A CHEMICAL ANALYSIS OF FIBER IN FEEDS AND ITS APPLICATION TO FEEDING STUDIES AND PURE CULTURE ANALYSIS

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ABSTRACT

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Sodium chlorite treatment, a technique of plant research for 30 years, is the oxidation of the phenyl ring of lignin without removal of polysaccharides. This method was applied to the analysis of forages and feeds and compared to permanganate lignin. One gram samples were despersed in 0.5% ammonium oxalate, boiled for 2 hr and filtered. The extracted fiber was resuspended in 1% acetic acid at 70 C, sodium chlorite (1.25 g) was added, and lignin was oxidized for a maximum length of 45 min. Oxidation was stopped by adding ascorbic acid, and the suspension was filtered and dried at 60 C for 48 hr. The difference in weight was defined as sodium chlorite lignin. Differences in lignin values were considerable with the various substrates, but sodium chlorite lignin values generally tended to be higher than permanganate lignin values. Sodium chlorite oxidation also removes some structural protein and may require pepsin-hydrochloric acid digestion prior to oxidation.

Gas-liquid chromatography methodology employed in this study

involved the hydrolysis of the hemicellulose of the plant cell wall with trifluoroacetic acid into individual monsaccharides of all 20 substrates. The monosaccharides are converted to the corresponding alditiol acetates which can be quantitated by GLC. Substrates were boiled in ammonium oxalate solution to remove cell wall cytoplasm and some uronic acids. Lignin was removed by sodium chlorite oxidation prior to hydrolysis. Uronic acids removed in the various extracts were determined by spectrometry. Values for lignin, cellulose, hemicellulose, hemicellulosic sugars and uronic acids were calculated. Hemicellulose values as determined by detergent analyses was, in general, higher than hemicellulose values determined by GLC. Cellulose values as determined after hydrolysis with trifluoroacetic acid yielded similar results as detergent cellulose in most substrates. Neutral detergent fiber values for all substrates were lower than ammonium oxalate fiber values and correlated significantly ($P<.05$).

Analysis of the neutral detergent fiber and acid detergent fiber fractions of the feeds and forages showed substantial hemicellulose losses in the cell walls treated with neutral detergent and recovery of hemicellulose in the cell walls treated with acid detergent.

Digestion trials with beef cattle, pigs and ponies were designed to examine the fiber component digestibility in each animal. Four beef cattle were fed a corn silage-supplemented diet for a 7 day collection period. Pigs were fed a corn-soybean meal diet during a 5 day collection period. Three ponies were limit-fed (80% ad libitum) alfalfa-grass hay and oats during a 7 day collection period. Feed and feces were collected and analyzed for NDF, ADF, and GLC fiber compon-

ents. Cattle digested 95.3% glucose, 54.5% galactose, 50.0% arabinose and 37.4% xylose. The digestibilities of individual sugars by the pig decreased in the following order: glucose, galactose, arabinose and xylose. Ponies digested 92.9% arabinose, 68.9% glucose, 68.6% xylose and 57.5% galactose. NDF digestibility was significantly correlated with the digestibility of ammonium oxalate fiber (r=0.92), ADF (r=0.96) and dry matter (r=0.92) in all species.

Two predominant rumen cellulolytic bacteria, Ruminococcus flavefaciens C94 and Bacteroides succinogenes S85 were cultivated with alfalfa, bromegrass, corn silage, cattle manure fiber, wheat straw and Whatman filter paper (no 1) as substrates. GLC analyses, before and after fermentation, showed that R. flavefaciens fermented a mean of 35.6% of the hemicellulose and 29.7% of the cellulose in the substrates, while B. succinogenes fermented a mean of 31.6% and 17.4%, respectively. Electron microscopy showed that there were some differences in the adherence of these species to wheat straw and filter paper and no adherence to cattle manure fiber. Arabinose and galactose composition of the cell wall was significantly ($P<.05$) correlated with utilization only with R. flavefaciens (r=0.87 and r=0.76, respectively.

The examination of fiber structure, digestibility in animals and utilization by bacteria was enhanced by GLC analyses and compared favorably to detergent fiber analyses.

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INTRODUCTION

In the disciplines of nutrition and waste management, there is a need for more definitive and descriptive methods of fiber analysis to interpret experiments with dietary fiber and waste residues. Previous methodology for fiber analysis can only be used for proximate analysis, therefore, it is desirable to develop a new laboratory procedure to examine the fiber structure of a feed, its effect in the gastrointestinal tract and the possibility of recycling many fibrous waste residues.

The word "fiber" is loosely defined as an element that gives texture or substance with a basic toughness. Many fibers have been described such as: crude (91), normal acid (216), acid detergent (194), neutral detergent (197) and dietary (187). Each of these methods can be used for an approximation of some fiber components, but can not be used to determine the effect of a particular fiber upon utilization of nutrients or microbial fermentation in the gastrointestinal tract.

In 1965, Salo (157) presented a review of carbohydrate and fiber techniques used for animal feed and feces. In this review, he introduced another method of fiber analysis referred to as fiber fractionation. This procedure varies widely between laboratories, but may consist of similar fractions such as: cellulose, hemicellulose, holocellulose, lignin, starch, water-soluble carbohydrates and fructosan.

Once again, it is difficult with this methodology to ascertain the effect of fiber in nutritional studies. In general, the approach to fiber chemistry by animal and human nutritionists has been one of availability and fractionation (64, 173, 174), whereas the botanists and cereal chemists have been more concerned with better understanding the complex structure of fiber through finite chemical analyses (11,34,53,61,182).

Each chemical analysis differs in design, however, the outcome is usually similar in that the procedure separates the fiber into individual monosaccharides, disaccharides or oligosaccharides for further study. The measurement of each fraction can be done gravimetrically (19), colorimetrically (157) or by chromatography (163).

The objectives of this study were: (1) to examine fiber more fully by developing and modifying the earlier techniques of Albersheim (11), (2) to examine the detergent fiber components of 20 forages and feeds and compare them to the fiber components of the developed technique, (3) to conduct feeding trials with beef cattle, swine and ponies to demonstrate the feasibility of the developed technique, (4) to conduct pure culture studies with two rumen cellulolytic bacterial species to examine mode of attack and (5) to use electron microscopy to further elucidate differences of techniques and bacterial attachment.

REVIEW OF LITERATURE

There exists today many definitions of fiber which depend upon the perspective of the particular field of study. The American College Dictionary (13) has eight definitions, of which none refer to a nutritional component. These definitions are: (1) a fine thread-like piece, as of cotton, jute or asbestos; (2) a slender filament; (3) filaments collectively; (4) matter composed of filaments; (5) fibrous structure; (6) character: moral fiber; (7) filamentous matter from the bast tissue or other parts of plants and (8) vulcanized fiber. This lack of definition of the most basic of sources typifies the understanding of fiber in nutritional sciences, i.e., many techniques and definitions exist with very little agreement. In 1682, Nehemiah Grew (189) wrote in his "The Anatomy of Plantes": "So in... plants: not only the threads of which the bladders (subsequently renamed cells): but also the single fibers... may sometimes with the help of a good glass (renamed microscope), be distinctly seen." Grew recognized the complexity of the fibrous structure within the plants he examined. The definition of what fiber is, has remained just as complex. Cereal chemists have defined plant fiber as cellulose (34,61) whereas botanists have described it as a dispersed phase of microfibrils packed round with a continuous matrix (10, 53, 182). The nutritional term of fiber has received a great deal

of attention by animal scientists. In the ruminant, it represents the plant cell wall which is utilized as an energy source by the rumen microflora and is extensively degraded whereas in the non-ruminant fiber represents the insoluble matter of plant cell walls indigestible by animal enzymes, but partially degraded by gastro-intestinal microflora. In either case, the plant fiber is determined with neutral (197) or acid detergent (195).

Human nutritionists have defined fiber in the diet many ways. Trowell (187,188) first defined dietary fiber as the plant food resistant to hydrolysis by human alimentary enzymes and composed of cellulose, hemicellulose and lignin. He continued by defining a second term called dietary fiber complex which represented the dietary fiber plus all chemical compounds associated with the structural polymers of the plant such as pectins, waxes, gums, minerals and non-available protein.

An alternative term, plantix, has been proposed by Spiller (176) which would represent cellulose, hemicellulose, mucilages, pectins, gums and lignin. Another term, coplantix, represented the plantix plus associated cell wall factors such as waxes, cutin, cell wall bound protein and minerals. It was suggested that the term fiber remain as a popular term whereas the term plantix remain as a scientific term. In partial agreement with this, it was proposed that the term fiber be changed to partially digestible plant polymers (PDPP), partially digestible biopolymers (PDB) or plantix (79).

Although the term dietary fiber has been very controversial, there is a general agreement in the qualitative definition of Trowell

(51,64,93,96,119), however, the definition excluded the possibility of microbial utilization (151). This controversy has been magnified with the difficulties in established analyses and failure to develop quantitative techniques to examine the plant cell wall more completely. Van Soest (205) suggested that there are two things which are not always compatible in fiber studies: (1) for research purposes one needs a detailed system of structural analysis that is definitive in character for the individual plant fiber sources and (2) for quality control, the methods must be rapid, convenient and must permit the handling of large numbers of samples. Southgate (175) has stated that any procedure for the measurement of fiber must represent the compromise between a complete fractionation and measurement of all the various species and a simplified system involving grouping of different compounds in some arbitrary and often empirical way. These compounds should include cellulose, hemicellulose, lignin and pectin as the primary sources of fiber.

Plant cell wall constituents

Cellulose. The most abundant constituent found in the plant cell wall is cellulose. Cellulose, a linear polymer of glucose, is found in nature in close association with other polysaccharides (47,75). It is the main constituent of the cell walls and serves therein as the primary structural element. Recognition of cellulose was first made by Payen in 1839 (219).

The ideal cellulose is a linear polymer composed of individual anhydroglucose units linked at the C-1 and C-4 of glucose through

glucosidic bonds with the beta configuration. The number of units range from about 100 to 10,000 or more (143,145). The glucan chains in the cellulose fibers are held tightly together by hydrogen bonds (10,78). These bonds form between the hydrogen atom of a hydroxyl group in one sugar unit and an oxygen atom on another unit. Linear molecules of cellulose are also held together by Van der Waals forces. Highly oriented cellulose is termed crystalline whereas randomly oriented cellulose is termed amorphous (46). Cellulose chains undergo a local motion in the amorphous regions of the polymer which involves the anhydroglucose rings. Hydrogen bonds result in rigidity of the crystalline regions which do not permit partial rotation of the anhydroglucose rings without breaking hydrogen bonds. Nuclear magnetic resonance and dielectric constant data have confirmed that reorientation of anhydroglucose of the cellulose chains is sufficiently rapid and vigorous at room temperature to disrupt the structure of cellulose. This apparently involves the amorphous regions, breaking the weak hydrogen bonds in amorphous regions which allow large scale changes in the physical properties of cellulose (78).

Crystallinity and lignification have been shown to be the most important determinants of the susceptibility of cellulosic materials to enzymatic conversion processes (47). Enzymes specific for the bonds in a glucan chain are not very effective in degrading the intact cellulose fiber (10). The accessibility of cellulose to chemical reagents and the extracellular enzymes or other metabolic catalysts of cellulolytic organisms is determined in part by its distribution within the cell wall and the nature of the structural

relationships among various cell wall constituents (47).

The classical method for the determination of cellulose was developed by Cross and Bevan in 1903 (60) and involved alternate chlorination and sodium sulfite extraction. Since then, many other methods have evolved using hypochlorite (141), potassium permanganate and ashing (211), sodium chlorite and trifluoroacetic acid (44), sodium hydroxide and ashing (144), nitric acid-acetic acid digestion (48) and hydrolysis to glucose in sulfuric acid (122). In any method, the effect upon the cellulose bonding is most important. Trifluoroacetic acid (T.F.A.A.) hydrolysis appeared not to hydrolyze cellulose, but only the hemicellulose chains (182). Potassium permanganate has been used widely in nutritional studies, however, the mode of action upon cellulose has never been fully elucidated. Other chemical treatments upon cellulose that have been shown to alter cellulose include hydrochloric acid, liquid ethylamine, ammonia (120) and hydrochloric acid in benzene (138).

Hemicellulose. Hemicelluloses are widely distributed in the vegetable kingdom and, next to cellulose, are undoubtedly the most abundant of the materials of plant origin (150). Hemicellulose is a name given by Schulze in 1891 (218) to a group of polysaccharides in plant material which dissolves in dilute alkaline solution. The term hemicellulose as used today has been given many definitions by different authors. Phillips (150) defined it as carbohydrate substances that are insoluble in boiling water but are soluble in dilute aqueous solutions of alkali. Reid and Wilkie (154) defined it as the polysaccharides in a plant tissue other than cellulose

which is extracted with alkali and hydrolyzed in acid. Nutritionists have looked at hemicellulose as a unit since the fractionation procedures have produced no meaningful nutritional division (204). The very name is a misnomer, since it stems from an erroneous early idea that hemicellulose is a carbohydrate in the process of being converted to a cellulose (204). Some hemicelluloses are relatively branched molecules and are therefore more soluble than a linear molecule such as cellulose (210).

Most hemicelluloses are heteroglycans containing two to six different types of sugar residues. Common heteroglycans include: arabinoxylans (53), arabinoglucuronoxylans (218), methylglucuronoxylans (168), glucans (139), galactoglucomannans (218), glucomannans (168) and arabinogalactans (218). Keegstra et al. (102) suggested that the plant cell wall is held together by non-covalent interactions between macromolecular components. He presented evidence that suggested that macromolecules of the wall are covalently cross-linked (except cellulose) and that the linkage between cellulose and other cell wall polymers has the strength of a covalent bond. Theander (183) divided plant structural polysaccharides into two main classes: (1) the fiber polysaccharides and (2) the matrix polysaccharides. The former compounds are largely crystalline and are present as microfibrils. These microfibrils, mostly cellulose, are held together by hydrogen bonds in cement of largely amorphous matrix polysaccharides, lignin and some protein. Matrix polysaccharides are usually separated into two groups: pectic polysaccharides and hemicelluloses.

The fractionation of hemicellulose has been difficult depending

upon the solubility of each polysaccharide and has been complicated with the fact that these polysaccharides are not uniform. This may be influenced by the molecular weight and distribution, shape, type and configuration of the functional groups (137). The more highly branched and heterogenous composed hemicelluloses are usually removable with a dilute alkaline solution whereas a more homogenous polysaccharide is removed by extraction with strong alkali (22,35). The most common method for hemicellulose extraction has been with a 10% alkaline solution and reprecipitation with ethanol. Five techniques for measuring hemicellulose have been described. The first involves the boiling of the cell wall in 12% hydrochloric acid with a subsequent determination of the furfural evolved (112). The second procedure is dependent upon the isolation of a holocellulose or delignified plant cell wall and hydrolysis of the hemicellulose in alkali (227). A third technique produces hydrolyzed sugars in acid and quantitation with paper chromatography and colorimetry (156, 178,179,180). The fourth technique has been used in many nutritional studies. It is based on the subtracted value between two fiber values, i.e., neutral and acid detergent fiber (82). The newest procedure is based upon a complete fractionation of the hemicellulose and involves delignification of the cell wall, acid hydrolysis, conversion to a volatile derivative and measurement on a gas chromatograph (44).

Lignin. Lignin, the third most abundant component of the plant cell wall, is widely distributed in the plant kingdom. Two classes, namely guaiacyl lignins and guaiacyl-syringyl lignins, for the gymno-

sperms and angiosperms respectively have been suggested (2). Lignin was first recognized as an encrusting material by Payen in 1838, but was later named by Schulze in 1865 (2).

It has been difficult to define lignin. Hartley (88) defined it as a polyphenolic polymer containing phenyl propane structure. Van Soest and Robertson (210) defined lignin as a condensed polymer of substituted phenyl propyl alcohols and acids. Definitions have tended to differ with point of view. Botanists have regarded lignin as a plastic, three-dimensional, substituted phenyl propane polymer. Wood chemists have defined it as a plastic substance giving distinctive properties to wood. Nutritionists have regarded lignin as a structural substance protecting plant cell walls from microbial degradation (203).

The structure and bonding in a particular lignin will depend upon the plant. Lignin has been shown to be intimately associated with the polysaccharides of the cell wall and has a covalent link with cellulose (88). It has also been shown to be linked with a hydroxyproline-rich protein (221) and hemicellulose (131,132). Lignin is relatively easily oxidized since it contains many ether linkages and polyphenolic substitutions. Most phenolic compounds in plants occur as glycosides or esters with carbohydrates (204).

There are several procedures which have been reported in the analysis of lignin. The earliest lignin procedure used fuming hydrochloric acid to dissolve the cell wall carbohydrates leaving lignin (204). A similar procedure was based upon the gravimetric removal of all the structural polysaccharides by hydrolysis in 72% sulfuric acid

followed by ashing (70, 210). A third method is that which was proposed by Morrison (127,128). Crude cell walls are heated in acetyl bromide which reacts with lignin to produce a series of soluble products which are then estimated by measuring the absorption at 280 nm. Two other methods of lignin determination have been developed involving a preliminary treatment of the plant material with acid detergent. Lignin is then determined by measuring the weight of material removed by heating the acid detergent fiber with a mixture of triethylene glycol and hydrochloric acid (65) or oxidation with potassium permanganate (211). Many of these procedures are complicated with artifacts (82,152,204,208). Lignin has also been determined by a newer technique which used sodium chlorite to oxidize lignin. Lignin is then calculated as a loss of weight (44).

Other constituents. Several other plant cell fractions have been reported. The abundance or scarcity of these constituents has been found to be dependent upon the plant. These constituents include: pectins, gums, mucilages, cutin, tannin, cell wall protein and cell wall minerals.

Pectin has been defined as a partially methoxylated polymer of galacturonic acid (111). It has been further defined as a group designation for those complex, colloidal carbohydrate substances which occur in or are prepared from plants and contain a large proportion of anhydrogalacturonic acid units which are thought to exist in a chain-like combination (59). The sugars found in pectin include arabinose, galactose, rhamnose, xylose, fucose, glucose, 2-O-methyl-

L-fucose and 2-O-methyl-D-xylose (59). Pectin is soluble in water, formamide, dimethyl sulfoxide, warm glycerols and ammonium oxalate. Pectin has been regarded as the material soluble in hot solutions of neutral chelating agents and consists of substituted polygalacturonic acid (204). They are part of the non-cellulosic carbohydrates of the cell wall which includes hemicellulosic sugars. Two structures containing pectic substances have been reported in sycamore primary cell walls (21). These structures, arabinogalactan and rhamnogalacturonan, are zig-zagged shape with $\alpha 1 \rightarrow 2$ and $\alpha 1 \rightarrow 4$ glycosidic bonds. It had been assumed that all uronic acid residues after hydrolysis in acid are present in the hydrolysates as aldobiouronic acids having D-xylosyl residues. Buchala and Wilkie (32) demonstrated that the proportion of uronic acids in the xylans from any one type of tissue decreases as the plant ages.

Waxes in leaf tissue have been examined with gas-liquid chromatography. Chemically, was consisted of a mixture of hydrocarbons, ketones, alkyl esters, aldehydes, primary alcohols and secondary alcohols. Wax in Clarkia elegans leaf tissue occurs in the form of smooth films, tubes, dendrites or plates depending upon the growth temperature. An artificial system used for the recrystallization of plant waxes has shown that waxes transported as individual fractions successively to the surface of a porous membrane develop as a composite arrangement of crystals whereas the same solutions delivered as an homogenous mixture form a uniform layer with a poorly defined structure. This suggested that individual components of waxes are responsible for particular structures

and that at the plant surface specific sites may exist for the exudation of each constituent.

There are many types of gums. These substances of plant origin which are obtained as exudations from the fruit, trunks or branches of trees that appear to serve a protective mechanism (99). They have been classified into three major categories according to the raw material or origin. They are plant exudates, seaweed extracts and the seed gums. Gums have described as water-soluble substances or colloids used as viscosity builders forming colloids or gels which can have cationic, anionic or non-ionic exchange properties (117). More specifically, plant gums are the neutral salts of complex polysaccharide acids composed of hexose residues, pentoses, uronic acids and methylpentose residues in diverse fashion (99). They have been distinguished by the fact that glucuronic acid is the acid component in them all. Mathison (115) has reported that rapeseed gum consisted of an aqueous emulsion of phospholipids with smaller amount of triglycerides and non-lipid material. Mangle gum has been reported to contain highly branched galactose-rhamnose chains with arabinose, glucuronic and galacturonic acids (160).

Plant mucilages, similar to gums, have been shown to be water-soluble polysaccharides which form colloids and have been precipitated by ammonium sulfate and sodium chloride. They do not form jellies like pectin. Jones and Smith (99) showed that if the mucilage is on the outside of the seed coating, extraction with water was sufficient, however, if the mucilage was in the endosperm or tuber, extraction by powdering was necessary.

Cutin has been defined as an insoluble biopolyester and a major component in fruits and vegetables (24). Gas-liquid chromatography-mass spectral data has revealed that the major component of the polymer are fatty acids. This polymer has been shown to be fairly resistant to biodegradation (24,208) and has been included as part of a crude lignin fraction. Tannins are defined as polyphenolic compounds which form insoluble complexes with proteins (87). Tannins have also been determined as part of the crude lignin fraction.

Little information has been reported about the secondary cell wall protein. Lamporte (107) has reported that there are high levels of hydroxyproline and disulphide bridges in primary cell walls. Paradies (147) reported that algae cell walls contained 10% to 15% protein which was high in glycine, alanine, aspartic acid and glutamic acid. Minerals also have been shown to be part of the cell wall. Plant cell walls that are secondarily thickened contain silicon and other metal cations (100). They have appeared to be integral with polyuronides and carboxyl and phenolic hydroxyl groups. Grass analyses indicated an accumulation of these minerals (206).

Historical perspective

Fiber analyses. For well over 150 years, the development of techniques for fiber analyses has been difficult. The original technique compared the nutritive values of different feedstuffs. This was called hay equivalents and was attributed to both Einhof and Thaer (190), although this has been highly debated (190). Neither of these scientists used water, alkali, acid or alcohol in

their techniques. The use of these chemicals is part of the crude fiber (CF) technique which was developed between 1800 to 1820 and first reported in corn analysis by Gorham in 1820 (83). This technique involved the treatment of a sample with dilute acid for 30 min and dilute alkali for another 30 min (91). The definition and procedure for crude fiber has remained variable at best. Subtraction of crude fiber, ash, crude protein and ether extract from 100 yielded a fraction called nitrogen-free extract (NFE). NFE represented a highly digestible fraction of the feed.

As early as 1907, data was reported upon losses due to chemical treatments found in the CF technique (89). Haywood (89) treated pure cotton cellulose with the acid and alkali of the CF procedure and reported 2.7% and 17.1% loss in weight, respectively, or 19.8% loss of cellulose.

This was followed by an analysis of the CF residue in 1935 by Norman (140), who found that 60% to 80% of the cellulose and 4% to 67% of the lignin was recovered in the CF fraction. He determined that the pentosans are digested during the 30 min acid digestion period and lignin was extensively removed during the 30 min alkaline digestion. They suggested an alternative procedure was needed to replace the acid and alkali because lignin exercises a direct effect upon the digestibility of the cell walls.

A further complication of the CF-NFE method showed that in many instances, the NFE of straws and grasses could contain as much as 90% of the hemicellulose, cellulose and lignin. This then made the NFE appear less digestible than CF in many cases.

Williams and Olmsted (224) reported that the crude fiber fraction contained variable amounts of lignin, hemicellulose and cellulose and represented the vegetable materials not attacked by digestive enzymes in the mammalian gut. They described an enzymatic procedure for crude fiber to decrease the variability of crude fiber.

A symposium in 1940 (25,149,150,185) showed that the NFE fraction contained a large amount of the cell wall constituents. Despite the awareness of inherent difficulties of the CF method, further modifications have been reported (94,217,220).

In 1946, Matrone et al. (116) suggested that cellulose could be used to evaluate feedstuffs because of its close association with other polysaccharides in the plant cell wall. This technique did not consider the importance of hemicellulose and lignin and remained a procedure in the determination of cellulose.

Paloheimo and Paloheimo in 1949 (146), described a separation of plant products into total vegetable membrane substances and enclosure substances. Total membrane substances represented cellulose, pentosans, hexopentosans, polyuronides, pectin, lignin, suberins and cutins, whereas the enclosure substances represented sugars, dextrins, starch, inulins, proteins, amino acids, amides, amines, lipids, alcohols, pigments, alkaloids, organic and inorganic salts and water. These researchers analyzed for membrane substances by lipid removal in 1:2 v/v ethanol:benzene, boiling in 0.05 N hydrochloric acid, drying and ashing. Protein was determined on each fraction for back calculation.

Walker and Hepburn in 1955 (216) proposed another analysis for the evaluation of roughages. This was called normal-acid-fiber (NAF) and was based upon their criticisms of the CF technique. Samples were extracted for 8 hr in 1:2 v/v ethanol:benzene, boiled in hot 1 N sulfuric acid, filtered and washed with ethanol and ether. NAF was determined by loss of weight after ashing at 550 C. Raymond et al. (153) reported a negative relationship between the percent NAF in the fecal organic matter and herbage organic matter digestibility. They also found 80% to 90% and 100% of the NAF fraction was accounted for as cellulose and lignin in herbage and feces, respectively. The value of NAF was greater than crude fiber in all cases examined (85). Digestibility of NAF was always lower than the digestibility of crude fiber (215). This was attributed to the higher concentration of lignin and cellulose. The variability of NAF due to protein and ash was mentioned. High correlations between the percent NAF in forages with dry matter digestibility were reported (98). A 3 hr digestion in acid, instead of 1 hr, was suggested to decrease variability.

A comprehensive analysis of grasses was reported in 1959 by Waite and Gorrod (213,214). This method involved extraction of the sample in 1:2 v/v ethanol:benzene, boiling in ammonium oxalate, digestion in pepsin-hydrochloric acid, oxidation with sodium chlorite, extraction with water, alkali and acid. Extracts were then analyzed for sugars by paper chromatography and colorimetry. This procedure was complicated by increased error through many transfers and lack of purity in the cellulose fraction. Individual sugars of the hemi-

cellulose were reported.

In the 1960's, Van Soest and others (194,195,196,197,198) examined the use of detergents in the analysis of fiber in forages. They suggested that fiber should represent substances resistant to animal enzymes. Forages were refluxed with 2% hexadecyltrimethylammonium bromide in 1 N sulfuric acid for 1 hr (194,195) in an attempt to lower the protein content of the fiber residue. The fraction retained after filtration was called acid detergent fiber (ADF) and was composed of cellulose, lignin, minerals and some nitrogen (196). Unheated forages retained 2% to 20% of the original nitrogen in the ADF.

It was also found that sodium lauryl sulfate removed large amounts of protein in forages (195,207). This was followed by the use of sodium lauryl sulfate in determining total plant cell walls. Samples were refluxed in a buffered solution of 3% sodium lauryl sulfate containing 1.86% disodium ethylenediaminetetraacetate for 1 hr. This fiber fraction was called cell wall constituents (CWC) or neutral detergent fiber (NDF). It was composed of hemicellulose, cellulose, lignin and some protein (197,207). NDF minus ADF was suggested as a calculation for hemicellulose (197).

Lignin was analyzed by digestion of ADF in 72% sulfuric acid (196). This was complicated with drying and heating procedures during the preparation of laboratory samples (197). Many digestibilities of lignin were reported using sulfuric acid (18,52,68,71 101, 178) which Van Soest attributed to the drying procedures examined. He reported that drying temperatures greater than 50 C

will increase the yield of lignin and fiber due to non-enzymatic browning (198). The nitrogen content of the ADF was suggested as a sensitive assay for non-enzymatic browning (198). Other complications with this procedure due to artifacts have been reported (123,204).

In an attempt to overcome this problem, an alternative procedure with potassium permanganate was suggested (211). In each forage examined, the permanganate lignin was higher than the corresponding lignin value obtained by sulfuric acid hydrolysis. The advantages of potassium permanganate appeared to be: (1) shorter time, (2) less powerful reagents and (3) fewer reagents. The complications appeared to be losses of cellulosic carbohydrates in young grasses and that polyphenolics, tannins, pigments and proteins in the ADF reacted with permanganate and increased the value of lignin (211). An alternative procedure was developed in 1973 (65). In this method, lignin was determined by measuring the weight of material removed by heating ADF with a mixture of triethylene glycol and hydrochloric acid.

The composition of CWC and ADF was examined by Colburn and Evans (16,38). CWC fractions from grasses contained 96.1%, 96.3% and 90.0% of the ADF, cellulose and lignin, respectively, and alfalfas contained 89.3%, 83.7% and 97.4%, respectively. An examination of ADF showed that 92.0% and 88.8% of cellulose was recovered from grasses and alfalfas, respectively. Hemicellulose values, as determined by NDF minus ADF, were not reported. Upon calculation, the values ranged from 5.5% to 30.3%. Calculation of the composition of their substrates on a dry matter basis showed 0.8% to 8.3% and

-0.6% to 1.3% of the hemicellulose value to be due to crude protein and ash contamination, respectively.

Several investigators examined the ADF fraction further (16,17, 207). Kim et al. (106) found that the percent ADF was higher than CF values in feces, silage and pellets. The ADF contained more lignin, but less pentosans and cellulose than the CF. Most of the lignin and cellulose was retained in the feed ADF, however, only 16% to 18% of the pentosans were recovered. Fecal ADF contained 9.7% pentosans.

Bailey and Ulyatt (17) prepared neutral and acid detergent residues from a range of grasses and clovers. He found that NDF consisted primarily of hemicellulose, cellulose, and lignin, however, ADF consisted of cellulose, lignin, some hemicellulose and up to 50% of the plant pectin in clovers. He suggested that the extraction time in the acid detergent fiber method be lengthened to 2 hr.

In 1969, Southgate (173,174) presented a more detailed fractionation system for fiber analysis. He proposed that the unavailable carbohydrates are those that are not hydrolyzed by any enzymes secreted into the digestive tract. He suggested that a small amount of fiber is utilized by the microbes of the digestive tract. Unavailable carbohydrates are measured after extraction in 85% methanol, treatment with enzymes and hydrolysis in 72% sulfuric acid. Hexoses, pentoses and uronic acids were analyzed by colorimetry and represented the sugars from the hemicellulose and cellulose. Available carbohydrates were determined by extraction of the sample in aqueous alcohol.

In an attempt to examine the structure of the cell wall and its relationship to ruminant nutrition, Morrison (127,128,129,131,132, 133,134) developed a series of techniques which determined lignin and lignin-carbohydrate complexes. He stated that different kinds of fiber have been reported and each one is characterized by its method of isolation. He suggested that the relevance of the term fiber is dubious since the chemical composition and ultimately the nutritional significance of different fibers is dependent upon many variables such as the method of isolation, species of plant and maturity of the plant. Morrison expressed that the composition of the plant cell wall ought to be characterized in a more specific way in terms of its individual components, namely lignin, cellulose, hemicellulose and pectin. He first developed a spectrophotometric technique to measure lignin as solubilized in acetyl bromide (127,128). Extraction with dimethylsulfoxide and alkali yielded fractions he called lignin-carbohydrate complexes (LCC) and lignin-hemicellulose complexes (LHC), respectively (128,129,132,133,134). Gas chromatographic analyses of alditol acetate derivatives of the hydrolyzed cell wall sugars (128, 129,132,133,134) were used to analyze the composition of both LCC and LHC.

Further characterization of plant tissues in foods, feeds and feedlot wastes through the use of gas-liquid chromatography (GLC) was proposed by Sloneker (164,170,171,172). He suggested that GLC had advantages over previous gravimetric and colorimetric procedures because individual aldoses could be measured directly and aldose content of different samples were readily visualized. Total neutral

carbohydrates were measured in samples after hydrolysis in 72% sulfuric acid at 30 C. This mixture was then diluted with water to 1 N and hydrolyzed at 120 C. Hydrolyzed sugars were reduced and acetylated with pyridine-acetic anhydride for 16 hr at 100 C. Sloneker (171,172) reported that 5% of the aldoses and 20% of the xyloses were degraded during hydrolysis.

In 1974 , simpler approach to the analyses of fiber was presented by Hellendoorn et al. (90). They proposed that dietary fiber represented the indigestible residue after treatment in pepsin and pancreatin and was calculated by subtracting crude protein, fat, available carbohydrate, ash and water from 100. They stated that the methods presented by Van Soest (68,194,195,196,197,198,207,211) and Southgate (173,174) were not physiological. Values of 10.4% and 56.0% for crude fiber and indigestible residue in wheat bran were reported, respectively.

Another method for the determination of fiber was developed by Elchazly and Thomas in 1976 (69). This method was tailored only for the determination of water-insoluble plant polymers. It was based on the use of amylolytic and proteolytic enzymes.

In 1977, Furda (79) proposed a fractionation system for both the water-soluble and insoluble polymers from plant residues which he termed partially digestible plant polymers (PDPP) or partially digestible biopolymers (PDB). He suggested that the fractionation of dietary fiber should provide the distinct fractions of relatively pure polymers with respect to their chemical structure and solubility. His method also involved amylolytic and proteolytic enzymes, followed

by delignification with sodium chlorite, solubilization in alkali and precipitation in alcohol. In disagreement with this approach, Van Soest and Robertson (210) stated that the soluble substances resistant to animal digestive enzymes should be distinct for analytical reasons.

The use of chemical procedures prepared in the field of botany had been avoided. Collings et al. (41) used sodium chlorite in dilute acid to examine the cell wall components further. They adapted the oxidation of lignin with sodium chlorite into a gravimetric procedure for the determination of lignin. They reported that lignin as oxidized by sodium chlorite left a white cell wall residue and had generally higher values when compared to lignin oxidized by potassium permanganate. Composition of individual cell wall sugars had not been examined. Collings and Yokoyama (44) in 1979 adapted the procedure of Albersheim et al. (11) to the hydrolysis of the cell wall and analyses using GLC. Individual hemicellulosic sugars were hydrolyzed in trifluoroacetic acid (T.F.A.A.), reduced in sodium borohydride, acetylated in acetic anhydride and examined by temperature programming by GLC. Cellulose was determined as the residue remaining after hydrolysis. Hemicellulose, determined by the detergent system, was overestimated in most cases, whereas, there was closer agreement between detergent cellulose and T.F.A.A. cellulose. Examination by electron microscopy showed partial losses of the cell wall after refluxing in neutral detergent and excessive unspecific attack of the cell wall after refluxing in acid detergent. It was suggested that these procedures could offer a more detailed examination of fiber which

could be used to determine the mode of action of the different fiber components.

Gas chromatography. Gunner et al.(86) first reported that carbohydrates, as non-volatile compounds, must be derivitized to a volatile compound before analysis by GLC. Both Gunner et al. (86) and Vanden Hevel and Horning (193) suggested the use of alditol acetates, a carbohydrate derivative, for GLC analyses. Since then, derivitization procedures of carbohydrates have consisted of : trimethylsilyl ethers (181), trimethylsilyaldonolactones (134), acetyl esters (170), dimethylsilyl ethers (166), acetylated aldonitriles (63) and trifluoroacetyl esters (67).

Sweeley et al. (181) presented a technique for the derivitization of carbohydrates to their trimethylsilyl derivatives. They stated that the anomeric pyranoside and furanoside forms of sugars were difficult to separate. They determined the optimal proportions of reagents needed for the maximum yield of each TMS derivative. A detailed investigation of this reaction on approximately 100 different carbohydrates demonstrated the utility of the TMS derivative.

In 1965, Sawardeker et al. (164) examined the use of alditol acetate derivatives further for the analysis of carbohydrates. They suggested that a derivitization method required that a volatile derivative be preparable in quantitative yield from each monosaccharide and that a mixture of derivatives be resolved completely. Another problem mentioned was that as many as four glycosides per sugar, resulting from anomeric and ring isomerization, could result in multiple peaks for each sugar. Sawardeker et al. (164) stated that reduction

of monosaccharides to their alditols and then separation of the alditol derivatives offered better possibilities for their quantitation. This procedure eliminated multiple peaks because alditols can not anomerize.

Morrison and Perry (134) also described the many problems of derivitization techniques of various sugars. They stated that two glycoses yielded the same glycitol upon alditol acetate derivitization. They suggested that the trimethylsilyl derivatives of aldono-1,4-lactones could be prepared from parent aldonic acids and analyzed by GLC. They applied this procedure to a mixture of sugars and reported that the method was both accurate and rapid.

Crowell and Burnett (49) reported that because water reacted with reagents during trimethylsilylation, it was necessary to employ drying procedures and the chromatogram of TMS derivatives was difficult because of anomerization of the many sugars analyzed. They found separation with alditol acetates superior to TMS derivatives. This was also confirmed by Dutton et al. (62).

Easterwood and Huff (63) presented data that prompted the need for more rapid and facile derivatization methodology. They developed a sugar derivative of acetylated aldonitrile and compared it with alditol acetates. They concluded the procedure of alditol acetates was a poor choice for the separation of sugar mixtures, however, their chromatogram indicated a poor alditol acetate derivitization technique.

Recently, two new procedures for the analysis of di- and trisaccharides were reported. Sugars were converted into their methox-

imes by a reaction with methoxylamine hydrochloride in pyridine followed by either esterification with acetic anhydride or by trifluoroacetylation with N-methyl-bis-trifluoroacetamide (166).

In 1968, Sloneker (170) reviewed the methods of derivitization for gas chromatography. The choice of which volatile derivative and of which particular column to use for analysis of sugars depended upon the complexity of the sugar mixture resolved. The rate at which free sugars degraded during acid hydrolysis depended upon four factors: (1) type of acid used, (2) concentration of acid, (3) temperature of hydrolysis and (4) length of hydrolysis.

The use of GLC techniques in the analysis of plant cell walls was first described in 1969 by Albersheim et al. (11). They examined the primary cell wall of pinto bean hypocotyl. Hemicellulosic sugars were separated by hydrolysis in trifluoroacetic acid at 121 C for 1 hr. This was followed by reduction, methylation and acetylation of the sugar mixture to their corresponding alditol acetates. The major advantages of this procedure appeared to be: (1) the T.F.A.A. used in the hydrolysis of polysaccharides is readily evaporated; (2) the entire procedure may be performed in a single test tube; (3) acetylation of alditols is catalyzed by sodium acetate, thus eliminating the need for pyridine; (4) the acetylation mixture may be injected directly into the gas chromatograph and (5) acetylation of the alditols was performed in a sealed tube which eliminated the need for heating under reflux. They also reported that a maximum 1 hr hydrolysis in acid was optimum before sugars, particularly xylose, would begin to decompose.

Also in 1969, Reid and Wilkie (155) reported the analyses of oat leaves using methylated glycoside derivatives. Hemicellulosic sugars were hydrolyzed in sulfuric acid at 100 C for 16 hr to 24 hr. Subsequent to this report, others from the same laboratory have examined the hemicellulosic sugars of oats (28,30,32,76,77), wheat (31) and bamboo (223) with alditol acetates.

Sloneker (171,172) developed a technique for complete sugar analyses in foods, feeds and feedlot wastes. Total neutral carbohydrates were measured in one sample after hydrolysis in sulfuric acid for 2 hr and derivitization to their corresponding alditol acetates. Cellulose was measured by an extraction procedure described by Matrone et al. (116). Total hemicellulose was determined by difference. He reported that 5% of the aldoses and 20% of the xylose were degraded during hydrolysis. Avicel, a microcrystalline cellulose, had 0.6% xylose and 1.0% mannose. He concluded that even with these complications, GLC had advantages over gravimetric and colorimetric procedures because individual aldoses were measured directly and because variations in the aldose content of different samples were readily visualized.

Since then, there has been no agreement upon which GLC technique should be used for lignified plant tissues. Woolard et al. (226) and Theander and Aman (184) used alditol acetates for sorghum grain and rapeseed meal respectively, whereas Ericsson et al. (74) used TMS derivatives for the analysis of soluble carbohydrates in pine needles and Morrison (133) suggested the use of aldononitriles for polysaccharide analysis.

In 1979, Collings and Yokoyama (44) described procedures for the study of fiber components in 15 lignified plant tissues. Tissues were delignified with sodium chlorite (41) and hydrolyzed with T.F.A. A. The hydrolyzed sugars are dirivatized to their corresponding alditol acetates and analyzed by GLC (11). Length of hydrolysis, chemical additives and other variables were examined. Comparisons between the detergent fiber analysis system and the gas chromatograph system were reported. They suggested that this system offered additional structural information about the capacity and structure of fiber or the plant cell wall.

Utilization of fiber by animals and bacteria. The development of fiber component analyses for nutritional studies have depended upon the particular organism being studied. Various approaches have included the isolation of: (1) the indigestible portion of the dietary fiber (195), (2) the most indigestible fraction of the plant cell wall (85,153,192,195,215,216), (3) the fractionated plant cell wall (157), and (4) the soluble and insoluble complex polymers associated with the plant cell wall (79,173,174).

Many animals have been shown to be able to utilize fiber components. Ruminants, such as cattle and sheep, have a large fermentation system in their rumen which contains bacteria and protozoa. This flora digests large quanities of the plant cell wall and produces protein and energy in the form of volatile fatty acids which can be used by the host animal (97). Nutritional studies have shown that the amount digested within a ruminant depends upon the type and maturity of forage, pelleting, flaking, composition of forage, micro-

flora, minerals, degree of lignification, degree of crystallinity, acetyl groups, particle size, etc. (15,46,174).

A comparison of fiber digestibility between sheep, rats and swine showed the highest amount digested was by sheep followed by pigs and rats (104,208). Utilization of fiber by non-ruminants has appeared to be due to cellulolytic utilizing bacteria in the gastro-intestinal tract (26,27). A cross-species comparison with three substrates showed that utilization of fiber decreased in the following order among species: sheep, swine, horse, voles and rats. The hemicellulose of the three substrates was more digestible in pigs and voles (103). Increasing amounts of fiber in the diet did not affect the digestibility of all detergent fractions in pigs, but decreased the digestibility of hemicellulose and cellulose in rats (105). The digestibility of ADF, cellulose and lignin decreased with increasing amounts of wheat middlings in the diet of pigs (39).

Other animals that have been shown to utilize fiber include: white-tailed deer (191), horses (204), veal calves (81), beaver (95), humans (93), rabbits (36), monkeys (125), and insects such as termites (114), locusts (124), grasshoppers (124) and shipworms (54).

The utilization of fiber in the intestinal tract of a monogastric animal and in the reticulo-rumen of ruminants has been shown to involve a close association with cellulolytic bacteria (26,27,97). Although fiber has been defined as the plant material resistant to intestinal enzymes (187,188), reports in many monogastric animals have shown extensive fiber utilization (26,27,39,40, 103,104, 105, 208,212).

In 1966, Dehority et al. (55) showed 60%, 75% and 80% of the hemicellulose from orchardgrass, alfalfa and timothy, respectively, was digested in vitro with rumen fluid. Hemicellulose digestibility was measured as the loss of total pentoses.

More specifically, Gaillard et al. (80) found that pasture plant hemicellulose consisted of three major polysaccharide types: (1) linear A hemicellulose - a water insoluble heteroxylan containing uronic acids, but only small amounts of arabinose, (2) linear B hemicellulose - a more soluble heteroxylan containing much more arabinose and less uronic acids than linear A, and (3) branched B hemicellulose a water soluble, highly branched polymer which, in addition to pentoses, is rich in galactose and uronic acids. They found that linear B hemicellulose was readily attacked by rumen bacteria in vivo. In grass-fed animals, grass linear A hemicellulose and branched B hemicellulose hydrolyzed faster than similar clover fractions. In clover-fed animals, clover and grass linear A hemicellulose and branched B hemicellulose hydrolyzed at the same rate.

Coen and Dehority (37) found that digestibility varied with maturity and type of plant and bacterial species. They reported that Bacteroides succinogenes S85 digested greater amounts of bromegrass, alfalfa or fescue hemicellulose when compared to Ruminococcus flavefaciens C94. Hemicellulose was measured as total pentoses.

In 1978, Dehority and Scott (56) reported Bacteroides succinogenes S85 was able to digest more cellulose than eight other rumen bacteria. They found only three bacteria of the eight examined were able to digest hemicellulose. Ruminococcus flavefaciens C94 digested

37.2% and 10.3% bromegrass and alfalfa hemicellulose whereas B. succinogenes S85 digested 13.3% and 0.0%, respectively.

Akin and Amos (8) examined forage cell wall after incubation with rumen contents with scanning electron microscopy (SEM) and transmission electron microscopy (TEM). They found that bacteria attacked the mesophyll and phloem of forages. Latham et al. (108) reported that B. succinogenes S85 adhered to the cut edges of ryegrass and to the intact mesophyll whereas R. flavefaciens C94 adhered to the cut edges of epidermal cell walls. They suggested that adhesion was similar, but there were different affinities for each cell wall.

Leatherwood (110) proposed that R. albus may have an affinity factor which is necessary to hold the hydrolytic factor of cellulase in position to the insoluble cellulose for multiple attacks to occur. Such a phenomenon may be required for hydrolysis of the cell walls more resistant to bacterial degradation where attachment precedes degradation.

Two modes of bacterial attack were visualized: (1) tunneling action and (2) erosion of surfaces. Dinsdale et al. (58) said there is a serious gap in the knowlege of cell wall digestion. No enzymes have been isolated and characterized which are capable of attacking highly ordered cellulose. The bulk of the cellulose in the cell walls of forages has been shown to have a moderate degree of order and digestion could be high depending upon the order of the cell wall (58).

Interest in fiber utilization in monogastrics, especially humans, has been increasing in recent years (27,158,212). Bryant (27) has identified hemicellulose fermenting bacterial species in human feces.

Previously to this, he reported significant cellulose utilizers were found in human feces (26). These bacteria have been identified as species of Bacteroides and Bifidobacterium.

The importance of the understanding of fiber utilization, structure and action in humans has increased over the last ten years. The action of fiber has been implicated in the following: diabetes, colon cancer, deep vein thrombosis, cardiovascular disease, diverticular disease, obesity, gallstones, appendicitis, hiatus hernia, hemorrhoids and others (177). Spiller et al. (177) mentioned a need for techniques to analyze fiber more completely than previous methods so to examine the mode of action of these diseases.

MATERIALS AND METHODS

Preparation of samples. Twenty feeds and forages were chosen to represent a broad cross-section of substrates (Table 1). Each was hand collected from controlled University plots, dried at 60 C for 48 hr, ground in a Wiley mill (1 mm screen) and stored in glass bottles. A second dry matter (A) on each sample was performed after grinding to account for water absorption (41,78). Fiber components as determined by the detergent method (195,200) were completed. Five to 10 trials with neutral and acid detergent were performed to collect one to two grams of the neutral detergent fiber and acid detergent fiber fractions of each substrate for further analyses. Crude protein (N x 6.25) was determined by the microkjeldahl method (14).

Delignification of plant samples. The sodium chlorite oxidation procedure as described by Green (84) was modified (41) to determine the lignin content in samples. A 1.00 g sample was weighed into a 600 ml Berzelius beaker, and 200 ml of a w/v 0.5% ammonium oxalate solution were added. The suspension was then heated to boiling (5 to 10 min) on a crude fiber reflux condenser. The heat was reduced to an even boil and refluxed for 2 hr timed from the onset of boiling. A sintered-glass crucible (#1 porosity) was preweighed and placed in a fiber manifold. The oxalate-fiber slurry was poured into the cruc-

TABLE 1. COMMON AND SCIENTIFIC NAMES AND DRY MATTER OF SUBSTRATES.

	Common name	Scientific name	Field dry matter, %
1.	Kentucky bluegrass	Poa pratensis	44.2
2.	Tall fescue	Festuca arundinacea	33.5
3.	Reed canarygrass-2nd cutting	Phalaris arundinacea	37.7
4.	Reed canarygrass-full bloom	Phalaris arundinacea	43.0
5.	Reed foxtail	Alopecurus pratensis	45.4
6.	Bromegrass	Bromis inermis	48.5
7.	Orchardgrass	Dactylis glomerata	46.3
8.	Quackgrass	Agropyron repens	45.8
9.	Wheat straw	Triticum aestivum	----[a]
10.	Wheat middlings	Triticum aestivum	90.1
11.	Wheat bran	Triticum aestivum	90.0
12.	Elodea	Elodea canadensis	8.8
13.	Algae	Cladophora fracta	12.5
14.	Manure fiber	----------[b]	
15.	Ladino clover	Trifolium repens	23.0
16.	Red clover	Trifolium pratense	20.7
17.	Crown vetch	Coronilla varia	30.0
18.	Alfalfa	Medicago sativa	31.2
19.	Birdsfoot trefoil	Lotus corniculatus	32.9
20.	Corn silage	Zea mays	31.2

[a] not available

[b] washed manure fiber from corn silage-fed beef steer.

ible, and a vacuum suction was applied. The residue was washed twice with 50 ml of hot distilled water. The crucible plus fiber was then dried for 48 hr at 60 C in a forced-air oven. The crucible plus fiber was weighed once after 30 min in the dessicator and once more after 30 min at room temperature. This was needed for measurement of water absorption of the fiber which was accounted for in later calculations. The fiber residue was quantitatively scraped from the crucible, weighed (B), then transferred into a 250 ml Phillips beaker, and 100 ml of a v/v 1% acetic acid solution were added. This mixture was heated to 70 C $\pm$ 1 C; then 1.00 g of sodium chlorite was added. This operation was performed under a hood because chlorine dioxide generated during the reaction is both toxic and explosive. Heating continued for 30 min with frequent stirring. After 30 min, an additional 0.25 g of sodium chlorite was added, and heating continued for an additional 15 min with frequent stirring. At the end of the total oxidation period (45 min) the solution was cooled, and enough ascorbic acid was added (up to 0.5 g) to stop the oxidation reaction. A color change in the solution from bright yellow to clear was indication of complete cessation of chlorite oxidation. The solution was poured into a preweighed sintered-glass crucible (#1 porosity), and the crucible and contents were dried at 60 C for 48 hr. The crucible was weighed after cooling for 30 min in a dessicator, and the following calculations were made. Weights obtained and calculations:

1. (A) Dry matter of ground sample.
2. (B) Oxalate fiber residue.
3. (C) (Wt. of crucible #1 + oxalate fiber) - Wt. of crucible#1=

Wt. of oxalate fiber at 0 min.

4. (D) (Wt. of crucible #1 + oxalate fiber) - Wt. of crucible #1 = Wt. of oxalate fiber at 30 min.

5. (E) (Wt. of crucible #2 + delignified residue) - Wt. of crucble #2 = Wt. of delignified residue.

6. Wt. of lignin = (BC/D) - E.

7. % Lignin in dry sample = (C) x (Wt. of lignin/(BC/C) x 100)/ (sample wt. x (A)).

Second dry matter of plant samples. Three 1.00 g samples of each plant sample were dried at 60 C for 48 hr and placed in a dessicator for 30 min. Dry matter was calculated and this value was used in all subsequent calculations. To demonstrate the absorption of water by the different fibers, three 1.00 g samples of solkafloc cellulose was dried at 60 C for 48 hr and placed in a dessicator for 30 min. Weights of individual samples were taken each minute for 30 min with the samples at room temperature. Dry matter of each sample was determined at minute intervals.

Preparation of alditol acetates. A 50 to 60 mg delignified sample (F) was transferred to a 18 x 150 mm heavy duty Wheaton tube[1] containing 10 ml of 1.0 N trifluoroacetic acid. Myo-inositol (5 to 7 mg) (G) was added to each tube as an internal standard. Each delignified sample was run in triplicate. The tubes were sealed with slotted stoppers and aluminum seals and hydrolyzed at 120 C for 60 min in an autoclave. After the hydrolysis, the samples were filtered through a preweighed sintered-glass crucible (#3). The filtrate was then collected and poured into a 50 ml round bottom flask. Total

[1]Wheaton Scientific blueprint no. 37350, Wheaton Scientific, Millville, N.J. 08332.

uronic acids were determined by colorimetric analysis of the ammonium oxalate and trifluoroacetic acid (T.F.A.A.) filtrates (23). The residue was washed with 50 ml deionized distilled water and dried for 24 hr at 60 C. The filtrate was evaporated to dryness under a stream of filtered air in a 60 C water bath. The hydrolyzed hemicellulosic sugars in the filtrate were reduced to their respective alditols with sodium borohydride (1.0 g) in 1 N ammonia (50 ml) for 1 hr with occasional swirling. The reduction was ceased with glacial acetic acid until the reaction stopped. 10 ml of methanol was then added and evaporated to dryness under a stream of filtered air in a 60 C water bath. Five more 10 ml methanol additions were added (11) and evaporated to dryness as before. Acetic anhydride (4 ml) was then added and the round bottom flask was sealed with a rubber stopper and wired down. The alditol mixture was then acetylated to their corresponding alditol acetates by heating at 120 C for 1 hr in an autoclave.

GLC quantification of alditol acetates. A 3 to 5 ul sample of the autoclaved mixture was injected into a gas chromatograph (Varian model 1800 Aerograph or Hewlett-Packard 5840A) equipped with a hydrogen flame ionization detector. A stainless steel column (120 x 0.3 cm) packed with 0.2% poly-ethylene glycol adipate, 0.2% poly-ethylene glycol succinate, and 0.4% silicone XF-1150 on Gas Chrom P (100 to 120 mesh) was used. Other GLC parameters were: column temperature, programmed between 135 C to 200 C with a 10 min holding at 135 C after injection of the sample, followed by 1 C/min increase in temperature; helium flow rate of 30 ml/min; injection temperature of 210 C; detector temperature of 250 C; and attenuation of 32X

and range of 1 mvolt. The following formula was used to calculate the percent hemicellulosic sugars and cellulose:

$$\% \text{ cellulose or } \% \text{ sugar} = \frac{(C) \times \frac{\text{Wt. of sugar or cellulose}}{BC/D}}{\text{Sample wt.} \times (A)} \times 100$$

Wt. of cellulose in (E) = JE/F.

Wt. of sugar in (E) = GIE/FH.

Where:

1. (F) Wt. of holocellulose sample (50 mg).
2. (G) Wt. of myo-inositol (5 to 7 mg).
3. (H) Myo-inositol GLC peak area.
4. (I) Sugar GLC peak area.
5. (J) Wt. of cellulose.

Trifluoroacetic acid hydrolysis. To determine the optimum hydrolysis time as specified by Albersheim et al. (11), samples of Reed canarygrass (full bloom) delignified tissue was prepared. 50 mg samples plus 5 to 7 mg myo-inositol were hydrolyzed in triplicate in 10 ml 1.0 N trifluoroacetic acid for 15, 30, 45, 60, 75 and 90 min.

Methanol additions. As shown by Albersheim et al. (11), five additions of methanol appeared to be sufficient to permit methylation of the hemicellulose sugars in sycamore cells. To test this in whole plants, samples of Reed canarygrass (full bloom) delignified tissue were prepared. 50 mg samples plus 5 to 7 mg myo-inositol were hydrolyzed in 10 ml 1.0 N trifluoroacetic acid for 60 min. After

collection of the filtrate, evaporation and reduction, one to six methanol additions (10 ml each) were tested.

Cellulose composition. Residual cellulose was collected from Reed canarygrass (full bloom) after hydrolysis in 1.0 N trifluoroacetic acid. (300 mg samples were hydrolyzed by the two-step sulfuric acid procedure as described by Adams (1). Hydrolysates were dried in a 60 C water bath and 5 to 7 mg myo-inositol was added. Each hydrolysate was reduced and acetylated as in the previous procedure and analyzed for the corresponding alditol acetates using GLC.

Neutral and acid detergent fiber analyses. The neutral detergent fiber and acid detergent fiber fractions of all twenty forage and feeds were treated with ammonium oxalate, sodium chlorite and trifluoroacetic acid as described before to examine the possible losses of polysaccharides with detergent treatment (17).

Other fiber analyses. The amino acid composition of wheat straw, wheat straw neutral detergent, acid detergent and ammonium oxalate fibers, and the delignified residue of wheat straw were done by ion-exchange chromatography. Samples of the ammonium oxalate fiber, delignified residue and T.F.A.A. cellulose were prepared from wheat straw, Kentucky bluegrass, bromegrass, ladino clover and alfalfa and analyzed for crude protein (14) and ash (14). Calculations of subsequent losses due to treatment were converted to a dry matter basis.

Pony digestion trial. Three ponies (Chester, Snipper and Elvis) of varying weights (146.1, 161.5 and 129.7 kg, respectively) were borrowed from the Michigan State University Endocrine Research Unit.

The ponies were fed an alfalfa hay-oats ration twice daily (0800 hr and 1700 hr) and preadjusted 7 days on the ration before a 7 day collection period began. All animals were maintained individually in 183 cm x 213 cm stalls with cement floors and no bedding. Feed contamination of the feces was kept to a minimum by picking any feed particles out of the feces. No attempt was made to moniter urine output. Feed and feces were collected three times daily and weighed. The unconsumed feed was weighed and recorded daily. Dry matter of the feces was calculated on each daily sampling. At the end of the collection period, the daily fecal samples were thoroughly mixed and a dry matter content was determined.

Beef cattle digestion trials. Two digestion trials were conducted with Hereford steers to determine the digestibility of fiber at the Beef Cattle Research Center at Michigan State University. In trial I, two Hereford steers (approximately 295 kg) were fed a corn silage-supplemented ration (30.9% D.M. and 13.5% crude protein). Each animal was preadjusted 7 days on the ration before a 4 day collection period began. Rations were mixed and fed ad libitum once daily. The unconsumed feed was weighed and recorded daily. All animals were housed in an environmentally controlled metabolism room and were maintained individually in 91 cm x 244 cm collection stalls. All steers had free access to water. Plastic bags were laid immediately behind each steer to collect the feces during the collection period. Feces were taken daily and 10% of the feces was collected and refrigerated. Dry matter of the feces was calculated on each daily sampling. At the end of the collection trial, the daily

fecal samples were thoroughly mixed and its dry matter content determined.

In trial II, two Hereford steers (approximately 270 kg) were fed a corn silage-supplemented ration (32.1% D.M. and 13.4% crude protein) on a restricted intake. Both animals were fed once daily and preadjusted 7 days on the ration before a 7 day collection period began. All other parameters were identical to trial I.

Swine digestion trials. Four starting Yorkshire pigs were housed in 60 cm x 80 cm individual cages at the Swine Research Center at Michigan State University. Feed in meal form (Table 2), and water were restricted to two 200 g meals with an equal weight of water mixed with the feed to form a gruel which the pigs rapidly consumed. This resulted in minimal contamination of excreta. Six growing and six finishing pigs were housed in 2.44 x 1.83 m elevated pens with expanded metal floors. Room temperature was held constant at 26.7 C with proper ventilation. Six Yorkshire growing pigs were randomly allotted to two groups of three pigs each with the average weight being 30 kg for pen I and 51 kg for pen II. Six Yorkshire finishing pigs were randomly allotted to two groups of three pigs each with the average weight being 89 kg for pen I and 80 kg for pen II. Feed in meal form (Table 2), and water were supplied ad libitum at all times.

Prior to collection, pigs were adjusted to the diet for 4 days. Collection periods were 4 days in each group of ad libitum-fed pigs and 8 days in individually meal-fed pigs. Chromic oxide (1% of the diet) was mixed into each diet at the beginning of each test. At

TABLE 2. COMPOSITION OF SWINE DIETS.

Item	Starter	Grower	Finisher
	%	%	%
Ground shelled corn	71.15	78.45	84.60
Dehulled soybean meal	25.00	18.00	12.00
Defluorinated phosphate	1.50	1.00	1.10
Calcium carbonate	0.60	0.80	0.70
Salt	0.50	0.50	0.50
MSU-Vitamin-Trace mineral mix[a]	0.50	0.50	0.50
Vitamin E-Se premix[b]	0.50	0.50	0.50
Antibiotic premix[cd]	0.25	0.25	0.25
	100.00	100.00	100.00

[a]Supplying the following vitamins and trace elements per kilogram of diet: vitamin A, 3300 IU; vitamin D, 660 IU; vitamin E, 5.5 IU; menadione sodium bisulfite, 2.2 mg; riboflavin, 3.3 mg; niacin, 17.6 mg; pantothenic acid, 13.2 mg; choline, 110 mg; vitamin B_{12} 19.8 ug; zinc, 74.8 mg; manganese, 37.4 mg; iodine, 2.75 mg; copper, 9.9 mg; iron, 59.4 mg.

[b]Supplying 5.5 IU of vitamin E and 0.1 ppm of selenium to the diet.

[c]Supplying 110 ppm of chlortetracycline, 110 ppm of sulfamethazine and 55 ppm of penicillin to the diet of starting and growing pigs.

[d]Supplying 22 ppm of chlortetracycline to the diet of finishing pigs.

the first appearance of the green marker in the feces, the pens were cleaned and feces were collected daily. Chromic oxide (1% of the diet) was fed again 4 days after the first addition of chromic oxide. At the appearance of this green marker, fecal collections were terminated. Feed intake was recorded. Fecal samples were collected, thoroughly mixed and weighed.

Digestion trial samples. Feed and fecal samples were ground through a Wiley mill (1 mm screen) and stored in glass bottles. Each sample was analyzed for the detergent fiber components (195,200,211), crude protein (14) and their components as described previously.

Pure culture analyses. Pure cultures of Ruminococcus flavefaciens C94 and Bacteroides succinogenes S85 were obtained from M.P. Bryant, University of Illinois. Six fiber samples were chosen to represent a broad cross-section of fiber types: alfalfa, bromegrass, corn silage, cattle manure fiber, wheat straw and Whatman #1 filter paper. All substrates were ground through a Wiley mill (0.5 mm screen), washed three times in distilled water, dried at 60 C for 96 hr and analyzed for their fiber components as determined by alditol acetates. Media was prepared in 500 ml round bottom flasks which contained: 0.6 g trypticase, 0.4g yeast extract, 0.2 ml 0.1% Reazurin, 15.0 ml of 0.6% dipotassium phosphate, 15.0 ml of mineral solution, 0.6 ml VFA solution, 2.0 ml 0.01% ferric chloride, 2.0 ml 0.01% cobalt chloride, 0.6 g substrate, 4.0 ml 2.5% cysteine sulfide and 10.0 ml 8.0% disodium carbonate. The mineral solution contained: 0.6% potassium phosphate, 1.2% ammonium sulfate, 1.2% sodium chloride, 0.25% magnesium sulfate and 0.16% calcium chloride. The VFA solution contained:

17.0 ml acetic acid, 6.0 ml propionic acid, 4.0 ml n-butyric acid, 1.0 ml isobutyric acid, 1.0 ml DL-methyl-n-butyric acid, 1.0 ml n-valeric acid, 1.0 ml isovaleric acid and 1.0 g phenylacetic acid. Each flask was inoculated with R.flavefaciens C94 or B. succinogenes S85 and incubated for 48 hr at 39 C on a stirring plate at low rotational speeds. At the end of the incubation, the pH was taken immediately and the media was centrifuged at 5000 x g for 20 min. A minute quantity (10 to 15 mg) of the fiber pellet was transferred to 5 ml of cold phosphate buffer (6.8), gently swirled and saved for further analyses. The remaining fiber pellet was washed six times with copius amounts of distilled water and recentrifuged. The final pellets were dried at 60 C for 96 hr, weighed and then analyzed for their fiber components as determined by alditol acetates. The percent fermented was determined by loss in weight divided by initial substrate dry weight.

Electron microscopy. Dried (60 C) wheat straw was ground through a Wiley mill (1 mm screen) and treated with neutral or acid detergent (82), ammonium oxalate, sodium chlorite (41) and trifluoroacetic acid (44). Each sample was adhered to aluminum stubs using double stick tape, coated with 200 to 300 A gold in a film-vac sputter coater, and observed in an ISI-Super III SEM operated at 15 kvolts at the Pesticide Electron Microscopy Laboratory at Michigan State University. Each sample was fixed in 5% glutaraldehyde in 0.1 M phosphate buffer on ice for 2 hr, followed by 2 hr in 1.0% osmium tetroxide in the same buffer. Fixed tissues were dehydrated in a graded ethanol series and embedded in epoxy resin (121).

Ultrathin sections were stained with 0.5% uranyl acetate and subsequently stained in 1.0% lead citrate. Sections were examined in a Phillips 300 transmission electron microscope.

Wet fecal samples were taken from a beef steer in digestion trial I. One sample was washed through fine screens and dried at 60 C while another was just dried at 60 C. The dried fecal sample was analyzed for its neutral detergent, acid detergent and ammonium oxalate fibers. Each fiber sample was then adhered to an aluminum stub using double stick tape, coated with 200 to 300 A gold in a film-vac sputter coater, and observed in an ISI-Super III SEM operated at 15 kvolts.

Samples from each pure culture incubation were taken and placed in cold phosphate buffer. Each sample was fixed in 2% glutaraldehyde and then with osmium tetroxide. Following fixation, the tissue was washed in phosphate buffer and dehydrated with ethanol. Samples were then subjected to critical point drying and mounted on stubs and coated with gold in a film-vac sputter coater. Each was then observed in an ISI-Super III SEM operated at 15 kvolts.

Soluble carbohydrates in corn silage. Fresh chopped corn silage was placed in 11 jug silos, closed and sealed with carbon dioxide. One jug was opened at the following time periods: 0,6,12,18,24,36, 48,72,96,144 and 312 hr. Three 100 g samples from each jug were weighed into beakers, washed with 300 ml deionized distilled water and pressed gently. This slurry was strained through two layer of cheese cloth and the pH was immediately taken. The liquid was then freeze-dried for 6 days in a Virtis model 25SRC freeze dryer. The

dried samples were then reduced and acetylated to the corresponding alditol acetates and analyzed by GLC as before.

Statistical analyses. The data within these trials was computed and analyzed on a Hewlett Packard 9825A computer. Correlation coefficients, analyses of variance tables and multiple comparison tests were computed as outlined by Zar (228).

RESULTS

Preparation of plant samples. Field dry matters of eight grasses, five legumes and seven other substrates are given in Table 1. Of the eight grasses, field dry matter ranged from a low of 33.5% in tall fescue to a high of 48.5% in bromegrass. Dry matter among the legumes ranged from a low of 20.7% in red clover to a high of 32.9% in birdsfoot trefoil. Among all substrates, wheat middlings and elodea had the highest and lowest dry matter with 90.1% and 8.8%, respectively.

The fiber fractions as determined by the detergent system of all substrates are shown in Table 3. Among all grasses, NDF ranged from 57.15% for Reed foxtail to 62.44% for tall fescue with an average of 59.54%. NDF in legumes ranged from 29.62% in red clover to 50.09% for birdsfoot trefoil with an average of 37.05%. Manure fiber had the highest NDF and red clover had the lowest NDF with 83.10% and 29.62%, respectively. The values for ADF ranged from 33.81% in Reed foxtail to 38.70% in orchardgrass in the grasses and from 21.48% in red clover to 37.70% in birdsfoot trefoil in the legumes. The average for grasses and legumes was 36.85% and 28.39%, respectively. Wheat straw and wheat bran had the highest and lowest ADF with 51.75% and 13.95%, respectively. Hemicellulose was determined by subtracting ADF from NDF. Hemicellulose ranged from 18.66% in orchardgrass to

TABLE 3. DETERGENT FIBER FRACTIONS OF SUBSTRATES.[a]

Substrate	NDF	ADF	Hemicellulose	Cellulose	Lignin	Silica
	%	%	%	%	%	%
1. Kentucky bluegrass	59.90	37.60	22.30	28.88	5.56	3.16
2. Tall fescue	62.44	38.50	23.94	29.88	3.13	5.63
3. Reed canarygrass-2nd cutting	58.24	34.87	23.37	24.71	5.33	4.74
4. Reed canarygrass-full bloom	59.61	34.74	24.87	27.98	5.85	1.57
5. Reed foxtail	57.15	33.81	23.34	25.92	4.46	3.79
6. Bromegrass	60.12	38.38	21.74	28.14	5.54	4.69
7. Orchardgrass	57.36	38.70	18.66	30.10	4.68	3.85
8. Quackgrass	61.50	38.20	23.30	22.47	4.98	10.76
9. Wheat straw	79.84	51.75	28.09	34.10	12.36	5.73
10. Wheat middlings	49.77	14.66	35.11	10.19	4.63	0.15
11. Wheat bran	52.25	13.95	38.30	9.93	4.18	0.17
12. Elodea	31.16	22.67	8.49	17.97	4.09	0.90
13. Algae	42.89	27.08	15.81	21.56	5.97	0.76
14. Manure fiber	83.10	51.35	31.75	40.04	9.61	1.71
15. Ladino clover	29.77	25.81	3.96	20.03	4.94	1.22
16. Red clover	29.62	21.48	8.14	16.43	3.96	1.08
17. Crown vetch	34.19	26.91	7.28	19.63	5.66	1.62
18. Alfalfa	41.60	30.06	11.54	23.67	6.21	0.26
19. Birdsfoot trefoil	50.09	37.70	12.39	28.94	8.73	0.30
20. Corn silage	61.98	27.60	34.38	24.46	4.18	0.00

[a]Each value represents the average of six determinations.

24.87% in Reed canarygrass-full bloom in the grasses and from 3.96% in ladino clover to 12.39% in birdsfoot trefoil in the legumes. Hemicellulose for all grasses and legumes was 22.69% and 8.66%, respectively. Wheat bran and ladino clover had the most and the least hemicellulose with 38.30% and 3.96%, respectively. Cellulose was determined after delignification and ashing. The average for grasses and legumes was 27.26% and 21.74%, respectively. Cellulose values ranged from 22.47% in quackgrass to 30.10% in orchardgrass in grasses and from 16.43% in red clover to 28.94% in birdsfoot trefoil in the legumes. Manure fiber and wheat bran had the highest and lowest cellulose values with 40.04% and 9.93%, respectively. Lignin, as determined by loss in weight with potassium permanganate oxidation, was highest in wheat straw and lowest in tall fescue with 12.36% and 3.13%, respectively. Silica was determined as the weight recovered after ashing. This ranged from 1.57% in Reed canarygrass-full bloom to 10.76% in quackgrass among the grasses and from 0.26% in alfalfa to 1.62% in crown vetch. The average silica content among grasses and legumes was 4.77% and 0.90%, respectively. The highest and lowest value among all substrates was 10.76% and 0.00% for quackgrass and corn silage, respectively.

The fiber fractions as determined by the proposed method consist of ammonium oxalate fiber (AOF), holocellulose (cellulose+hemicellulose+chlorite lignin), cellulose, hemicellulose and chlorite lignin. The values for the 20 substrates are presented in Table 4. AOF represents the cellulose, hemicellulose, lignin, some ash and protein of the plant cell wall. The AOF values for grasses ranged from

TABLE 4. MSU FIBER FRACTIONS OF SUBSTRATES.[a]

Substrate	AOF	Holocellulose	Lignin	Cellulose	Hemicellulose
	%	%	%	%	%
1. Kentucky bluegrass	73.58	55.15	6.24	36.21	18.94
2. Tall fescue	71.59	52.35	7.29	29.96	22.39
3. Reed canarygrass-2nd cutting	68.21	46.27	4.94	29.89	16.38
4. Reed canarygrass-full bloom	66.00	42.13	5.35	26.87	15.26
5. Reed foxtail	69.21	47.19	4.99	29.49	17.70
6. Bromegrass	69.81	43.50	5.54	28.17	15.33
7. Orchardgrass	70.05	46.22	6.73	31.84	14.38
8. Quackgrass	71.59	60.29	7.29	29.96	23.04
9. Wheat straw	84.14	66.21	7.72	46.63	19.58
10. Wheat middlings	64.81	49.02	7.08	15.73	33.29
11. Wheat bran	60.39	23.68	7.73	10.36	13.32
12. Elodea	60.66	31.45	3.65	21.21	10.24
13. Algae	62.99	29.84	15.22	25.94	3.90
14. Manure fiber	91.36	47.93	13.07	36.78	11.15
15. Ladino clover	56.57	31.13	6.52	18.95	12.18
16. Red clover	56.88	26.05	8.02	20.26	5.79
17. Crown vetch	76.41	54.88	8.00	24.61	22.27
18. Alfalfa	59.95	35.47	5.66	22.17	13.30
19. Birdsfoot trefoil	65.45	43.96	5.85	28.79	15.17
20. Corn silage	47.98	36.17	4.44	23.48	22.25

[a]Each value represents the average of three determinations.

66.00% in Reed canarygrass-full bloom to 73.58% in Kentucky bluegrass and for legumes ranged from 56.57% in ladino clover to 76.41% in crown vetch. The average among grasses and legumes was 70.01% and 63.05%, respectively. Manure fiber had the most AOF with 91.36% and corn silage had the least with 47.98%. Holocellulose represents the structural polysaccharides of the cell wall. This was highest in wheat straw and lowest in wheat bran with 66.21% and 23.68%, respectively. The values for grasses ranged from 43.50% in bromegrass to 60.29% in quackgrass and for legumes ranged from 26.05% in red clover to 54.88% in crown vetch. The average value for grasses and legumes was 49.14% and 38.30%, respectively. Lignin was determined by loss in weight after oxidation in sodium chlorite and v/v 1% acetic acid. Values ranged from 4.94% in Reed canarygrass-2nd cutting to 7.29% in both tall fescue and quackgrass among all grasses with an average of 6.05%. The values among legumes ranged from 5.66% in alfalfa to 8.02% in red clover with an average of 6.81%. Algae and elodea had the highest and lowest values of lignin with 15.22% and 3.65%, respectively. Values were, in general, higher than corresponding permanganate lignin values as shown in Table 3. With eleven of the samples analyzed, sodium chlorite lignin values were higher ($P<.05$) than its permanganate lignin value. There were no significant differences between the values for the remaining seven samples. Cellulose was determined by weighing the recovered substrate after hydrolysis in 1 N trifluoroacetic acid (T.F.A.A.). In grasses, these values varied from 26.87% in Reed canarygrass-full bloom to 36.21% in Kentucky bluegrass with an average of 30.30%.

The values among legumes varied from 18.95% in ladino clover to 28.79% in birdsfoot trefoil with an average of 27.88%. The highest and lowest value were found in wheat straw and wheat bran with 46.63% and 10.36%, respectively. A comparison of T.F.A.A. cellulose and detergent cellulose (Table 3 and 4) yielded similar results in most substrates except Kentucky bluegrass, Reed canarygrass-2nd cutting, Reed foxtail, quackgrass, wheat middlings, wheat straw, algae and crown vetch which have significantly ($P<.05$) higher cellulose values as determined by T.F.A.A. hydrolysis. Hemicellulose as determined in the proposed system was quantitated by gas-liquid chromatography. The values among grasses ranged from 14.38% in orchardgrass to 23.04% in quackgrass with an average of 17.93%. In legumes, the values ranged from 5.79% in red clover to 22.27% in crown vetch with an average of 13.74%. Wheat middlings contained the most hemicellulose with 33.29% while algae contained the least with 3.90%. A statistical comparison could not be done between T.F.A.A. hemicellulose and detergent hemicellulose (Table 3) because the detergent hemicellulose represented an approximation as determined by subtraction of ADF from NDF. However, 16 of the 20 substrates had higher values when determined by subtraction. Red clover, crown vetch, alfalfa and birdsfoot trefoil had higher values as determined by gas-liquid chromatography.

The relationship between NDF and AOF and with ADF were significantly correlated ($P<.001$) as shown in Table A1. AOF and ADF were significantly correlated ($P<.01$) as were detergent cellulose and MSU T.F.A.A. cellulose ($P<.001$). No relationship existed be-

tween detergent and MSU T.F.A.A. hemicellulose.

Hydrolysis of the delignified tissue with trifluoroacetic acid and derivitization to the corresponding alditol acetate yielded a pattern of the hemicellulosic sugars (Table 5) which include glucose, galactose, mannose, xylose, arabinose, rhamnose and possible ribose. In these calculations, uronic acids, which may include those from pectins, were included in the hemicellulose. Uronic acids in the ammonium oxalate and T.F.A.A. filtrates were determined by colorimetry. Xylose, arabinose and glucose were present in all substrates examined. Xylose was the highest in concentration in all substrates with the exceptions of elodea, algae, ladino clover, red clover and alfalfa. Uronic acids were the predominant hemicellulosic component in elodea, ladino clover, red clover and alfalfa whereas galactose was the predominant component of hemicellulose in algae. Mannose was found in wheat middlings, wheat bran, elodea, manure fiber, ladino clover, red clover, crown vetch, alfalfa, birdsfoot trefoil and corn silage. Rhamnose did not appear to be a common hemicellulosic component, however, it was found in Reed foxtail, ladino clover, crown vetch, alfalfa and birdsfoot trefoil.

The hemicellulosic sugars expressed as a percentage of the total hemicellulose are presented in Table 6. Uronic acids ranged from 1.86% in wheat middlings to 67.36% in red clover. The average for grasses and legumes were 12.04% and 37.59%, respectively. The concentration of glucose varied from 7.25% in red clover to 27.10% in wheat bran. The average for all grasses and legumes were 10.13% and 15.89%. Galactose ranged from 0.00% in manure fiber to 31.54% in

TABLE 5. INDIVIDUAL SUGARS OF THE HEMICELLULOSE FRACTION OF SUBSTRATES AS DETERMINED BY GAS CHROMATOGRAPHY.[a]

Substrate	Uronic acids	Glucose	Galactose	Mannose	Arabinose	Xylose	Rhamnose	Hemicellulose
	%	%	%	%	%	%	%	%
1. Kentucky bluegrass	2.50	1.58	0.98	0.00	2.77	11.11	0.00	18.94
2. Tall fescue	2.27	1.65	0.89	0.00	3.38	14.20	0.00	22.39
3. Reed canarygrass-2nd cutting	1.55	1.49	0.98	0.00	2.68	9.68	0.00	16.38
4. Reed canarygrass-full bloom	1.63	1.27	0.67	0.00	2.16	9.53	0.00	15.26
5. Reed foxtail	2.13	2.12	1.18	0.00	3.12	8.86	0.29	17.70
6. Bromegrass	1.78	1.72	0.71	0.00	2.41	8.71	0.00	15.33
7. Orchardgrass	2.73	1.50	0.48	0.00	1.89	7.78	0.00	14.38
8. Quackgrass	2.35	3.28	4.15	0.00	1.87	11.39	0.00	23.04
9. Wheat straw	1.50	2.01	0.52	0.00	2.20	13.35	0.00	19.58
10. Wheat middlings	0.62	8.36	0.60	0.41	9.23	14.07	0.00	33.29
11. Wheat bran	2.85	3.61	0.43	0.17	2.36	3.90	0.00	13.32
12. Elodea	5.01	2.38	0.76	0.33	0.52	1.05	0.19	10.24
13. Algae	0.75	0.68	1.23	0.00	0.75	0.49	0.00	3.90
14. Manure fiber	0.54	1.33	0.00	0.31	0.71	8.26	0.00	11.15
15. Ladino clover	5.29	1.25	1.21	0.52	0.96	2.56	0.39	12.18
16. Red clover	3.90	0.42	0.40	0.20	0.41	0.46	0.00	5.79
17. Crown vetch	4.64	3.52	2.44	0.50	1.91	9.13	0.13	22.27
18. Alfalfa	4.84	3.31	0.61	0.41	0.78	3.12	0.23	13.30
19. Birdsfoot trefoil	3.02	3.22	1.21	0.47	1.28	5.66	0.31	15.17
20. Corn silage	1.57	2.22	1.35	0.43	2.10	2.58	0.00	8.25

[a]Each value represents the average of three determinations.

TABLE 6. INDIVIDUAL SUGARS OF THE HEMICELLULOSE FRACTION AS EXPRESSED AS A PERCENT OF HEMICELLULOSE.[a]

Substrate	Uronic acids	Glucose	Galactose	Mannose	Arabinose	Xylose	Rhamnose
	% of Hemicellulose						
1. Kentucky bluegrass	13.20	8.34	5.17	0.00	14.63	58.66	0.00
2. Tall fescue	10.14	7.37	3.97	0.00	15.10	63.42	0.00
3. Reed canarygrass-2nd cutting	9.46	9.10	5.98	0.00	16.36	59.10	0.00
4. Reed canarygrass-full bloom	10.68	8.32	4.39	0.00	14.15	62.45	0.00
5. Reed foxtail	12.03	11.98	6.67	0.00	17.63	50.06	1.64
6. Bromegrass	11.61	11.22	4.63	0.00	15.72	56.82	0.00
7. Orchardgrass	18.98	10.43	3.34	0.00	13.14	54.10	0.00
8. Quackgrass	10.20	14.24	18.01	0.00	8.12	49.44	0.00
9. Wheat straw	7.66	10.27	2.66	0.00	11.24	68.18	0.00
10. Wheat middlings	1.86	25.11	1.80	1.23	27.73	42.26	0.00
11. Wheat bran	21.40	27.10	3.23	1.28	17.72	29.28	0.00
12. Elodea	48.93	23.24	7.42	3.22	5.08	10.25	0.00
13. Algae	19.23	17.43	31.54	0.00	19.23	12.56	0.00
14. Manure fiber	4.84	11.93	0.00	2.78	6.37	74.09	0.00
15. Ladino clover	43.43	10.26	9.93	4.27	7.88	21.02	3.20
16. Red clover	67.36	7.25	6.91	3.45	7.08	7.94	0.00
17. Crown vetch	20.84	15.81	10.96	2.25	8.58	41.00	0.58
18. Alfalfa	36.39	24.89	4.59	3.08	5.86	23.46	1.73
19. Birdsfoot trefoil	19.91	21.23	7.98	3.10	8.43	37.31	2.04
20. Corn silage	19.03	26.91	16.36	5.21	1.21	31.27	0.00

[a]Each value represents the average of three determinations.

algae. The average among grasses and legumes were 6.52% and 8.07%, respectively. Mannose was not found in 10 of the 20 substrates, most of which were in the Grass family. Rhamnose was found only in five substrates with the highest amount being in ladino clover. Correlation analyses showed (Table A2) that hemicellulose content was strongly correlated with glucose ($P<.01$), arabinose ($P<.001$) and xylose ($P<.001$) among all substrates. A strong relationship ($P<.001$) between arabinose and hemicellulose was observed only in the grasses (Table A3). Among the grasses, galactose and arabinose, xylose and galactose, and xylose and arabinose correlated significantly ($P<.001$). These relationships were not shown in legumes (Table A3).

A comparison between both fiber systems was made by adding the corresponding cellulose, hemicellulose and lignin into a total fiber fraction (Table 7). No specific trends were observed among the substrates. Ten of the detergent values were higher than MSU fiber values. Grass samples ranged from 50.75% in quackgrass to 58.70% in Reed canarygrass-full bloom to 61.39% in Kentucky bluegrass for the MSU fiber system. The average value among all grasses for detergent and MSU total fiber was 54.89% and 54.27%. Detergent total fiber in legumes ranged from 28.53% in red clover to 50.06% in birdsfoot trefoil with an average of 36.30%. The MSU values for legumes ranged from 34.09% in red clover to 54.88% in crown vetch with an average of 43.51%.

Ratios between individual fiber fractions are sometimes computed to predict digestibility. A comparison between the ratios obtained by the two methods is presented in Table 8. Hemicellulose/cellulose

TABLE 7. COMPARISON OF TOTAL FIBER VALUES OF DETERGENT FIBER AND MSU FIBER SYSTEMS.[a]

Substrate	Detergent	MSU
	%	%
1. Kentucky bluegrass	56.74	61.39
2. Tall fescue	56.95	59.64
3. Reed canarygrass-2nd cutting	53.41	51.21
4. Reed canarygrass-full bloom	58.70	47.48
5. Reed foxtail	53.72	52.18
6. Bromegrass	55.42	49.04
7. Orchardgrass	53.44	52.95
8. Quackgrass	50.75	60.29
9. Wheat straw	74.55	74.93
10. Wheat middlings	49.93	56.10
11. Wheat bran	52.41	31.41
12. Elodea	30.55	35.10
13. Algae	43.34	45.06
14. Manure fiber	81.80	61.00
15. Ladino clover	28.93	37.65
16. Red clover	28.53	34.09
17. Crown vetch	32.57	54.88
18. Alfalfa	41.42	41.13
19. Birdsfoot trefoil	50.06	49.80
20. Corn silage	63.02	36.17

[a]Total fiber represents the cellulose, hemicellulose and lignin fractions.

TABLE 8. RATIOS BETWEEN FIBER FRACTIONS IN DETERGENT AND MSU FIBER SYSTEMS.

Substrate	Detergent		MSU	
	Hemicellulose / Cellulose	Lignin / Cellulose	Hemicellulose / Cellulose	Lignin / Cellulose
1. Kentucky bluegrass	0.77	0.19	0.52	0.17
2. Tall fescue	0.80	0.10	0.75	0.24
3. Reed canarygrass-2nd cutting	0.95	0.22	0.55	0.17
4. Reed canarygrass-full bloom	0.89	0.21	0.57	0.20
5. Reed foxtail	0.90	0.17	0.60	0.17
6. Bromegrass	0.77	0.20	0.54	0.20
7. Orchardgrass	0.62	0.16	0.45	0.21
8. Quackgrass	1.04	0.22	0.77	0.24
9. Wheat straw	0.82	0.36	0.42	0.17
10. Wheat middlings	3.45	0.45	2.11	0.45
11. Wheat bran	3.86	0.42	1.29	0.75
12. Elodea	0.73	0.23	0.48	0.17
13. Algae	0.47	0.28	0.15	0.59
14. Manure fiber	0.79	0.24	0.30	0.36
15. Ladino clover	0.20	0.25	0.64	0.34
16. Red clover	0.50	0.24	0.29	0.40
17. Crown vetch	0.37	0.29	0.90	0.33
18. Alfalfa	0.49	0.26	0.59	0.26
19. Birdsfoot trefoil	0.43	0.30	0.53	0.20
20. Corn silage	1.41	0.17	0.95	0.19

Correlation coefficient between detergent and MSU hemicellulose/cellulose ratios had a r value of 0.53, ($P < .02$).

Correlation coefficient between detergent and MSU lignin/cellulose ratios had a r value of 0.76, ($P < .001$).

ratios calculated from detergent fiber values ranged from 0.62 to 1.04 among grasses and from 0.20 to 0.50 among legumes. The same ratio calculated from MSU fiber values ranged from 0.45 to 0.77 in grasses and from 0.29 to 0.90 in legumes. The MSU hemicellulose/cellulose ratios for grasses were all lower than their corresponding detergent value. A comparison of ratios in legumes showed that all MSU values were higher than the corresponding detergent values. Detergent lignin/cellulose ratios in grasses ranged from 0.10 to 0.22 and in legumes from 0.25 to 0.30. MSU ratios ranged from 0.17 to 0.24 in grasses and from 0.20 to 0.34 in legumes.

Uronic acids as determined by colorimetry are shown in Table 9. Most of the uronic acids were found in the ammonium oxalate filtrate with the exceptions of wheat straw and manure fiber. More uronic acids were found in the T.F.A.A. filtrate for these two substrates. Grass uronic acids ranged from 1.07% to 2.51% whereas legumes were higher and ranged from 2.69% to 4.34%. The T.F.A.A. filtrates contained fewer uronic acids, but ranged from 0.22% to 0.83% in grasses nd 0.33% to 0.50% in legumes.

Second dry matter in plant samples. Results of determinations of dry matter of solkafloc cellulose indicate an increase in weight of 3.4% after samples were dried and then weighed each minute for 30 min at room temperature as shown in Figure 1.

Trifluoroacetic acid hydrolysis. A hydrolysis time of 60 min showed the maximum yield of hemicellulose and decreased with a hydrolysis time greater than 60 min as shown in Figure 2. Both arabinose and xylose were degraded at hydrolysis times greater than 60

TABLE 9. URONIC ACIDS IN AMMONIUM OXALATE AND T.F.A.A. FILTRATES OF SUBSTRATES.[a]

Substrate	Ammonium oxalate	T.F.A.A.	Total
	%	%	%
1. Kentucky bluegrass	1.67	0.83	2.50
2. Tall fescue	2.05	0.22	2.27
3. Reed canarygrass-2nd cutting	1.07	0.48	1.55
4. Reed canarygrass-full bloom	1.17	0.49	1.63
5. Reed foxtail	1.61	0.52	2.13
6. Bromegrass	1.28	0.50	1.78
7. Orchardgrass	2.51	0.22	2.73
8. Quackgrass	1.89	0.46	2.35
9. Wheat straw	0.74	0.76	1.50
10. Wheat middlings	0.40	0.22	0.62
11. Wheat bran	2.49	0.36	2.85
12. Elodea	4.57	0.44	5.01
13. Algae	0.66	0.09	0.75
14. Manure fiber	0.15	0.39	0.54
15. Ladino clover	4.95	0.34	5.29
16. Red clover	3.90	0.37	4.27
17. Crown vetch	4.23	0.41	4.64
18. Alfalfa	4.34	0.50	4.84
19. Birdsfoot trefoil	2.69	0.33	3.02
20. Corn silage	1.15	0.42	1.57

[a]Each value represents the average of three determinations.

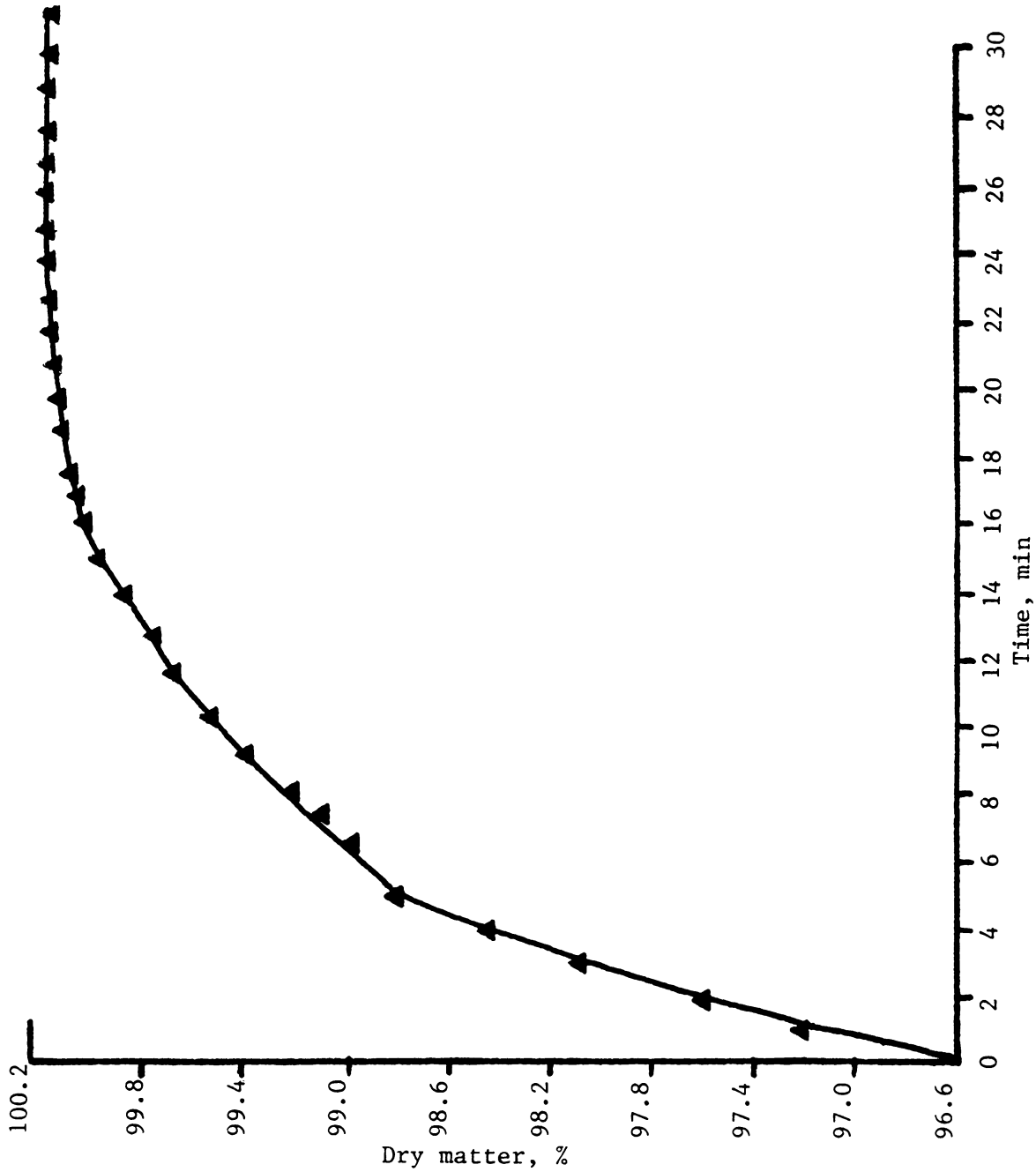

FIGURE 1. CHANGES IN DRY MATTER WITH TIME AT ROOM TEMPERATURE.

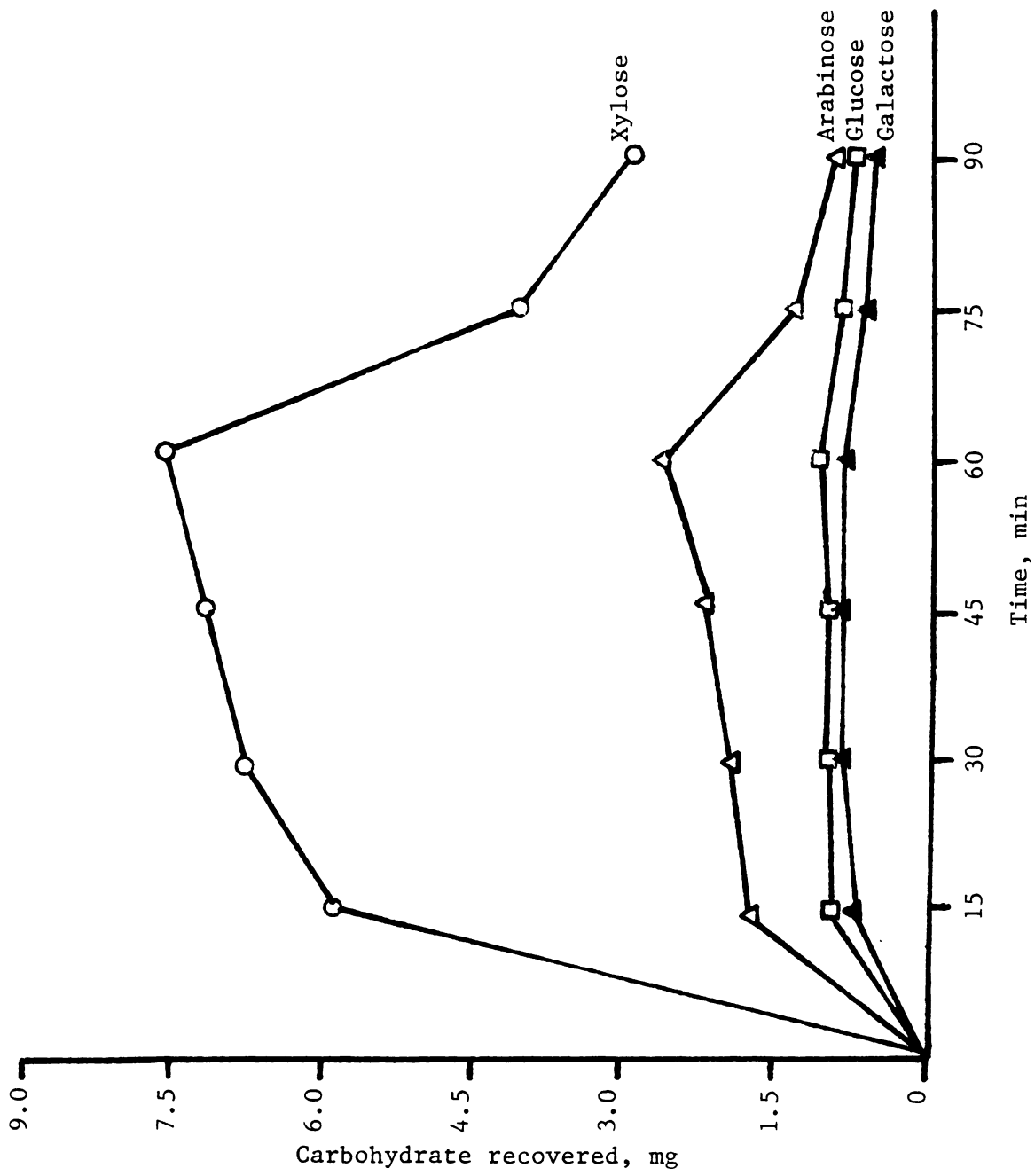

FIGURE 2. LENGTH OF HYDROLYSIS TIME AND SUGAR YIELD.

min of hydrolysis at which time cellulose concentration remained constant.

Methanol additions. The effect of different amounts of methanol upon hemicellulosic sugar yield is presented in Figure 3. Maximum sugar yield was apparent at two methanol additions and stayed the same with additional methanol.

Cellulose composition. Samples of delignified Reed canarygrass-full bloom were hydrolyzed in T.F.A.A. These were then hydrolyzed in sulfuric acid to analyze for sugar composition by GLC. Cellulose obtained by T.F.A.A. hydrolysis contained 97% glucose and 3% unidentified.

Neutral and acid detergent fiber analyses. The effect of refluxing the 20 substrates in neutral detergent upon cell wall carbohydrates is presented in Table 10. Among the grasses, 82.6% of the cellulose, 53.3% of the glucose, 35.3% of the galactose, 61.3% of the arabinose, 67.9% of the xylose, 2.5% of the uronic acids and 55.5% of the hemicellulose were recovered. Cellulose varied from 73.0% to 93.0% recovered and hemicellulose varied from 14.9% to 79.3% recovered in the grasses. Among the legumes, 80.0% of the cellulose, 100.4% of the glucose, 30.4% of the galactose, 38.8% of the arabinose, 55.4% of the xylose, 4.2% of the uronic acids and 26.5% of the hemicellulose were recovered. Cellulose varied from 54.5% to 95.1% recovered and hemicellulose from 11.4% to 37.5% recovered. Among all substrates, recovery of the individual fiber components was extremely varied. Cellulose ranged from 70.3% in red clover to 100.0% recovered in wheat bran. Glucose ranged from 0.0% in Reed canarygrass-full bloom

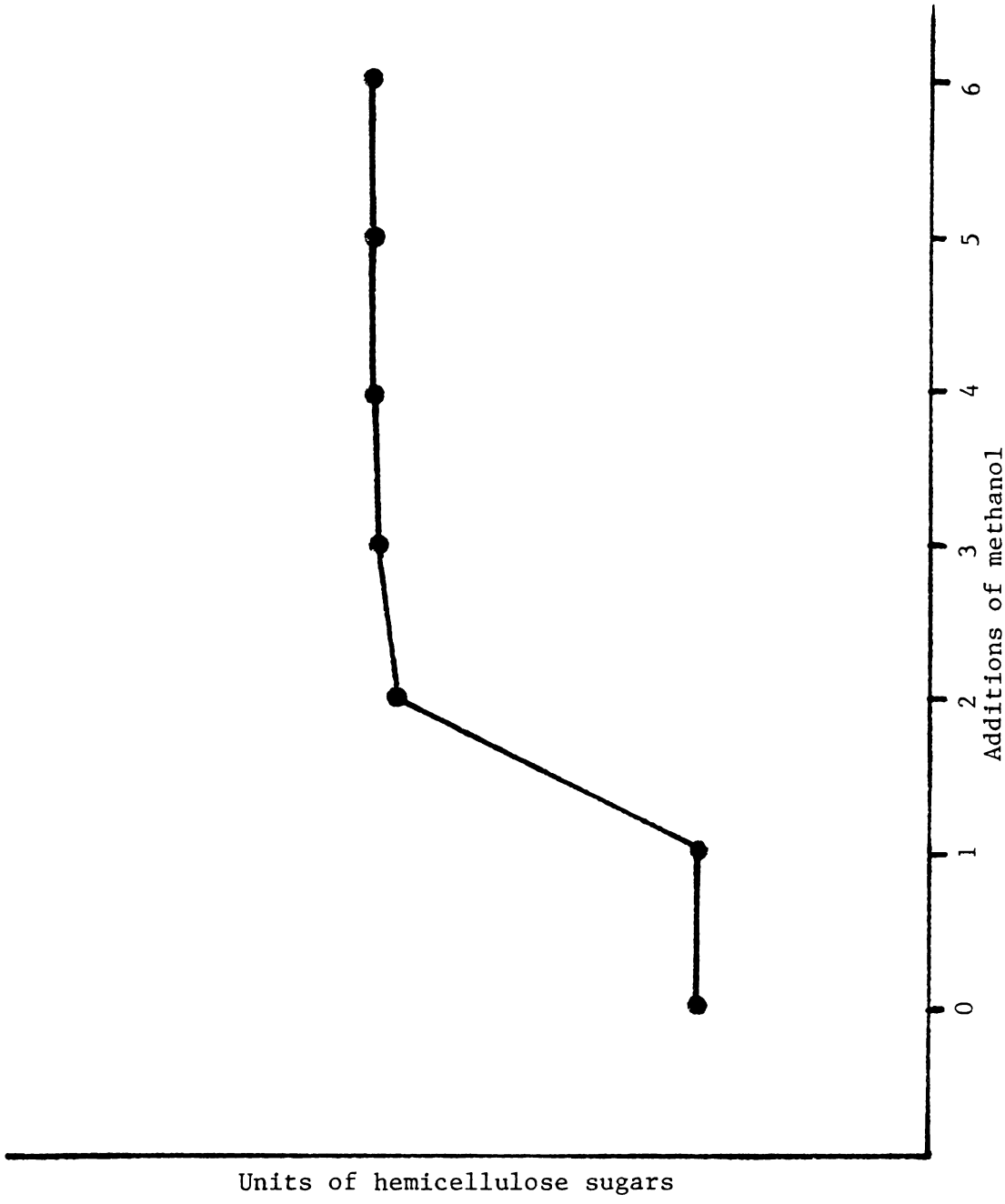

FIGURE 3. YIELD OF HEMICELLULOSIC SUGARS AS AFFECTED BY METHANOL ADDITIONS.

TABLE 10. ALDITOL ACETATE ANALYSIS OF NEUTRAL DETERGENT FIBER FRACTION OF SUBSTRATES AND PERCENT RECOVERY OF EACH COMPONENT.[abc]

Substrate	Cellulose		Glucose		Galactose		Arabinose		Xylose		Uronic acids		Hemicellulose	
	% D.M.													
1. Kentucky bluegrass	26.43	(73.0)	0.57	(36.1)	0.26	(26.5)	0.35	(12.6)	1.46	(13.1)	0.20	(8.0)	2.84	(14.9)
2. Tall fescue	27.86	(93.0)	1.86	(100.0)	0.90	(100.0)	2.45	(72.5)	12.74	(89.7)	0.00	(0.0)	17.75	(79.1)
3. Reed canarygrass-2nd cutting	24.44	(81.8)	0.64	(43.0)	0.33	(33.7)	0.76	(28.4)	3.87	(40.0)	0.18	(11.6)	5.78	(35.3)
4. Reed canarygrass-full bloom	23.03	(85.7)	0.00	(0.0)	0.00	(0.0)	1.28	(59.3)	6.00	(62.3)	0.00	(0.0)	7.28	(47.7)
5. Reed foxtail	21.86	(74.2)	0.94	(44.3)	0.47	(39.8)	1.70	(54.5)	7.90	(89.2)	0.00	(0.0)	11.24	(63.5)
6. Bromegrass	24.22	(86.0)	1.13	(65.7)	0.39	(54.9)	2.08	(86.3)	7.44	(85.4)	0.00	(0.0)	11.08	(72.2)
7. Orchardgrass	24.31	(76.4)	1.78	(100.0)	0.13	(27.1)	1.86	(76.4)	6.95	(89.3)	0.00	(0.0)	10.72	(74.6)
8. Quackgrass	27.08	(90.4)	1.74	(53.1)	0.00	(0.0)	2.30	(100.0)	8.49	(74.5)	0.00	(0.0)	12.43	(54.0)
9. Wheat straw	37.44	(80.3)	2.03	(100.0)	0.59	(100.0)	1.84	(83.6)	10.38	(100.0)	0.75	(50.0)	17.84	(91.1)
10. Wheat middlings	15.40	(97.9)	1.65	(19.7)	0.08	(13.3)	4.86	(52.7)	8.38	(59.6)	0.00	(0.0)	14.97	(44.9)
11. Wheat bran	10.61	(100.0)	0.97	(26.9)	0.28	(65.2)	3.27	(83.9)	2.97	(125.0)	0.00	(0.0)	7.49	(56.2)
12. Elodea	14.89	(70.2)	1.97	(82.8)	0.37	(48.7)	0.22	(29.3)	0.74	(70.5)	1.77	(35.3)	3.51	(34.3)
13. Algae	23.37	(97.8)	1.72	(105.9)	0.45	(36.6)	0.50	(66.7)	0.47	(100.0)	0.00	(0.0)	2.46	(63.1)
14. Manure fiber	36.08	(98.1)	1.55	(100.0)	0.44	(100.0)	1.52	(100.0)	11.77	(100.0)	0.00	(0.0)	15.28	(100.0)
15. Ladino clover	18.01	(95.1)	0.77	(61.6)	0.43	(35.5)	0.41	(42.7)	1.52	(100.0)	0.72	(13.6)	3.47	(28.5)
16. Red clover	14.24	(70.3)	1.09	(259.3)	0.19	(47.5)	0.17	(41.5)	0.27	(58.7)	0.00	(0.0)	1.87	(32.3)
17. Crown vetch	22.50	(91.4)	1.72	(48.9)	0.39	(16.0)	0.27	(14.1)	2.43	(26.6)	0.39	(8.4)	5.12	(23.0)
18. Alfalfa	19.62	(88.5)	1.82	(60.0)	0.00	(0.0)	0.52	(66.7)	2.65	(85.0)	0.62	(12.8)	4.99	(37.5)
19. Birdsfoot trefoil	15.68	(54.5)	0.40	(72.4)	0.64	(52.9)	0.32	(29.0)	0.37	(6.5)	0.00	(0.0)	1.73	(11.4)
20. Corn silage	22.02	(93.8)	3.80	(90.1)	0.00	(0.0)	2.08	(49.3)	10.05	(79.9)	0.00	(0.0)	15.93	(71.6)

[a]Values in parentheses represent % of original material recovered after boiling in neutral detergent.

[b]Rhamnose was detected in elodea (0.14%).

[c]Mannose was detected in Reed canarygrass-2nd cutting (0.16%), elodea (0.21%), algae (0.32%), ladino clover (0.34%), red clover (0.15%) and crown vetch (0.31%).

to 259.3% recovered in red clover. Tall fescue, orchardgrass, wheat straw, algae and manure fiber had all the hemicellulosic glucose recovered in their corresponding neutral detergent fiber fractions. Alfalfa, corn silage and quackgrass neutral detergent fibers had 0.0% galactose recovered whereas 100.0% recovered in tall fescue, wheat straw and manure fiber. Arabinose recovery ranged from 12.6% in Kentucky bluegrass to 100.0% in quackgrass and manure fiber. Recovery of xylose was 100.0% in wheat straw, algae, manure fiber and ladino clover whereas birdsfoot trefoil NDF contained only 6.5% of the original xylose. Only seven of the 20 substrates retained any uronic acids in the NDF. Wheat straw NDF retained the most uronic acids with 50.0% recovered whereas elodea NDF contained the most uronic acids on a dry matter basis with 1.77%. Hemicellulose recovery in the NDF ranged from 11.4% in birdsfoot trefoil to 100.0% in manure fiber.

The fiber components of the 20 substrates acid detergent fiber fractions are presented in Table 11. The grasses ADF fractions contained from 83.1% to 99.0% of the original cellulose and 10.8% to 31.5% of the hemicellulose. Among the grass hemicellulosic sugars, 68.6% of the glucose, 46.3% of the galactose, 0.0% of the arabinose, 15.3% of the xylose and 15.8% of the uronic acids were recovered. Among all substrates, cellulose recovery ranged from 64.5% in wheat middlings to 100.0% in alfalfa. Glucose ranged from 0.0% recovered in bromegrass to 189.1% recovered in elodea. Six substrates of 19 substrates had 100.0% or greater recovered. Ladino clover ADF and red clover ADF retained 118.4% and 109.5% of the galactose, respectively. Of the 20 substrates, only red clover retained any of the hemi-

TABLE 11. ALDITOL ACETATE ANALYSIS OF ACID DETERGENT FIBER FRACTION OF SUBSTRATES AND PERCENT RECOVERY OF EACH COMPONENT.[a,b]

Substrate	Cellulose		Glucose		Galactose		Arabinose		Xylose		Uronic acids		Hemicellulose	
	% D.M.													
1. Kentucky bluegrass	30.57	(84.4)	1.23	(77.9)	0.81	(82.7)	0.00	(0.0)	1.41	(12.7)	0.49	(19.6)	3.94	(20.8)
2. Tall fescue	28.22	(94.2)	1.56	(94.5)	0.00	(0.0)	0.00	(0.0)	1.41	(9.9)	0.38	(16.8)	3.35	(15.0)
3. Reed canarygrass-2nd cutting	24.82	(83.0)	1.58	(100.0)	0.00	(0.0)	0.00	(0.0)	2.89	(29.9)	0.32	(20.7)	4.79	(29.2)
4. Reed canarygrass-full bloom	25.48	(94.8)	1.21	(95.3)	0.69	(100.0)	0.00	(0.0)	2.90	(30.4)	0.00	(0.0)	4.80	(31.5)
5. Reed foxtail	24.49	(83.1)	1.13	(53.3)	1.00	(84.8)	0.00	(0.0)	0.25	(2.8)	0.63	(29.6)	3.01	(17.0)
6. Bromegrass	27.90	(99.0)	0.00	(0.0)	0.00	(0.0)	0.00	(0.0)	2.60	(29.9)	0.35	(19.7)	2.95	(19.2)
7. Orchardgrass	26.37	(82.8)	1.65	(100.0)	1.65	(100.0)	0.00	(0.0)	0.31	(4.0)	0.55	(20.2)	3.03	(21.1)
8. Quackgrass	26.95	(89.9)	0.90	(27.4)	1.29	(2.6)	0.00	(0.0)	0.30	(2.6)	0.00	(0.0)	2.49	(10.8)
9. Wheat straw	34.52	(74.0)	1.79	(89.1)	0.52	(100.0)	0.00	(0.0)	1.91	(14.3)	0.46	(30.7)	4.68	(23.9)
10. Wheat middlings	10.15	(64.5)	1.43	(17.1)	0.17	(28.3)	0.00	(0.0)	0.13	(0.9)	0.16	(25.8)	1.89	(5.7)
11. Wheat bran	9.48	(91.5)	0.76	(21.1)	0.20	(3.9)	0.00	(0.0)	0.15	(3.9)	0.12	(4.2)	1.53	(11.5)
12. Elodea	17.68	(83.4)	2.40	(189.1)	1.67	(100.0)	0.00	(0.0)	0.10	(9.5)	0.20	(4.0)	4.37	(42.7)
13. Algae	19.06	(73.5)	0.67	(98.5)	1.10	(89.4)	0.00	(0.0)	0.00	(0.0)	0.81	(108.0)	2.58	(66.2)
14. Manure fiber	35.54	(93.9)	1.88	(141.4)	1.59	------[c]	0.00	(0.0)	2.73	(33.1)	0.00	(0.0)	6.20	(55.6)
15. Ladino clover	15.70	(82.9)	1.21	(96.8)	1.48	(118.4)	0.00	(0.0)	0.54	(21.1)	0.98	(18.3)	4.21	(34.6)
16. Red clover	14.47	(71.4)	0.42	(100.0)	0.43	(109.5)	0.41	(100.0)	0.42	(91.3)	1.24	(31.8)	2.94	(50.8)
17. Crown vetch	17.89	(72.7)	0.60	(17.1)	0.79	(32.4)	0.00	(0.0)	0.20	(2.2)	0.00	(0.0)	1.59	(7.1)
18. Alfalfa	22.27	(100.0)	1.47	(44.4)	0.00	(0.0)	0.00	(0.0)	1.21	(38.9)	0.42	(8.7)	3.10	(23.3)
19. Birdsfoot trefoil	25.77	(89.5)	2.10	(65.2)	0.00	(0.0)	0.00	(0.0)	0.31	(5.5)	0.54	(17.9)	2.95	(19.5)
20. Corn silage	19.50	(83.0)	1.42	(33.7)	0.00	(0.0)	0.00	(0.0)	0.23	(1.8)	0.17	(10.8)	1.82	(8.2)

[a] Values in parentheses represent % of original material recovered after boiling in acid detergent.

[b] No mannose was detected in any acid detergent fiber sample.

[c] No galactose was found in the original manure fiber sample.

cellulosic arabinose. Xylose recovery ranged from 0.0% in algae ADF to 91.3% in red clover ADF. Four substrates contained 0.0% uronic acids in the ADF. These included Reed canarygrass-full bloom, quackgrass, manure fiber and crown vetch. Algae ADF contained 108.0% of the original uronic acids.

Protein and ash composition. The crude protein and ash composition of wheat straw, bromegrass, alfalfa, Kentucky bluegrass and ladino clover and their fiber fractions as determined by the proposed MSU method are presented in Table 12. After refluxing in ammonium oxalate, 60% to 74% and 16% to 40% of the crude protein and ash, respectively, were recovered in the AOF fractions. After delignification with sodium chlorite, 39% to 52% and 15% to 38% of crude protein and ash were recovered, respectively. Of individual samples, 30% of the wheat straw crude protein was lost after delignification or 1.3% of the dry matter. Of the remaining substrates, 2.1%, 3.5%, 2.1% and 5.2% of the dry matter was lost after oxidation in sodium chlorite. Sodium chlorite oxidation did not affect ash content of the fiber residue, with 1% to 2% of the original ash lost. After trifluoroacetic acid hydrolysis, no crude protein and ash was found in the recovered cellulose.

Amino acid composition of wheat straw and wheat straw NDF, ADF, AOF and the delignified residue are presented in Table 13 and 14. Glutamic acid and methionine were the highest and lowest amino acids among all fractions. Wheat straw NDF contained a higher amount of proline and alanine and a lower amount of arginine than intact wheat straw (Table 13) when expressed on a nitrogen basis. Similarly,

TABLE 12. PROTEIN AND ASH CONTENT IN MSU FIBER FRACTIONS.[a]

Substrate	Original sample	Ammonium oxalate fiber	Delignified residue	Cellulose
Protein	%	%	%	%
Wheat straw	4.19	2.89 (69)	1.65 (39)	0.00
Bromegrass	9.42	6.95 (74)	4.89 (52)	0.00
Alfalfa	16.47	11.86 (72)	8.43 (51)	0.00
Kentucky bluegrass	10.07	6.02 (60)	3.91 (39)	0.00
Ladino clover	25.91	17.92 (70)	12.85 (50)	0.00
Ash[b]				
Wheat straw	8.74	3.48 (40)	3.29 (38)	0.00
Bromegrass	10.10	3.01 (30)	2.97 (29)	0.00
Alfalfa	7.43	2.76 (37)	2.65 (36)	0.00
Kentucky bluegrass	8.24	1.29 (16)	1.26 (15)	0.00
Ladino clover	10.90	4.22 (39)	3.99 (37)	0.00

[a]Value in parentheses is the % retained of protein(N x 6.25) or ash from a dry sample.

[b]Each value represents the % ash(920 F).

TABLE 13. AMINO ACID COMPOSITION IN DETERGENT AND MSU FIBER FRACTIONS OF WHEAT STRAW (G/16 G N).

Amino acid	Original sample	NDF	ADF	AOF	Delignified residue
Aspartic acid	8.83	8.84	8.84	8.67	10.16
Threonine	4.93	4.71	4.44	4.21	6.07
Serine	5.82	5.25	4.97	5.16	8.22
Glutamic acid	11.70	11.47	11.39	10.02	15.62
Proline	5.53	8.55	8.09	5.53	6.57
Glycine	5.74	5.72	5.69	5.16	7.03
Alanine	8.18	9.83	8.12	6.00	7.96
Valine	7.56	7.62	8.01	6.15	8.38
Methionine	0.53	0.46	0.73	0.51	0.26
Isoleucine	4.54	4.77	4.94	4.69	5.56
Leucine	8.80	8.63	6.81	8.73	10.96
Tyrosine	2.45	2.55	2.27	1.90	0.00
Phenylalanine	5.42	5.31	4.75	4.57	7.18
Lysine	9.25	8.86	4.20	7.38	5.21
Histidine	2.64	1.70	1.54	1.81	0.94
Arginine	5.91	4.46	2.22	4.22	5.76

TABLE 14. AMINO ACID COMPOSITION AND LOSS IN DETERGENT AND MSU FIBER FRACTIONS OF WHEAT STRAW (MG/G D.M.).

Amino acid	Original sample	NDF	ADF	AOF	Delignified residue
Aspartic acid	2.90	0.73	0.45	2.22	1.13
Threonine	1.62	0.39	0.22	1.08	0.68
Serine	1.91	0.44	0.25	1.32	0.92
Glutamic acid	3.84	0.95	0.57	2.57	1.74
Proline	1.81	0.71	0.40	1.42	0.73
Glycine	1.88	0.48	0.28	1.32	0.78
Alanine	2.68	0.81	0.41	1.54	0.89
Valine	2.49	0.63	0.40	1.58	0.94
Methionine	0.17	0.04	0.04	0.13	0.03
Isoleucine	1.50	0.40	0.25	1.20	0.62
Leucine	2.90	0.72	0.34	2.24	1.22
Tyrosine	0.81	0.22	0.11	0.49	0.00
Phenylalanine	1.79	0.44	0.24	1.17	0.80
Lysine	3.05	0.73	0.21	1.89	0.58
Histidine	0.87	0.14	0.08	0.46	0.11
Arginine	1.95	0.37	0.11	1.09	0.64
Total	31.19	8.21	4.37	21.73	11.81
Recovery, %		26.32	14.01	69.67	37.86

wheat straw ADF had lower serine, leucine, phenylalanine, lysine, histidine and arginine. AOF had lower serine, glycine, alanine, valine, tyrosine, phenylalanine, lysine, histidine and arginine but higher proline. After delignification, wheat straw contained higher amounts of 10 of 16 amino acids, but had lesser amounts of lysine and histidine on a nitrogen basis. When amino acids were expressed on a dry matter basis, it was found that 26.32%, 14.01%, 69.67% and 37.86% of the total amino acids were recovered in the NDF, ADF, AOF and delignified residue, respectively. Glutamic acid was in the highest concentration among all fiber fractions. Methionine was the lowest in all fractions with the exception of the delignified residue which had no tyrosine.

Electron microscopy of fiber fractions. Scanning and transmission electron microscopy was used in order to examine the effects of neutral detergent, acid detergent, ammonium oxalate, trifluoroacetic acid and sodium chlorite treatment of the cell walls of wheat straw. The surface of intact plant cells is coated with debris (Figure 4A). Both neutral detergent and ammonium oxalate appear to rid the plant cell wall of cell cytoplasm and debris (Figure 4B and 5A). Small frequent disruptions were seen in the cell wall of wheat straw NDF (Figure 4B). The cell wall of ammonium oxalate treated wheat straw appeared to have opened cell walls (Figure 5A) with typical flaking of the cell wall (Figure 5B), but no apparent losses. Acid detergent has been reported to contain cellulose, lignin and some ash. The chemical mode of attack appeared to be localized (Figure 6A and 6B) with many ruptured cell walls. TEM studies showed that hemicellulose

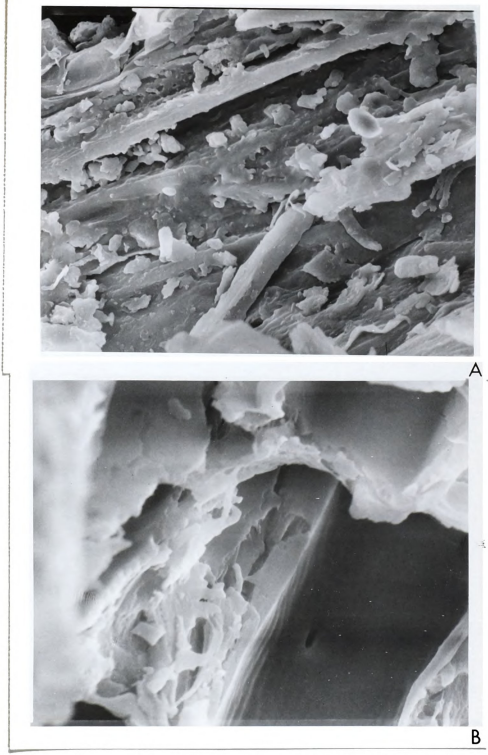

FIGURE 4. EFFECT OF NEUTRAL DETERGENT TREATMENT ON WHEAT STRAW. A. Control sample. Surface is coated with debris. No open cell apparent: X1000. B. Section treated with neutral detergent. Open cells are quite apparent. The wall is intact with small disruptions (arrows) evident: X3000.

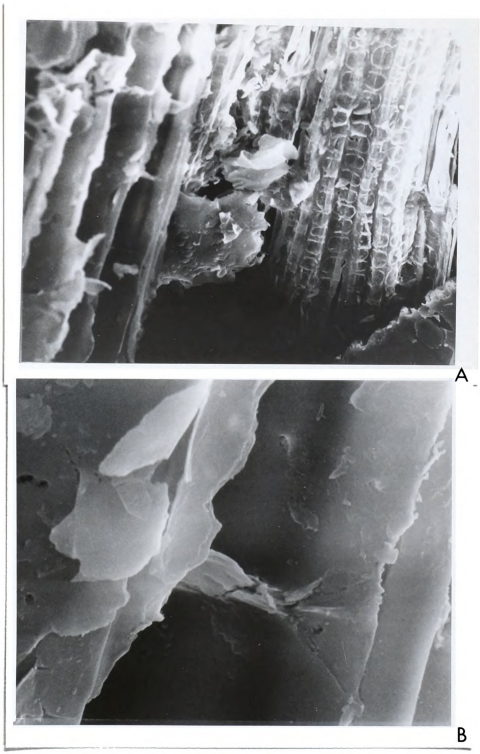

FIGURE 5. EFFECT OF AMMONIUM OXALATE TREATMENT ON WHEAT STRAW. A. Section treated with ammonium oxalate. Open cells are quite apparent: X400. B. Section treated with ammonium oxalate. Cell wall is smooth and glossy with small typical flaking (arrows): X3000.

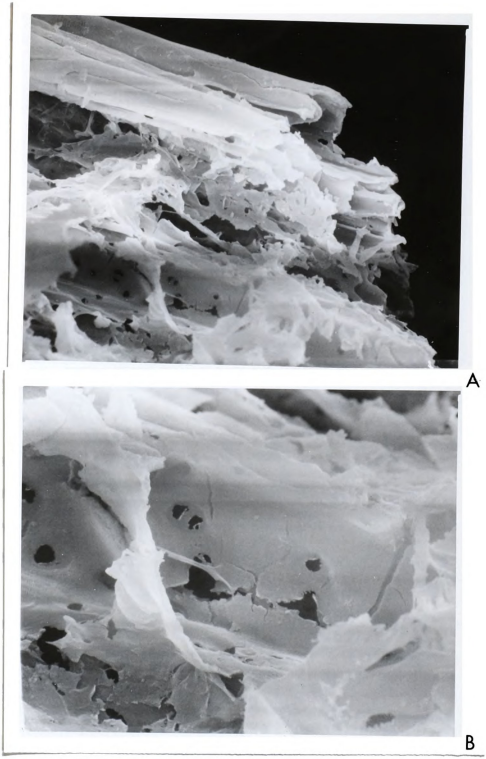

FIGURE 6. EFFECT OF ACID DETERGENT TREATMENT ON WHEAT STRAW. A. Section treated with acid detergent: X1000. B. Close-up of section A . Ruptured cell walls are apparent (arrows): X3000.

was disrupted (Figure 7A) and was clearly present in wheat straw ADF (Figure 7B). Delignification of wheat straw showed no disruptions and appeared to be evenly extracted (Figure 8B). After trifluoroacetic acid hydrolysis, cellulose sheets were clearly evident (Figure 8B).

Pony digestion trial. Dry matter and crude protein digestibility in three ponies is presented in Table 15. Oat intake was kept constant but hay intake was monitored. The amount of dry matter digested was highest in Elvis and lowest in Snipper. The apparent dry matter digestibility was highest in Elvis with 68.1% digested and lowest in Chester with 54.8% digested. Crude protein digestibility was similar to dry matter digestibility in all three ponies.

The fiber composition of the diets fed to the ponies and feces are presented in Table 16. Hay, oats and feces contained higher AOF when compared to the corresponding NDF. Large differences were observed in the hemicellulose content of all samples between the detergent and MSU T.F.A.A. fiber methods. Detergent hemicellulose was 24.26% of the oat dry matter whereas hemicellulose as measured by GLC was 5.36%. This hemicellulose consisted of arabinose, xylose, galactose , glucose and traces of mannose. GLC examination of the feces showed similar concentrations of glucose and galactose, but the concentration of xylose was higher in Elvis' feces when compared to the other two ponies. No arabinose or mannose was found in the feces. Lignin, as determined by oxidation in sodium chlorite, was significantly higher ($P<.05$) than all permanganate lignin values.

The digestibility of each fiber fraction in each pony is pre-

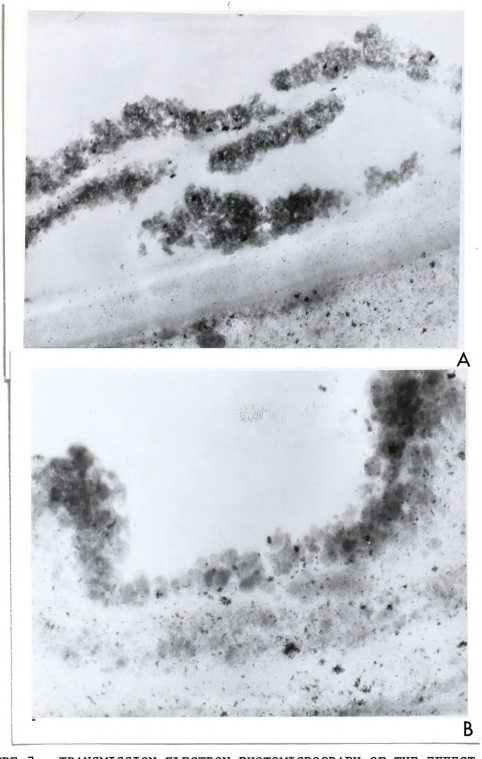

FIGURE 7. TRANSMISSION ELECTRON PHOTOMICROGRAPH OF THE EFFECT OF ACID DETERGENT ON WHEAT STRAW.
A. Hemicellulose disruption clearly evident (arrows): TEM X40,000.
B. Hemicellulose residue (arrows): TEM X60,000.

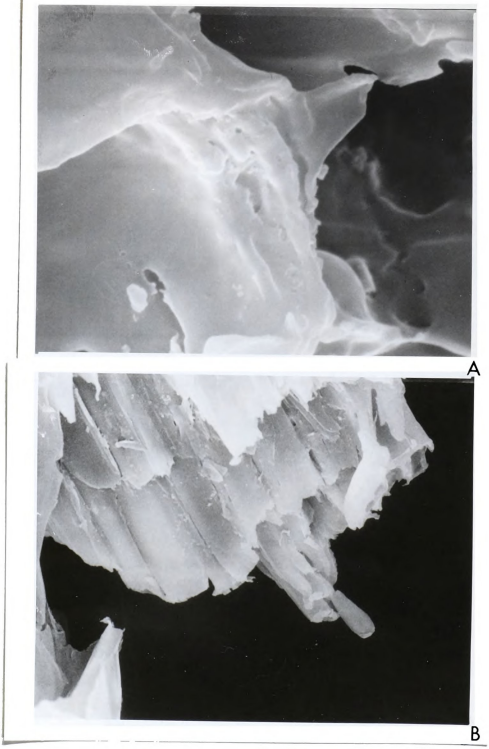

FIGURE 8. EFFECT OF SODIUM CHLORITE AND TRIFLUOROACETIC ACID ON WHEAT STRAW.
A. Section treated with sodium chlorite. Cell wall very much intact: X10,000. B. Section treated with trifluoroacetic acid. Cellulose sheets clearly evident (arrows): X1000.

TABLE 15. APPARENT DRY MATTER AND CRUDE PROTEIN DIGESTIBILITY IN THE PONY

Item	Animal		
	Chester	Snipper	Elvis
Dry matter			
Hay intake, g/day	1809.2	1330.6	1384.9
Oat intake, g/day	837.7	840.4	837.7
Total intake, g/day	2646.9	2171.0	2222.6
Dry feces, g/day	1196.0	908.0	708.1
Digested, g/day	1450.9	1263.0	1514.5
Apparent digestibility, %	54.8	58.2	68.1
Crude protein			
Hay intake, g/day	201.5	145.6	154.3
Oat intake, g/day	149.6	150.1	149.6
Total intake, g/day	351.1	295.7	303.9
Dry feces, g/day	160.0	106.9	95.1
Digested, g/day	191.1	188.8	206.8
Apparent digestibility, %	54.4	63.9	68.7

TABLE 16. FIBER COMPOSITION OF FEED AND FECES IN PONY DIGESTION TRIAL.[a]

Fraction	Feed		Feces		
	Oats	Hay	Chester	Snipper	Elvis
Ammonium oxalate fiber	34.45	71.34	77.64	77.19	76.27
Neutral detergent fiber	29.62	60.62	66.23	66.21	61.19
Acid detergent fiber	5.36	35.85	40.47	44.35	39.17
Detergent hemicellulose[b]	24.26	24.77	25.75	21.86	22.02
Detergent cellulose	4.40	29.08	26.98	27.88	27.64
T.F.A.A. hemicellulose	5.36	13.88	6.92	6.69	14.51
T.F.A.A. cellulose	5.50	28.09	30.14	29.37	31.26
Permanganate lignin	0.34	6.36	11.50	10.25	9.15
Chlorite lignin	1.05	8.28	12.14	13.14	12.98
Hemicellulosic sugars					
Arabinose	0.23	0.91	0.00	0.00	0.00
Xylose	1.19	6.21	1.45	1.47	8.90
Mannose	tr	tr	0.00	0.00	0.00
Galactose	0.74	3.66	3.03	2.69	2.66
Glucose	3.20	3.10	2.44	2.53	2.37
Detergent holocellulose[c]	28.66	53.85	52.73	49.74	49.66
T.F.A.A. holocellulose[d]	10.86	41.97	37.06	36.06	45.77
Detergent total fiber[e]	29.00	60.21	64.23	59.99	58.81
T.F.A.A. total fiber[f]	17.91	50.25	49.20	49.20	56.75

[a]Each value represents the average of three determinations.

[b-f]For definition, see Table A7.

sented in Table 17. Digestibility of AOF, NDF and ADF was higher in Elvis than Chester and Snipper. Digestibility of detergent hemicellulose was 52.7%, 62.8% and 71.5% whereas the digestibility of T.F.A.A. hemicellulose was 72.0%, 73.6% and 56.7%, for Chester, Snipper and Elvis, respectively. Detergent and T.F.A.A. cellulose digestibilities were very similar with the digestibility by Elvis the highest in both cases. Lignin, a phenylpropane polymer, appeared to be digested by Elvis using either potassium permanganate or sodium chlorite to determine lignin. Arabinose digestibility was similar in Chester and Snipper, but lower in Elvis with 78.6%. Similarly, xylose digestibility was lower in Elvis than in Chester and Snipper. Both galactose and glucose digestibility were higher in Elvis with 66.9% and 75.9% digested, respectively. All mannose was digested by each pony. The digestibility of both holocelluloses were similar in Chester and Snipper, however, detergent and T.F.A.A. holocellulose digestibilities of Elvis were 64.3% and 51.8%, respectively. Digestibility of total fiber as determined with detergent or T.F.A.A. were similar in Chester and Snipper, but Elvis digested 58.8% and 52.5% of the detergent and T.F.A.A. holocellulose, respectively. Of all the digestibility coefficients, there was a strong significant correlation ($P<.001$) between NDF and AOF, AOF and DM and NDF and DM (Table A4).

Beef cattle digestion trials. Within each trial, intake, fecal and digestion of dry matter and crude protein were similar between animals as indicated by similar digestibilities within trials (Table 18). Dry matter digestibility was higher in Trial I as was crude protein digestibility when compared to the animals in Trial II.

TABLE 17. APPARENT DIGESTIBILITY OF FIBER FRACTIONS IN THE PONY.[a]

Fraction	Animal		
	Chester	Snipper	Elvis
	%	%	%
Ammonium oxalate fiber	41.2	43.4	57.7
Neutral detergent fiber	41.1	43.0	60.2
Acid detergent fiber	30.2	22.9	48.8
Detergent hemicellulose	52.7	62.8	71.5
Detergent cellulose	40.9	40.3	55.5
T.F.A.A. hemicellulose	72.0	73.6	56.7
T.F.A.A. cellulose	35.0	36.5	49.1
Permanganate lignin	-16.6	-6.4	28.7
Chlorite lignin	3.6	0.0	18.3
Hemicellulosic sugars			
Arabinose	100.0	100.0	78.6
Xylose	85.9	85.6	34.3
Mannose	100.0	100.0	100.0
Galactose	50.0	55.5	66.9
Glucose	64.8	66.2	75.9
Detergent holocellulose	47.2	52.8	64.3
T.F.A.A. holocellulose	49.3	49.6	51.8
Detergent total fiber	42.3	47.8	58.8
T.F.A.A. total fiber	44.4	45.5	52.5

[a]Diet fed to the ponies.

TABLE 18. APPARENT DRY MATTER AND CRUDE PROTEIN DIGESTIBILITY IN BEEF CATTLE DIGESTION TRIALS.

Item	Trial I		Trial II	
	Steer #153	Steer #187	Steer #528	Steer #992
Dry matter				
Intake, kg/day	8.28	7.49	4.68	4.49
Feces, kg/day	1.80	1.72	1.47	1.25
Digested, kg/day	6.48	5.77	3.21	3.24
Digestibility, %	78.3	77.0	68.6	72.2
Crude protein				
Intake, kg/day	1.12	1.01	0.44	0.42
Feces, kg/day	0.28	0.27	0.19	0.18
Digested, kg/day	0.84	0.74	0.25	0.26
Digestibility, %	75.0	73.3	55.3	56.5

The fiber composition of feed and feces is presented in Table 19. The feed in Trial I was higher in each fiber component than the feed in Trial II. NDF was lower than AOF in both feeds and feces as was the feed T.F.A.A. hemicellulose when compared to feed detergent hemicellulose. Detergent cellulose was significantly ($P<.05$) higher than T.F.A.A. cellulose in the two feeds, but not in the feces. Detergent holocellulose and total fiber were higher than the corresponding T.F.A.A. holocellulose and total fiber. The feed in Trial I had higher arabinose, xylose and galactose than the feed in Trial II. This corresponded with an increase in arabinose, xylose and galactose in all fecal samples.

The apparent digestibility of each fiber component was higher in Trial I than in Trial II with the exception of glucose digestibility which was higher in Trial II (Table 20). AOF and NDF, detergent and T.F.A.A. hemicellulose and detergent and T.F.A.A. digestibilities were similar and each pair were correlated significantly ($P<.05$) as shown in Table A4. More arabinose, xylose and galactose were digested in Trial I than Trial II. Glucose digestibilities in the two trials were very similar. T.F.A.A. holocellulose and total fiber digestibilities were lower than their corresponding detergent values in both trials.

Feces samples of the animals in Trial I were examined by scanning electron microscopy. Feces were washed and dried at 60 C and refluxed in neutral and acid detergent and ammonium oxalate. Dried samples contained debris with some bacteria evident (Figure 9A). Washing the feces before drying appeared to rid the sample of bac-

TABLE 19. COMPOSITION OF FEED AND FECES IN BEEF CATTLE TRIALS.

Fraction	Trial I			Trial II		
	Feed	Feces, 153	Feces, 187	Feed	Feces, 528	Feces, 992
Ammonium oxalate fiber	59.59	75.33	70.44	54.17	75.30	75.27
Neutral detergent fiber	51.13	64.36	61.45	46.47	61.62	58.75
Acid detergent fiber	27.72	37.56	33.52	23.94	36.84	34.49
Detergent hemicellulose[c]	23.41	26.80	27.93	22.53	24.78	24.46
Detergent cellulose	21.73	25.18	21.66	20.24	23.65	21.34
T.F.A.A. hemicellulose	15.72	18.68	18.66	8.45	10.68	10.58
T.F.A.A. cellulose	18.43	22.44	21.12	17.16	21.45	20.29
Permanganate lignin	5.73	8.58	8.65	3.06	8.57	9.19
Chlorite lignin	11.25	17.23	15.90	9.34	16.03	17.04
Hemicellulosic sugars						
Arabinose	0.94	1.12	0.92	0.32	0.51	0.65
Xylose	7.73	12.88	12.83	4.07	8.61	8.54
Galactose	2.69	3.35	3.95	0.48	0.75	0.74
Glucose	4.36	1.34	0.96	4.58	0.81	0.66
Detergent holocellulose[d]	45.14	51.98	48.59	42.77	48.43	45.80
T.F.A.A. holocellulose[e]	34.15	41.12	39.78	25.61	32.13	30.87
Detergent total fiber[f]	50.87	60.56	58.24	45.83	57.00	54.79
T.F.A.A. total fiber[g]	45.40	58.35	55.68	34.95	48.16	47.91

[a]Each value represents the average of three determinations.

[b]Trial I and Trial II diets consisted of corn silage-soybean meal supplemented rations: Trial I (13.5% crude protein) and Trial II (13.4% crude protein).

[c-g]For definition, see Table A7.

TABLE 20. APPARENT DIGESTIBILITY OF FIBER FRACTIONS IN BEEF CATTLE.

Item	Trial I		Trial II	
	Steer #153	Steer #187	Steer #528	Steer #992
	----------Digestibility, %----------			
Ammonium oxalate fiber	72.5	72.8	56.3	61.3
Neutral detergent fiber	72.7	72.4	58.3	64.8
Acid detergent fiber	70.5	72.2	51.6	59.4
Detergent hemicellulose	75.1	72.6	65.5	70.1
Detergent cellulose	74.8	77.1	63.3	70.6
T.F.A.A. hemicellulose	74.2	72.7	60.3	65.2
T.F.A.A. cellulose	73.5	73.7	60.9	67.0
Permanganate lignin	67.5	65.3	11.9	16.1
Chlorite lignin	66.7	67.6	46.0	49.2
Hemicellulosic sugars				
Arabinose	74.3	77.1	53.3	46.7
Xylose	63.8	61.8	33.2	41.5
Galactose	73.1	66.2	50.0	59.1
Glucose	93.4	94.8	94.4	96.1
Detergent holocellulose	75.0	74.8	64.4	70.3
T.F.A.A. holocellulose	73.8	73.3	60.6	66.4
Detergent total fiber	74.1	73.7	60.9	66.7
T.F.A.A. total fiber	72.1	71.8	56.7	61.8

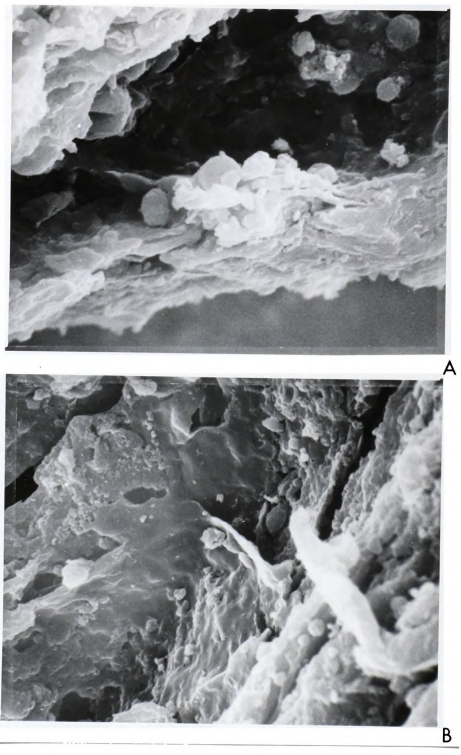

FIGURE 9. EFFECT OF WASHING AND DRYING ON FIBER OF BEEF CATTLE FECES. A. Dried sample (60 C). Bacteria evident in some debris (arrows): X7000. B. Washed and dried sample (60 C). Absence of bacteria: X5000.

teria (Figure 9B). Neutral detergent fiber from feces was completely absent of bacteria (Figure 10A). Feces boiled in acid detergent were absent of bacteria but were fragmented and disrupted (Figure 10B). Ammonium oxalate treated fecal samples were free of most debris and bacteria, but not completely (Figure 11A and 11B).

Swine digestion trials. Dry matter and crude protein intake was identical in starting pigs (Table 21). The amount digested of either crude protein or dry matter was similar between pigs and is reflected in similar digestibilities. Dry matter digestibility ranged from 91.0% to 91.8% whereas crude protein digestibility ranged from 89.8% to 92.9%.

Fiber analysis of feed and feces are presented in Table 22. AOF was significantly ($P<.05$) higher than NDF in all samples. Fecal AOF values ranged from 45.47% in pig 119-13 to 58.86% in pig 119-1. Fecal NDF values were highest in pig 119-13 with 40.21% and lowest in pig 119-1 with 35.09%. Detergent hemicellulose values for starting pig feces ranged from 19.34% to 25.63%. T.F.A.A. hemicellulose were much lower in comparison and ranged from 6.68% to 7.96%. Detergent cellulose values were very similar between pigs with the exception of pig 119-10. Values for detergent cellulose ranged from 10.31% to 11.73%. Cellulose as determined with T.F.A.A. was significantly higher ($P<.05$) than corresponding detergent cellulose values. Chlorite lignin values were higher ($P<.05$) than permanganate lignin values in all samples and were more variable. They ranged from 5.40% to 12.70% whereas permanganate lignin in fecal samples ranged from 1.83% to 2.55%. Glucose was the highest hemicellulosic sugar in the feed

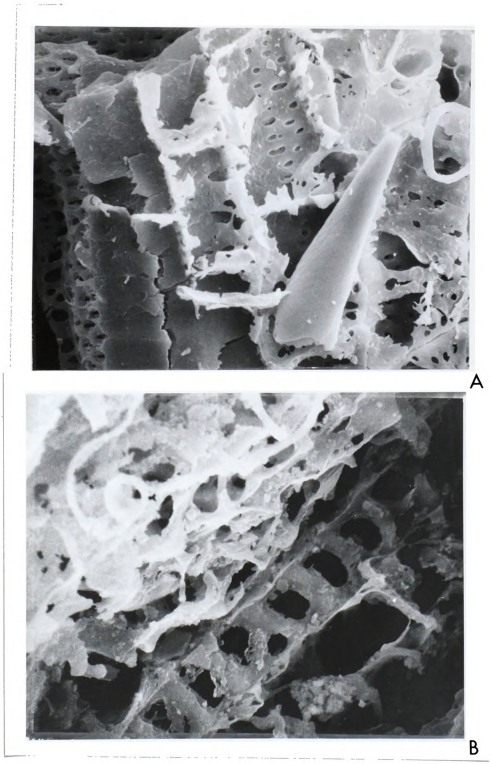

FIGURE 10. EFFECT OF NEUTRAL AND ACID DETERGENT TREATMENT ON FIBER OF BEEF CATTLE FECES.
A. Section treated with neutral detergent. Cell wall free of bacteria: X700. B. Section treated with acid detergent. Cell wall fragmented: X2700.

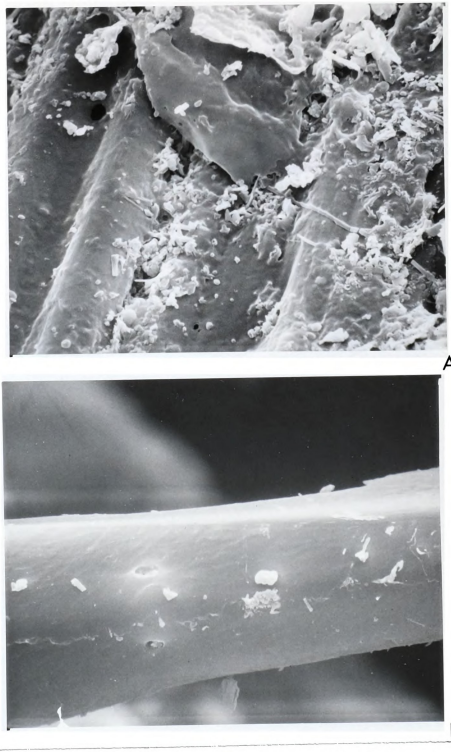

FIGURE 11. EFFECT OF AMMONIUM OXALATE TREATMENT ON FIBER OF BEEF CATTLE FECES.
A. Cell wall smooth with some debris: X2700. B. Cell wall smooth with bacteria attached (arrows): X2700.

TABLE 21. FIBER COMPONENT COMPOSITION OF FEED AND FECES IN STARTER PIGS.[a]

Item	Feed	Feces from animal # 119-1	119-10	Y6-1	119-13
	%	%	%	%	%
Ammonium oxalate fiber	30.52	58.86	57.91	49.10	45.47
Neutral detergent fiber	12.14	35.09	37.96	38.52	40.21
Acid detergent fiber	4.09	15.75	13.67	14.54	14.58
Detergent hemicellulose[b]	8.05	19.34	24.29	23.98	25.63
Detergent cellulose	3.29	11.73	10.31	11.49	11.50
T.F.A.A. hemicellulose	2.78	7.28	7.45	7.96	6.68
T.F.A.A. cellulose	4.33	16.28	14.01	12.49	12.71
Permanganate lignin	0.52	2.43	2.00	2.55	1.83
Chlorite lignin	5.66	6.10	12.70	9.74	5.40
Hemicellulosic sugars					
Arabinose	0.25	0.54	1.10	1.98	1.71
Xylose	0.50	1.59	4.14	4.62	3.76
Mannose	tr	0.00	0.00	0.00	0.00
Galactose	0.38	2.69	1.04	0.68	0.56
Glucose	1.65	2.46	1.17	0.68	0.65
Detergent holocellulose[c]	11.34	31.07	34.60	35.47	37.13
T.F.A.A. holocellulose[d]	7.11	23.56	21.46	20.45	19.39
Detergent total fiber[e]	11.86	33.50	36.60	38.02	38.96
T.F.A.A. total fiber[f]	12.77	29.66	34.16	30.19	24.79

[a]Each value represents the average of three determinations.

[b-g]For definition, see Table A 7 .

TABLE 22. APPARENT DRY MATTER AND CRUDE PROTEIN DIGESTIBILITY IN STARTER PIGS.

Item	Animal #			
	119-10	119-1	119-13	Y6-1
Dry matter				
Intake, g/day	366	366	366	366
Feces, g/day	30	33	31	32
Digested, g/day	336	333	335	334
Digestibility, %	91.8	91.0	91.5	91.3
Crude protein				
Intake, g/day	71.2	71.2	71.2	71.2
Feces, g/day	6.8	5.1	6.4	7.3
Digested, g/day	64.4	65.1	64.8	63.9
Digestibility, %	90.5	92.9	90.9	89.8

whereas xylose was the highest hemicellulosic sugar in three of four fecal samples. Galactose and glucose content of all feces were very similar, however, galactose and glucose were much higher in pig 119-1 than in the other fecal samples. Mannose was found in trace amounts in feed samples, but was not found in the feces. Detergent holocellulose values for all samples were greater than corresponding T.F.A.A. holocellulose values. Detergent total fiber was also greater than T.F.A.A. total fiber in feces, but not in the feed.

The digestibilities of each fiber fraction in starting pigs are presented in Table 23. The digestibility of AOF ranged from 82.6% to 87.4% with an average of 85.1% among all four pigs. NDF digestibility was lower than AOF digestibility and ranged from 71.8% to 74.4% with an average of 72.6%. Acid detergent fiber digestibilities were lower than corresponding NDF values and was highest in pig 119-10. NDF digestibility was correlated with both AOF digestibility ($P<.05$) and ADF digestibility ($P<.001$) as shown in Table A4. Detergent hemicellulose digestibility ranged from 73.0% in pig 119-13 to 78.3% in pig 119-1. T.F.A.A. hemicellulose digestibility ranged from 74.9% in pig Y6-1 to 79.1% in pig 119-13. The average hemicellulose digestibility was 75.2% and 77.1% for the detergent and MSU T.F.A.A. methods, respectively. Cellulose digestibility ranged from 67.9% to 74.3% and from 63.8% to 73.5% for the detergent and MSU T.F.A.A. methods, respectively. Cellulose digestibility as determined with detergent and T.F.A.A. for the four pigs was 70.5% and 70.5%, respectively. Chlorite lignin digestibilities was higher in each pig than were the corresponding digestibilities of permanganate lignin. Mannose was

TABLE 23. APPARENT DIGESTIBILITY OF FIBER FRACTIONS IN STARTER PIGS.

Item	Animal #			
	119-10	119-1	119-13	Y6-1
	Digestibility %			
Ammonium oxalate fiber	84.4	82.6	87.4	85.9
Neutral detergent fiber	74.4	71.8	72.0	72.3
Acid detergent fiber	72.6	65.3	69.8	68.9
Detergent hemicellulose	75.3	78.3	73.0	74.0
Detergent cellulose	74.3	67.9	70.4	69.4
T.F.A.A. hemicellulose	78.0	76.4	79.1	74.9
T.F.A.A. cellulose	71.7	63.8	73.5	73.1
Permanganate lignin	68.4	57.9	70.0	56.8
Chlorite lignin	81.6	90.3	91.9	84.9
Hemicellulosic sugars				
Arabinose	52.3	80.4	40.2	31.5
Xylose	32.2	71.6	36.1	19.0
Mannose	100.0	100.0	100.0	100.0
Galactose	97.7	36.7	87.8	84.2
Glucose	94.2	86.6	96.7	84.2
Detergent holocellulose	75.0	75.3	72.2	72.7
T.F.A.A. holocellulose	74.3	68.9	76.0	73.8
Detergent total fiber	74.7	74.5	72.1	73.3
T.F.A.A. total fiber	71.1	70.6	69.0	69.1

found in trace amounts in the feed and none in the feces so its apparent digestibility was 100.0% in all four pigs. In three of the four pigs, galactose and glucose were highly digestible, but in pig 119-1, galactose digestibility was depressed and arabinose and xylose digestibilities were increased. Xylose digestibility was lowest in the other three pigs. Total fiber digestibility was 73.8% and 73.3% as determined by detergent and T.F.A.A., respectively. Detergent total fiber digestibilities were greater than T.F.A.A. total fiber digestibilities. Total fiber digestion for all four pigs was 73.7% and 69.9% for detergent and T.F.A.A., respectively.

Fiber component composition of feed and feces of growing pigs are presented in Table 24. AOF values for feed and feces are greater than corresponding NDF values. Pen I AOF, ADF, detergent cellulose, T.F.A.A. cellulose and chlorite lignin values were higher than Pen II values. Pen I NDF, detergent hemicellulose, T.F.A.A. hemicellulose and permanganate lignin values were lower than Pen II values. Feed samples are high in hemicellulosic glucose. Pen I fecal samples had higher hemicellulosic glucose than any other sugar whereas xylose is the highest sugar in Pen II. Detergent holocellulose was higher than T.F.A.A. holocellulose in all samples. Pen II detergent holocellulose was higher than Pen I, however, Pen I and Pen II T.F.A.A. holocellulose values were similar. Detergent total fiber was larger in Pen II, but T.F.A.A. total fiber was higher in Pen I.

Dry matter and crude protein intakes were higher for the growing pigs in Pen II (Table 25). The pigs in Pen II were heavier (51 kg) and consumed 2718 g/pig/day of feed compared to 1171 g/pig/day by the

TABLE 24. FIBER COMPONENT COMPOSITION OF FEED AND FECES IN GROWER PIGS.[a]

Item	Feed	Feces	
		Pen I	Pen II
	%	%	%
Ammonium oxalate fiber	33.40	54.83	52.40
Neutral detergent fiber	15.70	35.90	41.64
Acid detergent fiber	4.41	13.21	12.66
Detergent hemicellulose[b]	11.29	22.69	28.98
Detergent cellulose	3.02	9.70	8.81
T.F.A.A. hemicellulose	6.05	0.81	1.81
T.F.A.A. cellulose	2.46	9.29	8.33
Permanganate lignin	1.37	1.61	2.34
Chlorite lignin	3.90	20.67	13.69
Hemicellulosic sugars			
Arabinose	0.16	0.00	0.23
Xylose	0.34	0.11	0.96
Mannose	tr	0.00	0.00
Galactose	1.07	0.33	0.24
Glucose	4.48	0.37	0.38
Detergent holocellulose[c]	14.31	32.39	37.79
T.F.A.A. holocellulose[d]	8.51	10.10	10.14
Detergent total fiber[e]	15.68	34.00	40.13
T.F.A.A. total fiber[f]	12.41	30.77	23.83

[a]Each value represents the average of three determinations.

[b-f]For definition, see Table A7.

TABLE 25. APPARENT DRY MATTER AND CRUDE PROTEIN DIGESTIBILITY IN GROWER PIGS.

Item	Pen I	Pen II
Dry matter		
Intake, g/pig/day	1171	2718
Feces, g/pig/day	299	310
Digested,g/pig/day	1472	2408
Digestibility, %	82.6	88.6
Crude protein		
Intake, g/pig/day	341.8	524.6
Feces, g/pig/day	72.8	71.2
Digested, g/pig/day	269.0	453.4
Digestibility, %	78.5	86.4

lighter (30 kg) pigs in Pen I. Dry matter and crude protein digestibility was also higher in Pen II.

The digestibility of each fiber fraction is presented in Table 26. The digestibility of AOF, NDF, ADF, detergent hemicellulose, detergent cellulose, T.F.A.A. cellulose and chlorite lignin were higher in Pen II when compared to Pen I. T.F.A.A. hemicellulose digestibility and permanganate lignin digestibilities were similar between pens. AOF digestibility was higher than NDF digestibility in both pens. Detergent cellulose digestibility was higher than cellulose digestibility as determined with T.F.A.A. in both pens. T.F.A.A. hemicellulose was almost completely digested in both pens with 97.7% and 96.6% for Pen I and II, respectively. Of the hemicellulosic sugars, arabinose and mannose were completely digested in Pen I. Digestibility decreased in the following order in Pen I: glucose, galactose and xylose. Mannose was completely digested by the pigs in Pen II, however, xylose and arabinose digestibilities were depressed compared to Pen I. In total, holocellulose and total fiber as determined with T.F.A.A. were more digestible than similar detergent fractions in both pens.

Fiber component composition of feed and feces of finishing pigs are presented in Table 27. Fecal NDF, ADF, detergent hemicellulose, detergent cellulose and chlorite lignin were higher in Pen I than Pen II. Fecal AOF, T.F.A.A. cellulose and permanganate lignin were similar in both pens. Fecal hemicellulose determined with T.F.A.A. was higher in Pen II. Of the hemicellulosic sugars, xylose was in the highest concentration in the feed. Only trace amounts of mannose

TABLE 26. APPARENT DIGESTIBILITY OF FIBER FRACTIONS IN GROWER PIGS.

Item	Pen I	Pen II
	%	%
Ammonium oxalate fiber	72.3	82.1
Neutral detergent fiber	61.3	69.8
Acid detergent fiber	49.4	67.3
Detergent hemicellulose	43.4	70.7
Detergent cellulose	45.8	66.7
T.F.A.A. hemicellulose	97.7	96.6
T.F.A.A. cellulose	36.2	61.4
Permanganate lignin	80.2	80.5
Chlorite lignin	10.5	59.9
Hemicellulosic sugars		
Arabinose	100.0	83.7
Xylose	94.5	67.8
Mannose	100.0	100.0
Galactose	94.8	97.5
Glucose	98.6	99.0
Detergent holocellulose	44.2	69.8
T.F.A.A. holocellulose	80.0	86.4
Detergent total fiber	48.6	70.8
T.F.A.A. total fiber	54.8	78.1

TABLE 27. FIBER COMPONENT COMPOSITION OF FEED AND FECES IN FINISHER PIGS.[a]

Item	Feed	Feces	
		Pen I	Pen II
	%	%	%
Ammonium oxalate fiber	23.03	66.92	66.71
Neutral detergent fiber	14.96	55.57	41.98
Acid detergent fiber	4.25	23.20	17.27
Detergent hemicellulose[b]	10.71	32.37	24.71
Detergent cellulose	2.73	8.55	7.92
T.F.A.A. hemicellulose	3.77	6.45	8.94
T.F.A.A. cellulose	3.71	11.45	11.73
Permanganate lignin	1.48	2.48	2.65
Chlorite lignin	4.82	21.60	9.46
Hemicellulosic sugars			
Arabinose	0.72	1.07	1.79
Xylose	1.40	3.28	4.09
Mannose	tr	0.00	0.00
Galactose	0.59	1.36	1.88
Glucose	1.06	0.74	1.18
Detergent holocellulose[c]	13.44	40.92	32.63
T.F.A.A. holocellulose[d]	7.48	17.90	20.67
Detergent total fiber[e]	14.92	43.40	35.28
T.F.A.A. total fiber[f]	12.30	39.50	30.13

[a]Each value represents the average of three determinations.

[b-f]For definition, see Table A 7 .

were found. Xylose was also the highest hemicellulosic sugar in both fecal samples. Holocellulose and total fiber as determined with detergent were higher than similar fractions determined with T.F.A.A. in all fractions.

Dry matter and crude protein intakes for finishing pigs are presented in Table 28. Pigs in Pen II consumed more than pigs in Pen I. This was accompanied with an increase in feces and the amount digested by the pigs in Pen II. Digestibilities of dry matter and crude protein were similar between pens.

The digestibility of each fraction in finishing pigs is shown in Table 29. The digestibilities of AOF, detergent cellulose, T.F.A.A. cellulose and permanganate in Pen I were slightly higher than in Pen II. NDF, ADF, detergent hemicellulose, T.F.A.A. hemicellulose and chlorite lignin digestibilities were higher in Pen II pigs. Of the hemicellulosic sugars, mannose was completely digested. Individual sugar digestibilities were always higher in Pen I but decreased in the following order in both pens: mannose, glucose, arabinose, and xylose and galactose. T.F.A.A. holocellulose differed from detergent holocellulose in Pen I with 77.1% to 70.% digested, respectively, and in Pen II with 69.4% to 73.1% digested, respectively. T.F.A.A. total fiber digestibility was higher in Pen I but lower in Pen II when compared to corresponding detergent total fiber digestibility.

Pure culture studies. Six substrates were chosen and inoculated with either R. flavefaciens C94 or B. succinogenes S85. In all cases, approximately 0.6 g was weighed into each flask. After the 48 hr fermentation period, the substrates were recovered by differential centri-

TABLE 28. APPARENT DRY MATTER AND CRUDE PROTEIN DIGESTIBILITY IN FINISHER PIGS.

Item	Pen I	Pen II
Dry matter		
Intake, g/pig/day	2455	3181
Feces, g/pig/day	235	352
Digested, g/pig/day	2220	2829
Digestibility, %	88.9	90.4
Crude protein		
Intake, g/pig/day	356.0	462.3
Feces, g/pig/day	54.4	73.2
Digested, g/pig/day	301.6	389.1
Digestibility, %	84.7	84.2

TABLE 29. APPARENT DIGESTIBILITY OF FIBER FRACTIONS IN FINISHER PIGS.

Item	Pen I	Pen II
	%	%
Ammmonium oxalate fiber	72.2	70.4
Neutral detergent fiber	64.4	69.0
Acid detergent fiber	47.8	55.0
Detergent hemicellulose	71.1	74.5
Detergent cellulose	70.0	67.8
T.F.A.A. hemicellulose	83.6	88.5
T.F.A.A. cellulose	70.5	65.0
Permanganate lignin	84.0	80.2
Chlorite lignin	57.1	78.3
Hemicellulosic sugars		
Arabinose	85.8	74.6
Xylose	77.6	67.7
Mannose	100.0	100.0
Galactose	77.9	64.7
Glucose	93.3	87.7
Detergent holocellulose	70.9	73.1
T.F.A.A. holocellulose	77.1	69.4
Detergent total fiber	69.3	79.2
T.F.A.A. total fiber	72.2	73.8

fugation. Recovery of substrates fermented by R. flavefaciens ranged from 229 mg to 525 mg and by B. succinogenes ranged from 357 mg to 595 mg (Table 30). Corn silage was the preferred fermentation substrate by both R. flavefaciens and B. succinogenes over the other five substrates. Manure fiber was the least fermented by R.flavefaciens and filter paper was the least fermented by B. succinogenes.

The fiber components of the substrates before and after fermentation are presented in Tables 31 and 32. Whatman filter paper contained the most cellulose with 91.04% and wheat straw contained the most hemicellulose with 22.33%. Of the hemicellulosic sugars, xylose was the highest in four substrates. Glucose was the highest in alfalfa and Whatman filter paper. No mannose was found in Kentucky bluegrass, wheat straw and Whatman filter paper. Arabinose and galactose were absent in Whatman filter paper and manure fiber, respectively. Of the recovered substrates, filter paper had the most cellulose with both bacteria. Wheat straw had the most hemicellulose among the substrates fermented by B. succinogenes whereas corn silage had the most among the substrates fermented by R. flavefaciens. Of the substrates fermented by R. flavefaciens, xylose was the highest in Kentucky bluegrass, corn silage and manure fiber. Glucose was the highest in wheat straw, alfalfa and filter paper. Of the hemicellulosic sugars, xylose was the highest in all substrates fermented by B. succinogenes. No mannose was recovered in any of the substrates.

The percent fermented of the six substrates by each bacteria are presented in Table 33. Cellulose fermented ranged from a high of 45.2% in corn silage to a low of 21.5% with R. flavefaciens, but

TABLE 30. FERMENTATION OF SUBSTRATES BY PURE CULTURES OF RUMEN CELLULOLYTIC BACTERIA.

Item	Kentucky bluegrass	Wheat straw	Alfalfa	Corn silage	Manure fiber	Whatman no. 1 filter paper
R. flavefaciens, C-94						
Input, mg	606	611	606	612	613	613
Recovery, mg	474	428	353	229	525	474
Fermented, mg	132	183	253	383	88	339
Fermented, %	21.8	30.0	41.8	62.6	14.4	22.7
B. succinogenes, S-85						
Input, mg	611	611	636	639	604	614
Recovery, mg	522	589	492	357	552	595
Fermented, mg	89	22	144	282	52	19
Fermented, %	14.6	3.6	22.6	44.1	8.6	3.1

TABLE 31. COMPOSITION OF SUBSTRATES USED IN PURE CULTURE ANALYSIS.[ab]

Substrate	Cellulose	Hemicellulose	Glucose	Galactose	Mannose	Arabinose	Xylose
Kentucky bluegrass	43.94	18.50	1.78	1.10	0.00	3.12	12.50
Wheat Straw	49.27	22.33	3.02	0.64	0.00	2.71	16.50
Alfalfa	34.86	13.07	5.26	0.97	0.65	1.24	4.95
Corn silage	29.69	9.79	3.44	1.67	0.53	0.15	4.00
Manure fiber	41.71	13.21	1.66	0.00	0.39	0.88	10.28
Whatman no. 1 filter paper	91.04	8.50	3.95	1.22	0.00	0.00	3.35

[a]Each value represents the average of three determinations.

[b]Each substrate was washed three times in distilled water and dried at 60 C.

TABLE 32. COMPOSITIONAL ANALYSIS OF RECOVERED SUBSTRATES AFTER FERMENTATION BY RUMEN CELLULOLYTIC BACTERIA.

Item	Kentucky bluegrass	Wheat straw	Alfalfa	Corn silage	Manure fiber	Whatman no. 1 filter paper
	%	%	%	%	%	%
R. flavefaciens, C-94						
Cellulose	42.41	47.58	37.61	41.82	35.24	84.21
Hemicellulose	11.91	16.36	13.26	19.23	10.60	9.18
Glucose	1.70	6.84	6.04	6.62	0.96	6.56
Galactose	0.80	4.16	3.12	2.39	0.38	2.02
Mannose	0.00	0.00	0.00	0.00	0.00	0.00
Arabinose	1.09	0.68	0.10	1.10	0.76	0.00
Xylose	8.32	4.61	3.95	9.12	8.50	0.60
B. succinogenes, S-85						
Cellulose	37.79	37.35	39.40	40.06	35.01	86.46
Hemicellulose	19.35	18.34	4.65	13.14	12.46	7.63
Glucose	4.57	5.11	1.67	6.17	0.59	2.35
Galactose	2.20	3.42	0.44	0.50	0.50	0.00
Mannose	0.00	0.00	0.00	0.00	0.00	0.00
Arabinose	2.41	0.94	0.00	0.30	1.16	0.00
Xylose	10.17	8.87	2.54	6.17	10.21	5.28

TABLE 33. PERCENT FERMENTED OF MSU FIBER FRACTIONS IN PURE CULTURE ANALYSIS.

Item	Kentucky bluegrass	Wheat straw	Alfalfa	Corn silage	Manure fiber	Whatman no. 1 filter paper
	%	%	%	%	%	%
R. flavefaciens, C94						
Cellulose	22.1	29.4	35.3	45.2	24.6	21.5
Hemicellulose	49.6	48.7	40.9	26.5	31.3	16.5
Glucose	25.3	0.0	33.1	28.6	50.5	0.0
Galactose	43.1	0.0	0.0	46.4	----	28.0
Mannose	----	----	100.0	100.0	100.0	----
Arabinose	72.7	100.0	95.3	0.0	26.0	----
Xylose	47.9	97.5	53.5	14.7	29.2	86.2
B. succinogenes, S85						
Cellulose	23.7	5.1	20.2	21.3	24.2	9.9
Hemicellulose	10.6	20.8	72.5	25.0	13.8	47.0
Glucose	0.0	0.0	75.4	0.0	67.5	42.3
Galactose	0.0	0.0	64.9	79.9	----	100.0
Mannose	----	----	100.0	100.0	100.0	----
Arabinose	34.0	66.6	100.0	0.0	0.0	----
Xylose	30.5	48.2	60.3	13.8	9.2	26.5

ranged from a high of 23.2% in manure fiber to a low of 5.1% in B. succinogenes. In four of the six substrates, R. flavefaciens fermented more substrate hemicellulose than B. succinogenes. Alfalfa hemicellulose fermented was higher with B. succinogenes with 72.5% fermented compared to R. flavefaciens with 40.9%. Filter paper hemicellulose was more fermentable with B. succinogenes compared in R. flavefaciens with 47.0% and 16.5% fermented, respectively. Fermentation of hemicellulosic glucose ranged from 0.0% to 50.5% and 0.0% to 75.4% with R. flavefaciens and B. succinogenes, respectively. Galactose fermented ranged from 0.0% to 46.4% in R. flavefaciens compared to 0.0% to 100.0% with B. succinogenes. Mannose was completely fermented in all substrates that contained mannose. The fermentation of arabinose in R. flavefaciens ranged from 0.0% to 100.0% and in B. succinogenes from 0.0% to 100.0%. Fermentation of xylose by R. flavefaciens was higher in all substrates with the exception of alfalfa. Xylose fermented ranged from 14.7% to 97.5% with R. flavefaciens and ranged from 9.2% to 60.3% with B. succinogenes. Among all substrates fermented by R. flavefaciens, arabinose and galactose content of the cell wall was significantly ($P<.05$) correlated with fermentability (Table A6).

Electron microscopy. R. flavefaciens and B. succinogenes attachment to plant cell walls were examined with electron microscopy. R. flavefaciens adhered on the surface of wheat straw near exudated debris (Figure 12A). Chains of bacteria were found on the surface with exudated debris (figure 12B). Adherence of R. flavefaciens on alfalfa appeared near the ends of fibers and surface (Figure 13A). Debris was

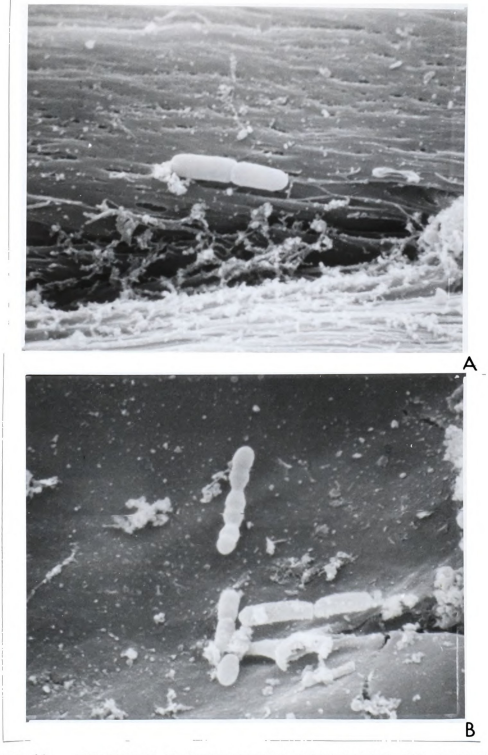

FIGURE 12. ATTACHMENT OF RUMINOCOCCUS FLAVEFACIENS C94 ON WHEAT STRAW.
A. Surface attachments frequently near exudated debris: X10,000.
B. Chains on surface with exudated debris: X10,000.

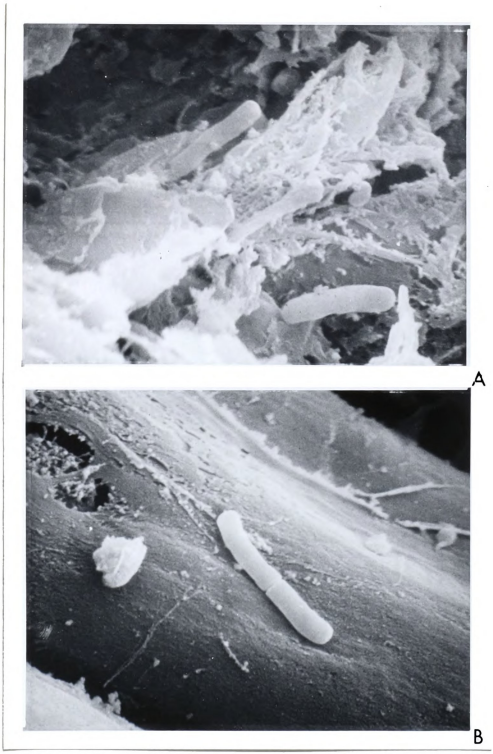

FIGURE 13. ATTACHMENT OF RUMINOCOCCUS FLAVEFACIENS C94 ON ALFALFA. A. Attachment of bacteria near ends of fibers: X10,000. B. Surface attached with very little debris. Particles are present (arrows): X10,000.

scarce on alfalfa compared to wheat straw, but particles are present (Figure 13B). Stringlike particles were observed with adherence of R. flavefaciens to corn silage (Figures 14A and 14B). R. flavefaciens was not found to adhere to filter paper and cattle fiber.

B. succinogenes adhered near the rough surface of alfalfa (Figures 15A and 15B). The bacteria mostly attached parallel to the cell wall (Figure 15A). Adherence of B. succinogenes to filter paper was predominately on the surface (Figure 16A) whereas the bacteria attached to Kentucky bluegrass primarily at the end of the fibers (Figure 16B). B. succinogenes did not adhere to cattle fiber and wheat straw.

Soluble carbohydrates in corn silage. The changes in pH, dry matter and soluble carbohydrates in corn silage over time are presented in Table 34. PH decreased from 5.5 to 3.6 at the end of 312 hr. Dry matter fluctuated between silos and showed no specific change over time. Xylose increased from 0.06% at 0 hr to 0.29% at 24 hr and then decreased to 0.04% by 96 hr. Mannose fluctuated between silos, but was highest at 72 hr and lowest at 312 hr. Glucose appeared to increase with time from 4.71% at 0 hr to 11.37% at 24 hr and then decreased to 0.00% at 144 hr. Total sugars also increased and then decreased with 6.58% at 0 hr to 15.31% at 36 hr and then to 0.71% at 312 hr.

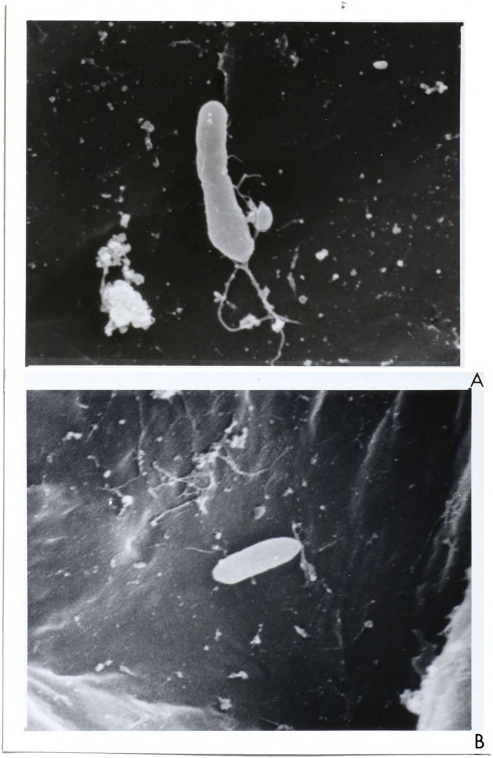

FIGURE 14. ATTACHMENT OF RUMINOCOCCUS FLAVEFACIENS C94 ON CORN SILAGE. A. Surface attachment upon stringlike particle: X10,000. B. Bacteria appeared anchored to stringlike particle: X10,000.

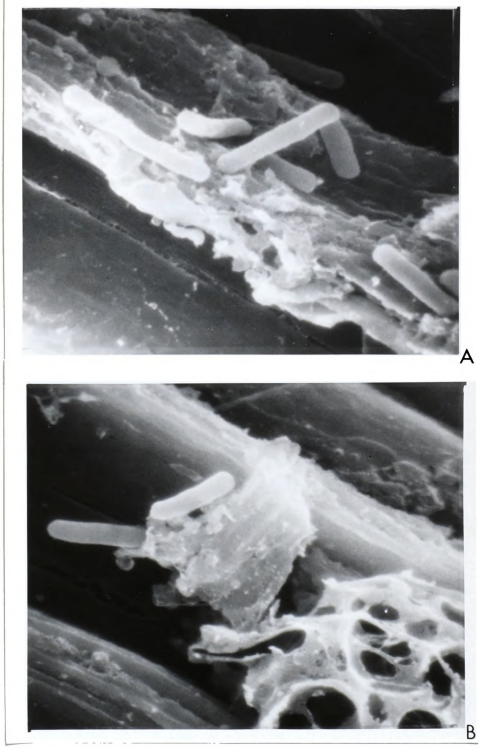

FIGURE 15. ATTACHMENT OF BACTEROIDES SUCCINOGENES S85 ON ALFALFA. A. Mostly parallel attachment evident with exception of one bacteria near perpendicular attachment: X8,000. B. Attachment near rough surface: X8,000.

FIGURE 16. ATTACHMENT OF BACTEROIDES SUCCINOGENES S85 ON WHATMAN FILTER PAPER AND KENTUCKY BLUEGRASS.
A. Whatman filter paper no. 1. Attachment was predominately on surface: X8,900. B. Kentucky bluegrass. Attachment at end of fibers: X8,900.

TABLE 34. SOLUBLE SUGARS, PH AND DRY MATTER OF CORN SILAGE OVER TIME.[a]

Time	pH	D.M.	Xylose	Mannose	Glucose	Total
hr		%	%	%	%	%
0	5.5	33.7	0.06	1.81	4.71	6.58
6	5.6	36.2	0.17	3.20	5.30	8.67
12	5.6	35.3	0.21	3.70	8.64	12.55
18	5.2	33.3	0.28	1.44	8.28	10.00
24	4.8	33.9	0.29	2.64	11.37	14.30
36	4.3	35.3	0.15	4.32	10.84	15.31
48	3.9	32.2	0.06	4.72	4.46	9.24
72	3.7	32.7	0.05	8.10	2.79	10.94
96	3.7	32.8	0.04	4.62	2.21	6.87
144	3.8	33.9	0.04	1.25	0.00	1.29
312	3.6	34.1	0.04	0.67	0.00	0.71

[a]Each value is expressed as a percent of the freeze-dried sample.

DISCUSSION

Fiber analyses. The study of the plant cell is of great importance and has long been the object of extensive research in nutrition. Various authors have expressed the need for definitive nomenclature in order to better interpret the metabolic role of fiber in nutrition (177, 186).

Typical values for fibrous components in the 20 feeds and forages as determined by neutral detergent (NDF), acid detergent (ADF), potassium permanganate or detergent cellulose, detergent hemicellulose and permanganate lignin were presented in Table 3. These methods are relatively easy to perform and the values obtained describe the general physical structure of the various feeds and forages. Neutral detergent fiber represents the plant cell walls minus pectins (17), some insoluble ash (203) and possibly some arabinose (19). Acid detergent fiber includes the cellulose, lignin and insoluble ash (82). ADF has been shown to contain some hemicellulosic sugars (17,106). The difference between the two detergent fiber fractions is an estimate of hemicellulose which can be either overestimated or underestimated depending on the substrate (203). Oxidation of lignin in the acid detergent fiber with potassium permanganate and ashing at 493 C will yield values for lignin, cellulose and insoluble ash.

Studies have been conducted to examine the variability of the

detergent method between laboratories (202). Results indicated problems with filtration, uniform weighing procedure, and reliable preparation of asbestos used in the preparation of lignin. The results in Table 3 were similar to many previous reports (175, 178, 179, 195, 201, 208) with some exceptions. The values reported for cellulose and hemicellulose for orchardgrass were higher and lower, respectively, than values reported by Van Soest (202, 206, 208). Red clover NDF has been reported to be as high as 66% (202) compared to 26.62% in the sample in Table 3. Ladino clover values were similar to previous reports (202, 206, 208). The value for alfalfa NDF in Table 3 was similar to the values reported by Van Soest (199) and Van Soest and Mertens (209), but they reported 17.9% lignin-cutin in their alfalfa samples. They also reported a value of 49% NDF in wheat straw compared to our higher value of 79.84%. Grasses have also been shown to accumulate silica as did the samples in this study, especially quackgrass (206). In general, most of the values for the detergent fiber fractions reported in Table 3 were similar to previous reports. Part of the differences in values could be attributed to chemical technique, species and variety of the plant examined, maturity of the plant examined and sieve size of the ground sample.

Determination of fiber in feeds and forages involves the disruption of the plant cell walls to rid the plant of cytoplasm by digestion with neutral detergent (82) or with ammonium oxalate (41, 167, 213, 214). Upon comparison of the neutral detergent fiber (Table 3) and ammonium oxalate fiber values (Table 4) for the 20 feeds and forages, all NDF values were significantly higher ($P<.05$)

than their corresponding NDF values. A significant ($P<.001$) relationship between AOF and NDF in substrates was observed (Table A1). Marked differences were observed in ladino clover and red clover values. These differences in cell wall values for the substrates may have been due to losses in pectins and uronic acids (17), insoluble ash (38,204), possible losses of arabinose (19) or might represent differences in protein and ash content.

After delignification of the cell wall with sodium chlorite (41), each substrate was hydrolyzed in trifluoroacetic acid (T.F.A.A.). The percent cellulose in each substrate was determined after collecting the residue from T.F.A.A. hydrolysis. A comparison of detergent cellulose (Table 3) and T.F.A.A. cellulose (Table 4) yielded similar results in most substrates except Kentucky bluegrass, Reed canarygrass-2nd cutting, Reed foxtail, quackgrass, wheat middlings, wheat straw, algae and crown vetch which have significantly ($P<.05$) higher cellulose values as determined by T.F.A.A. hydrolysis. These differences could be due to the possible action of potassium permanganate on cellulose (66). Sullivan (178) compared 'natural' cellulose (116) and 'true' cellulose (135) and found similar differences in Kentucky bluegrass, orchardgrass and alfalfa.

A comparison of permanganate lignin and sodium chlorite lignin has shown that in general sodium chlorite lignin determinations tended to be higher in value (41). The differences between the two lignin values in part may be due to the varied complexity of the structural components of the different forages and feeds, the nature of the fiber residue after acid detergent or ammonium oxalate treatment, and

the mechanism of attack of the two oxidizing agents. Talmadge et al. (182) has described the complexity of the carbohydrate structure in the hemicellulose sugars. Lignin to carbohydrate bonding also has been investigated extensively (129, 131, 132, 221). Recently, it has been shown that the primary cell wall of wood contains bonds between lignin and hydroxyproline (222). These physical and chemical carbohydrate interactions are expected to vary among forages and feeds and will contribute to variations with a standardized analytical procedure. The starting fiber residue, either acid detergent or ammonium oxalate, also should have considerable influence on lignin. Extraction with acid detergent will remove hemicellulose leaving behind a fraction containing lignocellulose and insoluble ash (195, 196, 211). The treatment also may result in the loss of lignin-hemicellulose complexes although this effect has not been examined. Extraction with ammonium oxalate is a milder process and removes cell wall cytoplasm and some uronic acids. This leaves the holocellulose fraction virtually intact. Lastly, the mechanism of attack by chlorite and permanganate on lignin may be different, especially in regards to the starting residue. Permanganate is a stronger oxidizing agent than sodium chlorite, and its exact reaction on lignin is not known. It is believed also to effect carbohydrate components (66). Sodium chlorite oxidation of lignin, although not fully elucidated, has been examined more thoroughly (20, 57, 159) and has been used for many years in botany studies (28, 31). These results may be significant in balance studies for forage quality and passage studies with lignin as an internal marker. Indeterminate values for lignin erroneously could suggest high lignin

digestibility (39, 40) or apparent breakdown (92).

Hydrolysis of the delignified tissue with trifluoroacetic acid (T.F.A.A.) and derivatization to the corresponding alditol acetate yields a pattern of the hemicellulose sugars which include glucose, galactose, mannose, xylose, arabinose, rhamnose and possibly ribose. In these calculations, uronic acids, which may include those from pectins, were included in the hemicellulose. Uronic acids are also found as a component of the cell wall (32). Xylose, galactose and glucose were apparent in all substrates examined (Table 5). The value of wheat bran hemicellulose (Table 5) was higher than previous reports (161), but much lower than detergent hemicellulose (Table 3). Other techniques have been used to examine the structure of the plant cell wall using paper chromatography (179), gas-liquid chromatography (28, 29, 154, 155, 169, 179, 171) and densitometry (22). Binger et al. hydrolyzed the hemicellulose of orchardgrass and reported 15.6% xylose, 10.9% glucose, 4.1% arabinose, 2.4% galactose and 3.1% uronic acids. This is substantially higher than the value for orchardgrass reported in Table 5. Sullivan et al. (180) used a similar technique and found similar distributions of hemicellulosic sugars in orchardgrass, Reed canarygrass, tall fescue and Kentucky bluegrass as presented in Table 6. Orchardgrass hemicellulose was examined by colorimetry after extraction in ethanol:benzene, ammonium oxalate, sodium chlorite, alkali, and acid (213). Buchala and Wilkie (31, 32) investigated wheat straw hemicellulose with gas chromatography and reported that as the plant matures, xylose increased, and arabinose, uronic acids and glucose decreased. The results for wheat straw (31, 32) and orchardgrass (213)

were similar to the hemicellulose values as determined by GLC presented in Table 5.

The xylose:arabinose ratio has been used as an indicator of plant maturity, i.e., the ratio increases with age of the plant (31, 31). The similarity in xylose and arabinose content and in the xylose:arabinose ratio in grasses indicates possible structural similarities not apparent in other substrates. The hemicellulosic sugar composition of three hemicellulose fractions (linear A, linear B and branched B) were examined in ryegrass and red clover. These studies enhanced the understanding of _in vitro_ rumen digestibilities of the same substrates by examining the cell wall more closely (80).

Several investigators have indicated possible losses and complications in the detergent fiber system (17,19,106,130,152,204). Analysis of the NDF and ADF fractions of 20 feeds and forages showed that these fractions were not as exact as was first suggested (200). 55.2% of the grass hemicellulose and 26.5% of the legume hemicellulose was recovered in the neutral detergent fiber (Table 10). The grass ADF fractions contained 10.8% to 31.5% of the hemicellulose whereas the legume ADF fractions contained 7.1% to 50.8% of the original hemicellulose (Table 11). Kim _et al._ (106) compared ADF and CF fractions in feeds and feces and found 12% to 16% of the original pentosans in the feed ADF and 9.7% in fecal ADF. ADF has also been criticized for having more than cellulose and lignin and NDF has been criticized for having the bulk of the pectic substances removed in clovers (17). Clover ADF was reported to contain 15% uronic acids (17).

Scanning electron microscopy was used in order to examine the

effects of neutral detergent, acid detergent, ammonium oxalate, trifluoroacetic acid and sodium chlorite treatment of cell walls of wheat straw. Both neutral detergent and ammonium oxalate have been described to rid the plant cell wall of cell cytoplasm and debris (Figure 4B and 5A). Neutral detergent (a solution of sodium lauryl sulfate, disodium dihydrogen ethylenediaminetetraacetate dihydrate, disodium hydrogen phosphate, sodium borate dechydrate and ethylene glycol) appeared to open the plant cells of wheat straw as was seen in Figure 4B. Small frequent disruptions(arrows) were seen in the cell wall which may be due to the possible losses of uronic acids (17), insoluble ash (38) or arabinose (19). The cell wall of ammonium oxalate treated wheat straw (Figure 5A and 5B) appeared to have opened cell walls (arrows) with typical flaking of the cell wall, but no apparent losses. Both neutral detergent and ammonium oxalate appeared to rid the cell wall of debris.

The structure of hemicellulose in many plants has been examined extensively (21,182) and illustrated as cross-linked chains of sugars evenly distributed over a core of cellulose sheets. Acid detergent has been reported to remove the hemicellulose leaving cellulose, lignin and some ash. The chemical mode of attack as seen in Figure 6A and 6B appeared to be very localized (arrows) and may not remove all the hemicellulose. Akin et al. (9) reported acid detergent broke up cells, removing only non-lignified tissues. They defined cellulose as a linear array of D-glucopyranose units linked by β 1-4 glycosidic linkages with a degree of polymerization of 300 to 2500 units. Cellulose with a degree of polymerization less than 300 would be removed with meso-

phyll, phloem, bundle sheath and epidermal cell walls. They indicated that the removal of large numbers of cell walls by treatment with acid detergent is not consistent with the cellulose recovery reported by Colburn and Evans (38). In further studies, Barton and Akin (19) reported that ADF differed for representative grasses from warm and cool climates. In bermudagrass, some digestible tissues remained in the ADF. NDF was essentially the cell walls in an undegraded form in both bermudagrass and tall fescue.

The action of sodium chlorite is to oxidize the lignin of a plant cell wall without extracting carbohydrates from cellulose and hemicellulose. Figure 8A shows the cell wall of sodium chlorite delignified wheat straw. The cell wall showed no disruptions and appeared to be evenly extracted. After trifluoroacetic acid hydrolysis, cellulose is collected in a crucible, dried and weighed. Figure 8B illustrates wheat straw T.F.A.A. cellulose with typical cellulose sheets (arrows).

The use of detergent in analyzing for fiber in feeds and forages offered quick, easy and repeatable methodology, however, possible losses and complications have been described. The chemical action of acid detergent appears to be unsatisfactory for yielding a fraction of only cellulose as the major carbohydrate component, however, it does provide a repeatable measure of fiber in animal rations. The alditol acetate derivatization system for feeds and forages can be used to analyze for cellulose, hemicellulose, hemicellulosic sugars and uronic acids and lignin. It offers additional structural information about the fiber components in the plant cell wall and compares favorably

with the detergent system (Table 7 and 8). Previous investigators (28,29,31,32,118,170,171,223) have attempted to analyze plant cell walls for individual components as determined by gas-liquid chromatography. The preparation of the plant cell wall varied between investigator, but each used sulfuric acid to hydrolyze the hemicellulose to individual sugars. In addition to hydrolyzing hemicellulose, sulfuric acid hydrolyzed cellulose to individual glucose units, so an additional method was used to account for cellulose in each sample. Meinert and Delmer (118) and Talmadge et al. (182) reported that 97% of the cellulose was resistant to hydrolysis in trifluoroacetic acid (T.F.A.A.). Our results showed that the cellulose recovered after T.F.A.A. hydrolysis contained 97% glucose, no ash and no protein. Morrison (129) used trifluoroacetic acid to hydrolyze plant cell walls, but the hydrolysis time (16 hr) used appears to be too long according to Albersheim et al. (11) and our results as reported in Figure 2. Lignin has been shown to be in close association with cellulose, hemicellulose and cell wall protein (88,129,131, 132,221,222). Hydrolysis of the cell wall in sulfuric acid or in T.F.A.A. before delignification has appeared unsatisfactory (170). Many investigators have chosen to delignify with sodium chlorite (28, 29,31,32,72,223), although Barton (20) stated that lignin treated with 72% sulfuric acid dissolved readily. Delignification in permanganate appears to be too harsh due to possible losses in cellulose (66), but sodium chlorite appears mild enough not to attack plant cell wall structural carbohydrates (3,4,5). The results presented in Table 5 indicated losses in cell wall carbohydrates with neutral detergent.

It then may be important to use a less harsh solution such as ammonium oxalate to remove cell wall cytoplasm. Waite and Gorrod (213) used ammonium oxalate and sodium chlorite to isolate plant cell walls however continued their hemicellulosic analysis with a hydrolysis in sulfuric acid for 2 hr. The use of sodium chlorite for the determination of lignin would be enhanced if the isolated cell walls were free of protein. The data in Tables 12, 13 and 14 indicate loss in protein after treatment with sodium chlorite. Waite and Gorrod (213) suggested a digestion in pepsin-hydrochloric acid which should be examined further. Cell wall fractionation methods have involved many transfers of the plant material (20,135,136,154,1554,171,213) without calculation of water absorption (Figure 1) by the plant cell wall. This may be a further complication of some fractionation procedures. Fractionation of plant cell walls using sodium chlorite for delignification and GLC for sugar analysis appears to have advantages over previous techniques in time (42,44), cost (Table A8) and overall additional information about the plant cell wall. Some complications do exist, however, with further modifications these can be overcome. The detergent fiber system has been modified (165) to overcome filtration problems and should remain for quick product evaluation, for evaluation of feeds and forages or for pilot nutritional studies.

Fiber digestibility trials. The digestion of fiber by many animals has been extensively studied. The development of fiber component analyses for nutritional studies have depended upon the particular organism being studied. Early studies used the crude fiber (CF) method (91), but this eventually proved unsatisfactory (25,89,91,113,

123,140,149,150,185,224). Cellulose, a major constituent of the plant cell wall, was next used to predict fiber digestibility (116). Matrone et al. (116) suggested that cellulose could be used to evaluate feedstuffs because of its close association with other polysaccharides in the plant cell wall. This technique did not consider the importance of hemicellulose and lignin to the digestibility of fiber.

Walker and Hepburn (216) first proposed in a series of articles the use of a fiber fraction they called normal-acid fiber (NAF). This fraction represented cellulose, lignin, some protein and some ash. They found NAF digestibility was always lower than the digestibility of crude fiber (215). High correlations between the percent NAF in forages with dry matter digestibility were reported (98). This method failed to acknowledge the importance of hemicellulose as a major constituent of fiber and had a variable composition of protein and ash.

Van Soest and others (194,195,196,197,198,200,207,209,211) suggested the use of detergents in the analysis of fiber. They proposed that fiber should represent substances resistant to animal enzymes. Total fiber was extracted in neutral detergent in a fraction called neutral detergent fiber (NDF) or cell wall constituents (CWC). Another fraction representing lignocellulose called acid detergent fiber (ADF) was isolated in acid detergent. This fraction represents a residue which is closely associated with indigestibility (195,196). Cellulose, lignin and insoluble ash (silica) are determined after oxidation with potassium permanganate and ashing. Hemicellulose is

determined as the difference between the NDF and ADF. This fraction is just an approximation, but has been used as a quantitative value in the literature. Several problems exist with each fraction, which have been mentioned earlier, that suggest that these methods are not completely satisfactory in fiber evaluation studies. It does provide quick, easy and repeatable methodology for digestion trials, however, lignin digestibilities (18,39,40,52,68,71,101,178) have been reported and the hemicellulose value does not help evaluate the effects of this fraction upon digestibility.

Fiber as determined by ammonium oxalate significantly correlated with neutral detergent fiber of all 20 substrates (Table A1). The digestibility of AOF was also significantly ($P<.005$) correlated with NDF digestibility in all species of animals tested (Table A4). Examination of feed and feces samples in the digestion trials showed that AOF values were always higher than NDF values. This is probably due to higher protein and ash content of AOF (Table 14). It may be advisable to treat the AOF residue with pepsin-hydrochloric acid (73,213) to decrease the protein content. This remains to be tested.

Large differences were observed in hemicellulose values in all digestion trials. Hemicellulose as determined by detergent analyses appeared too high in comparison to the corresponding T.F.A.A hemicellulose value. It might be suggested that T.F.A.A. hemicellulose underestimates hemicellulose due to improper hydrolysis, methylation, acetylation, ammonium oxalate digestion or sodium chlorite oxidation. Examination of these parameters suggest no substantial losses of hemicellulosic sugars. Hydrolysis in trifluoroacetic acid longer than

60 min appears to cause breakdown of sugars (Figure 2) which agrees with a previous report (11). The collected residue contains 97% glucose and appears to represent the cellulose of the cell wall because very little glucose was found in the hemicellulosic sugars. This cellulose value was, in general, similar to cellulose as determined by oxidation in potassium permanganate. Ammonium oxalate has been used for cell wall cytoplasm extraction because of its mild action. Uronic acids were not determined in the digestion trials because of their reported high digestibility (214). Sodium chlorite was also used because of its mild oxidation of the cell wall. Very little of the hemicellulosic sugars are removed (19,31,32,57,159). It appears that the higher hemicellulose values for the detergent system are due to the suggested inacurracies stated above (8,17,38, 42,43,44,106) which need to be further elucidated. Detergent holocellulose and total fiber, when calculated by summation of fiber fractions, were always higher than corresponding MSU T.F.A.A. values. This is largely due to the higher hemicellulose values determined by these methods.

The digestibility of hemicellulose fractions did not correlate in all species (Table A4), however, they did correlate in the beef cattle and swine digestion trials. Hemicellulose digestibility, as analyzed by GLC, also examines the digestibility of each individual component. Arabinose digestibility was the highest of all sugars in the ponies whereas glucose digestibility was highest in the beef cattle. Glucose digestibility varied with age in pigs, but in general was the highest. All sugars were readily digested by pigs, but in

the starter pigs, xylose and arabinose digestibility was lower. Hemicellulose as a percent digestible versus actual amount digested was highest in the pigs. This is in agreement with previous research (103) in the examination of fiber digestibility of swine, voles, sheep, horses and rats. Morris and Bacon (126) followed the procedures of Sloneker (171) with sulfuric acid and reported that the digestibilities of hemicellulosic sugars found in grass decreased in the following order: arabinose, galactose, glucose and xylose. This is similar to the digestibilities reported in Table 20, although in corn silage, glucose was the most digestible. These differences could be due to cell wall structure, animals, microflora or analysis technique.

Lignin content, as determined by either potassium permanganate or sodium chlorite, were not satisfactory in digestion trials. Calculated digestibilities for lignin were high in each trial which suggests the lack of reliability of it use as a marker in passage studies. This is in agreement with previous studies which have reported high lignin digestibility (18,39,40,52,68,71,101,178). Ammonium oxalate treated with pepsin-hydrochloric acid (213) may prove to rid the error in chlorite lignin due to protein. Lignin as calculated by methoxyl number does not appear satisfactory in dogs or cows (50). Acetyl bromide may prove to be a better alternative to gravimetric methods.

Fiber has been approached as: (1) the indigestible portion of the dietary fiber (207), (2) the most indigestible fraction of the plant cell wall (98,153,207,215,216), (3) the fractionated plant cell

wall (157) and (4) the soluble and insoluble complex polymers associated with the plant cell wall (19,173,174). It appears unlikely that any chemical procedure will yield a totally indigestible fraction and still examine all fiber components. ADF and NAF were supposed to be highly indigestible, but high digestibilities have been reported in sheep (104), horses (104,105), swine (39,40,105), vole (103), rats (105) and the digestion trials described above. Fiber has also been reported as that which is resistant to gastrointestinal enzymes (186), however, this ignores the possible utilization of fiber components by intestinal bacteria (26,27,158,212). It therefore appears to be adviseable to fractionate the plant cell wall into chemically distinct components and examine each of these individually in digestion trials and in vitro studies. Many distinct components could be examined, but cellulose, hemicellulose, lignin and pectin are of major importance. Fiber as determined with detergents could remain for quality control or for pilot nutritional studies whereas GLC components as determined with trifluoroacetic acid could be used to determine the effect of a particular fibrous substrate upon utilization of nutrients or microbial fermentation in the gastrointestinal tract.

Pure culture. The utilization of fiber in the intestinal tract of a monogastric animal and in the reticulo-rumen of ruminants has been shown to involve a close association with cellulolytic bacteria (6,7,26,27,97). The utilization of a particular substrate depends upon plant cell wall structure (55,56,80). Coen and Dehority (37) found that digestibility varied with maturity and type of plant and bacterial species. They reported that B. succinogenes digested

greater amounts of hemicellulose when compared to R. flavefaciens. R. flavefaciens digested more hemicellulose in four of the six substrates when compared to B. succinogenes as shown in Table 33. Hemicellulose digested B. succinogenes was greater in filter paper and alfalfa. Coen and Dehority (37) determined hemicellulose as total pentosans. In disagreement with their previous report, R. flavefaciens digested 32.2% and 10.3% of bromegrass and alfalfa hemicellulose whereas B. succinogenes digested 13.3% and 0.0%, respectively (56). They also reported B. succinogenes was able to digest more cellulose than eight other rumen bacteria. In disagreement with these results, cellulose fermented as shown in Table 33 was greater in R. flavefaciens than B. succinogenes. Manure fiber and filter paper were least digested by R. flavefaciens and B. succinogenes, respectively. Arabinose and galactose content of the cell wall was significantly correlated ($P<.05$) with fermentability in R. flavefaciens (Table A6). No relationship was found in B. succinogenes (45).

Electron microscopy studies indicated surface adherence of both bacteria onto the plant cell walls. Two modes of bacterial attack were visualized by Dinsdale et al. (58): (1) tunneling and (2) erosion of surfaces. R. flavefaciens adhered on the surface of wheat straw near exudated debris (Figure 12A). Chains of bacteria were found on the surface with exudated debris (Figure 12B). Attachment of R. flavefaciens on alfalfa appeared near the ends of fibers and surface (Figure 13A). Stringlike particles were observed with attachment of R. flavefaciens to corn silage (Figure 14B). R. flavefaciens did not adhere onto filter paper and cattle fiber. B. succinogenes

adhered onto the surface of alfalfa (Figure 15A and 15B). Adherence of B. succinogenes to filter paper was predominately on the surface (Figure 16A) whereas the bacteria adhered to Kentucky bluegrass primarily at the end of the fibers. B. succinogenes did not attack cattle fiber and wheat straw.

Latham et al. (108,109) reported that B. succinogenes adhered to only the cut edges of ryegrass and to intact mesophyll whereas R. flavefaciens adhered to cut edges of epidermal cell walls. They suggested that adhesion was similar, but there were different affinities for each cell wall.

Leatherwood (110) proposed R. albus may have an affinity factor which is necessary to hold the factor of cellulase in position to the insoluble cellulose for multiple attacks to occur. Such a phenonomenon may be required for hydrolysis of the walls more resistent to bacterial degradation where attachment precedes degradation.

The results of the fermentation of R. flavefaciens suggest the use of arabinose and galactose in preference to other hemicellulosic sugars. It was observed that R. flavefaciens did not attach to filter paper and cattle fiber, two substrates which we found to be low in arabinose and galactose. It may also be possible that arabinose and galactose facilitates the attachment of R. flavefaciens, leading to the attack on the cell wall. This may be the affinity factor mentioned by Latham et al. (108,109). The appearance of stringlike debris near to attached bacteria appear similar to waxes seen by electron microscopy. Other possibilities include lectins such as in Rhizobia (53) or by sugars as in E. coli (12, 142). All of these remain to

be proven in further studies.

GLC analyses of plant cell walls fermented in pure culture indicate preference of hemicellulosic arabinose and galactose by R. flavefaciens. GLC analyses appears to enhance the study of the utilization of fiber by rumen cellulolytic bacteria.

Corn silage analyses. GLC analyses of the freeze-dried soluble sugars shown in Table 34 indicated solubilization of sugars up to 24 hr then utilization of these sugars for the remaining fermentation. PH steadily decreased during fermentation. Variability between samples makes it difficult to discern trends however most of the sugars are utilized by the end of the fermentation. GLC analyses of soluble sugars can be an aid to understanding silage fermentation, but further studies need to be designed to study this further.

CONCLUSIONS

Within the limits of the experimental conditions and procedures of the experiments presented herein, the results of this study have led the auther to make the following conclusions:

1. Gas-liquid chromatographic (GLC) analyses of fiber components of 20 feeds and forages was comparable to the fiber component values determined by the detergent fiber system with the exception of hemicellulose.

2. GLC analyses of the detergent fiber fractions of the 20 feeds and forages indicated substantial losses of cell wall components treated with neutral detergent and substantial hemicellulosic sugars recovered in cell walls treated with acid detergent.

3. Lignin, as determined by gravimetric methods, appears to be unreliable as a marker for digestibility or passage studies. Chlorite lignin values may prove adequate if samples are first treated with pepsin-hydrochloric acid. Sodium chlorite is adequate for delignification of the cell wall for further structural analyses.

4. GLC analyses of fiber components in digestion studies with beef cattle, pigs and ponies enhance the examination of fiber and are comparable with detergent fiber digestibility values.

5. Direct fiber component analyses by GLC appears to be a useful technique for determining the composition of fiber, differentiat-

ing the availability of fiber components, and characterizing the specificity of the attacking bacterial species.

6. R. flavefaciens C94 may have a preference for cell walls which contain arabinose and galactose.

7. Examination of freeze-dried soluble carbohydrates of corn silage indicates solubilization of sugars up to 24 hr of fermentation and utilization thereafter.

APPENDIX

TABLE A1. CORRELATION COEFFICIENTS OF FIBER FRACTIONS OF ALL SUBSTRATES.

Comparison	r	P level
NDF x ADF	0.75	0.001
AOF x NDF	0.64	0.01
AOF x ADF	0.74	0.001
Detergent hemicellulose x MSU hemicellulose	0.31	ns
Detergent cellulose x MSU cellulose	0.87	0.001

TABLE A2. CORRELATION COEFFICIENTS OF MSU T.F.A.A. FIBER FRACTIONS OF ALL SUBSTRATES.

Comparison	r	P level
Hemicellulose x glucose	0.58	0.01
Hemicellulose x arabinose	0.83	0.001
Hemicellulose x xylose	0.86	0.001
Glucose x arabinose	0.71	0.001
Rhamnose x uronic acids	0.64	0.01
Rhamnose x mannose	0.55	0.01
Xylose x arabinose	0.67	0.001

TABLE A3. CORRELATION COEFFICIENTS OF MSU T.F.A.A. FIBER FRACTIONS IN GRASSES AND LEGUMES.

Comparison	Grasses		Legumes	
	r	P level	r	P level
Hemicellulose x glucose	0.66	0.05	0.83	0.05
Hemicellulose x galactose	0.70	0.05	0.91	0.005
Hemicellulose x arabinose	0.30	ns	0.97	0.001
Hemicellulose x xylose	0.86	0.005	0.97	0.001
Galactose x glucose	0.95	0.001	0.54	ns
Galactose x arabinose	-0.35	ns	0.97	0.001
Xylose x glucose	0.21	ns	0.80	0.05
Xylose x galactose	0.30	ns	0.92	0.005
Xylose x arabinose	0.50	ns	0.98	0.001

TABLE A4. CORRELATION COEFFICIENTS OF FIBER DIGESTIBILITY COEFFICIENTS.

Comparison	Pony	Pigs	Cattle	All species
NDF x AOF	0.99^{****}	0.80^{*}	0.99^{***}	0.92^{***}
NDF x ADF	0.93	0.92^{***}	0.99^{***}	0.96^{***}
ADF x AOF	0.92	0.93^{***}	0.99^{***}	0.87^{***}
MSU hemicellulose x Detergent hemicellulose	-0.79^{****}	0.70^{*}	0.97^{**}	0.12^{***}
AOF x DM	0.99^{****}	0.59^{**}	0.99^{***}	0.92^{***}
NDF x DM	0.99^{****}	0.89^{**}	0.99^{***}	0.86^{***}

Interaction of variables significant: $^{*}(P < .05)$, $^{**}(P < .02)$, $^{***}(P < .005)$ and $^{****}(P < .001)$.

TABLE A5. CORRELATION COEFFICIENTS OF FIBER FRACTIONS FERMENTED IN PURE CULTURE ANALYSIS.

Comparison	R. flavefaciens, C-94		B. succinogenes, S-85	
	r	P level	r	P level
Hemicellulose x arabinose	0.90	0.01	0.53	ns
Glucose x xylose	0.84	0.05	0.13	ns
Arabinose x xylose	0.40	ns	0.96	0.001

TABLE A6. CORRELATION COEFFICIENTS BETWEEN FIBER COMPONENTS IN SAMPLE AND DIGESTIBILITY IN PURE CULTURE ANALYSIS.

Comparison	R. flavefaciens, C-94		B. succinogenes, S-85	
	r	P level	r	P level
Cellulose in x cellulose dig.	-0.64	ns	-0.59	ns
Hemicellulose in x hemicellulose dig.	0.90	0.01	-0.40	ns
Glucose in x glucose dig.	-0.28	ns	0.32	ns
Arabinose in x arabinose dig.	0.87	0.05	0.70	ns
Galactose in x galactose dig.	0.76	0.05	0.52	ns
Xylose in x xylose dig.	0.34	ns	0.19	ns

TABLE A7. ABBREVIATIONS AND DEFINITIONS.

1.	ADF	Acid detergent fiber
2.	AOF	Ammonium oxalate residue or fiber
3.	B.	Bacteroides
4.	CF	Crude fiber
5.	CWC	Cell wall constituents
6.	CWS	Cell wall solubles
7.	Detergent hemicellulose	NDF minus ADF
8.	Detergent holocellulose	Detergent hemicellulose plus detergent cellulose
9.	Detergent total fiber	Detergent holocellulos plus permanganate lignin
10.	GLC	Gas-liquid chromatography
11.	HPLC	High performance liquid chromatography
12.	LCC	Lignin-carbohydrate complex
13.	LHC	Lignin-hemicellulose complex
14.	NAF	Normal acid fiber
15.	NFE	Nitrogen-free extract
16.	PDB	Partially digestible biopolymers
17.	PDPP	Partially digestible plant polymers
18.	R.	Ruminococcus
19.	SEM	Scanning electron microscopy
20.	TEM	Transmission electron microscopy
21.	T.F.A.A	Trifluoroacetic acid
22.	T.F.A.A. holocellulose	T.F.A.A. hemicellulose plus T.F.A.A. cellulose
23.	T.F.A.A. total fiber	T.F.A.A. holocellulose plus chlorite lignin
24.	TMS	Trimethylsilyl

TABLE A8. COST COMPARISON BETWEEN THE DETERGENT FIBER SYSTEM AND THE MSU T.F.A.A. FIBER SYSTEM.

Detergent		MSU T.F.A.A.	
Chemical	Cost/10 samples, $	Chemical	Cost/10 samples, $
Sodium lauryl sulfate	0.81	Ammonium oxalate	0.10
Disodium dihydrogen EDTA	0.88	Trifluoroacetic acid	2.03
Disodium hydrogen phosphate	0.08	Methanol	1.31
Sodium borate decahydrate	0.05	Acetic anhydride	0.55
Decalin	1.08	Sodium chlorite	0.04
Ethylene glycol	0.04	Glacial acetic acid	0.12
Hexadecyltrimethyl-bromide	1.40	Ascorbic acid	0.63
Sulfuric acid	0.10		
Potassium permanganate	0.18		
Ferrous nitrite	0.02		
Glacial acetic acid	5.97		
Potassium acetate	0.02		
Tertiary butanol	4.57		
Ethanol (95%)	1.49		
Oxalic acid dehydrate	0.07		
Hydrochloric acid	0.24		
Ethanol (80%)	1.70		
Total cost	18.70		4.78

All chemicals were purchase from Fisher Scientific Co. with the exception of sodium chlorite, which was purchased from ROC/RIC.

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VITA

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1976-1979	Graduate work in Animal Husbandry, Michigan State University, East Lansing, Michigan
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1978	Graduate competition award (First place) at Midwestern Animal Science Society meetings, Carbondale, Illinois
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