectives of the research project or client need.

Fiber concentration. Determining the concentration of fiber in a feed is the most common goal of fiber analysis. The proximate or Weende system of analysis, in which fiber concentration is measured as crude fiber (CF), is the oldest method still in use today (Henneberg and Stohmann 1859). Cellulose is a gravimetric method. Under this analytical scheme, a sample is sequentially refluxed in dilute base followed by dilute acid. The resulting residue was originally thought to represent the indigestible portion of feed. In actuality, it is composed primarily of cellulose and variable proportions of the sample’s noncellulosic polysaccharides and lignin.

The CF method severely underestimates the total plant cell wall content of a feed and recovers only a portion of wall polysaccharides and lignin (Van Soest 1994). The CF method continues to be used today because it is an official AOAC method of feed analysis, a large data base has been accumulated for a wide variety of feeds, and it is an easy method of analysis.

In ruminant nutrition, the neutral detergent fiber (NDF) method developed by Van Soest has largely replaced CF (Van Soest 1963b). Neutral detergent fiber, like CF, uses chemical extraction (with a neutral detergent solution under reflux) followed by gravimetric determination of the fiber residue. Neutral detergent fiber is considered to be the entire fiber fraction of the feed, but it is known to underestimate cell wall concentration because most of the pectic substances in the wall are solubilized (Van Soest 1994). As a result, NDF is a poor estimate of cell wall concentration for the pectin-rich legumes. Heat-damaged proteins in processed feeds are also retained in NDF, which will overestimate fiber content. These shortcomings of NDF as a method to determine cell wall concentration are certainly a problem if one is interested in the plant cell wall as a biological structure, but as pointed out by Van Soest (1994) these inconsistencies are not of concern if fiber is defined as the incompletely digestible fraction of feeds. Although widely used for fiber analysis of ruminant feeds, the NDF procedure is not an official AOAC method. Acid detergent fiber (ADF) is a portion of the plant fiber (Van Soest 1963a). Acid detergent fiber includes the cellulose and lignin from cell walls and variable amounts of xylans and other components. Acid detergent fiber is an AOAC-approved method of analysis. A common variation of the ADF method is to use NDF as a pretreatment (Van Soest and Robertson 1980).

The Prosky dietary fiber (DF) procedure is the third major gravimetric method for measuring fiber concentration (Prosky et al. 1984). This method utilizes a series of enzymatic and chemical pretreatments followed by precipitation in 80% ethanol to isolate a fiber residue. Unlike NDF and CF, the DF method retains all the cell wall components. Starch and protein removal can be a problem in concentrate and processed feeds. Incomplete removal of these substances will result in DF overestimating cell wall concentration. The Prosky method has been recognized as an official AOAC method.

Fiber composition. The Uppsala method of fiber analysis is similar to the Prosky DF method but has the added advantage of providing compositional data (Theander et al. 1995). After pretreatment to remove non-wall components, the cell wall polysaccharides are hydrolyzed with sulfuric acid, and the neutral sugar components are identified and quantified by chromatography (generally gas chromatography). The acidic cell wall sugars, galacturonic and glucuronic acids, are measured colorimetrically in the hydrolysate. Klason lignin is the residue remaining after acid hydrolysis. Fiber concentration is calculated as the sum of all of the individual components.

Methods of Fiber Analysis

The Uppsala method recently received AOAC approval. Many analytical methods exist for the measurement of specific cell wall components. In ruminant nutrition, forage cellulose and hemicellulose concentrations are commonly estimated as ADF minus sulfuric acid detergent lignin (ADL) and as NDF minus ADF, respectively (Van Soest and Robertson 1980). Cellulose concentrations are overestimated by ADF minus ADL to the extent that xylans are present in ADF and underestimated by heat-damaged protein contamination of ADL. Similarly, hemicellulose estimates based on NDF minus ADF are overestimated by non-extracted protein in NDF, and
residual xylans in ADF cause underestimates of hemicellulose. The Crampton-Maynard method is thought to provide a better measurement of cellulose in forages (Morrison 1980), and trifluoroacetic acid hydrolysis of NDF can be used to determine the hemicellulosic sugars (Moore and Hatfield 1994). Pectin content of forages can be calculated from the summation of uronic acids and the major pectic neutral sugars, but this approach suffers from the inability to separately measure galacturonic and glucuronic acid residues. Alternatively, pectin can be extracted and determined gravimetrically or after hydrolysis to its sugar components. Very sophisticated extraction schemes have been developed to isolate specific classes of polysaccharides (Moore and Hatfield 1994). These isolated polysaccharides can be characterized for sugar composition, linkage patterns by methylation, and specific molecular structures using NMR. Although more data on the specific polysaccharide structures in forage cell walls are becoming available, these data are not directly utilized in ruminant nutrition.

Many methods of lignin analysis have been developed because of lignin’s negative association with digestibility. Acid detergent lignin is the most common lignin method in ruminant nutrition, but increasing evidence indicates it underestimates lignin due to solubilization of some lignin at the ADF step in the procedure (Lowry et al. 1994). Klasson lignin is the oldest lignin method and has been considered inappropriate for forages because of protein contamination. Recent results indicate this concern is unnecessary, and Klasson lignin may be the best method available for estimating lignin content (Hatfield et al. 1994). The previous two lignin methods measure the residue remaining after acid hydrolysis. The permanganate, sodium chlorite and acetyl bromide lignin methods solubilize the lignin and measure lignin as either weight loss or by spectrometry (Collings et al. 1978, Morrison 1972, Van Soest and Wine 1968). These oxidative methods are susceptible to incomplete solubilization of lignin or interference by other solubilized wall components. Lignin composition can be determined by nitrobenzene oxidation, thioacidolysis and pyrolysis of forages (Lapiere 1993). Chromatography is required for identification of the lignin degradation products from these methods, and they all suffer from lack of information on completeness of recovery of the lignin components. The ester-linked ferulic and p-coumaric acids in the cell wall can be extracted by alkaline hydrolysis at low temperature (Hartley 1972). Those ferulate molecules involved in ether cross-links between wall polysaccharides and lignin are hydrolyzed by high temperature alkaline treatment (Iiyama et al. 1990). Dioxane-HCl treatment is an alternative method for cleaving etherified hydroxyxycinnamic acids (Scalbert et al. 1985). Chromatography is used to identify and quantify these hydroxycinnamic acids.

**FUTURE TRENDS IN FIBER ANALYSIS**

Semi-automated methods of analysis (Ankom® Fiber Analyzer, Ankom Technology Corp., Fairport, NY, and Fibertec I, Perstorp Analytical, Silver Spring, MD) have been developed for NDF and ADF determination to increase sample handling capacity. Robotic sample preparation systems and autosamplers for gas chromatography and HPLC are used routinely to increase analysis capacity for the compositional methods. Even greater efficiencies in sample capacity have been realized through the use of near-infrared spectroscopy (NIRS) to almost instantaneously predict fiber concentration and composition of dried and ground forage samples (Martin et al. 1985). Near-infrared spectroscopy technology depends on the correlation of near-infrared reflectance spectra of samples with actual analytical measurements of the constituents by other methods. Although many chemical entities have been successfully analyzed using NIRS, this method is limited by the quality of the reference analytical methods and similarity of the sample to the calibration samples. Although not all methods of analysis have been streamlined to the same extent, it is unlikely that automation limitations will determine choice of fiber methods in the future.

In the research setting, most scientists are more interested in comparing treatments than generating absolute values. The opposite situation exists in the feed analysis industry. The plethora of methods and variations on specific methods developed in the research environment cannot be transferred to industry. Standardization of methods through organizations such as AOAC and the National Forage Testing Association will be needed even more in the future as producers utilize forage testing more extensively as an aid in diet formulation. Establishment of an “official” method of NDF analysis is probably the most critical need because of the central role NDF values have assumed in ruminant diet formulation. Similarly, although I have not discussed methods of measuring fiber digestibility, it will be necessary to standardize an in vitro or in situ method for this forage trait.

Sophisticated measurements of cell wall composition and structure will become more prevalent in research settings where information on cell wall development and composition and the mechanisms of ruminal degradation of cell walls is the objective. Use of these analytical methods in research more closely related to applied animal nutrition will be limited. Before they are widely adopted, these methods must demonstr-
strate that they provide information that can improve the accuracy with which we predict animal performance. Examples of possible improvements would be if inclusion of pectin in the fiber fraction increases predictive power for intake or if polysaccharide composition information improves understanding of rates and extent of fiber digestion. These more sophisticated analytical methods may see their first practical use in the plant improvement field, in which genetic changes are targeted. Specific molecular components should be more closely tied to genes than empirical traits such as NDF.

LITERATURE CITED


