DEVELOPMENT OF A COMPREHENSIVE SYSTEM OF FEED ANALYSES AND ITS APPLICATION TO FORAGES [1]

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FEEDSTUFFS are subjected to chemical analyses primarily for quality control and nutritive evaluation, but such analyses are also important in accounting for particular nutritive properties of individual feeds. Hence, they represent important complements to feeding trials and nutritional experiments.

The Weende system of proximate analysis has been generally used for all of the foregoing objectives in all of nutrition—human, nonruminant and ruminant—for more than 100 years. However, there has been much dissatisfaction with this system, particularly with the crude fiber method and the calculation of nitrogen-free extract (NFE), and from time to time efforts have been made to find a suitable replacement. In general, there has been a conservative tendency to continue to rely on established procedures despite their obvious limitations, and the crude-fiber method remains as the one officially recommended by the Association of Official Analytical Chemists.

Recent efforts to find a replacement for crude fiber have originated in the ruminant field, where the amount and nutritional quality of fiber is of particular importance. A project at Beltsville emphasizing summative or comprehensive systems of analysis (Ely and Moore, 1955; Van Soest, 1966b) has been in existence for 18 years and has been associated since 1960 with the NE-24 regional project, Nutritive Evaluation of Forages. While the work in NE-24 emphasizes evaluation with animals, the Beltsville project and another one at the United States Regional Pasture Laboratory, University Park, Pennsylvania, are concerned solely with chemical work on forage and feces samples collected in experiments by other members of the technical committee. The Pennsylvania project has made important contributions to the knowledge of lignin and hemicellulose.

Definition and Characterization of Fiber. Many failures to achieve the replacement of crude fiber have been due to inadequate understanding of the meaning and purpose of fiber determinations. Conflicting aims have caused some of the difficulty. For example, the definition of fiber as a chemically uniform substance like cellulose cannot be reconciled with the view that fiber represents the least digestible part of the forage and therefore includes lignin. Originally crude fiber (Thaer, 1809; Henneberg, 1859) was regarded as a non-nutritive residue, but the imperfect crude-fiber methodology has allowed most of the lignin and hemicellulose to be extracted into the NFE, which is supposed to represent available carbohydrates.

Before work on a practical method for fiber can begin, a clear nutritional definition of fiber must be established to the exclusion of other definitions—viz., chemical, textile —and basic experimental work must proceed to establish which chemical fractions properly belong in an ideal nutritional fiber procedure. Such a definition can take the place of a primary standard, which forms the basis of most analytical methods. A primary standard is not possible in the case of fiber because there is no single homogeneous substance that has a similar nutritional meaning.

Classification of the chemical fractions of forages and feedstuffs according to their nutritive availability to animals is presented in table 1. Proteins, soluble carbohydrates, and other constituents of the plant cell in category A are generally available to ruminants and nonruminants alike. However, components in category B are either of limited availability or not available at all. Enzymes that hydrolyze cellulose and hemicellulose are not secreted by higher animals, and a considerable difference exists between ruminants and nonruminants in their ability to utilize these structural carbohydrates. Utilization is possible only through microbial fermentation, which gives ruminants a considerable advantage. Definitive evidence that such a division of dry matter of forages (table 1) is justified for ruminants is presented in the following discussion.

Test of Nutritional Uniformity. An im-

[1] Invited paper presented at the 58th Annual Meeting of the American Society of Animal Science, New Brunswick, New Jersey.

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TABLE 1. CLASSIFICATION OF FORAGE FRACTIONS ACCORDING TO NUTRITIVE CHARACTERISTICS[a]

Class	Fraction	Nutritional availability	
		Ruminant	Nonruminant
Category A (Cellular contents)	Sugars, soluble carbohydrates, starch	Complete	Complete
	Pectin	Complete	High
	Nonprotein nitrogen	High	High
	Protein	High	High
	Lipids	High	High
	Other solubles	High	High
Category B (Cell wall)	Hemicellulose	Partial	Low
	Cellulose	Partial	Low
	Heat-damaged protein	Indigestible	Indigestible
	Lignin	Indigestible	Indigestible

[a] From Van Soest (1966a).

portant approach to the problem of deciding the proper division of forage dry matter according to nutritive availability is that of Lucas *et al.* (1961). This test is designed to determine whether the nutritional availability of a given chemical fraction remains the same in different species of forage and as a given species of forage matures. Nutritional availability is defined in the same sense as true digestibility and has been suggested as a more appropriate term (Kleiber, 1964). The reasoning behind this approach is that if forage dry matter could be resolved into components of uniform availability, nutritive evaluation of forages would become a simple matter of summing the amount of each component times its availability.

In the Lucas test, the digestible amount of the component to be tested (digestibility coefficient $\times$ forage content) is regressed on percent of the component in the dry matter of forage. Theory holds that the regression constant is an estimate of endogenous excretion of the component, which is assumed to be constant for the forages examined, while the regression slope is an estimate of the average true digestibility. Height of correlation and standard deviation of the regression coefficient are estimates of the uniform nutritional availability of chemical components of different forages. Graphical examples of the Lucas test applied to forage fractions are shown in figures 1, 2 and 3. Discussion of the significance of these presentations follows.

Nutritive Uniformity of Forage Nitrogen and Cellular Contents. The Lucas test is based on the classical technique used mainly in nonruminant studies, whereby true digestibility and endogenous excretion of protein are ascertained by adding the test protein to a basal diet at different levels. Endogenous protein is calculated as that excreted at zero protein intake; the availability of the test protein is calculated as a partial digestion coefficient (Blaxter and Mitchell, 1948: Mitchell, 1964). Application of the classical technique to ruminant nutrition yields availabilities of forage protein 90% or higher (Turk *et al.*, 1934; Elliot and Fokkema, 1960; Elliot and Topps, 1963). These levels of availability are in agreement with enzymatic data, which show similar digestibilities (Armitage *et al.*, 1948; Van Soest and Wine, 1967).

When the Lucas test was applied to a selected group of 19 NE-24 forages (including 11 grasses and 8 legumes of 8 species with a digestibility range of 45 to 80%) average true digestibility of nitrogen was 93 (table 2) with a low standard deviation. Similar results have been reported for nitrogen in S-45 studies at North Carolina (Lucas *et al.*, 1961).

A small amount (ca 7%) of forage nitrogen is combined in the lignin fraction and is unavailable. This nitrogen appears as part of the cell wall and is not extracted into the cellular contents. The amount of this nitrogen can be markedly increased by heating damp forage. The indigestibility is the result of an actual chemical combination of nitrogen in the apparent lignin fraction by the Maillard reaction (Van Soest, 1965c).

What has been stated concerning the high availability of protein applies to the cellular contents as well. An average true digestibility

of 98% can be applied to forages and concentrates, as shown in figure 1. The regression slope has a low standard deviation (table 2). The endogenous excretion of dry matter amounts to an average of 12.9% of intake, and this value represents the differential between true and apparent digestibility of dry matter.

The most important point to be noted about the cellular contents is that this fraction represents the maximum portion of the forage and is completely available. This will amount to more than 60% of the dry matter of forages and up to 90% of the dry matter in concentrates, as shown in figure 1. The feces of forage-fed ruminants are devoid of water-soluble carbohydrates (Jarrige, 1965). The cellular contents of the forage have no chemical identity with the corresponding soluble matter in the feces. The cellular contents are not encrusted or lignified, and correlations of apparent digestibility with indices of lignification are not significant (table 2). This does not exclude the possibility of a decline in digestibility of concentrates and seed-bearing forages because of the passage of whole kernels or because of high intakes and increased rate of passage.

Apparent digestibilities of nitrogen and cellular content are a function of forage content relative to endogenous output. One must reject the hypothesis of Drapala *et al* (1947) that availability of protein and other cell components is lowered by entrapment in lignified cells. The attempt by Watson and Horton (1936) to demonstrate entrapment of protein by showing increased solubility of fecal nitrogen upon grinding the feces is

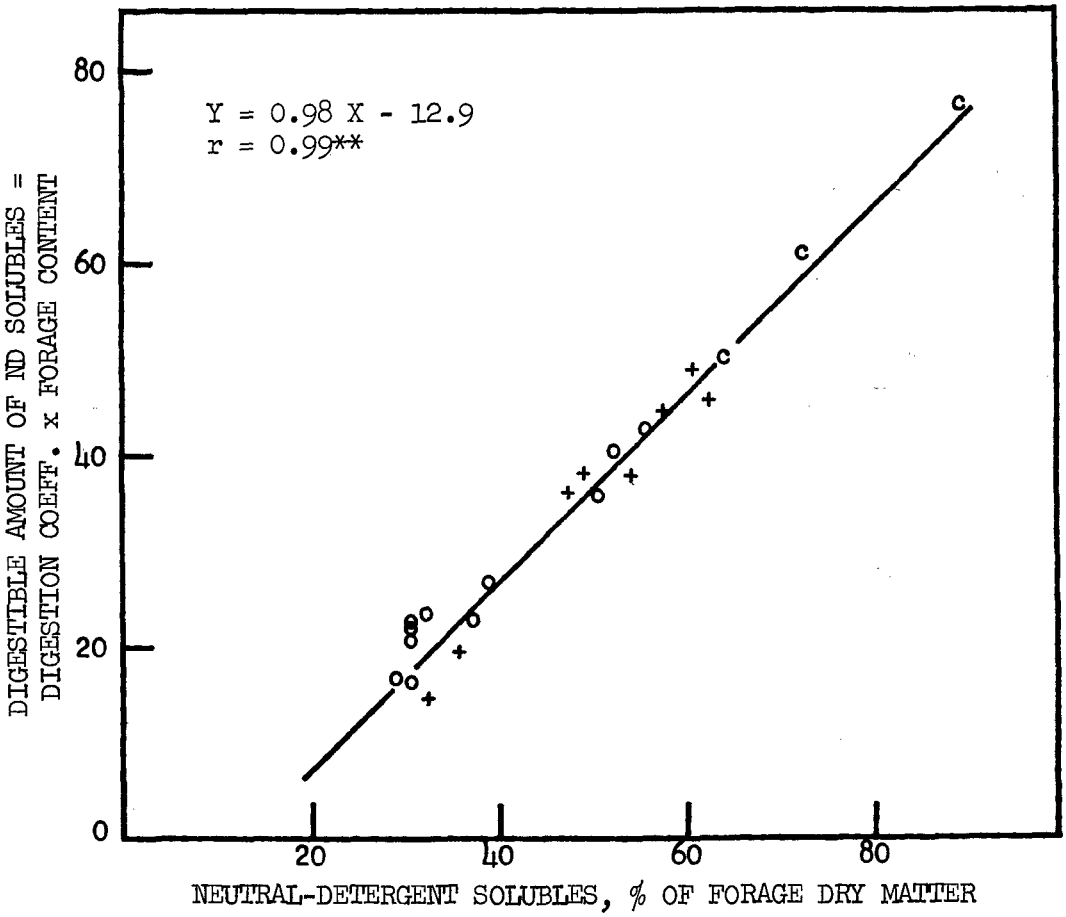

Figure 1. Relationship between the digestible amount of cellular contents and total amount in the dry matter, measured as dry matter soluble in neutral detergent (ND). Grasses denoted by (o); legumes (+) and concentrates (c).

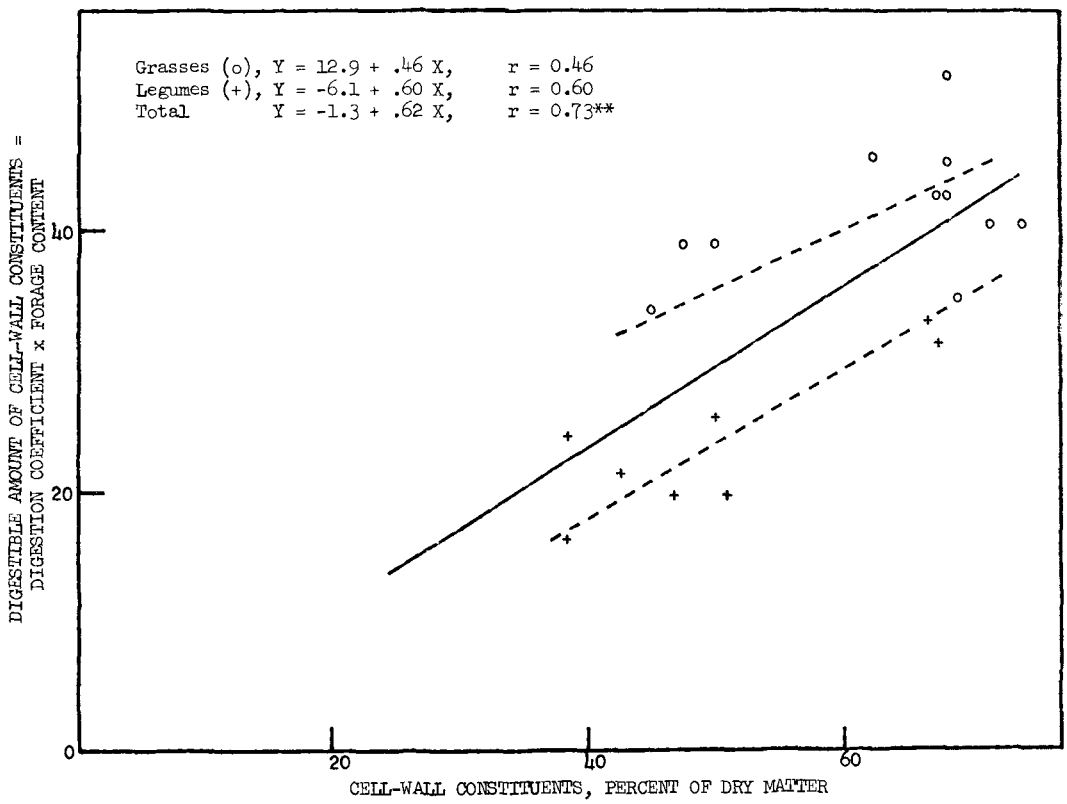

Figure 2. Relationship between the digestible amount of cell walls and the total content in the dry matter.

greatly confused by the failure to recognize that most of the protein in the feces of ruminants is of bacterial origin (Virtanen, 1966) and is resistant to pepsin digestion (Van Soest *et al.*, 1966).

Nonuniform Fractions. The remainder of the forage dry matter, apart from the cellular contents—namely, the cell wall—is not nutritively uniform. Figure 2 shows a marked separation, based on the results of the Lucas test, of legumes and grasses and the development of an interaction between them. Not only does the digestibility of the structural carbohydrates of the cell wall decline with the maturity of the forage, but also these components tend to form an increasing proportion of the dry matter of the plant with age. The studies on the components of the cell wall described below show that resolution of the cell wall into nutritively uniform components is not possible. The consequence of this nonuniformity is to invalidate the individual use of single fractions to predict dry matter digestibility.

Cellulose and Hemicellulose. It is well known that the digestibility of cellulose declines with plant maturation. The same decline occurs in the case of hemicellulose, but this fact is less well recognized because of the ready solubility of hemicellulose in dilute acids and bases and its longstanding inclusion in the NFE. Solubility has been often argued as a basis for nutritive availability (Dehority and Johnson, 1964). However, in the case of hemicellulose the facts do not agree with the hypothesis. Sullivan (1966) has shown that in many forages digestibility of hemicellulose is less than that of cellulose, but is in any event closely related to cellulose digestibility and to the lignification in the plant.

When the Lucas test is applied to cellulose and hemicellulose, nonuniformity is apparent from the magnitude of the standard deviation of the regression slopes shown in table 2. Hemicellulose might be mistaken for a uniform fraction if size of correlation (0.94) were the only criterion. The high correlation

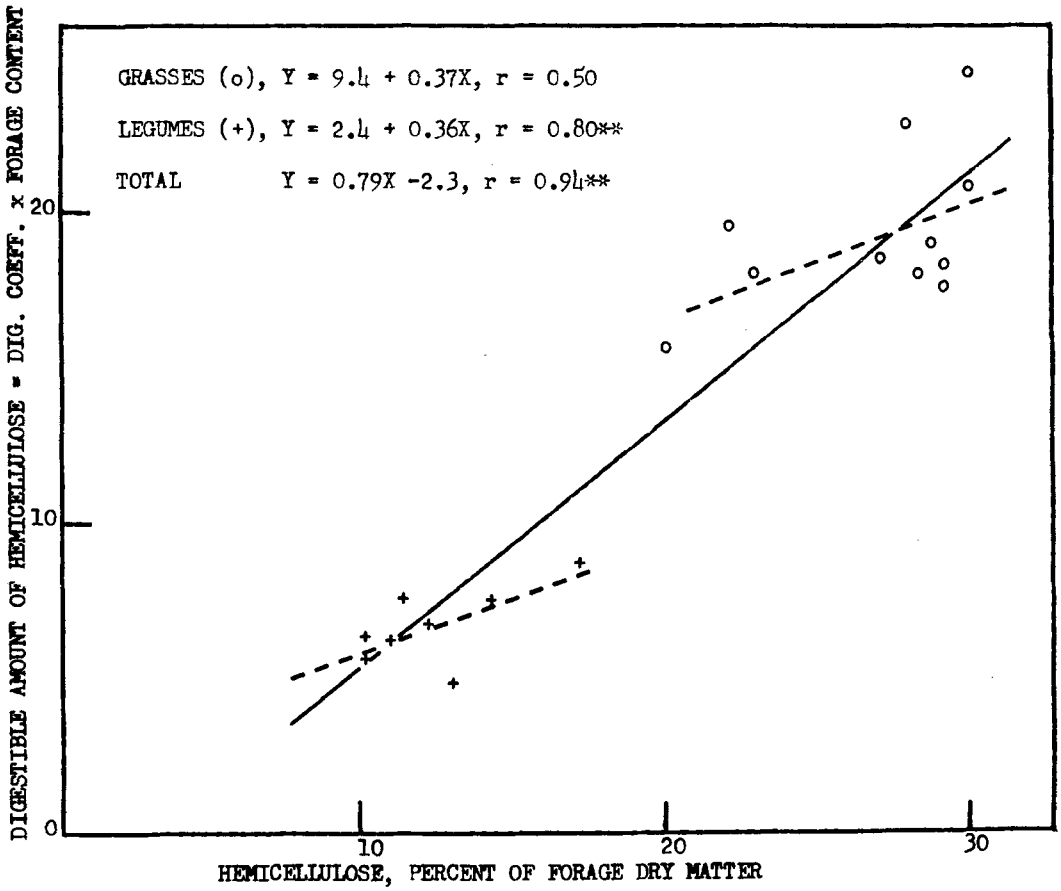

Figure 3. Relationship between the digestible amount of hemicellulose and the content in the dry matter.

TABLE 2. CORRELATIONS OF DIGESTIBLE AMOUNT WITH FORAGE CONTENT, ESTIMATES OF AVERAGE TRUE DIGESTIBILITY, AND CORRELATIONS OF APPARENT DIGESTIBILITY WITH INDICES OF LIGNIFICATION FOR DIFFERENT FORAGE FRACTIONS[a]

Fraction	Correlation of dig. amount with content	Estimated true digestibility[b]	Correlations of app. dig. with		
			Lignin/ cell wall	Lignin/ ADF[c]	Log lignin/ ADF[c]
		%			
N×6.25	0.99**	93± 3.1	—.14	—.16	—.21
Cellular contents	0.99**	98± 2.5	—.14	—.15	—.21
Cellulose	0.67**	50±13.5	—.83**	—.91**	—.93**
Hemicellulose	0.94**	79± 6.7	—.85**	—.86**	—.90**
Holocellulose	0.83**	73±11.7	—.87**	—.93**	—.96**
Acid-detergent fiber (ADF)	0.50*	30±12.6	—.86**	—.93**	—.95**
Cell wall	0.73**	62±14.1	—.90**	—.95**	—.98**

[a] From Van Soest and Moore (1965).
[b] Slope of the regression of digestible amount on forage content.
[c] Acid-detergent fiber.
* $P<.05$.
** $P<.01$.

results from a very large interaction between legumes and grasses, as shown in figure 3. Legumes are characterized by low content and digestibility of hemicellulose, while grasses are high in both respects. Individual regression slopes do not reveal these differences.

Cellulose and hemicellulose are highly correlated with indices of lignification (table 2). Hemicellulose appears to have a special relation to lignin. Evidence exists for chemical linkage of lignin with uronides and xylan (Bolker, 1963; Brauns and Brauns, 1960), and it is interesting that the digestibility of xylose is very much less than that of arabinose in the same forages (Gaillard, 1962; Lyford *et al.*, 1963). Burdick and Sullivan (1963) have shown that the rate of hydrolysis of xylan by acid is closely related to lignification and that other sugars of the cellulose and hemicellulose are not so well related. Hemicellulose becomes soluble in water upon delignification (Brauns and Brauns, 1960; Sullivan *et al.*, 1960).

One final observation needs to be made concerning cellulose and hemicellulose in order to dispel a persistent misconception regarding the value of cellulose as an estimate of structural carbohydrate. The point concerns the relationship (figure 4) between the amount of cellulose and the amount of hemicellulose in grasses and legumes. While grasses and legumes tend to have similar cellulose contents, grasses may contain up to four times the amount of hemicellulose found in legumes. The use of cellulose to estimate structural carbohydrate is particularly dangerous when applied to mixed species of

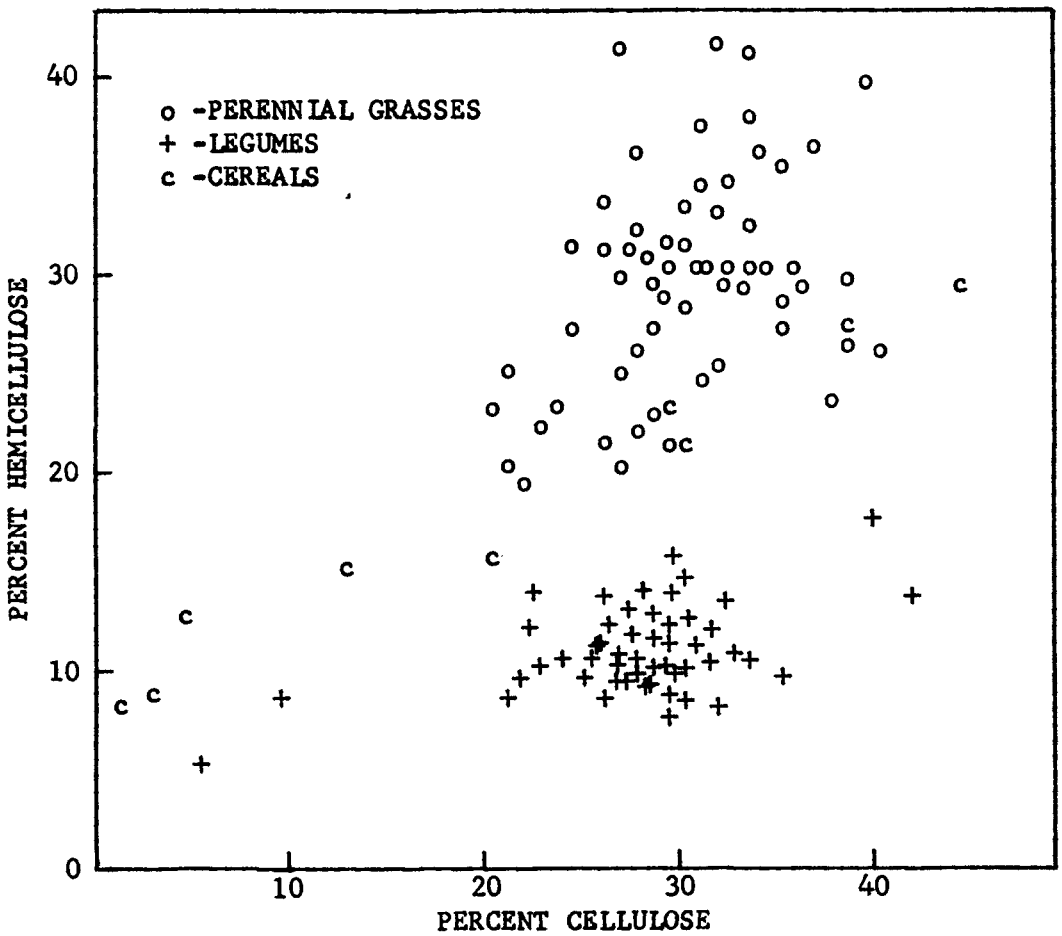

Figure 4. Scattergram showing the contrasting proportions of cellulose and hemicellulose in grasses and legumes.

forages. The nature of the scatter in figure 4 recalls similar relationships of lignin with cellulose and the cell wall (Van Soest, 1965b).

Hemicellulose has long been overlooked as an important component of forages, and yet it appears to be one of the most important fractions relating to the nutritive character of grasses and legumes.

Holocellulose, Acid-Detergent Fiber, Lignin and the Cell Wall. Cellulose and hemicellulose are so similar in their nutritive properties that it is not surprising that the combined fraction, holocellulose, is—in regard to digestibility—highly correlated with the indices of lignification (table 2). Although holocellulose is not a uniform fraction according to the Lucas test, there is no reason why it cannot represent a different kind of uniformity—as the total structural carbohydrate (Ely and Moore, 1955), the availability of which is a function of lignification and structural features of the cell wall.

To carry the matter of combination further, it can be noted that the acid-detergent fiber represents essentially the sum of cellulose and lignin (Colburn and Evans, 1965). This combination also appears, like holocellulose, to be uniform with respect to lignification (table 2). Lignin itself, in contrast to the structural carbohydrates, appears uniform in the Lucas test, having a regression slope of -2% and a standard error of 3.7. This indicates an availability not significantly different from zero. The cell wall represents the combination of holocellulose and lignin as a sum, and it is apparent from the correlations with indices of lignification (table 2) that the whole cell wall can be treated as a unit.

The measures of lignification presented in table 2 are of importance. If the cellular contents are entirely available and lignin is physically combined in the cell wall with the structural carbohydrates, presentation of lignin values as percent of the wall or one of its components is to be recommended (Van Soest and Moore, 1965). When ratios of lignin to crude fiber, cell wall, or acid-detergent fiber are correlated with digestibilities of cell-wall components, very high values result (Van Soest and Moore, 1965). All of these regressions are curvilinear, as revealed by correlations using the logarithm of the lignin content of acid-detergent fiber (table 2). The acid-detergent fiber base tends to yield slightly higher correlations, probably because of the reduction in sampling error brought about by the determination of lignin through the use of prepared acid-detergent fiber.

The importance of being able to treat the whole cell wall as a single entity is that it represents the entire portion of the dry matter not conforming to the Lucas test but demonstrably uniform with respect to structural features of the cell wall, particularly lignification. The entire remaining portion of the dry matter, represented as cellular contents, is completely available. Consequently, the whole forage dry matter can be divided into two fractions that are uniform in different ways.

Estimation of Digestibility. Digestibility can be calculated by means of a summative system based on the foregoing division. An example of such a calculation for an average alfalfa hay (*Medicago sativa*) is shown in table 3. Forage is analyzed for cell walls (Van Soest and Wine, 1967), acid-detergent fiber, and lignin (Van Soest, 1963). Digestibility of the cell wall or true digestibility can be determined by means of an *in vitro* procedure (Van Soest *et al.*, 1966) or, less accurately, from the lignin content of acid-detergent fiber (X) in the equation: $Y=147.3-78.9 \log X$, where Y is digestibility of the cell wall (Van Soest, 1965a). Digestibility of the cellular contents and the bacterial and endogenous excretion can be treated as constants. The composition and digestibilities of the fractions are multiplied and summed as shown in table 3.

TABLE 3. SUMMATIVE RELATIONSHIP BETWEEN TRUE AND APPARENT DIGESTIBILITY: EXAMPLE OF AN ALFALFA HAY [a]

Constituent	Percent in dry matter	True digestion coefficient	Digestible amount
Cell contents (neutral detergent soluble)	60	98	58.8
Cell-wall constituents	40	45	18.0
Total (true digestibility)			76.8
Endogenous and bacterial matter (as percent of intake)			−12.9
Apparent digestibility			63.9

[a] From Van Soest (1966b).

Caution must be used in applying the lignification regression to the evaluation of the cell walls of unusual forages, particularly the coarser mature grasses, because of undetermined factors which affect cell-wall digestibility. For example, silicification may be important in oats (*Avena sativa*), rice (*Oryza sativa*) and Reed canarygrass (*Phalaris arun-*

dinacea) (Van Soest and Jones, unpublished data, U. S. Department of Agriculture). Toxic factors may exist in these and other forages (Roe and Mottershead, 1962; Smart *et al.*, 1961).

It is not surprising that *in vitro* rumen procedures, particularly that of Tilley and Terry (1963) yield more accurate estimates of digestibility than do chemical methods (Hi Kon Oh *et al.*, 1966). This superiority of *in vitro* techniques should be expected because the digesting bacteria are sensitive to undetermined factors influencing the extent of digestion. However, it is the duty of chemistry to discover and elucidate the factors determining the availability of forage components.

In the summative system, an attempt has been made to present a calculation procedure that recognizes cause and effect relationships between chemical components and availability and is amenable to the discovery and incorporation of new factors. If the endogenous and bacterial excretion as well as the true digestibilities of the cellular contents, cell wall and dry matter are all determined experimentally in a total collection digestion trial (Van Soest, 1966a), one can arrive at a partition of the apparent digestion coefficient into the portions arising from different sources. Such a partition can be successfully related to the type of animal and conditions of feeding.

It must be kept in mind that true digestibility of cellular contents and the bacterial and endogenous excretion are in reality biological variables. In highly standardized digestion trials, these factors tend to be more or less constant, thus allowing the reproduction of digestion coefficients; but under practical feeding conditions variability may be important.

The true digestibility of cellular contents may decline with increasing intake, particularly in forages of high starch content (Karr *et al.*, 1965). The bacterial and endogenous excretion tends to be higher in cattle than in sheep (Van Soest *et al.*, 1966). The excretion may not be constant with level of intake, and can be an important factor in the decline of apparent digestibility with increasing level of intake in dairy cattle (Brown, 1966; Van Soest, 1966a).

Correlations of Nutritive Value and Chemical Components. Most prediction equations for nutritive value are based on regressions of digestibility, voluntary intake, or nutritive value index with one or more chemical components. This usage requires some comment in the light of the present discussion.

When digestibility is regressed on a chemical component, it is tacitly assumed that the nutritive availability is actually affected by the amount of that constituent. For example, the use of crude protein to estimate digestibility of dry matter is based on the hope that the decline in protein content is uniformly associated with the decline in digestibility and maturity of the forage. Crude protein is not likely to be a very reliable predictor because it is much affected by nitrogen fertilization and relative differences in content among legume and grass species. Basically, however, it is not logically sound to expect the amount of an available component such as protein to form a fixed relationship with the digestibility of the cell wall or any fiber component whose availability is governed by a different set of factors. Conversely, the use of crude fiber to estimate digestibility of protein or cellular contents is equally invalid.

A similar instance is that of lignin, which is often used to predict dry-matter digestibility. One assumes that the availability of whole dry matter is influenced by lignification. However, as has been pointed out, the entire cellular contents are available and are not lignified at all. The cellular contents of many forages, particularly legumes, may exceed 60% of the dry matter. This, then, constitutes a serious limitation to the use of lignin as the sole predictor of whole forage values.

Because of the relationship between lignin and the rest of forage dry matter, there is a different regression of lignin on dry-matter digestibility for every species of forage (Sullivan, 1959, 1964). The differentiation is particularly marked in the case of the plant families (Van Soest, 1964).

A third instance of error exists in the use of cellulose digestibility as an indicator of dry-matter digestibility. This is a common feature of many *in vitro* rumen procedures. Here again the assumption that digestibility of dry matter is affected by the availability of cellulose is false for the same reason outlined for lignin and crude fiber—because a major part of the dry matter is controlled by an unrelated set of factors. It is probable that many of the failures of *in vitro* rumen cellulose digestion to achieve correct estimation of forage nutritive value are related to the misuse of cellulose and not to faulty *in vitro* technique. The superiority of the Tilley tech-

nique (Hi Kon Oh *et al.*, 1966), based on acid-pepsin insoluble dry matter, can be readily understood, because this technique is essentially an enzymatic preparation of undigested cell walls (Van Soest *et al.*, 1966).

Cellulose digestibility is a valid measure of the availability of the cell wall or of hemicellulose, but not of the completely available cellular contents. Obviously, if there is no fixed relationship between cell-wall digestibility and the ratio between cell-wall and cellular contents, variability must result in any regression based on a single chemical component. Proportions of lignin to cellulose (Maymone, 1962), cellulose to hemicellulose (figure 4), lignin to cell wall, and relative proportion of cell wall are highly species-oriented (Van Soest, 1965b). Many high correlations between constituents and nutritive value result when date of cut is the main variable or when all of the forages are of a given species. While correlations are highly significant, the corresponding regression equations possess high standard errors (Sullivan, 1964). Combining of forage groups of different species leads to large interspecies interactions, which most often tend to cause correlations to drop and standard errors to increase. Occasionally the interactions are so large that correlations are increased to high significance—as in the relationships shown in figure 3, or that between lignin and voluntary intake, described in a previous paper (Van Soest, 1965b). One must conclude that size of correlation is an inefficient tool for discerning basic relationships and for comparing various procedures for the evaluation of forages.

A more fruitful approach is to design experiments which will establish or eliminate possible causative relationships between compositions and nutritive value. The generation of new and alternative hypotheses to challenge the patently accepted ones should accomplish this (Chamberlin, 1965; Platt, 1964). Only after a clearer understanding of the factors involved is obtained will reliable prediction of the nutritive value of forages be possible. Progress in forage research, as in any science, is dependent on basic knowledge which leads to understanding of true relationships.

Summary

Forage dry matter can be divided into two fractions on the basis of nutritional availability. The first fraction corresponds to the cellular contents and is composed of lipids, soluble carbohydrates, most protein and other water-soluble matter. This fraction is essentially available, but its digestibility appears incomplete because of the excretion of fecal non-cell-wall matter, which is principally of endogenous and bacterial origin.

The second fraction corresponds to the plant cell wall, the availability of which is controlled by structural features that link cellulose, hemicellulose and lignin together. Of the cell-wall components, hemicellulose is a large and variable fraction which prevents crude fiber, cellulose, or lignin from being a good estimator of the content of the plant cell wall. The plant cell wall corresponds to what can be nutritionally defined as a total fiber fraction. The nutritive availability of the cell-wall fraction is not uniform among forages.

The dual nutritive character of plant dry matter contraindicates the use of single factors to predict whole dry-matter digestibility.

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