SYMPOSIUM: CARBOHYDRATE METHODOLOGY, METABOLISM, AND NUTRITIONAL IMPLICATIONS IN DAIRY CATTLE

Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition

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ABSTRACT

There is a need to standardize the NDF procedure. Procedures have varied because of the use of different amylases in attempts to remove starch interference. The original *Bacillus subtilis* enzyme Type IIIA (XIA) no longer is available and has been replaced by a less effective enzyme. For fiber work, a new enzyme[1] has received AOAC approval and is rapidly displacing other amylases in analytical work. This enzyme is available from Sigma (Number A3306; Sigma Chemical Co., St. Louis, MO). The original publications for NDF and ADF (43, 53) and the Agricultural Handbook 379 (14) are obsolete and of historical interest only. Up to date procedures should be followed. Triethylene glycol has replaced 2-ethoxyethanol because of reported toxicity. Considerable development in regard to fiber methods has occurred over the past 5 yr because of a redefinition of dietary fiber for man and monogastric animals that includes lignin and all polysaccharides resistant to mammalian digestive enzymes. In addition to NDF, new improved methods for total dietary fiber and nonstarch polysaccharides including pectin and β-glucans now are available. The latter are also of interest in rumen fermentation. Unlike starch, their fermentations are like that of cellulose but faster and yield no lactic acid. Physical and biological properties of carbohydrate fractions are more important than their intrinsic composition.
(Key words: dietary fiber, neutral detergent fiber, nonstarch polysaccharides)

Abbreviation key: AD = acid detergent, AIA = acid-insoluble ash, ND = neutral detergent, NSC = nonstructural carbohydrates, NSP = nonstarch polysaccharides.

INTRODUCTION

Refining dietary balances has provided an important way of optimizing animal production. Such progress has been most advanced with the monogastric species, poultry and swine. However, in the case of ruminants, progress has been slower because of the great modifying influence of rumen fermentation by rumen organisms, which have a fiber requirement and alter the amino acid balance. Fermentation in the rumen modifies the actual diet received by the ruminant animal, and the balancing of diets for ruminants must also consider fiber quality and the rumen microbial requirements. In addition, fiber is not a nutritionally, chemically, or physically uniform material, which adds another dimension of complexity. Any system that sets fixed values for dietary fiber requirements is inadequate because rumen size and level of intake and production affect that requirement. Another factor affecting the fiber requirement is particle size, because two of the major functions of fiber are to stimulate rumination and ensalivation and to form a normal rumen mat that functions as a filtering system and prevents too rapid passage of particles and loss of nutrients. Thus, particle

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[1]The heat stable amylase, formerly Number 5426, has been changed by Sigma as of July 1991. The original procedure required .2 ml of this enzyme. The replacement, Number A3306, is four times stronger, and 50 µl are used per sample.

TABLE 1. Modifications of NDF.[1]

Source	Conditions	Reference cited in Mascarenhas-Ferreira (25)
Van Soest and Wine (53)	ND boil 1 h plus sulfite and decalin	A
Fonnesbeck (10)	Pepsin pH 2.2, 40 h at 55°C, boil detergent pH 3.5	
King and Taverner (21)	ND boil 2 h	
Schaller (37)	Pretreatment[2] with hog pancreas enzyme	
Robertson and Van Soest (36), Mongeau and Brassard (32)	ND boil 30 min, remove add amylase, reboil 30 m[3], delete decalin ± sulfite	B
Giger et al. (13)	Boil in H_2O 30 min, incubate 80°C with amylase 30 min, ND boil 1 h[3]	C
Wainman et al. (54)	Overnight with amylase 38°C ND boil 1 h[3]	D
McQueen and Nicholson (29)	Similar to D but with less enzyme b[3]	E
Wainman (54) modified by Mascarenhas-Ferreira et al. (25)	Incubate with amylase overnight, ND boil 1 h[3]	F
ibid.	Incubate 30 min at RT[4] ND boil 1 h[3]	G
Mascarenhas-Ferreira et al. (25)	ND without EDTA + enzyme 15 min RT[4], boil 1 h, add EDTA 5 min before end[3]	H
ibid.	Same as H except ND + EDTA used throughout[3]	I
Sills et al. (38)	Gel starch, amylase pH 4.6 overnight 38°C; ND boil 1 h	
Jeraci et al. (17)	Add .25 ml Termamyl 120[5] to ND boil 1 h[3]	
Van Soest and Robertson (51)	Soak samples in 8*M* urea + .2 ml Termamyl[5] for 3 h at RT[4], dilute with ND solution boil 1 and filter	

[1]ND = Neutral detergent.

[2]Uses sulfite and decalin and approved by the American Association on Cereal Chemists (37).

[3]Sulfite and decalin omitted.

[4]Room temperature.

[5]Sigma amylase Number 5426, page 390, 1990 catalogue (Sigma Chemical Co., St. Louis, MO).

size also is involved in the rumen buffering system along with nonstructural carbohydrates (NSC) and fiber quality.

METHODS FOR NDF

The insoluble fiber in feed includes the crosslinked matrix of the plant cell wall and, as coarse fiber, forms the rumen mat that stimulates rumen function. It is measured most conveniently as NDF, which includes cellulose, hemicellulose, and lignin as the major components. The original NDF method was applied to forages, and its subsequent application to starchy foods and feeds revealed interference by starch, thus presenting difficulties for the original neutral detergent (ND) method. Therefore, various modifications with amylases have been reported (Tables 1 and 2). Many of the commercial amylases that have been used contain other activities, including hemicellulase, β-glucanase, and protease (25). In some of the modified methods, the sample is incubated overnight at 20 to 35°C with amylase. With this longer incubation time, contaminating enzymes in the impure amylase preparations can degrade hemicellulosic components in the feed, giving low values of NDF, whereas inclusion of unwanted starch leads to high values (25). Enzymatic preparations from *Bacillus subtilis* have been most popular. However, their quality and availability has varied over the recent years. The amylase (Sigma Type XI is no longer available) contained sufficient hemicellulase to affect values of wheat bran and possibly wheat straw (Table 3). Avoidance of this problem was accomplished by conducting starch digestion at the highest possible temperature. A newer *B. subtilis* enzyme presently available from Sigma is of lower activity, and more enzyme has to be used. The *B. subtilis* amylase is limited to a temperature

TABLE 2. Description of two neutral-detergent (ND) methods using differing operating conditions and two amylases.

Reference	Conditions	Acronym
Jeraci et al. (17)	Single addition of heat stable α-amylase to ND solution, boil 60 min	ND-T
Robertson and Van Soest (36)	Boil sample for 50 ml of ND solution for 30 min and then add 50 ml of ND solution and *Bacillus subtilus* α-amylase, boil 30 min; filter and add *B. subtilus* α-amylase to crucible, incubate for 10 to 15 min	ND-S

of 80°C and is rapidly inactivated at 100°C. The α1-6 activity also is destroyed by EDTA because it is calcium-sensitive. In some cases, the enzyme was added during and after the refluxing step to remove the starch (36). These modified methods increased the assay time by only 5 to 20 min but increased the number of steps in the ND procedure.

This new heat stable α-amylase is stable to boiling detergent and is used to degrade starch in nondetergent chemical methods for dietary fiber (19, 35). This α-amylase, which has AOAC approval (Number A3306 in the dietary fiber kit; Sigma Chemical Co., St. Louis, MO), has been used effectively in the ND method (16). The use of high temperature with a short-term amylase treatment has the advantages of minimizing the effect of unwanted side activities and is a more rapid procedure.

We have compared ND procedures using either *B. subtilis* amylase (36) or heat stable amylases (Tables 2 and 3). Both amylases were effective in removing starch (16). Although wheat bran gave less NDF by the ND assay using the *B. subtilis* amylase, the starch content does not account for the difference (Table 3). The differences between NDF values for the samples cannot be attributed to starch; they probably reflect a loss in hemicellulose, as also noted by Mascarenhas-Ferreira et al. (25), who found even larger losses in hemicellulose with lower temperature treatments.

In the original ND method, starch removal was facilitated by using 2-ethoxyethanol. However, 2-ethoxyethanol (ethyleneglycol monoethyl ether or cellosolve) now is recognized as a health risk. Its use appears necessary for optimal removal of starch (16). Therefore, 2-ethoxyethanol should be replaced by a safer reagent. Use of triethylene glycol at the same concentration gives equivalent values and is on the safe list. Thus, even with the use of efficient amylases, addition of either 2-ethoxy-

TABLE 3. Evaluation of two amylases in two neutral detergent (ND) methods. Analysis of NDF and the content of starch in NDF.[1]

Sample	ND-S[2]			ND-T[3]		
	NDF	SEM[4]	Starch	NDF	SEM	Starch
	(% DM)					
Timothy hay	65.5	1.31	0	66.3	.98	0
Wheat straw	83.8	.11	0	85.0	.58	0
Alfalfa hay	46.8	.92	0	47.1	1.31	.2
Wheat bran, hard red	48.2	3.29	.2	54.5	3.53	.9
Corn silage	51.5	.10	.2	52.8	1.12	.7
Green peas	15.7	1.12	2.1	16.5	.21	1.1

[1]Adapted from Jeraci et al. (19), values corrected for water content.

[2]*Bacillus subtilis* amylase old Sigma Type XIA (Sigma Chemical Co., St. Louis, MO) is used in the ND-S method described in Table 2.

[3][Robertson and Van Soest (36)]; heat stable α-amylase is used in the ND-T method described in Table 2.

[4]Mean of three replicated experiments.

ethanol or triethylene glycol seems necessary for concentrate feeds (16).

The NDF method has been criticized for not recovering pectin, which has been regarded by some as part of the cell wall matrix. Although a botanical argument can be made, the evidence from fermentation with gut microorganisms and digestion trials is that pectin is unique in being completely and rapidly fermentable and therefore is not, in contrast with hemicellulose, a part of the crosslinked lignified matrix (45). Pectin also possesses a very high cation exchange, at least in the demethylated form. Our view is that when pectin deserves recognition, it should be determined as its own entity. It is, however, a part of the nonstarch polysaccharides (NSP) discussed in this paper. The method using *meta*-hydroxybiphenyl (6) modified by Bucher (7) is specific for galacturonic acid and is a relatively simple procedure that can be conducted along with other fiber procedures.

RECOMMENDED PROCEDURES

Procedure for NDF

Procedure A. A .5-g sample is heated to boiling in 100 ml of ND plus 50 μl of heat stable amylase (dietary fiber kit; Sigma catalogue Number A3306) added before the beaker is placed on heat. Sodium sulfite (.5 g), if used, is added at this point. Sample is boiled 1 h and filtered on pretared coarse sintered glass crucible or Whatman 54 paper (Whatman, Clifton, NJ). Because of varying soil contamination in forages and feeds, the ash content should be reported or excluded from the NDF. The starch-specific enzyme is stable to boiling, insensitive to EDTA, and approved by the AOAC. Samples should be ground through a 1-mm screen, but not finer, because overgrinding also can worsen filtration.

Procedure B. An alternate procedure for removing starch from the most difficult samples is as follows: first the sample is treated with 30 ml of 8 *M* urea plus 50 μl of amylase added to a 1-g sample; then it is stirred with a rod to break any lumps. The mixture may be heated briefly on a steam bath 80 to 90°C for 5 min. Then, it is incubated at room temperature for 4 h or overnight and diluted with 100 ml of ND solution; 50 μl of enzyme is added optionally, and the mixture is boiled for 1 h and handled as in procedure A.

Use of Sulfite

The use of sodium sulfite in the NDF procedure remains optional. Its purpose is to lower the protein level and remove keratinaceous residues of animal origin. Sulfite cleaves disulfide bonds and thus dissolves many crosslinked proteins. Its general use for ruminant feeds is discouraged, especially if the residues are to be used as an assay for ND insoluble protein, because the sulfite reaction is nonbiological. The ND and acid detergent (AD) insoluble proteins from animal products tend to be indigestible. Sulfite also attacks lignin and therefore should not be used in sequential analyses leading to lignin determination or when the residue is to be used for subsequent in vitro digestion with rumen organisms.

Lipid Interferences

Lipid contents above 10% are a problem for both ND and AD if a separate oil layer forms in the solution because the detergents are more soluble in the lipid phase than in water. High values of ADF and NDF result. Simple removal of lipid may be done by brief heating in ethanol and filtering on the pretared crucible to be used subsequently for NDF or ADF. Contents and crucible are boiled in the NDF or ADF reagent as in the sequential procedure (51).

Filtration

There also are some kinds of samples that frequently offer filtering problems. These will be minimized if proper filtering techniques are followed. The lowest possible vacuum pressure should be used. Liquid should not be added while vacuum is on. Pressure is released when adding liquid. Contents are allowed to settle at least 15 s before admitting vacuum. In this way, finer matter is filtered onto a settled mat. The very hottest water is used, and the crucible should not be allowed to cool. The solution is returned to the beaker and reheated if necessary. If a crucible clogs, positive pressure should be exerted from beneath to flush particles out of the filter plate. This is also a

recommended method for cleaning of crucibles. Crucibles can be tested after cleaning for the speed that liquid will flow. Inherently slowly filtering crucibles should be discarded.

Acid Detergent Fiber and Lignin

Acid detergent fiber is intended as a preparation for the determination of cellulose, lignin, ADIN, acid-insoluble ash (AIA), and silica. It is not a valid fiber fraction for nutritional use or for the prediction of digestibility. Summative systems are mechanistically valid and should replace empirical regressions (8, 45).

The ADF procedure was collaborated with the AOAC (44) and given first action. This procedure avoided the use of decalin. The Klason lignin procedure was collaborated at the same time. Since that time, the use of asbestos has been abandoned (36), allowing more flexible handling of sequential lignin procedures. Klason lignin is a better marker than permanganate lignin; however, sequential treatment, i.e., Klason lignin followed by treatment with permanganate, yields lignin by difference that is more recoverable in feces (51). The fraction resistant to both 72% (wt/wt) H_2SO_4 and permanganate is cutin, which is important in many seed hulls and bark.

Acid-Insoluble Ash

Neutral detergent reagent dissolves pectin and biogenic silica but not silicaceous soil minerals. On the other hand, AD precipitates pectic acid as the quaternary detergent salt and quantitatively recovers all silica (51). Acid-insoluble ash is conveniently measured as the residue from ADF after ashing at 525°C. It is a preferable procedure and shorter than that of Van Keulen and Young (42), which is liable to incomplete recovery of silica due to lack of sufficient acid dehydration (51). The insoluble ash after lignin determination by either $KMnO_4$ or Klason procedures is identical to that of the original ADF, provided that asbestos or other filter aids are not used (49).

Sequential Analysis

The sequential analysis for fiber fractions is attractive because important interferences can be avoided and because the use of sample is more economical. Its principal advantage is that estimates of hemicellulose and cellulose by difference are more accurate in a sequential system. Hemicellulose estimated by subtraction of ADF from NDF will be too low when pectin is precipitated into the ADF. Biogenic silica has a similar effect because it is soluble in the ND reagents and insoluble in the AD reagent.

Sequential treatment cannot be applied universally because there are specific instances in which fractions of interest can be lost in the process. In particular, biogenic silica, AIA, some tannins, and ADIN are better done on a direct ADF. For tannins, a double sequential analysis can be performed (51) in which ND is followed by AD and, in parallel, AD followed by ND. Lignin values from these two sequences are compared on the two residues. Presence of insoluble tannins is indicated by higher values from the ND-AD sequence compared with the AD-ND sequence (51).

Sequential analysis can begin with total dietary fiber or with NDF as the difference between dietary fiber and NDF as water-soluble NSP. The difference should be corrected for ash and CP (N × 6.25). The crucible containing the fiber preparation can be analyzed sequentially using a Tecator (Helsingborg, Sweden) fiber apparatus or other fiber apparatus using Berzelius beakers. In either case, the same crucible accompanies the sample throughout the sequence. If Berzelius beakers are used, the crucible is placed on its side in the beaker, and the sample is boiled in 100 ml of reagent plus enough solution to cover the crucible. At the end of boiling, the crucible is removed with tongs, rinsed into the beaker, placed on the filter, and all liquid is passed through the crucible (51).

Total Dietary Fiber

The concept of total dietary fiber arose as a result of interest in fiber and human nutrition. It is defined as the polysaccharides and lignin resistant to mammalian digestive enzymes and thus is relevant to most monogastric animals with hindgut fermentation. The fractions not recovered in NDF but resistant to mammalian enzymes are defined as water-soluble NSP; they include some legitimate cell wall components, such as β-glucans and pectins, as well as

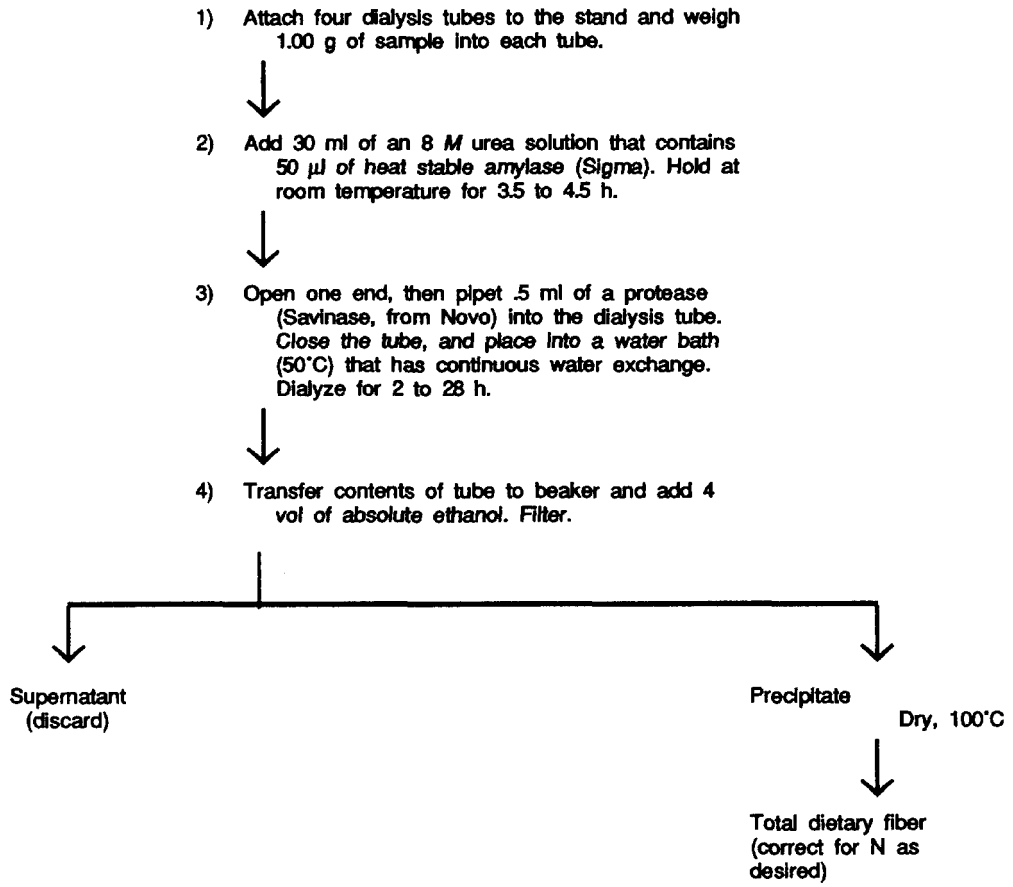

Figure 1. Diagram outlining the principal steps of the procedure for total dietary fiber (19). Courtesy of Association of Official Analytical Chemists, Washington, DC.

some storage polysaccharides, such as galactans in beans and sundry other gums and mucilages.

The definition of total fiber has led to appropriate enzymatic procedures isolating the fractions resistant to amylases and proteases. The first of these is the Asp procedure (35) adapted and collaborated by the AOAC. This method digested the heat-gelatinized sample with heat stable amylase, amyloglucosidase, and a protease. The final undigested fraction is precipitated by 4 vol ethanol. The residue is corrected for N × 6.25 and for ash.

The AOAC procedure offers many problems because hydrolyzed products remain in the solution. Their occlusion by ethanol precipitation is a major problem. Interference by Na and Ca salts (from the sample and the buffers used), which are insoluble in alcohol, often leads to more ash in the fibrous residue than present in the original sample. Volatile loss of ash components upon ashing at 525°C is prone to overestimation of fiber.

Because of these problems, various modifications of the method have been proposed. We have developed a new procedure involving urea-enzymatic dialysis, which avoids heat treatment and removes products via dialysis. The principle of the method depends on the extraordinary activity of the heat stable enzyme in 8 *M* urea. The schematic of the method is shown in Figure 1. Detailed information on the urea-enzymatic dialysis procedure is available (19).

TABLE 4. A listing of major carbohydrate in NSP[1] and NSC[2] (45).

Carbohydrate	Main occurrence	Water solubility	Classification[3]
Sucrose	All plants	+	nonstructural
Fructans	Temperate grass and composites	±	nonstructural
Starch	Cereal seeds	−	nonstructural (storage)
Galactans	Legume seeds (soybeans)	+	nonstructural, NSP (storage)
β-Glucans	Barley oats	+	structural, NSP
Pectins	Legumes and other dicots	±	structural, NSP

[1]Nonstarch polysaccharides.

[2]Nonstructural carbohydrates.

[3]From the aspect of function in the plant.

Method for Pectin

This procedure according to Bucher (7) is a modification of that of Blumenkrantz and Asboe-Hansen (6) and is improved with respect to the specificity for galacturonic acid over glucuronic acid. The procedure does not measure arabans that may be associated with pectin.

Reagents. Reagents include concentrated sulfuric acid (AR), sodium hydroxide solution (.5% NaOH, wt/wt), and *meta*-hydroxydiphenyl reagent [.15% *m*-phenylphenol, wt/vol (Eastman Kodak, Rochester, NY) in .5% NaOH, wt/wt].

Procedure. Aliquots (.5 ml) of sample solution containing 5 to 20 μg of uronic acid per aliquot are pipetted into test tubes (15 × 25 mm) in quadruplicate, and the tubes are placed in an ice bath for at least 10 min. Concentrated sulfuric acid (3 ml) at room temperature is pipetted into each tube, and the tubes are immediately returned to the ice bath for at least 5 min. The tubes are then mixed by vortexing and placed in an 80°C shaking water bath for exactly 8 min. The tubes are removed and cooled at room temperature. *Meta*-hydroxydiphenyl reagent (50 μl) is added to one pair of tubes, and NaOH solution (50 μl) is added to the second pair for a control. All tubes are vortexed for 10 s and held at room temperature for a few minutes to ensure complete color formation and to allow bubbles to dissipate. Absorbances are read at 520 nm in a spectrophotometer within 1 h of mixing (timing is important). The samples are corrected for the blank readings. The galacturonan concentration is calculated by reference to a galacturonic acid standard curve that follows the Beer-Lambert Law up to 35 μg/ml of sample solution (17.5 μg/aliquot). A set of standard galacturonic acid solutions is assayed simultaneously with each set of samples.

Nonstructural Carbohydrates and Nonstarch Polysaccharides

The more readily digestible carbohydrates in animal feeds lack a satisfactory system of classification. However, they are the major energy yielding components of feedstuffs. This lack of definition arises from their diversity and from the relative lack of basic research into their specific nutritive characteristics. Generally speaking, they comprise those carbohydrates not included in the cell wall matrix and are not recovered in NDF, and they include sugars, starches, fructans, galactans, pectins, β-glucans, etc. The sum of these is NSC. This value minus starch and sugars equals NSP. The NSP do not include native hemicelluloses and celluloses that ordinarily are a part of the lignified cell wall matrix, which recovers hemicellulose and cellulose, although their fermentation characteristics in the rumen are similar (41).

The NSC divide into sugars, starches, and the NSP (Table 4). Soluble carbohydrate is an ambiguous term because of the characteristics of starches, some of which are insoluble. Many carbohydrate chemists consider pectin in the structural group, but, for purposes of nutritional classification, it fits the NSP criteria. Pectins are important in grasses and cereals but are significant in dicotyledonous species, including forages and seed products. Legumes are the most important family.

The collective term soluble carbohydrates is not definitive, because some resistant starches,

for example, are quite insoluble and even indigestible (9). In speaking of solubility in reference to physical properties, ease and degree of solubility of nutrients in the rumen and in other parts of the digestive tract of farm animals have a major effect on dietary quality and digestive efficiency for both ruminants and nonruminants, although the respective physical factors affect these two groups of animals somewhat differently. The major factors affecting solubility and ease of digestion, but not necessarily intercorrelated, are crystallinity and macromolecular structure. Because these technical topics transcend the scope of this paper, some references are given (4, 9, 12, 39, 45).

The soluble NSC are digested rapidly and almost completely fermented in the rumen (90 to 100%). The insoluble, resistant starches may escape. It can be argued that an NSC value including pectin is more appropriate because it is a rapidly digested carbohydrate. This would be useful for estimating total rumen balance and output. The disadvantage is that dietary limits also need to be put on starch and sugars, because these components are liabilities for overproduction of lactic acid (41).

The general assumption that soluble substances are more easily and rapidly digested than insoluble ones is true only in a general sense. Some insoluble carbohydrates, e.g., unlignified amorphous cellulose in vegetable wastes, may be more rapidly fermented than some of the more soluble modified starches and hemicelluloses.

When large amounts of starch and sugar are added, the fermentation pathway can switch to a lactic acid production, which can lead to acidosis. However, other soluble NSP, such as pectins, arabans, and β-glucans, are not fermented to lactate (41). Hence, there is merit in distinguishing those feeds containing NSP because these can elicit good rumen efficiencies

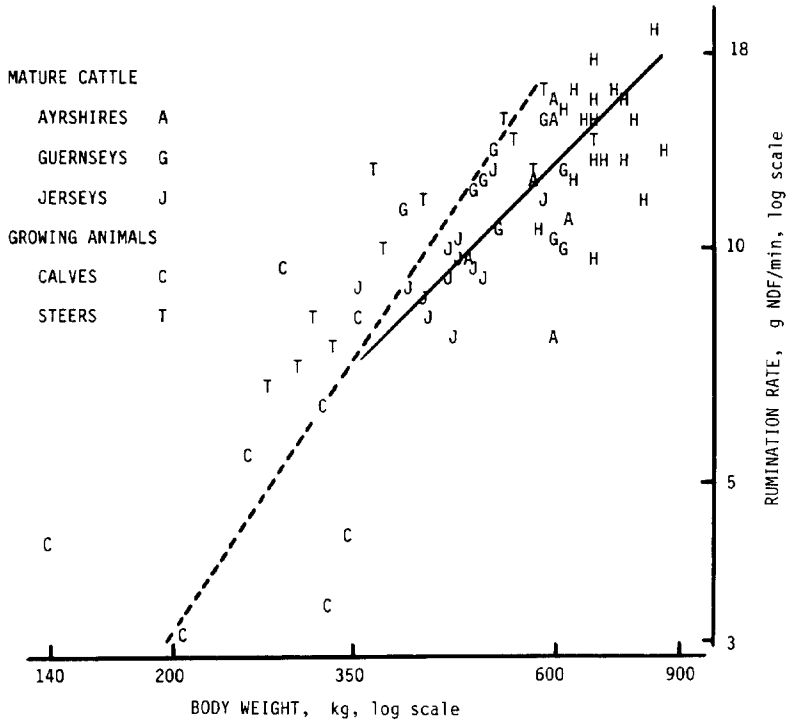

Figure 2. Logarithmic plot of rumination capacity (grams) of NDF per minute and BW of cattle. Regression slope for mature cattle is .95 and not significantly different from unity. Regression slope for immature and growing animals is 1.50. Data illustrate the greater chewing efficiency of larger animals. Calculated from data of Bae et al. (5) and Welch (55) and unpublished figures.

TABLE 5. The NDF and ADF equivalence for alfalfa and corn silage based on NRC (33).

Fiber Source	NRC[1]	Alfalfa composition	Diet level	Corn silage composition[2]	Diet level[3]
	(% DM)				
NDF	28	47	60	47	60
ADF	21	35	60	28	75

[1]Recommended minimum dietary level for cows in early lactation.

[2]Percentage of DM.

[3]Level necessary to provide fiber requirement from the forage sources.

without the problems associated with too much starch (46). The β-glucans that occur in oats and barley contribute to gumminess and are objectionable in poultry diets (3) but probably are beneficial in ruminant diets. See Jeraci and Lewis (18) for methods for β-glucans.

Because NDF and non-NDF carbohydrates represent the bulk of most feedstuffs, variation in the ratio of non-NDF carbohydrate to NDF carbohydrate has become a basis for ration adjustment (22). At least two systems have been proposed for dairy cattle that utilize aspects of this concept. One based on soluble carbohydrate has been patented (34), and another based on NDF (31) has been used for ensuring adequate rumination and efficient milk production. There is, at present, no system that takes into account the contrasting qualities of the so-called soluble carbohydrates, the NSC, or the NSP.

When the ratio of forage to concentrate is decreased (less than 50%) in the diet, balancing rations for NSC becomes important for high producing cows. This problem is more likely to occur with grass- or corn silage-based diets than with legume hay. Starch and sugars can be measured directly (22). The net fraction can be reasonably calculated by difference using one of two formulas (34): 1) NSC = 100 − (NDF + protein + fat = ash) or 2) NSC = 100 − [(NDF − NDF protein) + protein + fat + ash].

They have the disadvantage that NSP are included. They also may not work on silages in which sugars are replaced by fermentation products. The second equation recognizes that in some feedstuffs the protein is not totally extracted by detergent. The insoluble protein in NDF is the slowest to be degraded (52) and should therefore be excluded. This calculated NSC is quite close to determined starch and sugar values for many feeds but is larger when the feedstuff contains significant quantities of NSP, which includes pectins. Pectins are important in citrus, beet pulp, and legume forages but are low in grass forages. Oats, barley, rye, and triticale contain β-glucans. The NSP can be estimated by difference from total dietary fiber and NDF. The residues should be corrected for protein and ash.

The Fiber Requirement

Ruminants generally and dairy cattle in particular require adequate coarse insoluble fiber for normal rumen function and maintenance of normal milk fat test. Normal rumen function in dairy cattle is associated with adequate rumination and cellulose digestion. These maintain rumen pH and cellulolytic microorganisms that characteristically produce the higher acetate to propionate ratios needed for normal lipid metabolism in the cow. Daily rumination time is directly proportional to coarse NDF intake and related to body size (Figure 2) (5, 55). Other estimations of fiber, e.g., ADF or crude fiber are less well related, because only NDF quantitatively recovers insoluble matrix carbohydrates, including hemicellulose. The NDF is better related to intake and gastrointestinal fill than any other measure of fiber (30, 45), thus, the expectation that the fiber requirement is better expressed in terms of NDF rather than ADF or crude fiber. This point is illustrated by the experiments in terms of Welch (56) at the University of Vermont, who examined the ability of various forages to promote rumination. The best relationship was with intake of NDF that was correlated at .99 with chewing time.

The NRC requirements for dairy cattle (33), while allowing different levels of fiber relative

to production, set fixed levels of NDF relative to ADF for lactating cows. This system fails to reflect that the ratios of hemicellulose to cellulose vary widely between feed fiber sources, particularly in the all important corn silage and alfalfa that are the basis of many rations (Table 5). The recommendations are inconsistent with well-established knowledge. It is apparent that the NRC levels for ADF were based on alfalfa, because they do not fit corn silage. Use of the NRC recommendation for ADF will result in overfeeding of fiber in the case of corn silage, grass silages, and hays.

Although coarse NDF of any sort will satisfy the rumination requirement, the quality of that fiber has important effects on the rumen environment and on microbial efficiency. Beyond particle size and adequate levels of NDF, additional factors of buffering capacity, cation exchange, and fermentation rate are important feed and fiber properties needing consideration. Because NDF is not a uniform material, other physicochemical descriptions become important nutritional considerations. The quality of NSC and the proportion of starch and sugar relative to other NSP, such as pectin, have major influences on rumen microbes and efficiency. These factors, net fermentation rate, type and amount of fibrous and nonfibrous carbohydrates, along with the N and protein supply interact to affect rumen function and microbial efficiency.

Cation Exchange and Buffering

The most important feed characteristic besides particle size that contributes to net rumen buffering is the buffering capacity of the feed; this depends on cation exchange capacity of the fiber and, to some extent, on the fermentation of protein to ammonia. Ion-exchangeable groups in plant cell walls include carboxyl, amino, free aliphatic hydroxyls, and phenolic hydroxyls, all of which have some affinity for binding of metal ions (Table 6). Thus, the surface properties of fiber, hydration, and cation exchange are intercorrelated (r = ~.7) and are likely associated with short lag times and rapid rates of cell wall fermentation. Microbes that have negatively charged cell walls (23, 24) "recognize" fibrous particles through their exchangeable surface and form attachments (1), which require divalent cation liganding (probably magnesium). Cation exchange is the ability of fiber to bind metal ions on its surface in much the same way that clay minerals are able to hold cations in soil. The exchange serves as a bank, exchanging K^+, Ca^{++}, Na^+, and Mg^{++} for H^+ when pH drops and recharging when new cations become available as saliva and ingesta are mixed. An advantage of this regenerative bank is that ruminated fiber, as it passes down the digestive tract, contributes buffering action farther down the gut. If the pH rises in the rumen, the bank is recharged with metal ions, and the bound ions are prevented from washing out of the rumen by their attachment to coarse fiber.

TABLE 6. Correlation coefficients (r) of cation exchange capacity determined with copper (II) and praseodymium (III) at specified pH of batch-isolated neutral-detergent fibers (26).

Parameter	Cu (II) pH 3.5	Pr (III) pH 3.5	Pr (III) pH 7.0
Lignin, %	.76**	.69**	.84**
Hemicellulose, %	.49	.56†	.48
Cellulose, %	.11	.03	.16
N, %	.70*	.50†	.58†
Cu at pH 3.5		.96**	.95**
Pr at pH 3.5			.94**

†$P < .10$.
*$P < .05$.
**$P < .01$.

Mature legume forages are the most effective dietary ingredients for supplying exchangeable buffering capacity (Table 7), although some concentrate fibers are as good or better. Corn silage has only about one-third the capacity of alfalfa and also contains starch, which can promote lactic acid production.

The buffering capacity of feedstuffs derives in part from the physical effects that they elicit in the rumen and on rumination. Because the fermentation of carbohydrates leads inevitably to production of large amounts of VFA, their removal by absorption and the recycling of mineral ions are essential processes in the maintenance of pH and normal rumen environment. Fiber is among the more slowly digesting solid fractions and contributes most to the maintenance of normal rumen environment. More rapidly fermenting feeds yield organic acids at a faster rate, thus taxing the buffering system to a greater degree. Mature grasses are

TABLE 7. Cation exchange capacity (CEC) values for a range of foodstuffs and intakes of NDF and DM required to yield equivalent exchange capacity of 1 mol (100 g) calcium carbonate (27).

Feedstuff	NDF	CEC	Calcium carbonate equivalent	
			NDF Basis	DM Basis
	(%)	(meq/100 g)	(kg)	
Alfalfa hay	45	50	4	9
Birdsfoot trefoil	65	30	6	10
Coastal bermuda grass	70	11	17	25
Corn silage	44	15	13	30
Cottonseed meal	29	57	4	12
Distillers grains	50	35	6	11
Dried brewers grains	62	29	7	11
Guinea grass	72	22	10	13
Haycrop silage	43	25	8	19
Oats	37	17	12	31
Rapeseed meal	26	100	2	8
Reed canarygrass	49	21	4	12
Ryegrass	41	24	8	20
Safflower meal	60	20	10	16
Soybean meal	12	41	5	40
Sugar beet pulp	51	70	3	5
Sunflower meal	19	37	5	29
Timothy hay	63	30	7	11
Wheat straw	80	13	15	19

poor in exchange and buffering capacities but also ferment more slowly. Thus, supplementing grasses with starchy concentrate supplements that ferment faster and can yield lactic acid renders the rumen more susceptible to acidotic conditions that limit rumen efficiency and net feed intake. Under these conditions, grass-based forages are less efficient.

There are several systems for measuring cation exchange in plant cell walls. Direct measurement of H^+ exchange with acid is apt to degrade sensitive carbohydrate structures and produce artifacts, although direct titration gives good information (Figure 3). Our first values involved the use of lithium binding (50). Lithium is weakly bound, and variability is encountered in the washing procedures to remove unbound lithium. Calcium and barium proved unsatisfactory because of sulfate interference. Later values were obtained with Cu^{++} (28) via a modification of the procedure of Keijbets and Pilnik (20). This method is limited to measurement at pH 3.5 because of the instability of Cu^{++} at higher pH. More recently, the stronger binding rare earth ions (praseodymium and neodymium) have been applied in a new procedure (2). Values for cation ex-

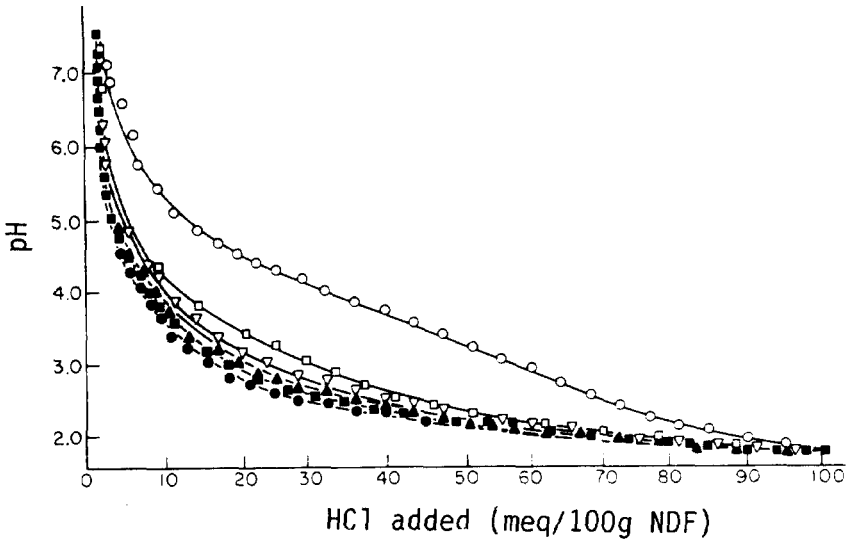

Figure 3. Titratable acidity of plant cell walls from pH 7.0 to 2.0 with .1*N* HCl. Symbols: ●, timothy hay; ■, oats; ▲, maize silage; □, wheat middlings; ○, alfalfa hay. Note the greater buffering capacity of alfalfa (27). Courtesy of the Journal of the Science of Food and Agriculture, Elsevier Science Publishers, Barking, Essek, England.

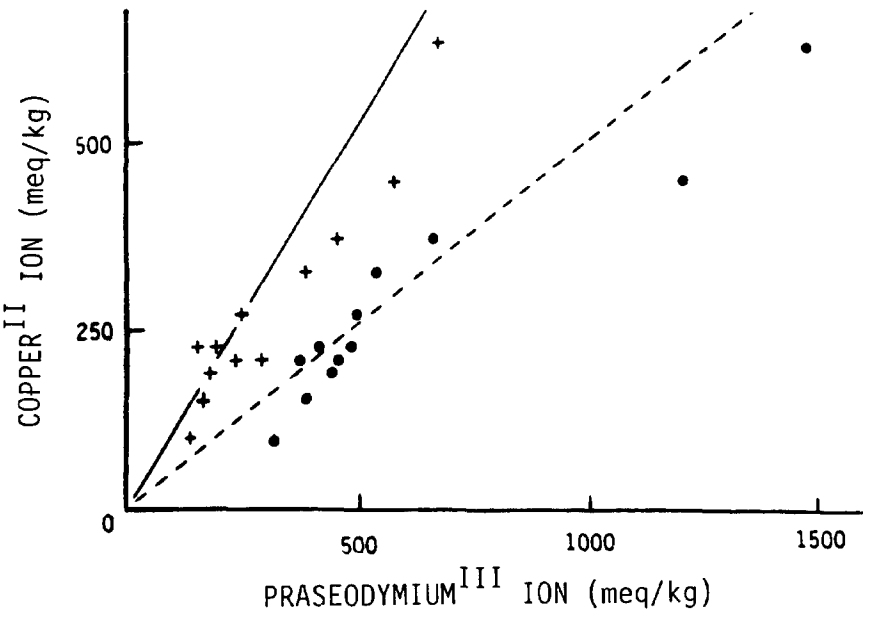

Figure 4. Comparison of three different measurements of cation exchange: copper II at pH 3.5 and praseodymium III at pH 3.5 (—) and 7.0 (- - -) (49). Courtesy of Walter de Gruyter Press, Berlin, Germany.

change capacity obtained by Cu and Pr are shown in Figure 4 and are approximately equal at pH 3.5; values at pH 7.0, obtainable only with Pr, are about double those at pH 3.5. These data are in agreement with Figure 3 data in that about half of the potential cation exchange lies between pH 3.5 and 7. Copper is attracted by unionized amino groups, for which the rare earth has little affinity. Both Cu and Pr have high affinities for phenolic groups, and the exchange values have high correlations with lignin content (Table 6).

The exchange retards mineral ion absorption and delays washout from the rumen to the extent that the exchange is associated with the coarse, lignified fiber. Thus, association with lignin helps maintain the reservoir of buffering exchangeable cations in the rumen. Lignin also is associated with crosslinking of cell wall carbohydrates and inhibits particle size breakdown by rumen organisms, thus, an essential feature of coarse fiber. It can have positive functions in the rumen in contrast with its dominant role in lowering digestibility. There probably is a lignin requirement for the rumen, but it cannot be so high as to limit availability of dietary energy excessively.

Rate of Fermentation

There are important relationships between rates of fermentation of the respective carbohydrates and microbial efficiencies, i.e., production of microbial protein per unit of feed digested in the rumen. Rate of fermentation sets the amount of feed energy per unit time for rumen bacteria. Faster digestion rates provide more food such that the effect is similar to that of plane of nutrition for animals, whereby the extra feed dilutes maintenance, leaving more for growth and production (40).

Rate of digestion has been measured by the ND modification of the Tilley and Terry in vitro rumen procedure (14), in which times of digestion are measured from 6 to 96 h (30). Combination of this information with expected intake and passage has led to the discount concept of calculating net energy (48) and overall ration balancing by matching carbohydrate and protein digestion rates in the Cornell carbohydrate protein model (11).

The range in digestion rates versus rumen microbial yield of various carbohydrates is shown in Figure 5. Cellulolytic bacteria are more efficient because of their lower mainte-

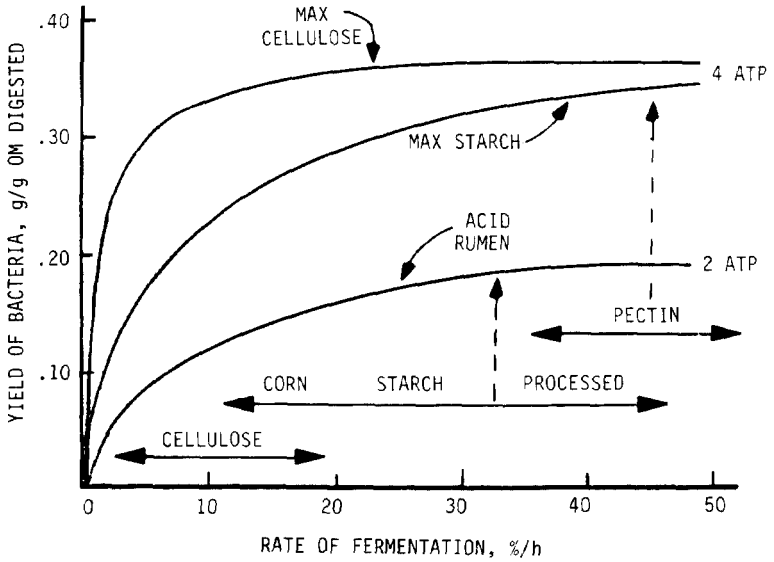

Figure 5. The relationship between the amount of microbial protein produced per unit of feed fermented in the rumen in relation to rate of fermentation. Bacteria in a normal rumen ferment carbohydrate to VFA with a yield of 4 ATP from 1 glucose. Lactic acid production (lower curve) is characteristic of acidic rumens and yields only 2 ATP/mol of glucose.

nance cost. The rate of digestion dilutes the maintenance cost for all rumen bacteria, and more rapidly fermenting carbohydrates improve rumen efficiency. When large amounts of starch are added to the diet, digestion rates in rumen fluid increase, and starch digesting organisms like *Streptococcus bovis* can switch from acetate production, when they get about 4 ATP per unit of glucose fermented, to lactate production when they get only 2 ATP per unit of glucose. In this case, the microorganisms tend to sacrifice the efficiency of ATP production for the sake of increased lactic acid, which makes the environment more favorable for their exclusive growth. *Streptococcus bovis* produces lactic acid when the pH is low, especially if the dilution rate will be slow. At low pH, growth rates of all organisms decrease, but cellulolytics are more adversely affected (47).

Pectin invariably is the most rapidly degraded complex carbohydrate, whereas starches and celluloses are quite variable according to source; hence, their quality reflects digestion rate. Selecting different carbohydrate sources to be complementary may be beneficial, provided that competition among substrates is not severe. Sugars and rapidly degrading starches appear to inhibit cellulose digestion, but pectins may impose this penalty. Pectin is high in citrus, beet pulp, and alfalfa, but there is very little pectin in most grasses and corn silage.

The character of pectin fermentation results not only from a lack of lactic acid output but also from the nature of the galacturonic acid structure that provides potential buffering through cation exchange and metal ion binding. Alfalfa contains 5 to 10% pectin as calcium pectate, and larger amounts occur in citrus and beet pulp as the methyl ester, which is hydrolyzed in the rumen to produce metal ion-binding capacity. In view of the faster fermentation rates of pectins, these physicochemical characteristics probably account for some of the "magic" effect observed when pectin-containing feeds are added to high starch diets.

Cereal grains are the basis for much animal feeding, and, because starch is the major component, starch quality affects feed efficiency. Starches vary in seeds according to physicochemical structure. Linear forms such as amylose are more crystalline and are digested more slowly. There is much genetic variation in cereal grains, which may account for differ-

ences between sources. For ease of degradation (45), uncooked starches rank in the following order: wheat, barley, oats greater than corn, and sorghum greater than legume. Response to processing is in approximately the reverse order (15). The potential rate of fermentation of all carbohydrates largely determines their fate in the digestive tract and the efficiency with which microbes can use them.

CONCLUSIONS

Fiber has come to be recognized as a required dietary ingredient for many herbivorous animal species and is necessary for normal rumen function in ruminants. Quality of fiber varies according to fermentability, particle size, and buffering capacity. Only coarse insoluble fiber is adequate for promoting rumen function. This corresponds to the NDF from forages, and NDF is the preferred measure for ruminant feeds and dietary balancing programs. Therefore, the standardization of procedures for NDF is of paramount importance. Recommended procedures have been provided.

Nonstructural carbohydrate in ruminant feeds also can have impact on dietary quality and microbial efficiency in the rumen, and computerized systems for using both NDF and the NSC have been proposed (11, 34). The NSC can be further subdivided into those carbohydrates (starch and sugar) capable of yielding lactic acid and those not yielding lactic acid, because lactic acid production has major impact on rumen efficiencies. The latter (NSP) include pectins, galactans, and β-glucans.

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