CHEMICAL AND PHYSICAL CHANGES ASSOCIATED WITH MATURITY OF DIFFERENT PLANTS AND ENHANCEMENT OF NUTRITIONAL VALUE BY CHEMICAL TREATMENT OF CROP RESIDUES

by

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(ABSTRACT)

Experiments were conducted to study the chemical changes and microanatomical characteristics associated with maturity, rate and extent of DM digestion, behavior and extent of tissue digestion and mode of microbial attack during simulated ruminal digestion. Barley and millet plants were collected at four different stages of development: vegetative, boot, heading and mature. Berseem was harvested at three different stages of development: vegetative, bud and full bloom. For the first three stages of grasses and legumes, the plants were separated into three botanical fractions: leaf blades (leaflets), leaf sheaths (petioles) and stems. Grasses were harvested at mature stage and barley

seeds were separated by thresher and millet seed by hand cutting the head. Straws were chopped in a hammer mill through a 2.5 cm screen and treated: 1) no added water (control), 40% added water, 2) alone or with 3) urea, 4) NH_4OH and 5) H_2O_2 at pH 11.5.

Grasses showed higher ($P<.05$) cell wall constituents, xylose, p-coumaric and ferulic acid and lower ($P<.05$) IVDMD values for stems and leaf sheaths than leaf blades. Legume showed higher ($P<.05$) values for cell wall constituents and xylose for stems, compared to petioles and leaflets.

At advanced maturity, scanning electron microscopy (SEM) showed increased lignification and decreased tissue digestion for leaf sheaths and stems, compared to leaf blades. Lignification was higher in millet leaf sheaths than barley leaf sheaths. Within berseem plant parts, petioles showed better digestion than leaflets and stems.

The contents of ADF, cellulose and lignin were increased ($P<.05$) and hemicellulose was decreased ($P<.05$) in alkaline H_2O_2-treated straws, compared to untreated, NH_3- and urea-treated straw. Total phenolic acids were decreased ($P<.05$) in treated, compared to untreated straw. In vitro DM digestibility was increased ($P<.05$) for all chemical treatments, with larger ($P<.05$) increase for NH_3 treatment than alkaline H_2O_2 treatment.

DEDICATION

The author wishes to dedicate this work to her mother, Saeeda Naseer.

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CHAPTER I

INTRODUCTION

Forages are herbaceous plants or plant parts, high in lignocellulosic materials, fed to domestic animals, usually ruminants. Ruminants, by virtue of symbiosis with microorganism in forestomach and large intestine, can utilize lignocellulosic-rich materials as feed. It has been estimated in the U.S. forages provide 80% of the feed units for ruminants (Hodgson, 1978).

Quality of forages, which is determined by the response of animals to consumption of forages, is an important factor associated with level of animal production. Nutritive value of forages is determined by chemical composition, digestibility and nature of digested products. Generally, the quality of forage declines with physiological maturity.

With advancing plant maturity, there is a decrease in crude protein and increase in structural carbohydrates and lignin. Voluntary intake and digestibility of forages is negatively correlated with cell wall components. Electron microscopic studies have revealed that microanatomical differences, site and extent of lignification and nature of phenolic compounds play significant roles in the biodegradation of forages.

In developing countries, the cultivable land is limited and the cost of grain is too high to feed substantial amounts to livestock. Cereal straws and other roughages are the main feed for ruminants. The low-protein and high-fiber content of straw contribute to their low nutritive value, hence limiting its use as ruminant feed. Chemical treatments have been used extensively to improve the nutritive value of cereal straws and other roughages.

Experiments were conducted to 1) evaluate the effect of plant maturity on chemical composition and rate and extent of DM digestion in different plant portions and botanical fractions, 2) study the microanatomical differences, extent of tissue digestion and mode of attack by rumen microbes, using SEM and TEM techniques, and 3) assess the effect of chemical treatments on the nutritive value of straws.

CHAPTER II

REVIEW OF LITERATURE

Forage plants consist of two main constituents, cell solubles and cell walls. The cell solubles include sugars, starch, protein, organic acids, lipids, pectins, and minerals, whereas the cell wall components include structural carbohydrates (cellulose and hemicellulose) and lignin. Evidence is also accumulating indicating the presence of phenolic monomers (2 to 5% of lignin) linked to the cell wall carbohydrates in esters and (or) glucosidic bonds (Harris and Hartley, 1976).

Plant Cell Walls

The cell wall is a dynamic structure, its composition and properties constantly changing in response to growth, stage of differentiation and environment (Northcote, 1972). In plants, cell walls are composed of three distinct layers, middle lamella, the primary wall and the secondary wall (Figure 1). The middle lamella, composed mainly of pectin, forms an amorphous intercellular layer between the primary walls. The primary wall is thin and develops first in growing plants. It contains loosely arranged cellulosic microfibrils embedded in a matrix of noncellulosic

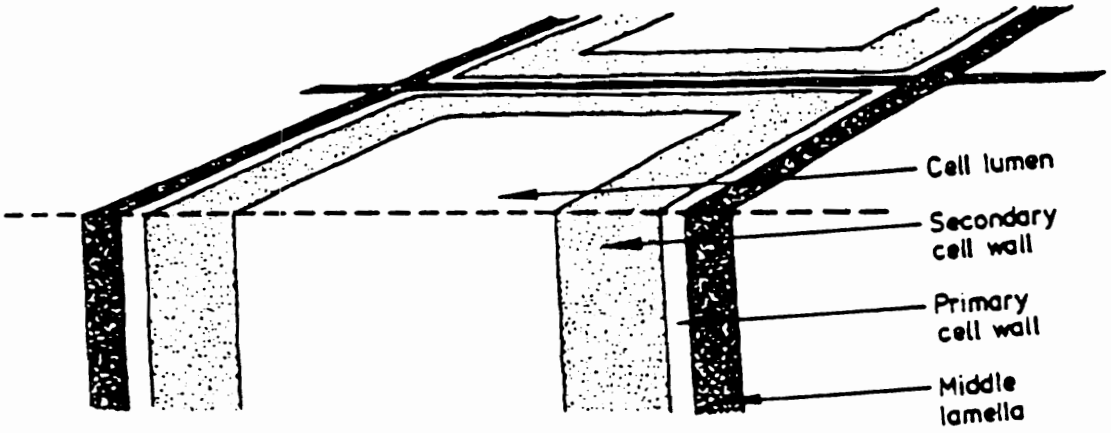

Figure 1. Schematic diagram of a mature plant cell wall (Theander and Aman, 1984).

polysaccharides (hemicellulose and pectin) and structural proteins (Albersheim, 1975). During the maturation of plants there is an increase in the thickening of the cell wall by deposition of highly oriented cellulose and hemicellulose, along with lignification to form secondary wall (Jones, 1970).

The plant cell wall consists of three types of structural polysaccharides, namely, cellulose, hemicelluloses and pectic polysaccharides, classified into two main groups, the matrix and the fiber polysaccharides (Northcote, 1972). The fiber polysaccharides are cystalline and present as cellulose microfibrils held together by various bonds in matrix polysaccharides (hemicellulose and pectin), lignin and some protein to form cell wall.

Cellulose. Cellulose, the most abundant carbohydrate, comprising 20 to 50% of the DM of most plants (Van Soest, 1973), is a linear polymer of β1, 4 linked D-glucose units (Bailey, 1973). Generally, cellulose is present in plant tissues as fibers composed of microfibrils, consisting of cellulose chains aligned along the microfibril axis and held together by inter- and intramolecular hydrogen bonding. This arrangement of microfibrils in a perfect three dimensional array gives rise to a crystal structure (Goodwin and Mercer, 1985). The cellulose crystallinity explains its mechanical

strength as well as its resistance both to biological degradation and chemical hydrolysis (Theander and Aman, 1984) and variation in its nutritive value (Van Soest, 1973).

Hemicelluloses. The term hemicelluloses denotes a wide variety of polysaccharides, namely, xylans, mannans and galactans (Wilkie, 1979). The hemicellulosic polysaccharides are composed of different monosaccharides, including xylose, arabinose, galactose, rhamnose, mannose, glucose, galacturonic acid, glucuronic acid, and the 4-O-methyl ether of glucuronic acid at highly variable concentrations.

Plant hemicelluloses are composed of two types of fractions, hemicellulose A, less bonded and often high in xylans, and hemicellulose B, branched fraction and often high in arabinose and (or) uronic acid (Wilkie, 1979).

Xylans from grasses and legumes generally have a backbone of β-1,4 linked xylopyranosyl units (Theander and Aman, 1984). They form the bulk of the hemicellulose in annual herbaceous plants (Bailey, 1973). The monosaccharides, L-arabinose, D-galactose, D-glucuronic acids and possibly D-glucose, are usually present as a single unit side chain and (or) at the terminus of the chain (Bailey, 1973). Linkages between arabinose and xylose are 1,3, while between uronic acid and xylose they are either 1,2, 1,3 or 1,4. Generally arabinose serves as the branch point in longer chains.

The structure of mannans, glucomannans and galactomannans, reveals a backbone chain of β-1,4 linked D-glucose and D-mannose, respectively (Goodwin and Mercer, 1984). These polymers are characteristic of legumes (Van Soest, 1984).

Variations in Herbages Polysaccharides. Variation in herbages exists at two levels, firstly in the types of polysaccharides, and secondly in the amounts of fractions (Bailey, 1973). Differences in the type and amount of polysaccharides may be attributed to: a) growth and development of plant (cell wall), b) plant botanical fractions (anatomy), c) types of plants (grasses, legumes, etc.), and d) agronomic conditions.

In young plants, short chain branched heteroxylans (hemicellulose-B) form the main bulk of hemicellulose (Morrison, 1980). Throughout growth and between plant species and varieties, the branched xylans show consistent composition. As the plant matures, the concentration of hemicellulose A increases, compared to hemicellulose-B fraction. The ratio of xylose to arabinose increases considerably during maturation in perennial ryegrass (Lolium perenne L. cv. S24), cocksfoot (Dactylis glomerata L. cv. S26), timothy (phleum pratense L. cv. Scots) and meadow fescue (Festuca pratensis L. cv. S215) (Morrison, 1980), Russian

wild rye (Elymus juceus Fisch.), smooth brome grass (Bromis inermis L.) and reed canary grass (Phalaris arundinacea L.) (Burritt et al., 1984), timothy (P. pratense L. cv. Bottnia II) (Lindgren et al., 1980), alfalfa (Medicago sativa L.) and orchardgrass (Dactylis glomerata L.) (Wedig et al., 1986). The increased concentration of xylose and increase in the ratio of xylose to arabinose (X:A) reflected an increase of linear xylan, relative to branched xylan, and also an increased proportion of linear hemicellulosic polymer to branched polymer (Morrison, 1980).

Gaillard (1965) reported that the hemicellulose-A fraction from grasses was composed of arabino-xylan and a low concentration of uronic acid. The hemicellulose-A fraction from legumes contained arabinose-free 4,0-methyl uronic acid xylan. The amount of hemicellulose-A fraction was higher in grass stem than legume stem (Whistler and Gillard, 1961). Further, they reported that xylans were responsible for lower digestibility of grass stems, compared to legume stems. Galacto- and glucomannans (Gaillard and Bailey, 1968), and fucose (Nevins et al., 1967) were found only in legumes. These hexoses are, however, also present in cereal seeds, thus imparting a high viscosity when cereals are cooked.

Differences in structural carbohydrates within grasses are due to the levels or proportions of total fractions

(Bailey, 1973). Generally, tropical grasses have higher levels of cellulose and hemicellulose than temperate grasses (Butterworth, 1967). Ojima and Isawa (1968) found differences in the proportions of xylose, arabinose, glucose and galactose, the hemicellulosic monosaccharides, between coastal bermuda grass (Cynodon dactylon L.) and perennial ryegrass (74:12:11:2 vs 50:15:30:2, respectively). They suggested that higher concentrations of xylose in tropical grasses are responsible for lower digestibility.

Lignin Biosynthesis. Lignin, a complex highly ramified polymer of phenylpropane (C_6-C_3) residues, is the second most important organic component of the cell wall. It is widely distributed in mature plants (Jung et al., 1983). Its occurrence is associated with the presence of supporting and conducting tissues in vascular plants (Sarkanen and Ludwig, 1971). In secondary cell walls the deposition of lignin in the polysaccharide components prevents the growth of plants and (or) plant parts (Northcote, 1972). Lignin does not appear to occur in primary cell walls, but primary precursors (phenolic monomers) are widely distributed (Harris and Hartley, 1980).

Lignin arises from the oxidative polymerization of three precursor alcohols, p-coumaryl, coniferyl and sinapyl alcohols (Sarkanen and Ludwig, 1971). Aromatic amino acids,

phenylalanine and tyrosine, give rise to the three precursor alcohols (Harkin, 1973). The pathway of lignin biosynthesis is well established. Phenylammonia lyase, which catalyzes the conversion of phenylalanine to cinnamic acid is present in almost all types of plants. Thus it plays a key role in initiating phenolic acid metabolism. Cinnamic acid is further decarboxylated by cinnamic acid-4-hydroxylase to p-coumaric acid. Brown and Neish (1956) suggested that in monocot grasses p-coumaric acid may also be formed from tyrosine deamination in the presence of tyrosine ammonia lyase.

Through a series of reactions involving hydroxylation, methylation and reduction, p-coumaric acid is converted to cinnamyl alcohols. The final step in lignin biosynthesis involves the oxidation of cinnamyl alcohols to free radicals by peroxidase/H_2O_2, followed by random non-enzymatic polymerization involving ether and C-C linkages (Vance et al., 1980). Enhanced lignin synthesis is often associated with lignin synthetase enzyme activity (Vance et al., 1980) and the production of H_2O_2 at appropriate sites (Stafford, 1962).

Occurrence of Lignin. Plant lignins are classified into three categories: gymnosperm (soft wood), angiosperm (hard wood) and monocotyledenous lignin (Nimz, 1972). Soft wood

lignins (~15 to 16% methoxyl content) are derived from coniferyl (80%), p-coumaryl (14%) and sinapyl alcohol (6%). In contrast, hard-wood lignins (~21 to 22% methoxyl content) contain coniferyl (56%), p-coumaryl (4%) and sinapyl alcohol (40%) (Nimz, 1972). Grass and herbage lignin (variable methoxyl content) contains mainly p-coumaryl units (Freudenberg and Harkin, 1963).

The three categories of lignins are best differentiated on the basis of nitrobenzene oxidation products. Sarkanen and Ludwig (1971) reported vanillin with some p-OH benzaldehyde from gymnosperms, syringaldehyde and vanillin from angiosperm, and significant amounts of all three aldehydes from grass lignin.

Variation in Herbages Lignin Content. Legumes, in general, contain higher concentrations of lignin than grasses (Van Soest, 1975). Jung et al. (1983) reported higher concentrations of syringaldehyde and vanillin followed by syringic acid, vanillic acid and protocatechuic acid when plant lignin was subjected to nitrobenzene oxidation, compared to low concentrations of alkali labile phenolic (ferulic and p-coumaric) acids. However, they did not find much difference in the composition of lignin between grasses and legumes. Quantities of alkali labile phenolic monomers were higher in grasses than legumes (Jung et al., 1983) and

may explain reason for the lack of response of leguminous plants to alkaline treatments (Jayasuriya and Owen, 1975). Within forages, the stem fractions showed a higher proportion of lignified cells than leaves (Thiago et al., 1982).

Phenolic Monomers. In all vascular plants, phenolic compounds are synthesized by two metabolic pathways (Geissman and Crout, 1969). The shikimate pathway (Figures 2 and 3) is the major route responsible for the synthesis of phenolic monomer precursors (Davis, 1955; Levey and Zucker, 1960) and lignin (Higuchi, 1977). The acetate malonate pathway, the second major route (Birch, 1957) gives rise to aromatic phenols such as gentisic acid, via the cyclization of polyketide chain (Geissman and Crout, 1969).

The aromatic amino acids, phenylalanine and tyrosine, are precursors of both benzoic acid derivatives (C_6- C_1), and phenyl propanoid compounds (C_6-C_3). The phenyl propanoids include p-coumaric, ferulic and sinapic acids (Conn, 1964). Benzoic acid derivatives include p-OH benzoic, vanillic, syringic, salicylic, protocatechuic and gallic acids.

Higuchi et al. (1967) reported that in mature graminaceous plants, p-coumaric and ferulic acid residues comprise 5 to 10% of the total phenolic components of the cell wall. Phenolic acids occur mostly in bound form rather than free acids. They are found mainly as ester linkages to

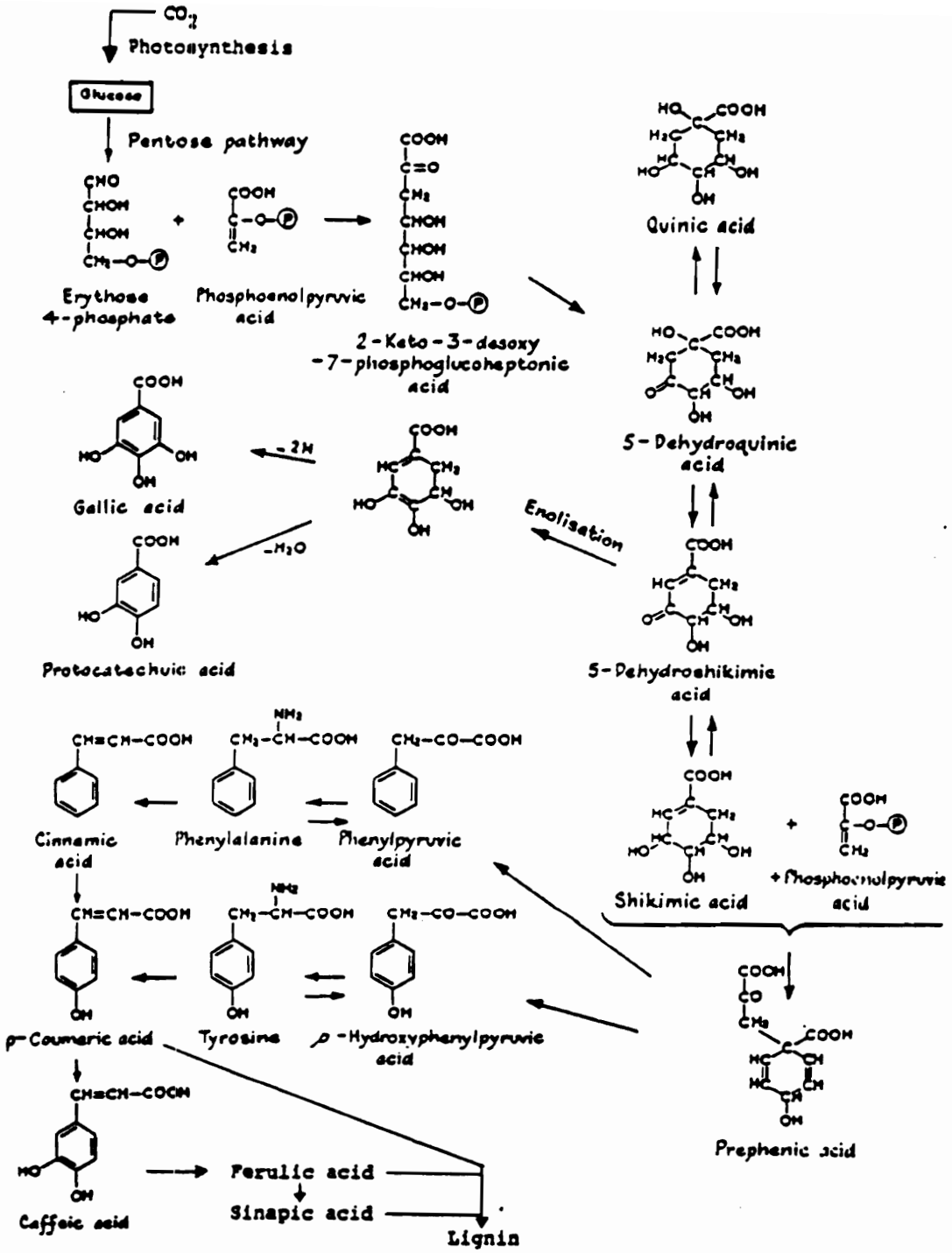

Figure 2. Schematic diagram of shikimate pathway. Adapted from Ribereau-Gayon (1972).

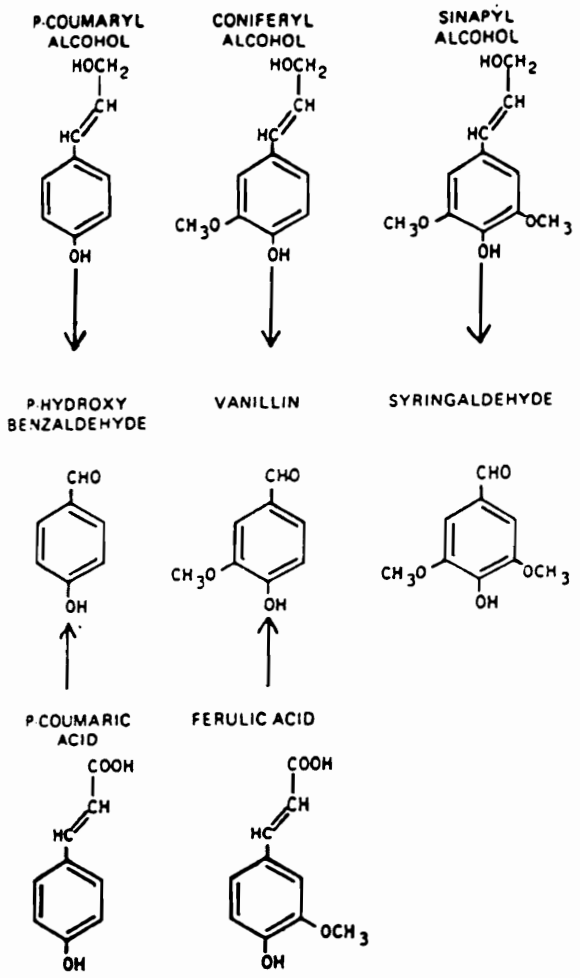

Figure 3. Relationship between cinnamyl alcohols, acids and their nitrobenzene oxidation products (Hartley, 1987).

hemicellulose (Higuchi et al., 1967), but glucosidic linkages (Harborne, 1980) and complexes with organic acids, lipids, terpenoids and alkaloids (Harborne, 1964, 1969) are also found.

Ferulic and p-coumaric are the major phenolic acids, representing 2.5% of the cell walls of temperate grasses (Hartley and Jones, 1977) and 5.1% of the cell walls of tropical grasses (Chaves et al., 1982).

Scanning electron micrographic studies showed the presence of phenolic acids not only in lignified cell walls (non-mesophyll) but also in non-lignified (mesophyll) cell walls of Italian ryegrass (Harris et al., 1980). No differences were found in the ferulic acid content of both types of cells (6 mg/g cell wall). However, the content of p-coumaric acid was found to be confined mainly to lignified cell walls (3 mg/g cell wall), compared to non-lignified cell walls (.4 mg/g cell wall).

Jung et al. (1984) analyzed immature and mature lucerne (Medicago sativa L.) and tall fescue (Festuca arundinacea L.) for their hydroxybenzoic and hydroxycinnamic acid contents. Fescue contained higher concentrations of alkali-labile phenolic monomers than lucerne. They also observed that contents of phenolic monomers varied with maturity in grasses but not in legumes.

Lignin-Carbohydrate Complexes. El-Basayoni et al. (1964) reported that esterification of hydroxycinnamic acids occurred with the aliphatic hydroxyls of a number of compounds such as glucose, quinic acid or anthocyanins.

The linkages of ferulic acid to hemicellulosic groups of cell wall polysaccharides in sugarcane bagasse (Kato et al., 1987), wheat bran (Smith and Hartley, 1983), maize cell walls (Kato and Nevins, 1985), Italian rye grass (Lolium multiflorum L.) (Hartley, 1973; Hartley et al., 1976) and perennial rye grass (Morrison, 1974) showed a direct ester link of ferulic acid to arabinose, which in turn was linked to xylose, indicating that it was derived from ferulolyted arabinoxylan.

Mueller-Harvey et al. (1986) reported two types of lignocellulosic compounds when barley straw was treated with oxyporus "cellulase", a mixture of polysaccharide hydrolases. The two compounds identified were: O-[5-O-(trans-p-coumaryl)-α-L-arabinofuranosyl]-(1→4)-O-β-D-xylopyranosyl-(1→4)-D-xylopyranose (PAXX) and O-[5-O-(trans-ferulolyl)-α-L-arabinofuranosyl]-(1→3)-O-β-xylopyranosyl-(1→4)-D-xylopyranose (FAXX).

Two major ferulolyted disaccharides of D-galactose and L-arabinose were extracted from Spinach (Fry, 1982). He concluded that ferulolytion of cell wall polymer was not a

random process, but occurred at very specific sites. The ferulate isolated from secondary cell walls of wheat endosperm contained a ferulolyted arabinoxylan (Neukom, 1976) and the same ferulate isolated from secondary cell walls of mature leaf blades of perennial rye grass was associated with xylose, arabinose and glucose (Hartley, 1973). Ferulate has been linked also through an amide bond to the N-terminus of a protein (Van Sumere et al., 1973).

Morrison (1974) postulated three different types of covalent bonding between lignin and carbohydrate. One cleaved on borohydride reduction, another cleaved by alkali and the third was resistant to alkali. Apart from these three linkages, two other bondings have been suggested, one acid labile and another cleaved by oxidation mechanism. Tanner and Morrison (1983) found two classes of lignin-carbohydrate complexes when ryegrass cell walls were digested with cellulose. The two classes differed in the ratio of individual monosaccharides.

Phenolic acids have two active groups, carboxyl and phenolic groups, are bifunctional and able to form ester or ether linkages (Hartley, 1972; Scalbert et al., 1985). The phenolic acids, free or bound, copolymerize with lignin forming alkali resistant bonds. The occurrence of phenolic acids with free carboxyl group suggested the possible exist-

ence of ether linkages between phenolic groups of phenolic monomers and lignin in cell wall.

Scalbert et al. (1985) studied the presence of ether linkages between phenolic acid and lignin in wheat straw. However, at least 93% of p-coumaric was alkali labile, thus linked by an ester bond to lignin, whereas 35 to 75% of ferulic acid was ether linked to lignin. This suggested that ferulic acid ethers might form cross-linkages between lignin and hemicellulose by simultaneous esterification of their carboxyl group to arabinose substituents of arabinoglucuroxylans (Smith and Hartley, 1983; Koshijima et al., 1983).

Dimers of Phenolic Acids. During the biosynthesis of lignin in grasses, the sugar esters of phenolic acids, including ferulic acid and p-coumaric acid, formed corresponding free phenolic alcohols followed by the oxidative coupling leading to lignin formation (Hartley and Jones, 1976).

Geissman and Neukom (1971) demonstrated in vitro formation of a low polymer, dimer of ferulic acid, from the oxidative polymerization of synthetic ferulic acid esters of polysaccharides in the presence of peroxidase/H_2O_2. Hartley (1973) reported the presence of diferulic acid in the cell walls of Italian rye grass. Three isomers of diferulic acid, trans, trans- cis, trans- and cis, cis-, were obtained from

the cell walls of Italian ryegrass on treatment with NaOH (Hartley and Jones, 1976). These isomers were apparently bound via ester links to the structural carbohydrates of the cell walls, involving one or both of their carboxyl groups.

Use of fluorescent microscopy (Fulcher et al., 1972; Harris and Hartley, 1976) also showed the presence of bound phenolic acids both in lignified and non-lignified cell walls of the grasses. Crosslinking of cell wall polysaccharides and phenolic acids through oxidative dimerization probably rendered the cross linked chains insoluble, thereby restricting the cell structure, and thus being partially responsible for the carbohydrates of the cell wall to be less easily attacked by microbial carbohydrases in the rumen (Hartley and Jones, 1978).

Nutritive Value of Forages

The ratio and proportion of cell solubles and cell wall components varied with number of factors: 1) climate, 2) temperature, 3) light, 4) humidity, 5) age and maturity, 6) fertilizer, and 7) soil condition (Alberda, 1965; Deinum, 1966).

Experimentation has confirmed that light intensity and N-fertilization (Deinum et al., 1968) exerted a positive influence on water-soluble carbohydrates, and hence, DM digestibility, whereas, high temperature (Wilson et al.,

1976) reduced DM digestibility and increased the cell wall carbohydrates and lignin content. Minson and Mcleod (1970), from field data, suggested that high temperature was one of the causes of poor digestibility in tropical grasses. Higher temperature enhanced the metabolic activity, which caused decrease in the pool size of cellular metabolites and hence increased the deposition of cell wall carbohydrates and lignin (Van Scest, 1982). A negative effect of high temperature was also observed in stems of alfalfa. However, no change was found in the digestibility values of alfalfa leaves. An increase in the leaf to stem ratio and little or no change in leaf digestibility counteracted the negative effect of lignification in legumes, resulting in variable effects (Kahnt, 1976). Other negative factors include silica, cutin and waxes.

Computing the effect of silica on digestibility values of grasses, Van Soest and Jones (1968) reported an average decline of 3.0 units of digestibility per unit of silica increase. Jones (1963) reported that grasses are active accumulators of silica in the cell walls of epidermis and vascular bundles of leaves. Scanning electron microscopy of big blue stem (Andropogan gerardi L.) and little bluestem (A. scoparius) (Brazle et al., 1979), smooth brome grass and tall fescue (Harbers et al., 1981) showed that silica re-

sisted microbial digestion by imposing a barrier to microbial attack. No effect of silica was observed in leguminous plants, probably due to increased lignin concentration (Van Soest, 1984).

Changes Associated with Maturity. Cell wall constituents account for a considerable proportion of the DM of grasses, especially at mature stages (Jones, 1970). Most of the energy derived by the ruminants is from structural-carbohydrate fermentation in the rumen. Waite (1964) reported an increased proportion of highly indigestible structural polysaccharide such as xylan with advancing maturity of grasses.

Danley and Vetter (1973) reported that with advancing maturity there were increases ($P<.01$) in DM and hemicellulose, and decreases in CP, estimated TDN and in vitro dry matter digestibility (IVDMD). Forage*maturity interactions were also noted for DM, ADF, cellulose, hemicellulose, lignin and IVDMD, suggesting a variable effect of advancing maturity among forages.

While comparing forages at same growth stage, Jones (1970) reported that perennial ryegrass varieties (S23 and S24) were higher in DM digestibility and lower in lignin than cocksfoot (S143 and S345) and timothy (S48 and S352). At the same level of DM digestibility, lower values of structural

carbohydrates and higher values of lignin were noted for cocksfoot. He concluded that differences in the digestibility between grass species at the same stage of growth may not be simply related to the content of any particular structural constituent.

Grass leaves are considered to be the most nutritious part among different botanical fractions (Burton et al., 1964). They reported that young top leaves were more palatable, higher in CP and lower in lignin than older leaves collected from the same culm of Gahi-1 pearl millet (Pennisetum americanum L.). Eighteen successive leaves from top to bottom of the culm of late maturing millet plants gradually decreased in digestible DM from 73.9 to 58.2%.

Relationship Between Digestibility and Cell Wall Components. The nutritive value of forage plants in terms of digestibility and feeding value are markedly influenced by stage of maturity of forages at time of harvest (Moxon et al., 1951; Trimberger et al., 1955). The variation in forage digestibility, however, is mainly associated with cell-wall components, especially structural carbohydrates, lignin and phenolic monomers (Van Soest, 1967).

Cellulose crystallinity is often regarded as a structural factor limiting IVDMD (Kamstra et al., 1958). However, no covalent linkages were found between cellulose and other

cell wall components. The only probable reason reported for limiting the cellulose digestibility with increasing maturity was the decreased accessibility of ruminal microorganisms (Jung and Fahey, 1983). However, insufficient data are available to support this claim.

The composition and structural organization of hemicelluloses were also responsible for limiting the digestibility of forage plants (Akin, 1980). Lindgren et al. (1980) reported that xylose concentration of timothy cell walls increased linearly ($P < .01$) with maturity and was the least digested sugar unit at all stages of maturity. Morrison (1982) pinpointed the presence of the arabinose side chain as the limiting factor in microbial digestion. A negative correlation ($r = -0.84$, $P < .01$) between percent xylose and IVDMD in three maturing grasses (reed canary grass, smooth brome grass, wild rye grass) was reported by Burritt et al. (1984). They proposed that the strong correlation between xylose concentration and IVDMD, and increased binding of xylose to lignin may be reflected in lowered digestibility values. Morrison (1974) reported that xylans were closely associated with lignin in lignin-carbohydrate complexes. Waite et al. (1964) studied the effect of maturity on apparent digestibility of ryegrass, cocksfoot and timothy. They observed that xylan digestibility decreased more during plant

growth than digestibility of cellulose or other hemicellulosic polymers.

The phenylpropanoid polymer, associated with the polysaccharide constituents of the cell wall, is the most commonly recognized limitation to the extent of forage and cell wall digestibility (Waldo et al., 1972). The low digestibility of tropical grasses as compared to temperate grasses is thought to be due to their high lignin concentration (French, 1957). Experiments have shown strong negative correlations between digestibility of cell wall and (or) DM with the lignin content in alfalfa (Sullivan, 1966), Reed canary grass (Bittner, 1983), Townsville stylo (tropical legume, Dekker et al., 1971) and Spear grass (Beveridge and Richards, 1973). They concluded that resistance of the polysaccharides to complete digestion by ruminal microbes was due to physical protection by lignification.

Jung and Vogel (1986) analyzed 194 samples of grasses to determine the relationship between forage digestibility and lignin concentration. Linear regression indicated that DM digestibility was inhibited to a lesser extent ($P<.05$) by lignin than cell wall digestibility. A comparison of linear and curvilinear models indicated that, for all digestibility measurements, the curvilinear model was a better ($P<.05$) description of the relationship with lignin concentration. It

was also concluded that the curvilinear model suggests a complex mode of inhibition by lignin.

Minson (1971) studied the effect of lignin on the OM and cell wall digestibilities of three cultivars of Panicum coloratum and Panicum maximum. Results revealed that the factors controlling OM digestibility were the percentage of both hemicellulose and cellulose contents present in the forages and the extent of their lignification. Chemical studies have shown that delignifying agents improved the digestibility of low-quality roughages by breaking the bond between lignin and structural carbohydrates (Klopfenstein, 1978).

Scanning electron microscopy of treated and untreated coastal bermuda grass blades (Spencer and Akin, 1980) and stems collected at two stages of maturity (Spencer et al., 1984) showed that alkali treatment enhanced degradation of sclerenchyma and affected separation of the lignified tissues into individual cells by removing the intercellular substances.

Allinson and Osbourn (1970) analyzed different varieties of ryegrass and legumes to study the relationship between IVDMD and lignin. They reported that variation between varieties and growth phases of ryegrasses and legumes were less closely related to the nature of cellulose-lignin complex

than the type of lignin fractions. Lignin varied in type based upon methoxylation at the 3- and 5-carbon portions of the aromatic ring of monomeric units, i.e., p-coumaryl, coniferyl and syringyl (Barton et al., 1983).

The variation in lignin type was reflected in differential responses of tissue types to microbial digestion when exposed for various incubation period (Akin and Burdick, 1981). Scanning electron microscopy of coastal bermuda grass leaf blades (Akin et al., 1977), alfalfa leaves (Brazle and Harbers, 1977), arrow leaf (Trifolium vesiculosum Savi cv. Amclo) and crimson clover (T. incarnatum L. cv. Dixie) (Akin and Robinson, 1982) and Panicum sp. (Akin et al., 1984) showed that non-lignified mesophyll and phloem cell walls were degraded extensively and rapidly followed by epidermis, bundle sheath and later sclerenchyma cells. However, xylem in vascular bundles and cuticle resisted degradation even after 72 h.

Reeves (1985) examined specifically the role of lignin composition in relation to digestibility. The best single predictor of cell wall or DM digestibility was found to be ADF and the percentage of vanillin in lignin oxidation products. With multiple lignin components, lignin composition was a better predictor of digestibility. Comparative studies of lignin in normal and brown midrib genotypes of corn

(Gymaluk et al., 1973) and sorghum (Bucholtz et al., 1980) provided further evidence that differences in lignin composition were related to differences in forage quality.

Rumen Microbial Degradation of Fiber

Ruminants are ubiquous and unique in their ability to utilize structural carbohydrates as their main energy source (Dehority, 1973). Forage polymers are degraded in the bovine and ovine rumen by cellulolytic bacteria and to a lesser extent by protozoa (Bryant, 1973; Prins and Clarke, 1980) and anaerobic phycomyceteous fungi (Orpin and Latcher, 1979).

The fundamentally important process of plant cell wall digestion depends upon colonization and digestion within the very complex microbial ecosystem of the rumen (Cheng et al., 1984; Latham et al., 1978). Recent elegant scanning electron microscopic work has shown that comminuted plant tissue particles entering the rumen are colonized by bacteria within 5 min (Cheng et al., 1977), by protozoa (Epidinium sp.) within 15 min (Amos and Akin, 1978; Bauchop, 1979) and by fungal sporangia within 2 h (Orpin, 1977; Bauchop, 1979).

Careful washings of plant material during preparation of samples for electron microscopy revealed that the vast majority of associated microorganisms were truly adherent (Cheng et al., 1984). The preservation and staining of bacterial cells showed a direct connection between the

colonizing bacteria and plant cell walls, mainly by the fibrous polysaccharide glycocalyces of bacteria (Cheng et al., 1977; Costerton and Cheng, 1982).

Bacteria. Microbial digestion of plant cell wall carbohydrates, summarized by Cheng et al. (1977) and Akin (1979) showed two distinct groups of bacteria involved in plant cell wall digestion. Bacteroides succinogenes (the gram negative rods), Ruminococcus albus and R. flavefaciens (the gram positive cocci) were the most active fiber digesting species.

Costerton et al. (1978) suggested that fibrous polysaccharide glycocalyx of bacteria, being most negatively charged, probably formed a polar bond with higher cell-wall polysaccharides. A thick slime layer is thus formed by cocci and a very thin layer by gram negative rods, which enables the bacteria to adhere closely to plant cell wall. The enzymes involved in cell wall digestion are closely associated with plant cell wall, however, a few in cell solubles are also available (Cheng et al., 1977).

Bacteroides succinogenes adhered tenaciously to plant cell wall (Groleau and Forsberg, 1981), thus, was quite able to attack more resisting substrates (Stewart et al., 1981). Transmission electron microscopy of tissue digestion of rapidly degraded orchard grass blades revealed that mesophyll, parenchyma bundle sheath and epidermal cell wall apparently

were degraded without direct attachment of bacteria, although bacteria were found near the cell walls undergoing digestion (Akin, 1980).

Protozoa. Tier (1926) was the first to suggest the involvement of ruminal protozoa in plant cell wall degradation. Hungate (1942) and Coleman et al. (1976) showed larger size entodiniomorphs played a role in cell-wall digestion, However, due to smaller proportion of entodiniomorphs population, their role as plant degraders remained debatable. Experiments with defaunated animals indicated an overall decrease in fiber digestibility, as compared to faunated animals when both groups were fed on roughage-based diets (Lindsay and Hogan, 1972; Joanny and Senand, 1979; Klopfenstein et al., 1966). Amos and Akin (1978) suggested that protozoa play a role in fiber digestion. However, their essentiality to cell wall degradation is debatable and unclear (Hungate, 1975).

Fungi. Orpin (1975) isolated anaerobic fungi from the rumen of sheep. Three species were identified and named: Neocallinastix frontallis, Piromonas communis and Sphaeromonas communis. Bauchop (1979), using scanning electron microscopy, demonstrated the presence of a large population of these fungi on plant fragments. Motile flagellate zoospores, often released from sporangium, moved

to specific sites, especially vascular tissues on plant fragments (Bauchop, 1981). It appeared that population size of fungi depend upon the type of diet and turnover time. Thus, the most fibrous diet appeared to support the largest population of fungi.

Zoospores invaded predominately the exposed tissue of damaged part or stomata (Orpin, 1977 and Bauchop, 1981). Scanning and transmission election microscopy revealed that rumen fungi preferably colonize the rigid hemicellulosic tissue of fiber (Bauchop, 1979). Unlike anaerobic bacteria, the rhizoids of rumen fungi penetrated the plant cell wall and caused an extensive degradation of plant tissue (Akin et al., 1983). Orpin and Letcher (1979) have shown N. frontallis as a cellulose and hemicellulose degrader.

Microbial Enzymes in Polysaccharide Fermentation. A wide variety of polysaccharide hydrolytic enzymes, cellulases (exo and endo-(1-4)β-gluconases, C_x and C_1, respectively), hemicellulases, pectin methyl esterases, pectin lyase and polygalacturases were isolated from a variety of bacterial populations (Groleau and Forsberg, 1981; Forsberg et al., 1981; Pettipher and Latham, 1979; Smith et al., 1973), protozoa (Amos and Akin, 1978) and fungi (Orpin and Latcher, 1979).

The soluble carbohydrates produced by the enzymatic activity of hydrolytic enzymes served either as the main energy source for the host animal and (or) may be utilized by the attacking microorganisms itself (Scheifinger and Wolin, 1973).

Degradation of Grasses and Legumes. Electron micrographic studies of microbes associated with the degradability of grasses and legumes documented differences in the type of microbes associated with plant types (Cheng et al., 1984). A large area of the surface of grass cell wall was found to be extensively colonized by pleomorphic, strongly adherent B. succinogenes (Akin et al., 1974; Akin and Amos, 1975; Forsberg et al., 1981; Latham et al., 1978b) and gram positive coccus, R. flavefaciens (Latham et al., 1978a).

Unlike the grass cell wall, the intercellular spaces between parenchyma cells in fresh whole legume leaves were initially colonized by discrete microcolonies of Lachnospira species (Cheng et al., 1979, 1980). However, the presence of B. succinogenes and R. flavefaciens in legume digestion were rarely observed. The mode of digestion also differed in both types of plants.

The degradation of grasses generally involved digestion pits at the site of microbial colonization, followed by the intercellular digestion (Akin et al., 1974; Latham et al.,

1978a). Legumes exhibited derangement of cell walls and ultimately damage in the vicinity of loosely adherent bacteria (Cheng et al., 1980). Not much information is available on legume cell wall digestion. Detailed examination of the bacterial digestion of grass cell wall using transmission electron microscopy indicated differential digestion pattern attributed to various chemicals and biological factors. The chemical factors, which included the chemical composition of structural carbohydrates and associated lignin and phenolic compound depend upon the genotype and maturity of grasses (Cheng et al., 1980).

Leaf anatomy is a good indicator of photosynthetic pathway in temperate and tropical grasses. Akin (1982b) suggested that variation in leaf anatomy between warm and cool grasses was reflected in different digestive patterns. The warm season grasses have more closely spaced vascular bundles and a distinct thick-walled parenchyma bundle sheath surrounding the vascular bundle (Kranz), whereas, cool season grasses have widely spaced vascular bundles and a less distinct bundle sheath (Hatch and Slack, 1970; Edwards and Walker, 1983). The mean tissue percentages of warm- and cool-season grasses and their degradability data revealed that mesophyll tissue digested easily, followed by less degradable sclerenchyma and bundle sheath cells (Akin et al.,

1984). However, vascular bundles, especially xylem, resisted microbial attack even after 72 h.

Cool season grasses showed better digestion than warm season grasses, when digested for the same period of time (Hanna et al., 1973; Akin, 1982). They suggested that the better digestion of cool season grasses is due to loosely arranged mesophyll tissue and slightly less thick-walled bundle sheath cells.

Akin and Burdick (1981) suggested that the variation in the degradation of same tissue type in two types of grasses resulted from the deposition of chlorine sulfite positive lignin in warm season grasses. Studies have indicated that p-coumaric acid occurred most commonly with plant fractions of low quality (Harris et al., 1980). A strong negative correlation between p-coumaric and digestibility was reported for warm (Chaves et al., 1982) and cool season grasses (Burritt et al., 1984).

Toxic Effect of Phenolic Acids on Biological System

Jurd et al. (1971) were the first to identify obtusastyrene (trans-4, cinnamyl phenol) as a potent natural bactericide and fungicide. They observed that obtusastyrene, dihydro-obtusastyrene and 2-cinnamyl phenol completely inhibited the growth of a variety of gram positive bacteria, yeasts and molds. Woodhead and Cooper-Driven (1978) reported

the importance of phenolic acids as deterrants to Locusta feeding in mature leaves of sorghum plants. They observed that individual phenolic acids showed no inhibitory effect except when present as a mixture. They found that sorghum contained a mixture of hydrolases that transformed "inactive" phenolic esters and glucosides into active phenolic acids.

A number of phenolic acids have also been identified as phytotoxins involved in allelopathic interactions (Rice, 1974). Ferulic acid, p-coumaric and vanillic acid were tested for their inhibitory effect on sorghum seed germination (Rasmussen and Einhellig, 1979). An equimolar combination of each of the three phenolics showed synergistic inhibition on seed germination.

Zemek et al. (1979) studied the antimicrobial properties of compounds with guaiacyl and syringyl structures, using model cultures of bacteria, yeast and yeast-like microorganisms. Structures containing a double bond in α, β position of the side chain and a methyl group at Γ position showed the most inhibitory activity.

Isolated rumen bacteria were examined for growth and their ability to degrade cellulose in the presence of hydroxycinnamic acids (ferulic and p-coumaric acids) and hydroxybenzoic acids (vanillin and 4-OH benzoic acid) (Chesson et al., 1982). Hydroxy cinnamic acids at or above

the concentration of 5 mM were the most toxic and suppressed the growth of the cellulolytic strains R. albus, R. flavefaciens and B. succinogenes. The extent of cellulose digestion was also substantially reduced at higher concentrations (>5 mM) of phenolic acids. They also observed a limited ability of cellulolytic strains to hydrogenate hydroxycinnamic acids. They postulated this ability of cellulolytic strains as an immediate defense against the toxic effect of phenolic acids.

Using light microscopy, Akin (1982) examined the toxic effect of individual phenolic acids on rumen bacterial growth, rumen protozoal motility, and cellulose and cell wall degradation. Addition of .1% p-coumaric acid to the media inhibited not only the growth of cellulose-utilizing bacteria but also xylan-utilizing bacteria by 50%. The percent motility of entodiniomorphs, but not the holotrichs, decreased more rapidly. Ferulic acid reduced the growth rate of rumen microorganisms. However, no effect of sinapic acid was observed. It was also noted that higher concentration (0.2%) of p-coumaric acid impeded the degradation of readily digestible tissues (epidermis and mesophyll in orchard grass), suggesting a toxic effect of phenolic acids toward cellulolytic bacteria.

Jung and Fahey (1983) and Jung (1985) studied the effect of different concentrations of phenolic acids (1, 5 and 9%) on cellulose digestibility. They reported a linear decrease in cellulose digestibility with increases of phenolic acids. Responses were variable depending upon phenolic acids and bacterial species. Salicylic acid had the greatest inhibitory effect on cellulose degradation ($P<.05$), followed by p-coumaric acid and vanillin. Ferulic acid had the least inhibitory effect among phenolic acids tested. Jung (1985) reported that hemicellulolytic bacteria exhibited more tolerance to phenolic toxicity than cellulolytic bacteria.

Varel and Jung (1986) and Bornemann et al. (1986) reported that cinnamic acid and vanillin depressed in vitro cellulose digestibility by 14 and 49%, respectively. Varel and Jung, (1986) reported higher viable count of cellulolytic (three fold) and hemicellulolytic (ten fold) bacteria in medium with phenolic acids, compared to medium without phenolic acids. They suggested that lower IVDMD values and higher viable counts indicated the inhibitory effect of phenolic acid on the attachment of the fibrolytic microorganism to fiber particles.

Akin and Rigsby (1985) reported that microbial digestion of lignified tissues of forages depend upon the colonization of bacteria and fungi. They conducted an experiment to as-

sess the negative effect of phenolic acids on tissue degradation. Addition of phenolic acids (0.1%) reduced the number of sporangia clustered on the cut edges of leaf blades by two to three times.

Nutritive Value of Cereal Straw

Cereal straws and other fibrous agricultural by-products (stovers, husks and cobs) constitute the major part of livestock feed in southeast Asia and developing countries in other parts of the world (Jackson, 1977).

The voluntary intake and digestibility of these fibrous residues are limited owing to their high lignin content (Klopfenstein and Owen, 1982), close physical and chemical associations between phenolic acids and polysaccharides (Harris and Hartley, 1978) and crystalline arrangement of native cellulose polymer (Northcote, 1972). In addition, the crude protein content is also very low, about 3.0 to 6.5% (Kossila, 1984).

Treatment Technologies. Numerous treatment technologies have been used extensively to upgrade the nutrient availability of lignocellulosic materials. Three major types of treatment methods are commonly used: 1) physical-mechanical (chopping, grinding, heat extrusion and Γ-irradiation), 2) chemical (hydrolytic agents, oxidizing agents), and 3) biological (fungal treatment) (Fahey, 1989).

The basic concept in physical treatment is to reduce the particle size of lignocellulosic feed and increase the surface area available for microbial attack.

Walker (1984) reported that mechanical comminution of coarse fibrous residues increases the voluntary intake partly by increasing feed density and partly by reducing chewing time. Pigden and Heaney (1969) confirmed that milling of low quality roughages increased IVDMD. This increase in IVDMD was suggested to be accomplished by the disruption of lignocellulosic bonds and decreased crystallinity of native cellulose polymer (Pew and Weyna, 1962).

The digestibility of poorly digested cereal straws can be improved by biological treatment using different types of white rot fungi (Zadrazil, 1977). Several strains of white rot fungi have the potential for selective delignification of lignocellulosic agricultural by-products (Agosin et al., 1987). This group of white-rot fungi include Stropharia rugosoannulata, Pleurotus spp and Abortiporus biennis.

The effect of biological treatment is influenced by a number of factors: fungal species (Zadrazil, 1977), cultivation time and temperature, water and air ratio in substrate (compaction), and preparation and composition of the substrate (Zadrazil and Brunnert, 1981).

Extensive research has been done on the chemical treatment of lignocellulosic material. The concept of chemical treatment is based mostly on the hydrolysis of lignocellulosic bonds and the oxidation of lignin compounds.

Treatment with NaOH of barley straw (Jayasuriya and Owens, 1975; Coombe et al., 1979), wheat straw (Singh et al., 1971) and corn stover (Oji and Mowat, 1977) improved DM digestibility and intake. Despite the fact that NaOH treatment improves the nutritive value of low quality forages, excess Na added to straw is excreted in urine and feces and aggravate soil salinity problems. In addition, high Na intake results in increased urinary excretion which increased bedding requirements and the potential for kidney damage.

A number of other alkaline agents have been tested for assessing their positive effect on the nutritive value of cereal straw. They include KOH (Wilkinson and Gonzalez-Santillana, 1978), $Ca(OH)_2$ (Owen and Nwadukwe, 1980), NH_3 (aqueous and anhydrous), and urea (Sundstol and Coxworth, 1984).

Tarkow and Feist (1969) suggested that a strong solution of alkaline reagents saponifies the ester linkages between substituted acetic acid and (or) phenolic acids and polysaccharides, and ester linkages between lignin and hemicelluloses. In addition, it reduces the strength of

intermediate hydrogen bonding in cellulose polymer (Whistle and Teng, 1970). They suggested that decreased bonding resulted in the unfolding of cellulose crystallinity, and hence, accounted for greater cellulose digestibility.

Lindberg et al. (1984) reported another notable change due to alkaline treatment. They indicated that xylans are probably translocated to a position in the material, where they are more easily accessible to microbial attack during ruminal digestion.

Ammoniation of low-quality roughages by aqueous and anhydrous NH_3 is the second most extensively used method for upgrading the quality of roughages (Sundstol et al., 1978). Anhydrous NH_3 (gas) has been used the most, however, the problems associated with its handling, cost and nonresponsiveness in straws below 30% moisture level have limited its use (Sundstol and Coxworth, 1984).

Oji et al. (1977) studied the effect of NH_3 (3%, DM basis) on the nutritive value of corn stover. Ammoniated stover showed increased DM intake ($P<.05$) and apparent digestibilities of DM, OM, ADF and cellulose ($P<.05$), compared to control. Hartley and Jones (1978) reported an increase of 14 units in IVDMD of barley straw treated with NH_4OH ($\cong$3.5% NH_3, DM basis) after 1 wk of treatment. Longer treatment times (4 and 13 wk) showed little or no additional

effect. The percent degradablity of cell walls was 85% higher, compared to untreated straw.

Moore and Latchenberg (1987) treated orchardgrass hay with anhydrous and aqueous NH_3 (3.6%, DM basis) at moisture levels of 15 and 30%. Hay treated with aqueous NH_3 at lower moisture levels showed better results than hay treated with anhydrous NH_3. However, the trend was reversed at higher moisture level, indicating the importance of moisture level for anhydrous NH_3 treatment. Average increases in the digestibility of DM, NDF, hemicellulose and cellulose were 182, 244, 198 and 230 g/kg, dry basis.

Increases in CP and DM digestibility were also reported for milo stalks (Bales et al., 1979), wheat straw (Solaiman et al., 1979) and whole crop barley and oat (Orskov et al., 1983) treated with NH_4OH ($\cong$3.5% NH_3, DM basis).

Urea is widely used as fertilizer, NPN source in livestock feed and also as a source of NH_3 in straw treatment. Unlike aqueous and anhydrous NH_3, it is safe and easier to handle and transport.

Oji and Mowat (1977) applied urea as a 50% solution ($\cong$5.3% urea, dry basis) to corn stover in polyethylene bags at room temperature. They reported that after 2 d of treatment 70% of the added urea was dissociated to NH_3 and completely decomposed by d 20.

Hadjipanayiotou (1982) reported that IVDMD of wheat straw sprayed with 4% urea solution reached maximum at 45 d post-treatment. The overall increase in digestibility of treated straw was 11 percentage units. In another experiment he found an increase in voluntary intake and DM digestibility of urea-treated straw (4%, DM basis) by 47 and 26%, respectively, over untreated straw. Similar results were reported by Ibbotson (1983) and Williams and Innes (1982).

Dias-Da-Silva and Sundstol (1986) fed diets based on untreated wheat straw, straw supplemented with urea and straw treated with urea (stacked or ensiled). Animals fed urea-supplemented straw showed better OM digestibility ($P<.05$) as compared to untreated straw. However, further increases were observed in animals fed treated straw. Digestibility of cell wall constituents was particularly enhanced by urea treatment. Between urea treatments (stacked vs ensiling), ensiled straw gave better results than stacked straw. Contradictory results were reported by Orskov et al. (1981). Barley straw was treated with anhydrous NH_3 and urea at 3.5% NH_3 levels, fresh basis. They reported that DM intake, OM digestibility and weight gain were lower for animals given urea-treated straw than straw treated with NH_3 (3.9 vs 5.9 kg/d; 47 vs 57%; -447 vs 324 g/d, respectively). Williams and Innes (1983) and Ibbotson (1983) commented that rate and extent of urea

hydrolysis and ammoniation of straw were influenced by the moisture content of straw, treatment temperature and treatment duration.

Jayasuriya and Perera (1982) reported that a maximum of 21 d are required to achieve an optimal treatment effect from urea-N. However, Ibrahim et al. (1983) found that this duration could be reduced to 5 d with the inclusion of a urease source. This suggested the importance of urease source and its activity in the breakdown of urea.

Certain strains of white rot fungi (Kirk et al., 1980) and some bacteria spp. (Holt and Jones, 1978) play an important role in the delignification of lignocellulosic materials. Tien and Kirk (1983) suggested that hydrogen peroxide excreted by the microorganisms may act as lignolytic agent.

Recently Gould (1984) demonstrated that alkaline solution of H_2O_2 can decrease the cellulose crystallinity, and solubilizes about 30 to 50% of lignin from lignocellulosic crop residues.

At alkaline pH (11.5), H_2O_2 dissociate to form the hydroperoxy (HOO^-) anion (Muhammed and Rao, 1957), which in turn reacts with undissociated H_2O_2 to form highly reactive hydroxy radicals ($\cdot OH$) and superoxide ($O_2^- \cdot$).

$$H_2O_2 + HOO^- \rightarrow \cdot OH + O_2^- \cdot + H_2O$$

In the presence of lignocellulosic materials, •OH and O_2^-• react rapidly with lignin and lignin model compounds (Gould, 1985) to form low molecular weight water-soluble oxidation products.

Kerley et al. (1985) conducted a series of experiments to study the treatment effect of alkaline H_2O_2 in improving the nutritive value of wheat straw. They reported an increase in DM intake and digestibility ($P<.05$) when lambs were fed diets containing 36 and 72% treated wheat straw. Cellulose digestibilities were increased from 56 to 85% and from 57 to 86% for diets containing low and high levels of treated wheat straw, respectively. Scanning electron microscopy of straw particles obtained from sheep fed treated wheat straw showed an improved and uniform attachment of microbial population, as compared to selective attachment in untreated digested samples.

In a follow-up experiment, Kerley et al. (1986) observed higher apparent DM, NDF and cellulose digestibilities in the rumen ($P<.05$) when wethers were fed alkaline H_2O_2 treated wheat straw diets than wethers fed on non-treated wheat straw diets.

Lewis et al. (1987) compared the effect of different alkaline reagents (NaOH, NH_4OH, NaOH + $Ca(OH)_2$, H_2O_2 and alkaline H_2O_2) on in sacco DM disappearance of wheat-straw.

All the treatments showed increases ($P<.05$) in DM disappearance in sacco. However, alkaline H_2O_2 treatment resulted in greater overall improvement, as compared to straw treated with NaOH alone, or with simultaneous addition of H_2O_2 (81 vs 62 and 69, respectively).

Atwell et al. (1989) found no differences in digestibilities of DM and OM, when wethers were fed diets based on alkaline H_2O_2 treated wheat straw alone, combination of treated straw and alfalfa hay and alfalfa hay alone. The authors concluded that results demonstrated the efficacy of the treatment procedure.

CHAPTER III

ANATOMICAL DIFFERENCES ASSOCIATED WITH PLANT MATURITY AND RUMEN MICROBIAL DEGRADATION OF LEAVES AND STEMS OF BARLEY, MILLET AND BERSEEM OBSERVED BY SCANNING AND TRANSMISSION ELECTRON MICROSCOPY

ABSTRACT

Barley, millet and berseem harvested at three stages of maturity were separated into three botanical fractions, leaf blades (leaflets), leaf sheaths (petioles) and stems to asess the effect of plant development and differences in the degradation pattern of specific tissue types when exposed to ruminal fluid, in vitro, for 6, 12 and 24 h. Anatomically, no marked differences were found in leaf blades of temperate grass (barley), whereas sheaths and stems showed increased proportion of lignified sclerenchyma cells with advanced maturity. Scanning electron microscopy indicated that barley blades were degraded extensively, compared to leaf sheaths and stems. Within plant parts, mesophyll and phloem of leaf blades, and sheaths and parenchyma of stems showed least resistance to microbial attack. Differences due to maturity occurred when barley plant parts were incubated with ruminal fluid for 24 h. Transmission electron microscopy indicated that thin primary cell walls of mesophyll and phloem were

degraded upon exposure of barley blades to microbes for 6 h. No degradation was observed on epidermis and xylem. The only anatomical difference between leaf blades and leaf sheaths of millet (tropical) plant was a higher proportion of lignified tissue in leaf sheaths. Leaf blades of millet showed better digestion than leaf sheaths. For stage II, leaf sheaths resisted microbial attack, compared to leaf blades. In berseem, similar responses were observed when leaflets, petioles and stems of three stages of maturity were incubated. Petioles showed better digestion than leaflets and stems. In stem, pith was totally degraded, but cuticle, vascular bundles and partly lignified interfasicular parenchyma resisted digestion.
(KEY WORDS: Electron Microscopy, Maturity, Anatomy, Tissue Digestion.)

INTRODUCTION

The quality of forages declines with increasing maturity. The forage constituents that are affected most are soluble and structural carbohydrates and lignin (Danley and Vetter, 1973). As the plant matures, the cell wall constituents increase and forage digestibility decreases (Van Soest, 1973). At advanced stages of maturity, the stems make up a larger proportion of the dry weight of forages (Brown et al.,

1968) and decrease more rapidly in digestibility than leaves (Pritchard et al., 1963; Beaty et al., 1977).

Nutritive value of forages for ruminants depends on the ability of microorganisms to colonize and digest the plant cell wall (Cheng et al., 1984) and to ferment the available carbohydrates (Akin et al., 1973). Data from chemical and in vitro studies indicate that chemical constituents of plants vary not only among different types of plants but also within the same plant and (or) plant parts (Van Soest, 1973).

In general, grasses adapted to tropical regions showed lower digestibility than grasses from temperate regions (Wilson et al., 1983). Legumes showed higher digestibility than grasses, however, a decline in digestibility occurred with maturity (Smith et al., 1975). Differences in forage digestibility are related to: relative proportion of different tissue types (Akin et al., 1983); arrangement of cells and lignified tissues (Akin, 1979); type and location of lignin (Stafford, 1962; Pigden, 1953); structural inhibitors such as silica, cutin and waxes (Brazle et al., 1979); and nature and extent of bonding between lignin and plant cell wall carbohydrates (Hartley and Jones, 1978).

Electron microscopy has been useful in providing information on plant cell wall degradation by rumen microorganisms (Harbers, 1985). Scanning electron microscopic investi-

gations of grasses have revealed that mesophyll and phloem were degraded more rapidly and easily than sclerenchyma and epidermal cells, whereas xylem resisted microbial digestion at 72 h (Akin and Burdick, 1975). Akin (1976), using the TEM, reported that during ruminal digestion grass cell walls were mostly colonized by pleomorphic Bacteroides succinogenes. Cheng et al. (1980) reported that legumes, in contrast to grasses, were colonized heavily by Lachnospira species, whereas, a few cells of Bacteroides succinogenes and Ruminococcus flavefaciens were also observed.

The objectives of the present study were to evaluate anatomical differences between leaf blades (leaflets), leaf sheaths (petioles) and stems at different stages of maturity of forages, to examine the mode of bacterial attachment to plant cell wall and assess the extent of degradation of specific tissue types by ruminal microorganisms.

EXPERIMENTAL PROCEDURES

'Wysor' barley (Hordeum vulgare L.), 'Tifleaf 1' millet (Pennisetum americanum L.) and 'Big bee' berseem (Trifolium alexandrinum L.) were cultivated at Blacksburg, VA in a 3 x 4 randomized block design. In the fall of 1987, the experimental area was fertilized with limestone (3,360 kg/ha), N (33.6 kg/ha), P_2O_5 (22.4 kg/ha) and K_2O (33.6 kg/ha), according to soil test recommendations. The entire exper-

imental plot, which covered an area of 68 x 55 m, was subdivided into 12 blocks. Each block covered an area of 19 x 11 m, with a 3 m wide buffer area between the blocks. In spring of 1988, blocks assigned for berseem and millet were sprayed with Paraquat[1] (2.29 L/ha) prior to their establishment. Millet was fertilized with NH_4NO_3 at a rate of 67.2 kg N/ha.

Sampling Procedures. The grasses were harvested at three stages of maturity; vegetative (nodes differentiating stage, 2-3 nodes; stage I), boot stage (inflorescence enclosed in flag leaf; stage II) and heading (100% heading; stage III). Plants from stages II and III were cut into three portions, whereas, stage I plants were kept as such. Each portion was designated as top, middle or bottom. The plants from stage I were collectively assumed as equivalent to middle portion of the other two stages.

The legume (berseem) plants were harvested at three stages of development also, vegetative (stage I), early bloom stage (stage II) and full bloom stage (stage III). The plants from stage II were divided into two equal portions, top and bottom, whereas plants from stage III were cut into

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[1] Chevron Chemical Co., Memphis, TN.

top, middle and bottom portions. The whole plant from stage I, and top portion of stage II were assumed to be equivalent to middle portion of stage III.

The middle portions of barley, millet and berseem were separated into three botanical fractions, leaf blades, leaf sheaths and stems for grasses, and leaflets, petioles and stems for legume.

For microscopic studies, 10 to 15 fresh, green plants were collected from each replicate and three botanical fractions from the middle portions were placed on dry ice in the field, and were later stored at -60 C. Fresh leaf blades (leaflets), leaf sheaths (petioles) and stems were cut into 5 mm sections and fixed in 4% paraformaldehyde-glutaraldehyde in .1 M sodium cacodylate buffer at pH 6.86. The fresh fixed specimens were used to assess the effect of freezing on the anatomy of plant parts.

In Vitro Incubation. Frozen specimens were thawed and cut into 3 to 5 mm sections. Top, bottom and marginal parts of leaf blades and leaf sheaths and nodal areas of culm were not included in the preparation of specimens.

Fifteen to 20 sections of each fraction were incubated with ruminal fluid-buffer mixture. The rumen fluid was collected from an Angus steer fed on a mixture of orchardgrass and alfalfa (1:1) hays, and was diluted with buffer (1:4,

respectively), as described by Tilley and Terry (1963). Sections were exposed to ruminal microbes for 6, 12 and 24 h at 39 C.

After incubation, plant sections were washed twice with water and .1 M sodium cacodylate buffer. Control and digested samples were fixed in a mixture of 4% paraformaldehyde-glutaraldehyde in .1 M sodium cacodylate buffer, pH 6.86, at 8 C for at least 24 h.

Scanning Electron Microscopy. Prefixed sections were dehydrated in a series of ethanol solutions (15, 25, 40 and 50%) for 5 min each and left overnight in 70% ethanol solution, and were later dehydrated in 80, 90, 95 and 100% ethanol solution, 10 min each. Sections were than placed in 100% acetone, dried over CO_2 in critical point drier (Ladd Research Industries). Liquid CO_2 was slowly fed into the chamber under pressure until the level reached three fourths of the chamber. Samples were soaked for 5 min, then flushed. The flushing procedure (fill, soak and vent) was repeated five times or until no fog appeared. The transitional fluid was then heated to 42 C, the chamber was then slowly vented at a rate of 46 kg/min while critical temperature was maintained until pressure reached 0 PSI.

Five to six sections of the dried specimens were then mounted on aluminium stubs with Delco No. 93 colloidal

silver[2]. and coated under vacuum with 20 nm of gold-palladium (Au-Pd). Samples were examined and micrographed with Phillips 505 scanning electron microscope at acceleration voltage of 15 kv. Results in the present study are based on the observations made on 5 to 6 sections scanned after Au-Pd coating.

Transmission Electron Microscopy. Pre-fixed incubated and control specimens were rinsed with .1 M Na-cacodylate buffer three times each for 15 min. The specimens were then postfixed in 2% Osmium tetraoxide in .1 M sodium cacodylate buffer for 1 h at room temperature. The samples were then rinsed with buffer three times, each for 15 min, dehydrated in graded ethanol-water series, and finally dried in 100% acetone. Dried specimens were then infiltrated with acetone: Spurr's epoxy resin in 3:1 (1 h), 1:1 (2 h), 1:3 and 0:1 (overnight), respectively. Specimens, kept in 100% resins, were embedded in plastic molds at 70 C for 20 h. The ultra thin sections of embedded samples were prepared on ultramicrotome and were collected on grids. The sections were stained with 2% uranyl acetate and 2% lead citrate, each for 5 min. The sections were examined on Zeiss TEM at ac-

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[2] 1603-02, Ted Pella Inc., Redding, CA.

celeration voltage of 80 kv. Eight to 10 ultrathin sections, which were prepared from two different sections of sample(s), were observed for discussing the results. The TEM electron micrograph(s) are representatives of the samples studied.

RESULTS AND DISCUSSION

Anatomical Characteristics of Barley. Cross sections of barley leaf blades revealed the characteristics of temperate plants (Figure 4 a). The leaf blades of barley showed a higher proportion of loosely arranged mesophyll cells than vascular bundles. Anatomically, not much difference was found between leaf blades of the three stages except that fewer of the mesophyll parenchyma cells showed increased deposition of cell wall materials with advanced maturity. The sheaths, which are botanically part of the leaf, showed higher amounts of highly lignified sclerenchyma cells on the adaxial side with advancing maturity, compared to blades (Figure 4 c). Wilkins (1972) reported increased proportion of sclerenchyma cells in leaf sheaths at advanced stages of maturity of cool-season grasses, compared to leaf blades. In the present study, no differences due to maturity were observed in the anatomical structure of stems (Figure 11 a and b).

Tissue Digestion of Barley. Scanning electron micrographs showed extensively collapsed thin-walled mesophyll

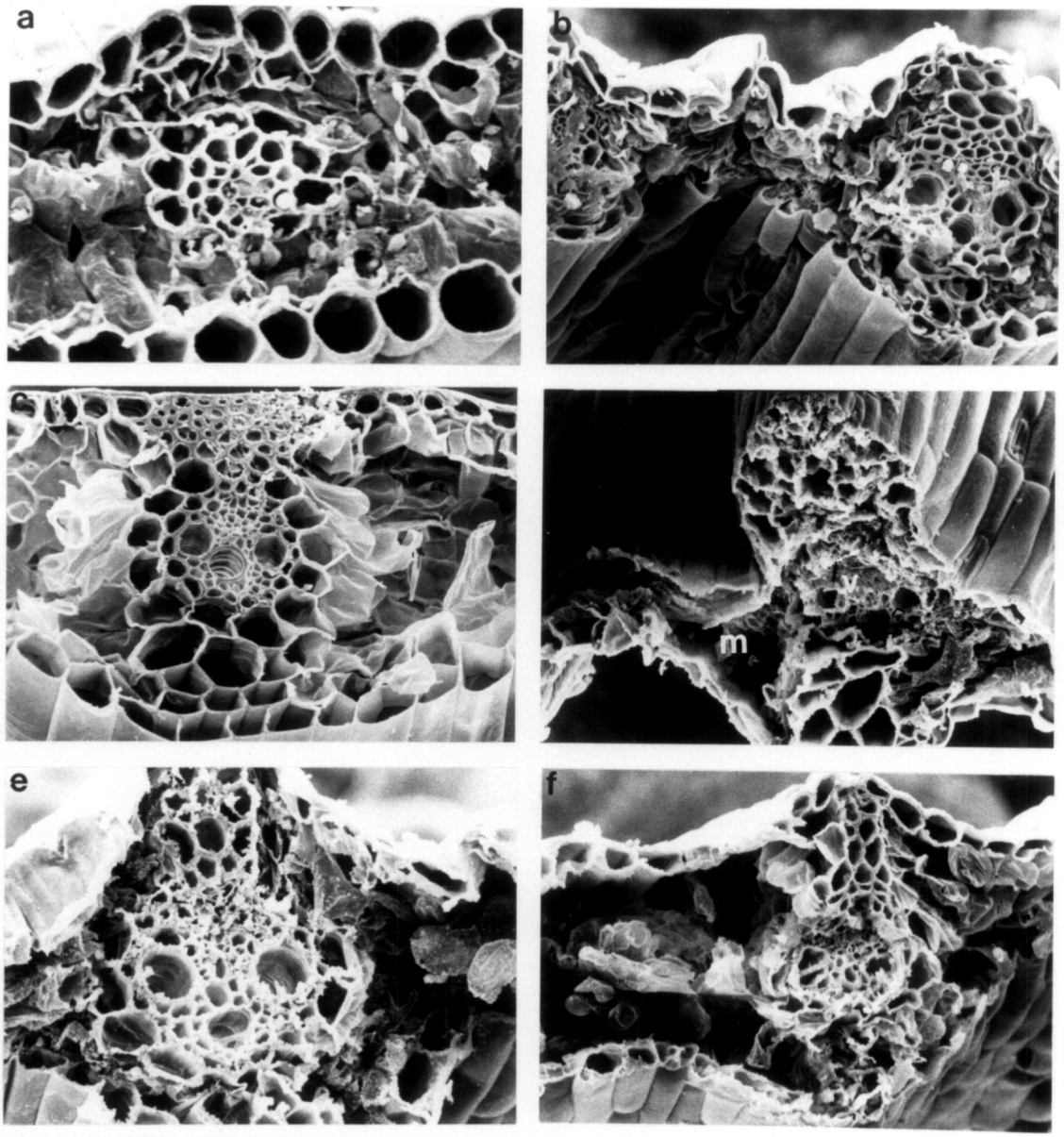

Figure 4. SEM. Control and digested barley leaf blades and sheaths. Caption on page 60.

cells in frozen undigested specimens but not in fresh fixed leaf blade sections (Figure 4 a and b). Barley blades of stage III were more resistant to microbial attack when incubated with rumen fluid for 6 h, compared to stages I and II sections (Figure 4 d, e and f). The mid-vein sections with large vascular bundles showed partial digestion of mesophyll, bundle sheath parenchyma and phloem in leaf blades digested for 6 h, for all three stages. A heavy microbial mat covered the specimens but no effect was evident in xylem, sclerenchyma and epidermal cells. Differences due to maturity, however, were clearly evident when leaf blades for all three stages were digested for 24 h (Figure 5 a, b and c). After 24 h microbial exposure, stage III specimens showed less degradation, compared to stages I and II. The specimens of stage I leaf blades showed extensive sloughing of tissue; the specimen lost its structural integrity. Even the xylem tissue in vascular bundles and epidermis were digested to an appreciable extent. However, not much difference was observed in the degradation pattern between stages II and III. The thick-walled xylem and epidermal cells resisted microbial attack but sclerenchyma cells and parenchyma bundle sheath cells were partially digested. These results agree with those of Hanna et al. (1973) who reported that leaf sections of Kentucky-31 and orchardgrass when incubated for 24 h with

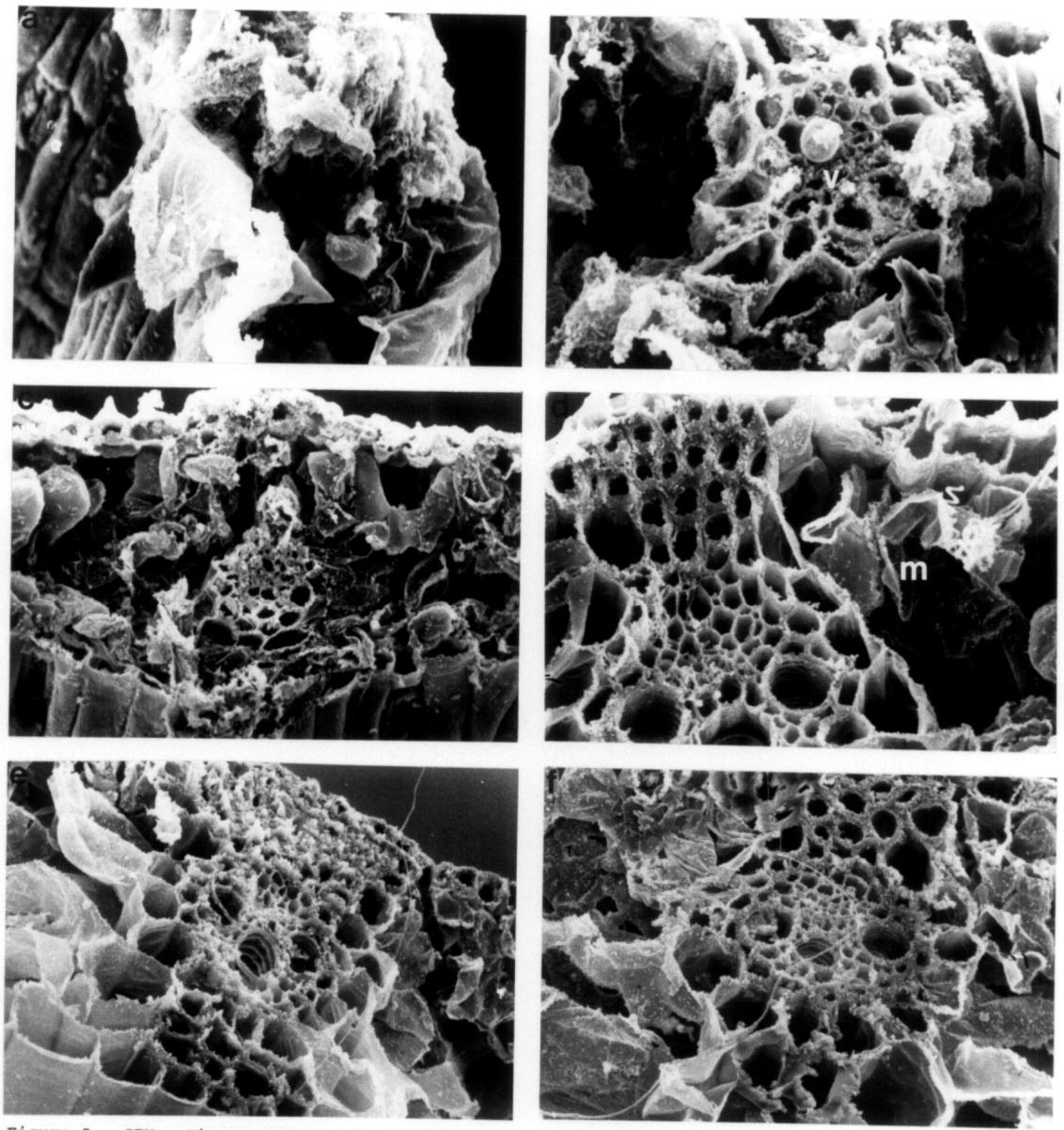

Figure 5. SEM. Digested barley leaf blades and leaf sheaths. Caption on page 60.

ruminal fluid, showed degradation of mesophyll, phloem and outer bundle sheath but not the inner bundle sheath cells. Similar results were reported by Akin et al. (1973) for tall fescue leaves. They reported that inner bundle sheaths were attacked and degraded extensively by 72 h. However, cutinized epidermis and lignified xylem resisted microbial attack. Stafford (1962) postulated three different types of lignin in timothy grass (Phleum pretense, a cool season grass). One is positive to acid phloroglucinol stain and usually is present in xylem tissue. The second showed positive response to chlorine-sulfite stain and usually stained sclerenchyma cells and thick-walled parenchyma bundle sheath cells. He reported a third type present in sclerenchyma cells of leaf blades behaved totally different from sclerenchyma cells of the rest of the plant. Acid phloroglucinol and chlorine-sulfite indicated the presence of coniferaldehyde and syringyl groups, respectively (Sarkanen, 1971). Akin et al. (1973) suggested that differences in lignin types of bundle sheath cells (acid phloroglucinol- and chlorine sulfite positive lignin) and the proportion of different tissues affected the rate of degradation. Comparing the digestion behavior of brome grass and tall fescue at 24, 48 and 72 h of incubation, Harbers et al. (1981) reported little change in the digestion pattern of

sclerenchyma cells. No change was observed in vascular bundles at three time periods. They suggested that maximum microbial digestion occurred by 24 h.

Microbial exposure of leaf sheaths for 6 h showed little digestion at all the three stages (Figure 5 d, e and f), although, lignified tissues appeared to be densely colonized by a microbial mat. The leaf sheaths of stage I, when incubated with ruminal fluid for 24 h showed extensive sloughing of tissue specimens, but epidermis appeared to be partly digested (Figure 6 a).

The leaf sheaths of stage II showed comparatively more digestion than stage III, when observed after 24 h of incubation (Figure 6 b, c and d). Mesophyll, parenchyma bundle sheath and phloem were totally removed, leaving a disrupted residue composed of undigested xylem and partly degraded epidermis and sclerenchyma cells. By 24 h, leaf sheaths of stage III showed more resistance to microbial attack than those of stages I and II. The specimen retained structural integrity and showed partial digestion only of phloem and mesophyll. No digestion of epidermis, xylem and bundle sheath was observed. Little information is available about the digestion pattern of leaf sheaths in temperate grasses. Akin et al. (1977) reported an increased proportion of chlorine-sulfite positive lignin in leaf sheaths of coastal

Figure 4. SEM. a) Fresh fixed leaf blade, stage I, loosely arranged mesophyll (arrow; x 555). b) Frozen fixed leaf blade, stage I, collapsed mesophyll (x 295). c) Leaf sheath, stage I, lignified sclerenchyma (s) cells above large vascular bundle (x 203). d) Leaf blade, stage I, partly digested mesophyll (m), bundle sheath (arrow) and phloem in vascular bundle (v) after 6 h microbial digestion (x 203). e) Leaf blade, stage II, digested for 6 h (x 281). f) Leaf blade, stage III, digested for 6 h (x 255).

Figure 5. SEM. a) Leaf blade, stage I, digested for 24 h, specimen lost structural integrity (x 405). b) Leaf blade, stage II, partially digested bundle sheath (arrow) and vascular bundle (v) after 24 h incubation (x 688). c) Leaf blade, stage III, partially digested after 24 h incubation (x 350). d) Leaf sheath, stage I, partially digested mesophyll (m) after 6 h incubation (x 435). e) Leaf sheath, stage II, resisted microbial attack after 6 h incubation (x 290). f) Leaf sheath, stage III, digested for 6 h (x 340).

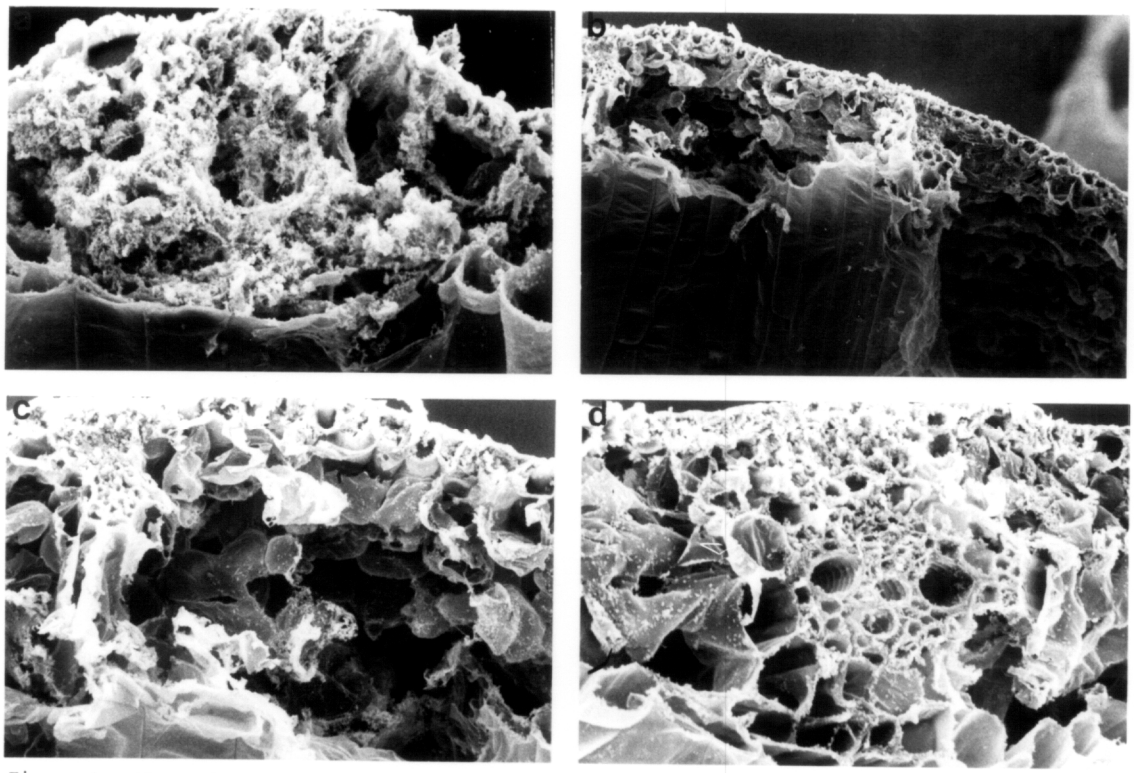

Figure 6. SEM. Digested barley leaf sheaths. Caption on page 67.

bermuda grass with advancing maturity. Scanning electron microscopy of digested leaf sheaths showed resistance to microbial attack. They suggested it was due to increased lignification. Terry and Tilley (1964) reported lower IVDMD of leaf sheaths in cool season grasses, compared to leaf blades.

Transmission electron microscopy of control and digested leaf blades incubated for 6 and 24 h with ruminal fluid showed sharp differences in the extent of colonization and digestion of cell walls of different tissues (Figure 7). The micrographs of leaf blades incubated for 6 h indicated that bacteria had degraded thin, primary cell walls of mesophyll and phloem prior to bundle sheath, epidermis and xylem (Figure 7 c and d). All the intracellular organelles were completely degraded. Broken fragments of chloroplasts were, however, found in few parenchyma cells. After 6 h of incubation, no differences were noticed in the microbial attack and degradation pattern of stage I and stage III leaf blades. No adhesion was observed between microbes and mesophyll or phloem cell walls.

Pectin constitutes a major portion of primary cell wall and matrix material in the intercellular spaces (Esau, 1965). The rapid microbial digestion of mesophyll seemed to be due to low degree of polymerization and cellulose crystallinity

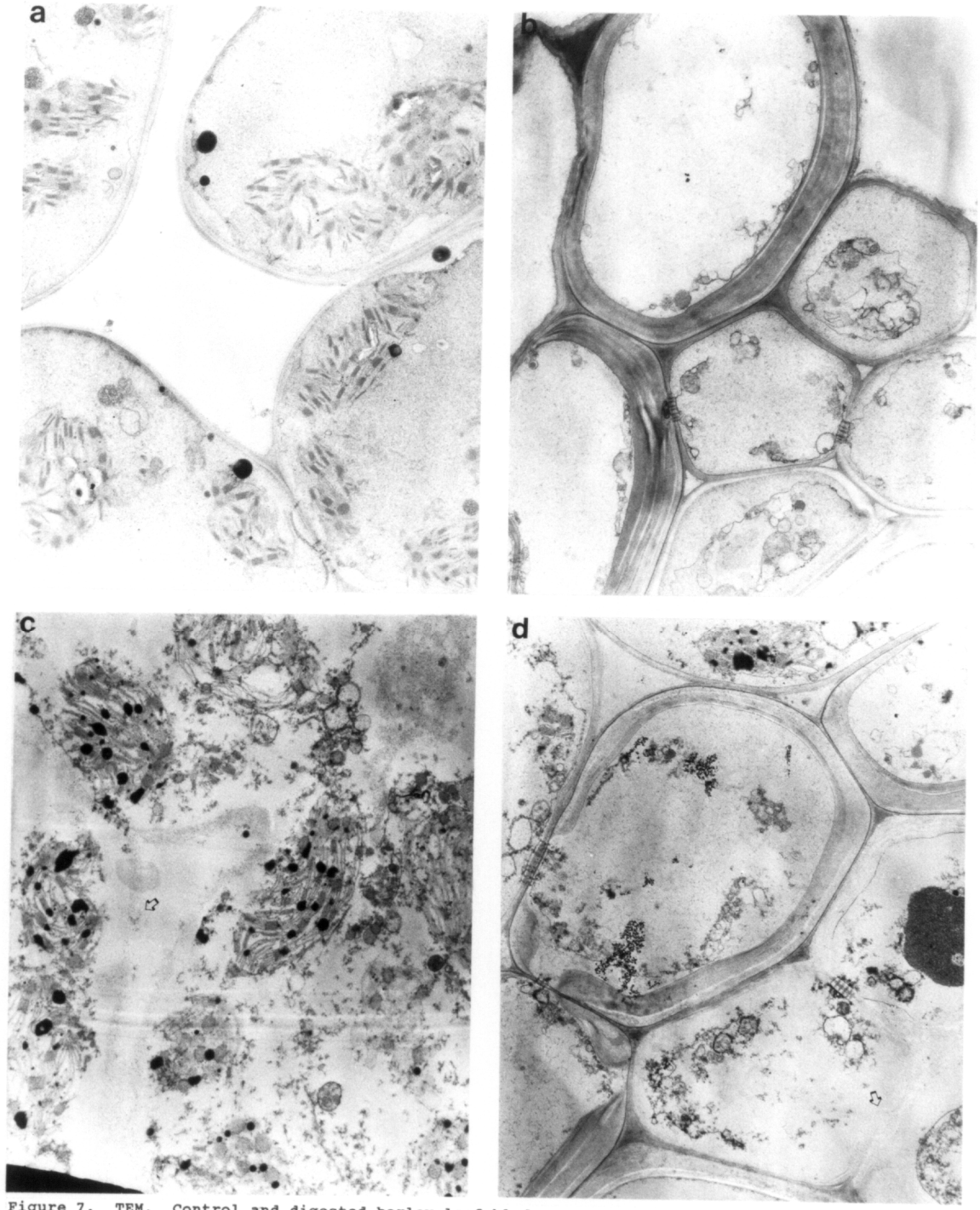

Figure 7. TEM. Control and digested barley leaf blades. Caption on page 67.

in primary cell walls (Shafizadeh and McGinnis, 1971). Akin et al. (1974) suggested that bacteria degraded thin primary cell walls apparently by extracellular enzymes and without any prior attachment. In present study, rumen microbes of diverse morphologies were found in inter- and intracellular spaces. The bacteria appeared to enter intracellular spaces through damaged or cut surfaces. Bacterial morphology indicated mainly the presence of cellulolytic microorganisms, cocci and rods (Figure 8 a, c and d). The cocci resembled mostly to Ruminococcus flavefaciens and the irregular pleomorphic cells resembled those of Bacteroide succinogenes, as studied by Akin et al. (1974) and Latham et al. (1978).

Akin (1980) reported that several morphological types of bacteria were involved in the attachment, close association and degradation of forage grass cell walls. The majority of cell wall degrading bacteria (>70%) consisted of two distinct types, (i) encapsulated cocci and (ii) irregular-shaped bacteria. Others included regular evenly-shaped bacilli and non-encapsulated cocci. The proportion of different bacteria reflects chemical composition and structural differences which depends upon genotype and maturity of different grasses (Cheng et al., 1984). Dinsdale et al. (1978) indicated that cocci-shaped bacteria were usually involved in the digestion of potentially digestible tissues,

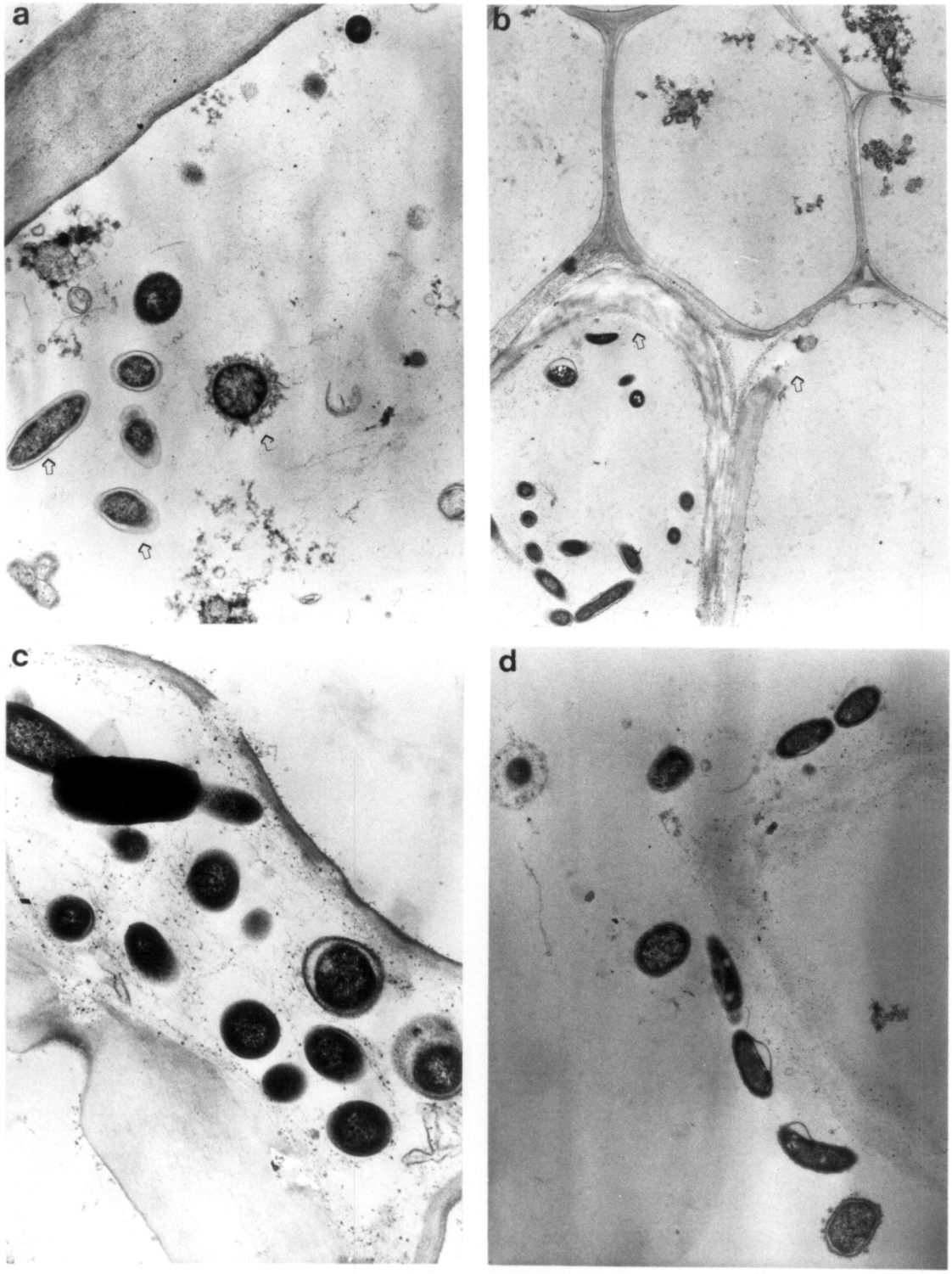

Figure 8. TEM. Digested barley leaf blades. Caption on page 67.

whereas the resistant residues elicit a population of rod-like organisms. Working with mixed cultures of Ruminococcus flavefaciens and Bacteroides succinogenes, Latham et al. (1978) observed that cocci predominate on epidermis and phloem and irregular pleomorphic Bacteroides predominate on mesophyll.

Studying the mode of attack, Dinsdale et al. (1978) visualized two distinct modes of bacterial attack on forage cell wall, either by tunneling within the forages or making very close contact, resulting in general erosion of the structure. They indicated that organisms responsible for the tunneling action were cocci, while the majority of rod-shaped bacteria and spirochete were associated with erosive action.

Despite the presence of microbes in the vicinity of epidermis, no digestion effect was observed (Figure 8 a). Even no effect was found on lignified sclerenchyma cells, outer bundle sheath and xylem in vascular bundles.

After 24 h of incubation, stage III leaf blades showed extensive digestion of parenchyma cells and partial hydrolysis of sclerenchyma and bundle sheath cells, indicated from the clearing zones and loss of election density (Figure 8 b and d). However, no adhesion or digestion effect of microbes and (or) their extracellular enzymes was noted on lignified xylem. Similar results were reported by Akin et

Figure 6. SEM. a) Leaf sheath, stage I, extensively digested after 24 h microbial exposure (x 345). b) Leaf sheath, stage II, partly digested sclerenchyma (s) and xylem (x) in vascular bundle after 24 h incubation (x 116). c) Specimen described in figure 6b at high magnification (x 303). d) Leaf sheath, stage III, partly digested mesophyll after 24 h incubation (x 350).

Figure 7. TEM. a) Control leaf blade, stage I, parenchyma cells (x 3,780). b) Control leaf blade, stage I, vascular bundle (x 4,700). c) Leaf blade, stage I, digested for 6 h indicated dissolved parenchyma cell walls (arrow; x 6,666). d) Leaf blade, stage I, partly digested parenchyma cell walls (arrow) close to vascular bundle (x 6,250).

Figure 8. TEM. a) Epidermal cell wall of leaf blade, stage I, after 6 h digestion shows bacteria (arrow) of diverse morphology in the vicinity, no bacterial adherence (x 13,335). b) Leaf blade, stage III, extensively digested parenchyma cell wall after 24 h incubation. Digestion indicated by clearing zones (arrow) without bacterial adherence (x 6,250). c) Intercellular space in mesophyll area, heavily colonized by microbes ater 6 h digestion (x 30,000). d) Leaf blade, stage III, digested for 24 h, bacteria aligned along the degraded parenchyma cell walls (x 13,335).

al. (1974) who found no bacterial attachment to or digestion of lignified xylem, even after 72 h of incubation. The non-responsiveness of lignified xylem and resistance of sclerenchyma, epidermis and bundle sheath cells appeared to be associated with multiple factors: type and amount of lignin (Stafford, 1962), the association of phenolic acids to β-glucans and (or) β-xylans (Hartley and Jones, 1977) and the presence of structural inhibitors, silica and cutin (Harbers et al., 1977).

Control and digested leaf sheaths observed by TEM revealed that incubation for 6 h did not have much effect on cell walls of different tissues (Figure 9). Immature leaf sheaths, digested with rumen microbes for 6 h (Figure 9 c and d), showed slightly better degradation of mesophyll cell walls, compared to mature leaf sheaths (Figure 10 a and b). Sections of parenchyma cell walls in the proximity of vascular bundles showed partial digestion of intercellular walls as appeared from loss of electron density and clearing zones. No effect was observed on the cell walls of lignified parenchyma bundle sheath.

Comparing micrographs of leaf blades and leaf sheaths of the same maturity, incubated for 6 h, it appeared that potentially digestible tissues of barley blades were more

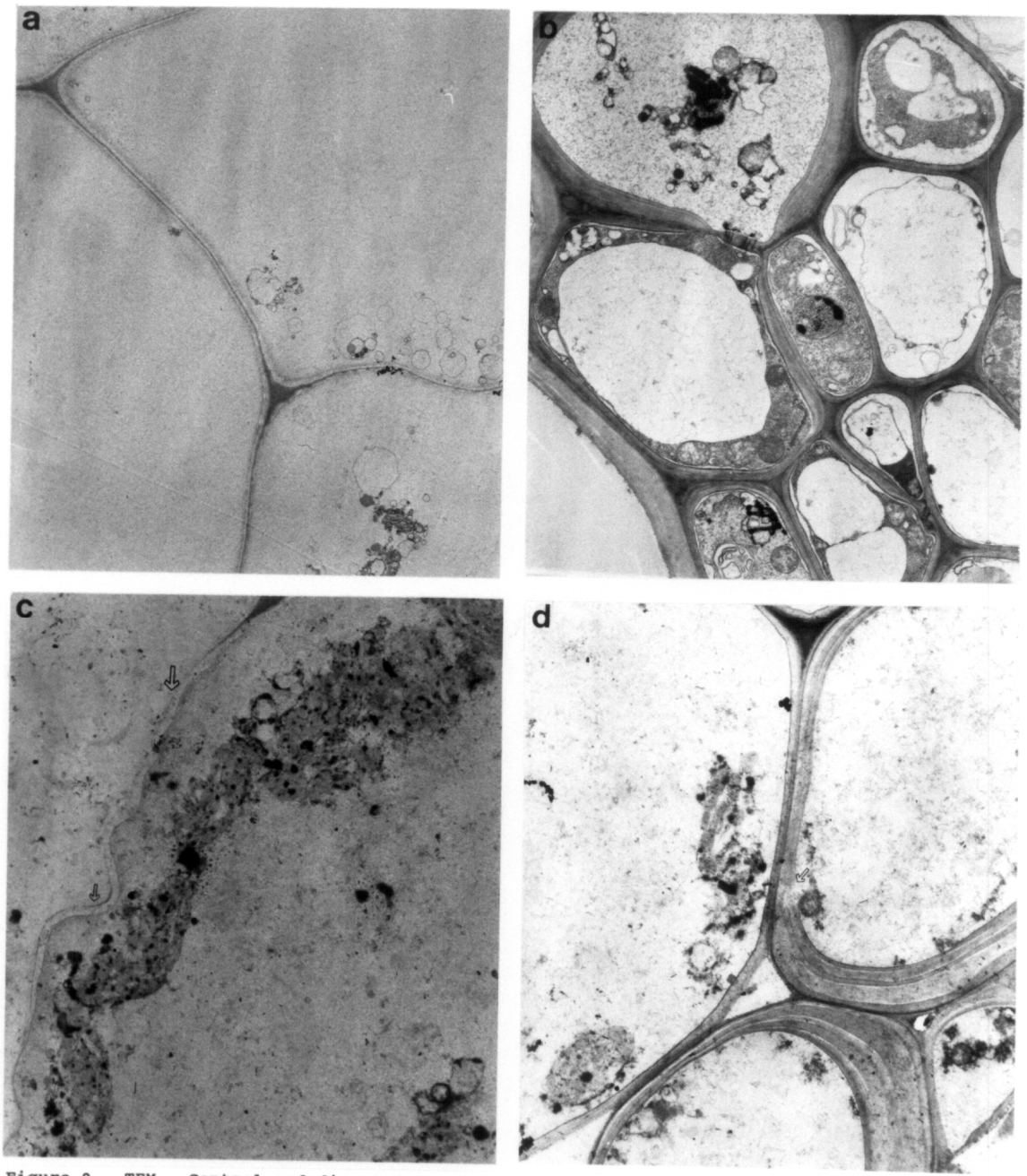

Figure 9. TEM. Control and digested barley leaf sheaths. Caption on page 73.

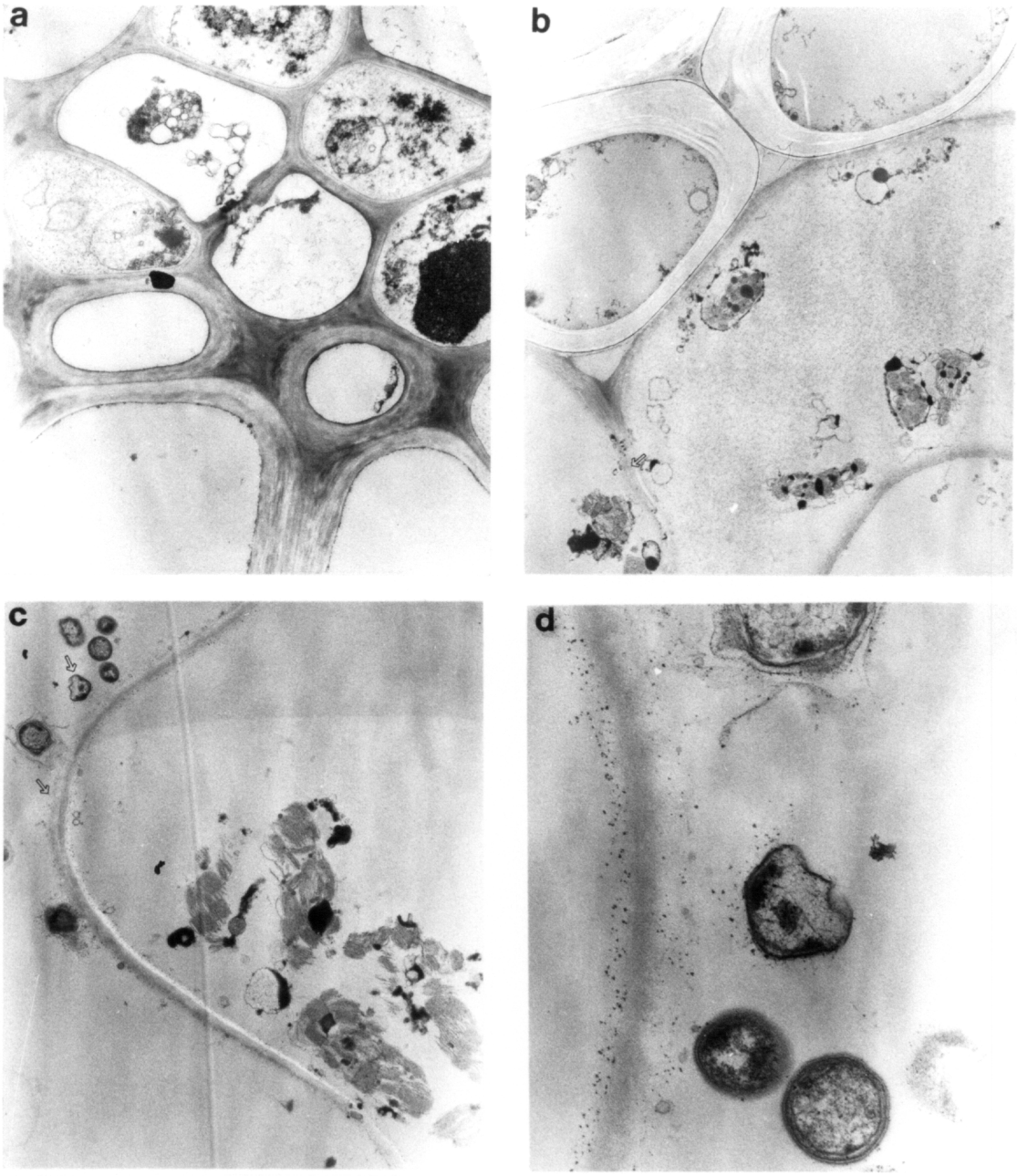

Figure 10. TEM. Digested barley leaf sheaths. Caption on page 73.

susceptible to microbial attack than leaf sheaths (Figures 7 to 10). These findings supported our observations on SEM.

After 24 h of incubation, cocci and irregular-shaped bacteria were found to be associated with parenchyma wall at the point of dissolution (Figure 10 c and d). Mature leaf sheaths digested for 24 h showed resistance, not only in lignified tissues (not shown), but also in non-lignified tissues (Figure 10 d). The parenchyma cell walls showed partial digestion, even in the area where bacteria were found in close association with cell wall. However, no adhesion was noted for bacteria and parenchyma cell walls of leaf sheaths. This resistance suggested the presence of chlorine sulfite positive lignin.

No differences due to maturity were observed in stem sections of stage II and III when incubated for 6 h with ruminal fluid (not shown). However, resistance to attack was more pronounced in stage III than stage II stem sections after 24 h incubation (Figure 11 c and f). Stem sections of stage II incubated for 24 h with ruminal fluid showed extensive digestion of phloem and parenchyma cells in cortex and pith region (Figure 11 c, d and e). The extensive digestion resulted in loss of structural integrity of specimen. Higher magnification of unhydrolyzed segments of stem sections showed remnants of unhydrolyzed thick-walled xylem cells,

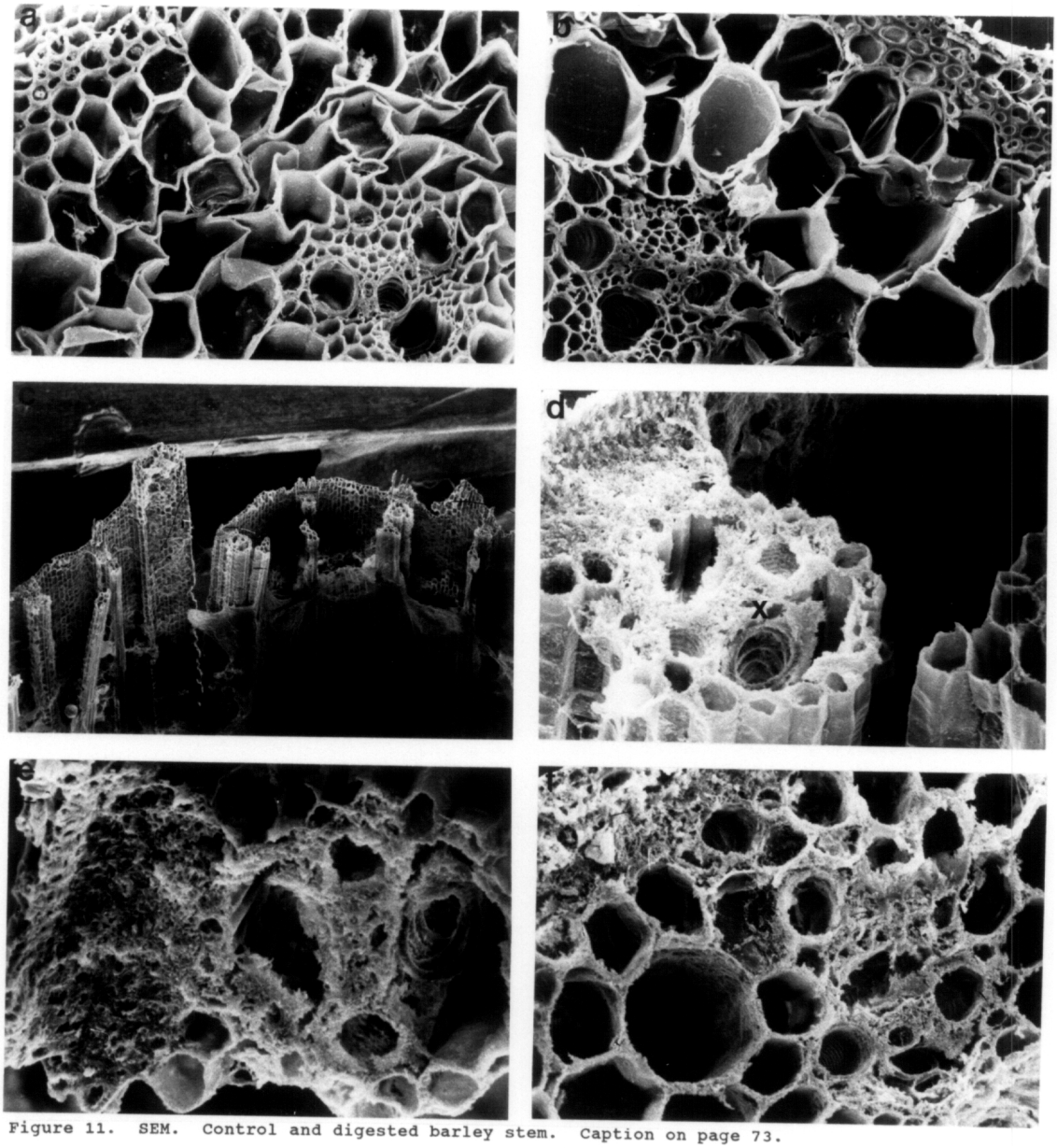

Figure 11. SEM. Control and digested barley stem. Caption on page 73.

Figure 9. TEM. a) Control leaf sheath, stage I, mesophyll cell walls (x 5,455). b) Vascular bundle of specimen described in figure 9a (x 2,500). c) Leaf sheath, stage I, partly dissolved parenchyma cell wall (arrow) after 6 h exposure to microbes (x 3,780). d) Leaf sheath, stage I, partly digested parenchyma bundle sheath after 6 h incubation (arrow; x 4,800).

Figure 10. TEM. a) Leaf sheath, stage III, no effect on vascular bundle after 6 h digestion (x 6,086). b) Leaf sheath, stage III, partly digested parenchyma cell walls after 6 h incubation (arrow; x 3,780). c) Leaf sheath, stage III, digested for 24 h, microbes in the vicinity of parenchyma cell wall (arrow; x 7,145). d) Higher magnification of figure 10c (x 30,000).

Figure 11. SEM. a) Control barley stem, stage II (x 284). b) Control barley stem, stage III (x 265). c) Barley stem, stage II, extensively damaged specimen after 24 h digestion (x 338). d) Residue of undigested xylem vessel (x) and sclerenchyma cells (s) of figure 11c (x 360). e) High magnification of figure 11d (x 545). f) Barley stem, stage III, partly digested phloem and parenchyma in cortex region after 24 h incubation (x 270).

partly digested lignified sclerenchyma band in cortex and epidermal cells (Figure 11 d), whereas a higher degree of resistance was observed in stage III stem sections with only partially hydrolyzed phloem, after 24 h incubation (Figure 11 f). Our findings are in agreement with the results reported by Pigden (1963), who found that advanced maturity in cool-season forage grasses resulted in increased proportion of acid phloroglucinol positive lignin in the sclerenchyma cells adjacent to vascular bundles resulting in increased resistance to microbial attack. Johnston and Waite (1965) indicated that larger cells, which formed the connective tissues between the major vascular bundles, also showed acid phloroglucinol positive lignin at advanced stages of maturity of temperate grasses. They suggested that increased lignification of stems caused lowered digestibility in cool-season grasses. Spencer et al. (1984) reported differences in the type of lignin in different cell walls of coastal bermuda grass at different stages of maturity. Epidermis, sclerenchyma ring and vascular tissue gave a positive reaction for lignin with acid phloroglucinol in top and bottom stem sections, whereas only bottom stem sections showed the presence of chlorine sulfite positive lignin in pith and cortex cells. They suggested that better resistance of bot-

tom stem sections were due to the presence of chlorine sulfite positive lignin in parenchyma and cortex cells.

The results of the present study indicated that stems showed more resistance to microbial attack than leaf blades and leaf sheaths. Leaf sheaths, showed more resistance to degradation than leaf blades. The SEM observations agreed with the general pattern of ease and extent of digestion of grasses as reported by Akin and Burdick (1975). They reported that mesophyll and phloem degraded at a faster rate than sclerenchyma and epidermal tissues, whereas vascular lignified tissues remained unhydrolyzed in mature tissues.

Anatomical Characteristics of Millet. Leaf blades of millet plant did not show much anatomical differences with maturity. Scanning electron microscopy revealed the typical anatomy of leaf blades of warm-season grasses, such as closely spaced vascular bundles, well developed parenchyma bundle sheath and relatively densely packed mesophyll (Figure 12 a and b). Most of the thin-walled parenchyma cells of mesophyll were replaced by lignified sclerenchyma cells. Akin and Burdick (1975) reported that bermuda grass possesed greater than 36% of leaf blade area as vascular tissue, of which the parenchyma bundle sheaths occupied greater than 50% of area. They observed that neither percentage nor sites of lignified tissues changed with maturity. No differences were

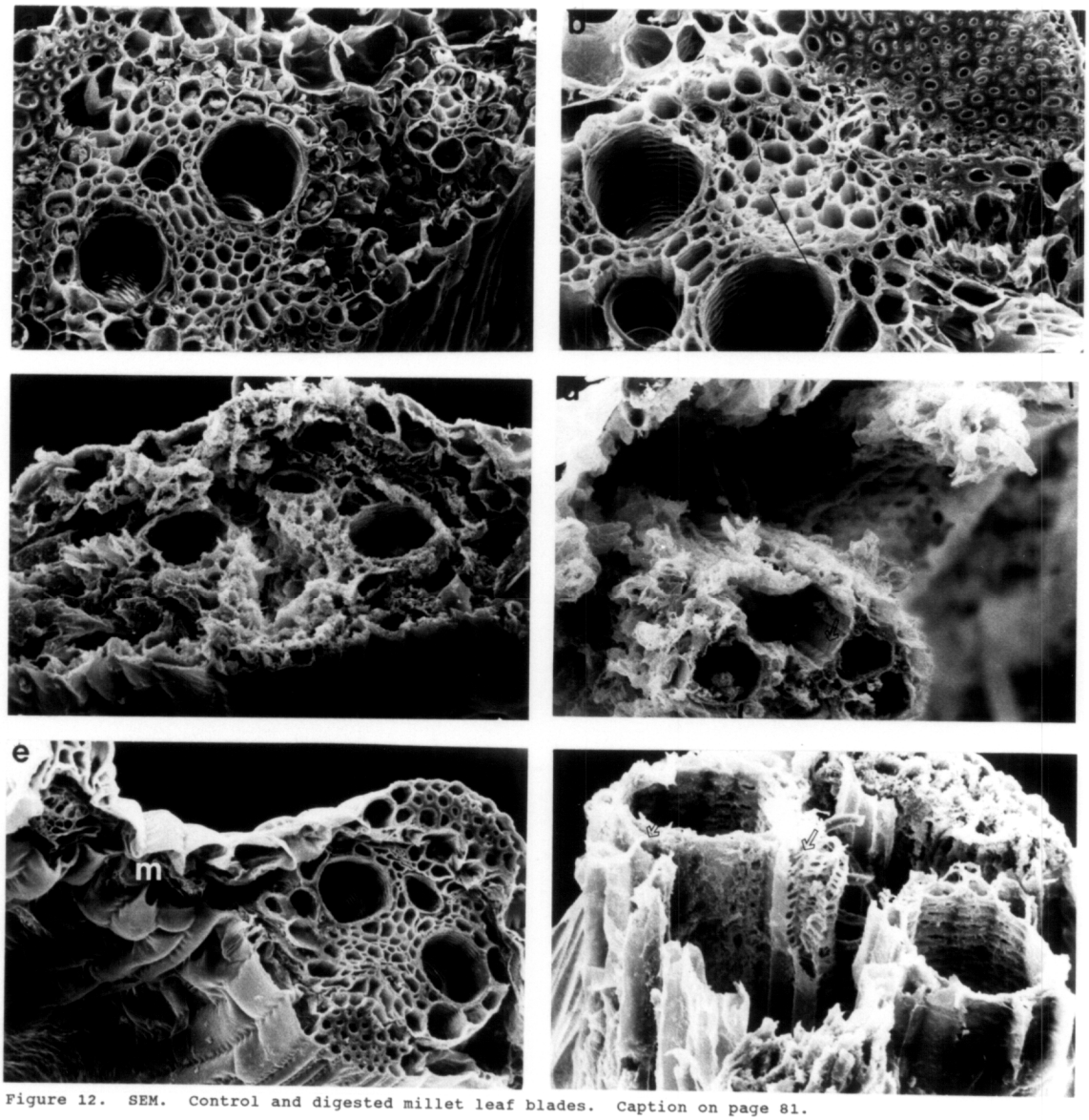

Figure 12. SEM. Control and digested millet leaf blades. Caption on page 81.

found in the anatomy of leaf sheaths of millet at two stages of maturity except increased proportion of lignified tissues with plant maturity (Figure 13 a and b).

Tissue Digestion of Millet. Scanning electron microscopy revealed differences in tissue digestion pattern when specimens were exposed to microbial attack for 6 and 24 h. Millet blades of stage II showed slightly better resistance to microbial attack than stage I (Figure 12 c and e). However, both the stages showed microbial attack, especially on nonlignified mesophyll cells and phloem. Specimens from stage I leaf blades showed partial digestion of epidermis and outer bundle sheath cells, whereas the epidermal and bundle sheath cells in stage II sections resisted microbial attack and remained unaffected. The extensive digestion of mesophyll cells resulted in overall distortion of specimen structure.

Effects due to maturity were not differentiated when leaf blades were incubated for 24 h (Figure 12 d and f). The specimens of stages I and II displayed extensive sloughing of tissues when exposed for 24 h. The mesophyll was totally removed from the depth of field of the SEM by 24 h. The epidermis was removed totally leaving a thin, pliable cuticle devoid of any epidermal tissue on either abaxial or adaxial sides. The residual mass consisted of partially degradable

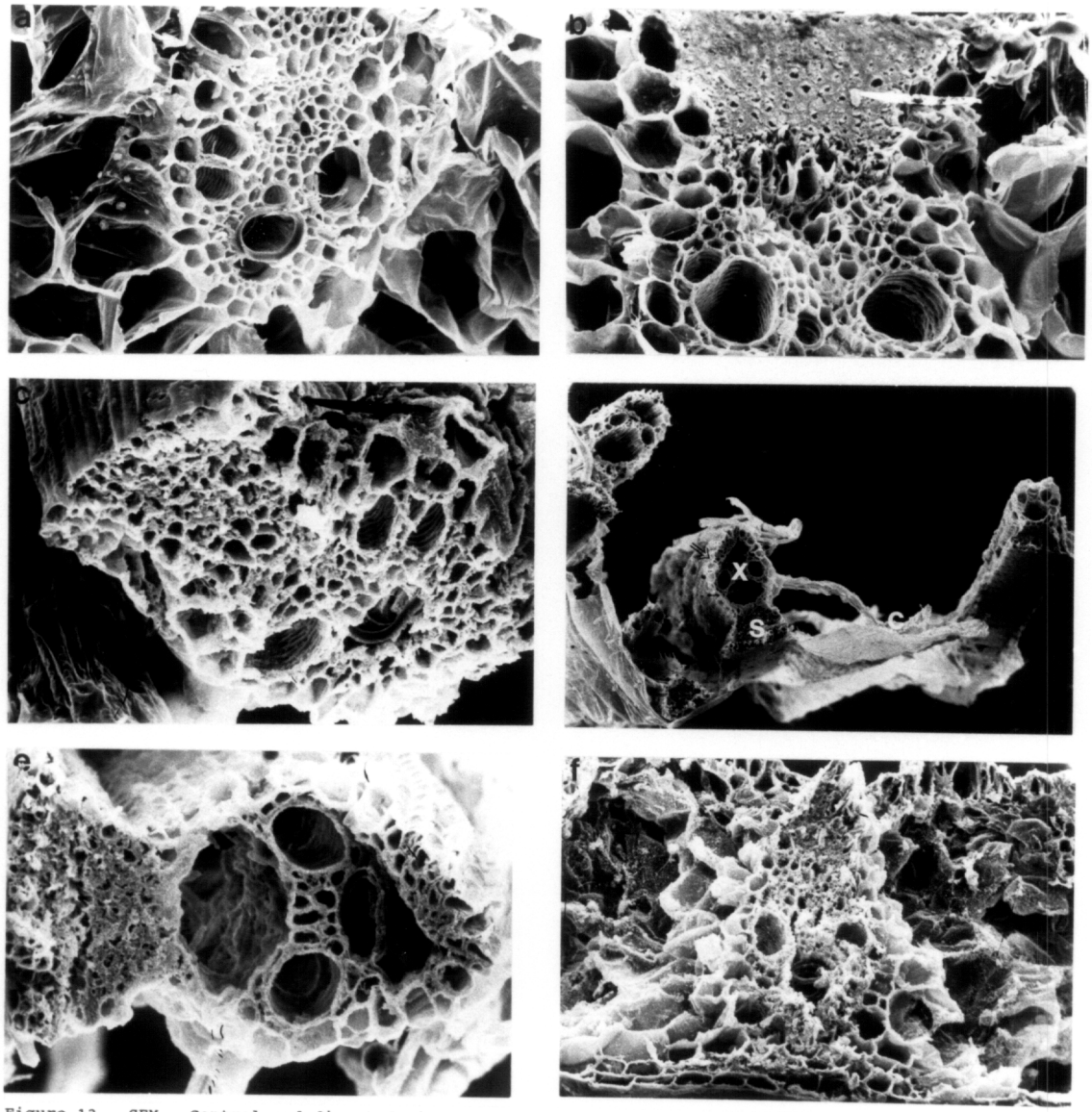

Figure 13. SEM. Control and digested millet leaf sheaths. Caption on page 81.

sclerenchyma cells and strands of unhydrolyzed vascular bundle of totally resistant xylem. By 24 h, not much difference was observed in the extent of tissue degradation between leaf blades of stages I and II. Pattern of tissue digestibility in our study was similar to that reported for bermuda grass, pearl millet (Hanna et al., 1973), old world blue stem, weeping love grass (Akin et al., 1983) corn and sorghum silage (Harbers and Thouvenelle, 1980). They reported that mesophyll, phloem and epidermis comprised the digestible cell wall types and xylem, portion of sclerenchyma and parenchyma bundle sheath as non-degradable cell or tissue types.

Akin et al. (1977) reported that tissues were removed equally from top and bottom leaf blades of bermuda grass at the selected time of incubation. Among different tissue types mesophyll and phloem were digested rapidly, whereas sclerenchyma showed partial digestion and xylem was highly resistant to microbial attack after 72 h incubation. They further reported that highly resistant xylem and inner bundle sheath stained positive to acid phloroglucinol, whereas partially digested sclerenchyma cells stained positive for lignin with chlorine sulfite stain.

Unlike leaf blades, the leaf sheath specimens did not show much digestion after 6 h of incubation (Figure 13 c). Even the potentially digestible tissue resisted microbial

attack. After 24 h of incubation, leaf sheaths of stage II showed more resistance to microbial attack, compared to stage I leaf sheaths (Figure 13 d and f). Microbial digestion of leaf sheath sections (stage I) resulted in complete removal of mesophyll, phloem and epidermal cells. On visual examination, the residual tissue appeared as thin strands of fibers connected by fine filaments. On SEM depth it appeared that strands were remnants of vascular bundles, especially of xylem vessels, connected by pliable cuticular layer (Figure 13 d). At higher magnification the residue showed remnants of partly digested bundle sheath, sclerenchyma and xylem (Figure 13 e). However, small vascular bundles exhibited better digestion effects.

Specimens of stage II leaf sheaths indicated resistance to microbial attack after 24 h. The specimens were found covered with a dense microbial mat. However, digestion effects were not visible, which indicated that increased lignification of tissue with maturity resisted microbial attack. Similar responses were observed in bermuda grass leaf sheaths by Akin et al. (1977). They suggested that high percentage of lignified tissues, parenchyma bundle sheath cells and vascular bundles, in leaf sheaths of bottom portions compared to top portions limit the rate of degradation.

Figure 12. SEM. a) Control frozen fixed leaf blade, stage I (x 242). b) Control frozen fixed leaf blade, stage II (x 288). c) Leaf blade, stage I, partially digested after 6 h incubation (x 288). d) Leaf blade, stage I shows extensive digestion with xylem (arrow) remaining intact after 24 incubation (x 203). e) Leaf blade, stage II, partly digested mesophyll (m) between vascular bundles after 6 h incubation (x 195). f) Leaf blade, stage II, extensive digestion except intact xylem vessel (arrow; x 402).

Figure 13. SEM. a) Control frozen fixed leaf sheath, stage I (x 290). b) Control frozen fixed leaf sheath, stage II (x 265). c) Leaf sheath, stage I, partly digested after 6 h incubation (x 315). d) Leaf sheath, stage I, digested for 24 h shows unhydrolyzed sclerenchyma (s), xylem (x), bundle sheath cells (arrow) and cuticle (c; x 71). e) High magnification of specimen 13 d (x 296). f) Leaf sheath, stage II, partly digested mesophyll (m) and phloem in vascular bundle (v) after 24 h incubation (x 150).

Anatomical Characteristics of Berseem. Berseem leaflet revealed the typical anatomy of herbaceous dicotyledon with a relatively undifferentiated mesophyll (Figure 14 a). The veins and hairs were found on the abaxial surface. The bulk of the leaflet consisted of mesophyll with some vascular tissues. No structural changes were observed when the plant matured from vegetative to boot stage. The petiole of berseem contains the same tissue as the stem, except that there were no signs of interfasicular cambial cells between vascular bundles (Figure 15 a and b). Akin and Robinson (1982) also observed no differences in the structure of arrow-leaf and crimson clover petioles at four stages of maturity, pre-bloom, early bloom, full bloom and mature (95% seed set) plants. Acid phloroglucinol positive lignin was found in vascular tissues. Anatomy of stem revealed the differences due to maturity (Figure 16 a and b). Increased deposition of lignin was observed in pith and cortex cells with advanced maturity.

Tissue Digestion of Berseem. Specimens incubated for 6 h showed rapid degradation of mesophyll tissue, but slightly less degradation occurred in mature specimens (Figure 14 b and c). The leaflet sections of small veins showed comparatively rapid and extensive digestion than midvein sections at all stages. Incubation for 6 h resulted in substantial

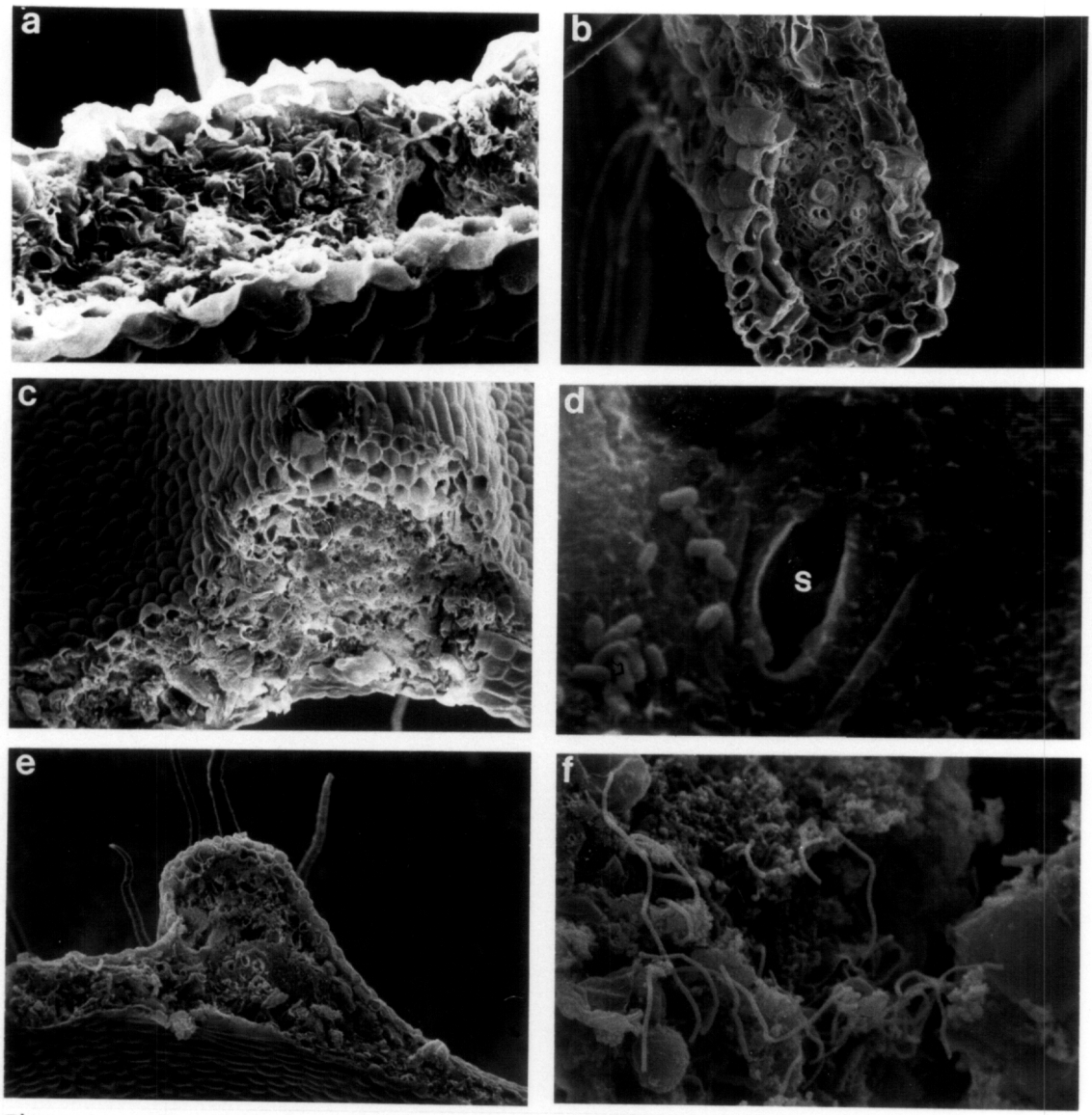

Figure 14. SEM. Control and digested berseem leaflets. Caption on page 89.

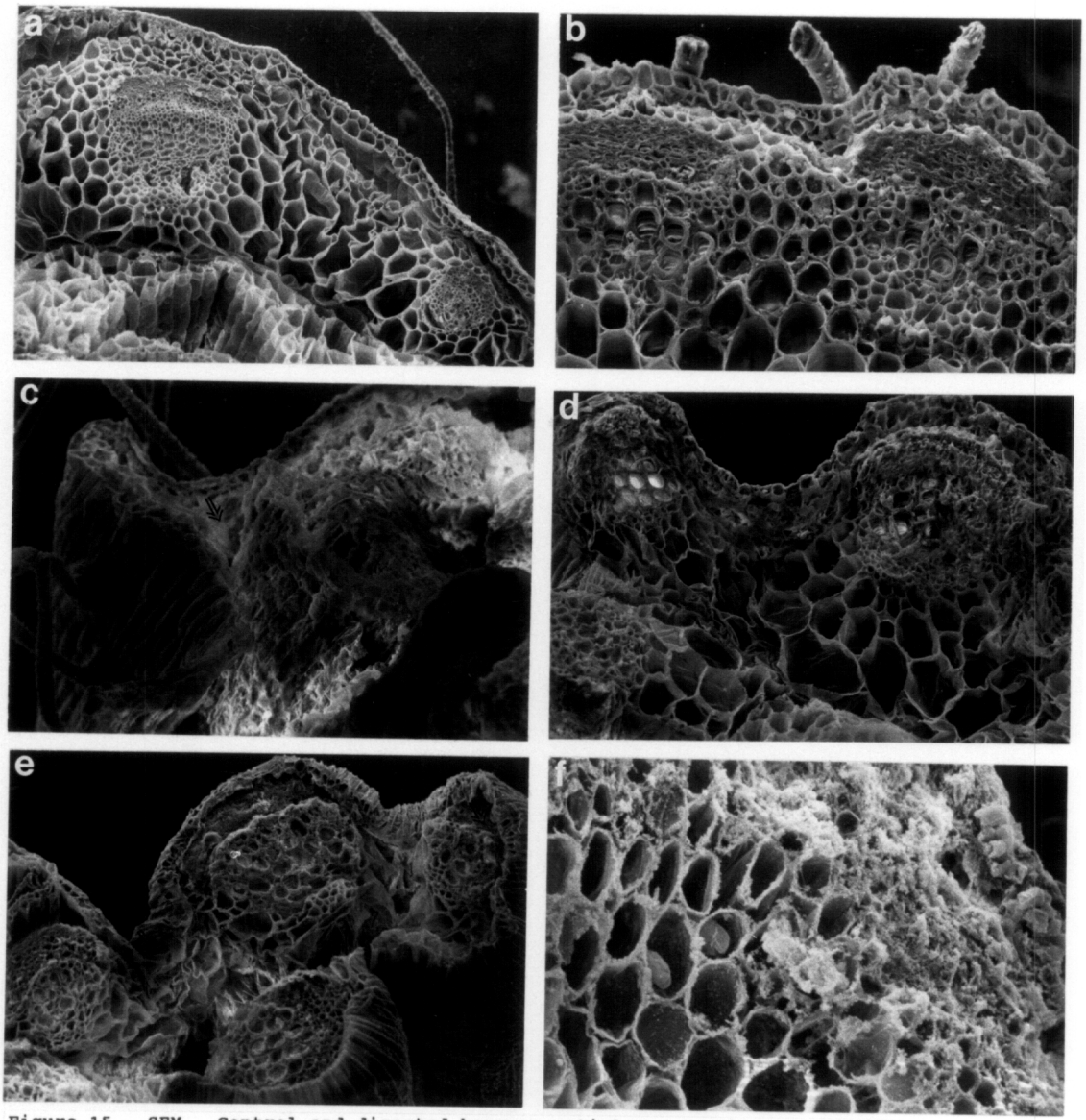

Figure 15. SEM. Control and digested berseem petioles. Caption on page 89.

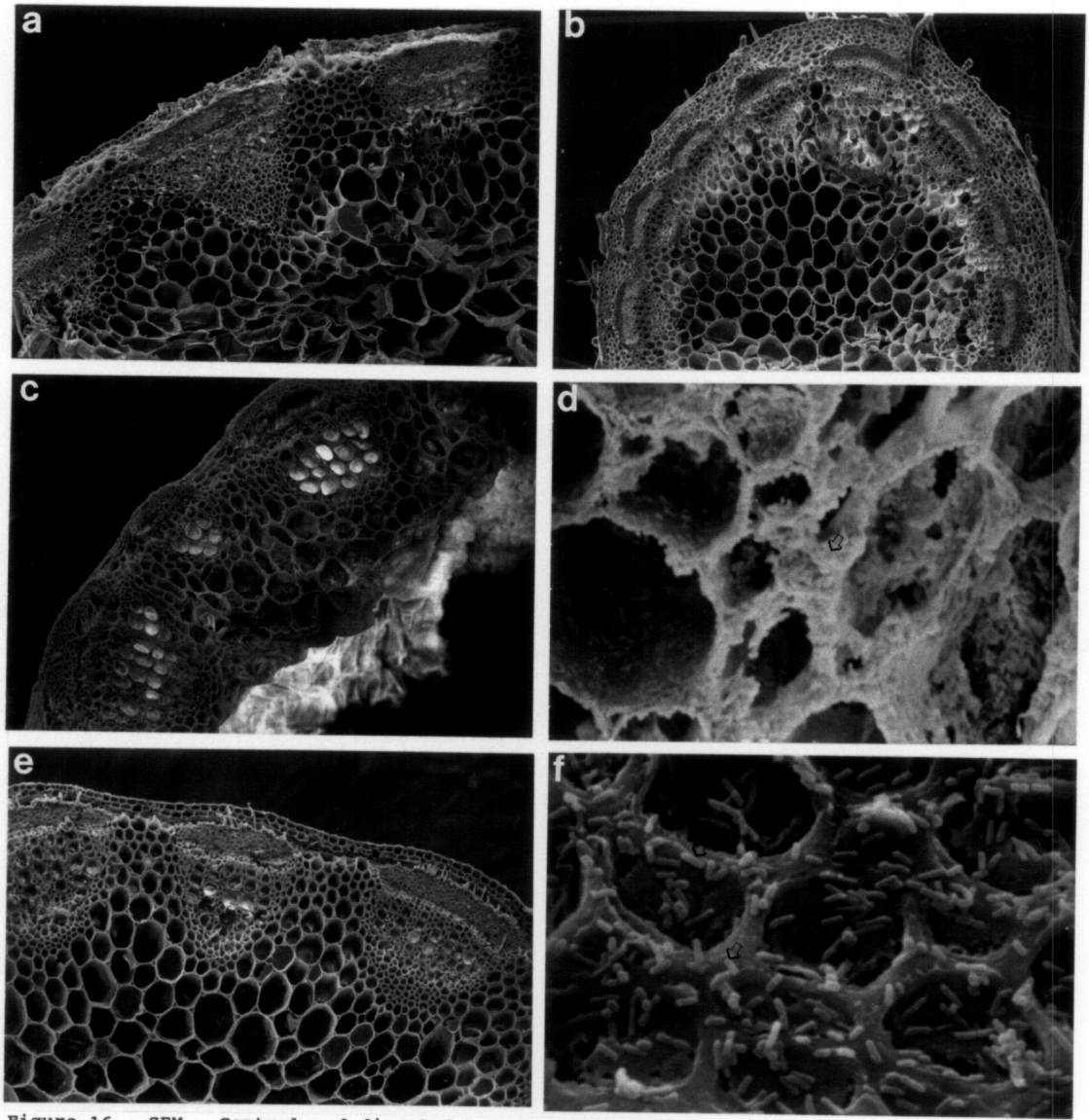

Figure 16. SEM. Control and digested berseem stem. Caption on page 91.

alterations in the specimens. Adaxial epidermis peeled off in large areas, exposing most of the mesophyll tissue to microbial attack and colonization. Fay et al. (1981) also observed such microbial effect on whole alfalfa leaves within 4 h of microbial exposure. They suggested that peeling off epidermis was due to the rapid production of fermentation gases, which were trapped and formed a "balloon" inside the epidermis. After a certain time, these "balloons" burst, leaving extensive mesophyll area directly exposed to subsequent, massive invasion by bacteria. Similar results were reported by Akin et al. (1982) when crimson clover and arrow leaf clover were digested for 6 h. They found that vascular tissue and cuticle resisted digestion, even at 72 h. Brazle and Harbers (1977) reported that exposure of alfalfa leaves to microbes for 12 h caused random sloughing and digestion of adaxial epidermis and mesophyll.

After 24 h of incubation, samples showed extensive digestion (Figure 14 e and f). However, differences due to maturity were reflected in the variable extent of digestion of large vascular bundles. Sections from stage I showed complete removal of mesophyll and epidermis, partly digested vascular bundles and undegraded cuticle, whereas stages II and III resisted microbial attack and showed partly digested mesophyll, connecting cells, epidermis and vascular bundles.

Brazle and Harbers (1977) reported complete degradation of mesophyll and epidermis and partial digestion of vascular bundles and cuticle in alfalfa hay leaflets exposed to microbial attack for 24 h.

Results from the present study indicated that digestion at cut edges were more pronounced than uncut marginal sides of berseem leaflets. Cheng et al. (1979) also reported that cutting the leaves before incubation enhanced the ability of Lachnospira multiparous to colonize and macerate the leaflets. Studying the sequence of events in the digestion of fresh legume leaves by rumen bacteria, Cheng et al. (1980) observed that invasion of intercellular spaces in legumes, which is mainly composed of pectin, seemed to occur before extensive hydrolysis of structural polymer of plant cell wall. Baker and Harris (1947) reported that pectinolytic bacteria gained access to intercellular spaces through stomata and damaged area. Chet et al. (1973) indicated that leaf leachates escaping through stomata exerted a chemotactic effect on Pseudomonas lachrymans. Fay et al. (1981) suggest that variability in rate and extent of tissue digestion in different species of legumes depend upon the nature of leachates, amount of antimicrobial substances in the epicuticular wax and the anatomical features of the forage tissues.

After 6 h of incubation, the petiole specimens of stage I showed rapid and extensive digestion, compared to stage III (Figure 15 c and d). By 24 h, substantial alterations were observed in petiole specimens of all three stages. Stage I specimens showed extensive digestion of parenchyma cells in pith and cortex region (Figure 15 e). The massive degradation of small vascular bundles resulted in complete loss of their structural integrity and differentiation of tissue types. Unlike stage I, stage II resisted microbial attack, as indicated from the rate and extent of vascular bundles digestion (Figure 15 f). However, parenchyma cells in pith and cortex region and epidermal cells showed microbial attack and digestion. At higher magnification, microbial colonization appeared on cut edges of lignified cortex cells and vascular bundles.

Differences in the digestion pattern of stem sections of stages I and III reflected the maturity effects. After 24 h of incubation with ruminal microbes, the stem sections of stage I showed massive digestion of parenchyma cells in cortex and pith, whereas vascular bundles were partially digested (Figure 16 c). Higher magnification revealed massive adherence and colonization of microbes, aligned in chains all over the cut edges of tissues (Figure 16 d). Cheng et al. (1980) suggested that Ruminococcus sp. and Bacteroides sp.

Figure 14. SEM. a) Control frozen fixed leaflet, stage I (x 194). b) Leaflet, stage I, showed no effect on vascular bundle after 6 h incubation (x 186). c) Leaflet, stage II, microbial mat covered the specimen after 6 h incubation (x 144). d) Leaflet, stage I, microbes (arrow) in the vicinity of stomata (s) after 24 h incubation (x 102). e) Leaflet, stage I, digested for 24 h (x 275). f) Leaflet, stage III, degraded mesophyll parenchyma cells extensively covered with chains of microbes (arrow) after 24 h incubation (x 828).

Figure 15. SEM. a) Control frozen fixed petiole, stage II (x 72). b) Control frozen fixed petiole, stage III (x 215). c) Petiole, stage I, completely degraded mesophyll (arrow) after 6 h incubation (x 172). d) Petiole, stage III, no apparent digestion effect after 6 h incubation (x 116). e) Petiole, stage I, incubated for 24 h, extensively digested mesophyll and phloem in vascular bundles (arrow; x 98). f) Petiole, stage III, microbial mat completely covered the specimen (x 168).

adhere to legume cell wall, by means of their glycocalyx, without producing pits. Thus, the bacterial penetration of legume cell wall proceeded by means of general disorganization, rather than by specific pit formation found in grass forage plants. Compared to stage I, stem sections of stage III resisted microbial attack (Figure 16 c and e). Stem sections of stage I showed extensively colonized thin-walled parenchyma cells and phloem in vascular bundles, compared to sparsely adhered microbes to cell walls in stage III stem (Figure 16 e and f). Our results agreed with the those of Akin and Robinson (1982), who reported that stem sections of full bloom arrow leaf and crimson clover showed resistance of interfasicular cambial cells in cortex region and lignified xylem to microbial attack after 24 h of incubation. However, the pre-bloom stem sections showed degradation of phloem and parenchyma pith cells. Brazle and Harbers (1977) reported that after 72 h of incubation, alfalfa hay stem sections showed massive digestion of epidermis and cortex, whereas, vascular bundles resisted digestion.

The differences in the degradation pattern as appeared from microscopic observation indicated that rate and extent of forage digestion depends upon anatomical differences, especially in leaves, stages of maturity and type and extent of lignification.

Figure 16. SEM. a) Control frozen fixed stem, stage II (x 56). (b) Control frozen fixed stem, stage III (x 51). c) Stem, stage I, partly disorganized tissues after 24 h incubation (x 50). d) Higher magnification of figure 16c. Parenchyma cells in cortex region heavily colonized by microbes (arrow; x 672). e) Stem, stage III, digested for 24 h (x 80). f) High magnification of figure 13e, sparsely colonized parenchyma cells in cortex region (arrow; x 2,430).

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CHAPTER IV

EFFECT OF PLANT MATURITY ON CHEMICAL COMPOSITION AND IN VITRO DRY MATTER DIGESTIBILITY OF BARLEY, MILLET AND BERSEEM

ABSTRACT

An experiment was conducted to assess the changes associated with plant maturity in the chemical components and the rate and extent of DM digestion in grasses and legume. Barley and millet were harvested at vegetative, boot and heading, and berseem, at vegetative, early bloom and full bloom. Plants were separated into three portions: top, middle and bottom. The middle portion was separated into three botanical fractions, leaf blades, leaf sheaths and stems for grasses and leaflets, petioles and stem for legume. Results for both grasses indicated higher ($P<.05$) contents of cell-wall constituents for stems, followed by leaf sheaths and leaf blades. The effect of maturity on cell-wall constituents was greater ($P<.05$) for stems and leaf sheaths, compared to leaf blades. A larger increase in xylose concentration and decrease in arabinose due to maturity was found in stems, followed by leaf sheaths and leaf blades. Higher values were found for p-coumaric acid, followed by ferulic acid with maturity for all botanical fractions. The concentration of p-coumaric acid and ratio of p-coumaric acid

to ferulic acid were higher in the tropical compared to the temperate grass. Results for in vitro DM digestion (IVDMD) in various botanical fractions of barley and millet indicated that leaf blades were digested at a faster rate, compared to leaf sheaths and stems.

No effect of maturity on cell-wall constituents was found in berseem. Leaflets had lower ($P<.05$) values for cell-wall constituents, compared to stems and petioles. Between stems and petioles, higher ($P<.05$) lignin was found for stems. Regardless of maturity, among all botanical fractions, a higher concentration of xylose was found for stems, compared to petioles and leaflets. Major alkali-labile phenolic monomers, p-coumaric and ferulic acids were not found in all botanical fractions at all stages of maturity. However, vanillin, vanillic acid and syringaldehyde were found, especially in immature plants. No differences ($P>.05$) were found in the rate and extent of dry matter digestion for all subparts of berseem.

(Keywords: Maturity, Grasses, Legume, IVDMD, Phenolic acids, Neutral sugars.)

INTRODUCTION

Herbaceous forages include tropical and temperate grasses and legumes. The cell-wall constituents of forages account for a considerable proportion of the DM, particularly

at mature stages of growth (Jones, 1970). The major structural polysaccharides of plants are cellulose, hemicellulose and pectin (Wedig et al., 1986).

Cellulose, composed of β-1, 4-pyranosidic chains of glucose, has a gross energy content equal to that of starch (Kerley et al., 1985). The digestibility and nutritive availability of cellulose varies with plant maturity. Allinson and Osbourn (1970) demonstrated a negative linear relationship between forage digestibility and lignin. However, in general, legumes which contained a high proportion of lignin showed higher digestibility than grasses (Van Soest, 1982). Comparison of linear and curvilinear models indicated that, for predicting forage quality, curvilinear models ($P<.05$) gave a better description of relationships with lignin, suggesting interactions between cell-wall components (Jung and Vogel, 1986).

Chemical analysis of forage cell wall indicates that lignin is covalently attached to xylose of hemicelluloses via ester linkages (Morrison, 1979). A strong negative correlation ($r = -.84$) was found between IVDMD and lignin of three maturing temperate grasses (Burritt et al., 1984). Brice and Morrison (1982) suggested that the extent to which xylans are substituted may influence the rate of digestion.

Plant phenolic acids and acetyl groups, although they constitute only 1 to 2% of plant DM, were suggested to have significant effect on cell-wall digestion (Higuchi et al., 1967). A wide variety of phenolic compounds are found in plants, however, benzoic and cinnamic acids are the most common (Jung, 1985). The cinnamic acids, which are abundantly available in forages, act as cross linking agents between lignin and hemicellulose. Complexes, consisting of ferulic and p-coumaric acids bound to xylan and glucans via alkali-labile ester linkages, have been isolated from forage cell walls (Hartley, 1972; Hartley et al., 1976). Studies on wood lignin have indicated alkali-labile ester bonds between lignin and phenolic moieties (Higuchi et al., 1967). Ether linkages between p-coumaric and lignin were also reported (Scalbert et al., 1983). Acetyl groups are found as substituents of plant cell walls (Bacon et al., 1981). Brice and Morrison (1982) reported that extent of substitution influences rate of digestion. Toxic effects of phenolic acids have been found on growth and cellulolytic activity of rumen bacteria (Chesson et al., 1982), protozoa (Akin, 1982) and fungi (Akin and Rigsby, 1985).

This study was conducted to 1) assess the variation associated with plant maturity on cell-wall constituents, alkali-labile phenolic acids, hemicellulosic monosaccharides

and rate and extent of DM digestion of botanical fractions and 2) establish relationship between IVDMD and chemical components.

MATERIALS AND METHODS

Two grasses (a tropical and a temperate) and one legume were established at Blacksburg, VA in a 3 x 4 randomized block design on a clayey silt loam soil. In the fall of 1987, the experimental area was fertilized with 3,360 kg limestone, 33.6 kg N, 22.4 kg P_2O_5 and 33.6 kg K_2O per hectare, according to soil test recommendations. The entire area was seeded with "Wysor" barley in fall of 1987. In spring of 1988, plots for berseem and millet were sprayed with Paraquat[1] (2.29 L/ha) to kill barley prior to establishment of these two forages. Big bee' berseem (Trifolium alexandrinum L.) was seeded in April, 1988, and 'Tifleaf 1' millet (Pennisetum americanum L.) was seeded in June, 1988.

The entire plot, which covered an area of 68 x 55 m, was subdivided into 12 blocks. Each block covered an area of 19 x 11 m. A buffer area of 3 m width was left between neighboring blocks. The blocking was done towards slope and treatments were assigned randomly to blocks. The blocks assigned to millet (tropical forage) were fertilized with NH_4NO_3 at a rate of 67.2 kg N/ha.

Sampling Procedures. Grasses were collected at four stages of development, vegetative (node differentiating, 2 to 3 nodes, stage I), boot (1/10 bloom, stage II), and heading (full bloom, stage III). Plants from stages II and III were cut into three portions, designated as top, middle and bottom portions, whereas, stage I plants were not separated and assumed as equivalent to middle portions of stages II and III.

Legume (berseem) was harvested at three stages of development, vegetative (stage I), bud (stage II) and full bloom (stage III). The plants from stage II were divided into two equal portions, top and bottom. Plants from stage III were cut into three equal portions; top, middle and bottom. The whole plants from stage I and top portion of stage II were assumed as equivalent to the middle portion of stage III.

The top, middle and bottom portions of barley and the middle portions of millet and berseem were separated into three botanical fractions, leaf blades, leaf sheaths and stems for grasses; and leaflets, petioles and stems for legume.

Sample Preparation. The portions and subparts of forages collected at different stages of maturity were dried in a forced draft oven at 60 C until constant weight and

ground through a Wiley mill using a 1 mm screen. The ground samples were stored in sample bottles until further analysis.

In Vitro Digestibility. One ruminally cannulated Angus steer was used for collection of ruminal fluid. The animal was fed a mixture of chopped alfalfa and orchard grass hays (1:1). The animal had access to water at all times except 2 h before rumen collection.

Ruminal ingesta was collected from the steer 2 h after the withdrawal of feed and water, filtered through eight layers of cheese cloth into a thermos and kept at 39 ± 1 C.

In vitro DM digestibility of dried (60 C) ground samples was determined using the two-stage Tilley and Terry (1963) technique. Each sample was incubated in triplicate for four periods of 12, 24, 48 and 96 h. The reaction was stopped by refrigerating the reaction tubes. The samples were centrifuged at 1250 g for 15 min. The supernatant was discarded and residue was dried at 100 C to a constant weight.

Chemical Analyses. The dried ground samples were analyzed for NDF (VanSoest and Wine, 1967), ADF (VanSoest, 1963), lignin and cellulose (VanSoest and Wine, 1968). Hemicellulose was calculated as the difference between NDF and ADF.

Preparation of Cell Wall Material. The cell walls were prepared from dried plants and (or) plant parts by the method

of Van Soest and Wine (1967), using boiling neutral detergent solution to remove cell solubles; no Na_2SO_3 and decahydronephthalene were used to prepare cell wall fraction.

The monosaccharide (neutral sugars) composition of hemicellulosic sugar in acid hydrolysate of plant cell walls were determined by gas chromatography as described by McGinnis (1982). Ten milligrams of isolated dried NDF were weighed into 5 ml reacti-vials[3] with vinyl-lined screw caps. Three milliliters of 1 N H_2SO_4 were added and samples were hydrolyzed at 100 C for 2 h in a reacti-therm[3]. Standard sugar solution was prepared by adding rhamnose, fucose and mannose, .2 mg/ml of each sugar in 1 N H_2SO_4, and arabinose, xylose and glucose, .5 mg/ml of each sugar in 1 N H_2SO_4.

Aldonitrite acetates were prepared by adding .4 ml of GC reagent (hydroxylamine•HCl, .5 g; methyl α-D glucopyranoside-internal standard, .1 g; methyl imidazole, 20 ml) in .2 ml solution of digested sample or standard solution. The reacti-vials were heated at 80 C for 10 min, later cooled to room temperature and 1 ml of acetic anhydride was added. The vials were held at room temperature, 1 ml of chloroform was added, mixed and washed twice with 1 ml of

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[3] Pierce, Rockford, IL.

water. The aqueous fraction was discarded and about .4 g of anhydrous Na_2SO_4 was added to the chloroform layer.

The derivatized sugars were chromatographed on Gowmac gas chromatograph (model 750) equipped with flame ionization detector and integrator (model 3390, Hewlett Packard). Gas liquid chromatography analysis was made with a 3.05 m x 3.2 mm i.d. glass column packed with 1% diethylene glycol adipate on chromosorb WHP (100-120 mesh)[4]. The injector and detector temperature were maintained at 240 C and column temperature at 195 C (isothermal). The flow rate of N_2 (carrier gas) and H_2 was 35 ml/min and of air, 300 ml/min. The concentration of sugars in the samples was calculated from peak areas relative to the peak areas of standard sugars using internal standard technique.

The alkali-labile phenolic acids were extracted according to the method of Ford and Hartley (1988). Cell walls (50 mg) were weighed in rubber stoppered tubes of 20 ml capacity. Two ml of 1 M NaOH were added, air was removed by flushing N_2 continuously, and the suspension was shaken at room temperature for 20 h. After alkaline hydrolysis, the filtrate was removed, using pasteur pipette, and the residue was

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[4] Supelco Inc., Bellefonte, PA.

washed with H_2O (2 x .5 ml). The filtrate and washings were collected and acidified with 2.5 M HCl and diluted to 5.0 ml with water.

Free phenolic acids and aldehydes were extracted with ethyl ether (3 x 5 ml). Ethyl ether extracts were collected and dried under N_2, and residues were stored over silica gel until further analysis. A mixture of standard phenolic acids and aldehydes (cis- and trans- p-coumaric acid, cis- and trans- ferulic acid, vanillic acid, vanillin, p-hydroxybenzaldehyde, p-hydroxybenzoic acid, and syringaldehyde) was prepared by adding 2 mg/100 μl of each acid or aldehyde in methanol, dried under N_2 and stored over silica gel.

Phenolic methyl esters were prepared by mixing the residues with 1 ml of diazomethane solution, and allowed to stand at room temperature for 1 h. Diazomethane solution was prepared according to the method of Arndt (1943). After 1 h, excess solution was evaporated under N_2 and the residue was redissolved in 100 μl of methanol. The derivatized samples and standards were injected in Perkin Elmer model 8500 gas chromatograph equipped with flame ionizing detector and GP-100 graphics printer. The derivatives were separated in a wall-coated open-tubular bonded phase fused silica column (SPB-1, 30 m x .25 mm i.d., .27 μm film thickness)[4]. The rate

of flow of helium carrier gas was 20 ml/min. Detector and injector temperatures were 325 and 350 C, respectively. Column oven temperature initially was at 115 C, kept isothermal for 10 min, programmed to rise at 2 C/min until it reached 135 C, held for 5 min, after which the rate of heating was increased to 25 C/min until a temperature of 300 C was achieved. The complete run time was 31 min.

The concentrations of phenolic acids and aldehydes were calculated from area of sample peak relative to area of standard peak.

Statistical Analyses. Statistical analyses were performed using the analysis of variance by general linear model procedure (SAS, 1982). For middle portion of all plants, block, maturity, subparts, maturity*block and maturity*subparts interactions were included in the model. The effect of maturity was tested with maturity*block interaction. For top and bottom portions of barley, block, maturity, subparts, portions, maturity*block, maturity*subparts, maturity*portions, portions*subparts and maturity*portions*subparts interactions were included in the model. The effect of maturity was tested with maturity*block interaction. For top and bottom portions of millet and berseem, block, maturity, portions, maturity*block and maturity*portion interactions were included in the model.

Maturity effect was tested with maturity*block interaction. For IVDMD studies, time was included in all the models. Comparison of least squares means were made by using Bonferonni's procedures (SAS, 1982).

RESULTS AND DISCUSSION

Changes in Cell Wall Constituents Associated with Maturity of Barley. Average values of three stages of maturity for different plant parts of barley middle portion indicated higher ($P<.05$) values for NDF, ADF, cellulose and lignin and lower hemicellulose, in stem samples than leaves (Table 1). Aman and Nordkvist (1983) reported higher values for cellulose and lignin and lower values for hemicellulose in barley stem than leaves. Leaf sheaths showed comparatively higher ($P<.05$) ADF and cellulose but lower ($P<.05$) hemicellulose content than leaf blades. Within subparts, the effects of maturity were more apparent. Neutral detergent fiber for leaf sheaths and stems was higher ($P<.05$) at heading stage than at primary growth stages (Table 1). The NDF values for leaf blades did not change consistently with maturation. The ADF and cellulose values of all subparts increased ($P<.05$) with advancing maturity. However, the change in ADF values for leaf blades and leaf sheaths were smaller than for stem. Subparts*maturity interactions ($P<.05$) were seen for NDF, ADF and cellulose.

TABLE 1. EFFECT OF PLANT MATURITY ON CELL WALL CONSTITUENTS OF BARLEY BOTANICAL FRACTIONS[a] (MIDDLE PORTION)

Subparts	Stage of maturity	NDF[bcd]	ADF[bde]	Cellulose[bde]	Hemicellulose[de]	Lignin[cd]
		----------%[f]----------				
Leaf blades	Vegetative	60.15	21.98[g]	16.66[g]	38.17	5.04
	Boot	55.58	23.01[g]	18.10[g]	32.57	4.28
	Heading	57.33	27.87[h]	21.27[h]	29.46	5.47
	Average	57.69	24.29	18.68	33.40	4.93
Leaf sheaths	Vegetative	56.71[i]	26.63[i]	20.57[i]	30.08	5.78
	Boot	54.32[i]	30.14[i]	25.50[j]	24.18	4.46
	Heading	70.53[j]	41.41[j]	32.90[k]	29.12	7.77
	Average	60.51	32.73	26.32	27.79	6.01
Stems	Vegetative	58.44[l]	26.80[l]	19.28[l]	31.64	6.82[l]
	Boot	59.35[l]	34.08[l]	30.54[m]	25.27	5.35[l]
	Heading	71.59[m]	48.45[m]	37.03[n]	23.14	11.27[m]
	Average	63.13	36.55	28.95	26.57	7.82
	SE	2.02	.59	.82	1.89	.92

[a]Each value represents the means for eight samples.
[b]Significant effect of subparts*maturity interaction (P<.05).
[c]Significant effect of subparts and maturity (P<.05).
[d]Means for leaf blades and leaf sheaths differ from means of stems (P<.05).
[e]Means for leaf blades differ from means of leaf sheaths (P<.05).
[f]DM basis.
[gh]For leaf blades, means within columns with different superscripts differ (P<.05).
[ijk]For leaf sheaths, means within columns with different superscripts differ (P<.05).
[lmn]For stems, means within columns with different superscripts differ (P<.05).

Average hemicellulose content decreased with maturity, although differences were not significant ($P>.05$). Highest deposition of lignin was in stem samples of heading stage ($P<.05$). Jones (1970) reported increases in structural carbohydrates and lignin of cocksfoot, timothy and ryegrass with physiological maturity. Similar results were reported by Kamstra et al. (1958), who analyzed orchard grass and timothy harvested at three different maturities.

Acid detergent fiber, cellulose and lignin, averaged over maturity stages, revealed highest values for stem and lowest for leaf blades (Table 2). The hemicellulose concentration was highest in leaf blades and lowest in stem. These results suggest an inverse relationship between hemicellulose and lignin content. In contrast to our findings, Morrison (1980) reported a strong correlation ($r = .87$) between hemicellulose and lignin for 10 temperate grasses. The total hemicellulose concentration generally increased with maturity, the increase being more pronounced in stem than leaves. He concluded that grasses with higher lignin content showed higher hemicellulose concentrations than the grasses with lower lignin concentration. In present study, the content of cellulose and lignin differed ($P<.05$) among plant subparts.

TABLE 2. EFFECT OF PLANT MATURITY ON CELL WALL CONSTITUENTS OF TOP AND BOTTOM OF PORTIONS OF BARLEY[a]

Subparts	Portion	Stage of maturity	NDF[bc]	ADF[bce]	Cellu-lose[bce]	Hemicel-lulose[cde]	Lignin[bce]
			----------%[f]----------				
Leaf blades	Top	Boot	53.77[g]	23.91[g]	16.91[g]	29.86[g]	4.39
		Heading	64.43[h]	23.51[g]	17.24[g]	40.92[h]	4.79
	Bottom	Boot	58.19[g]	24.23[g]	18.48[g]	33.96[g]	5.43
		Heading	72.95[i]	34.53[h]	24.32[h]	38.42[gh]	4.34
		Average	60.38	26.18	19.39	34.20	4.78
Leaf sheaths	Top	Boot	54.99[j]	27.83[j]	21.75[j]	27.17	5.16[j]
		Heading	64.72[k]	33.44[k]	27.58[k]	28.28	5.15[j]
	Bottom	Boot	58.13[j]	34.41[k]	27.24[k]	23.72	6.43[j]
		Heading	72.78[l]	46.96[l]	35.24[l]	25.82	9.07[k]
		Average	62.08	35.70	28.37	26.38	6.34
Stems	Top	Boot	43.43[m]	20.08[m]	15.83[m]	23.35	4.34[m]
		Heading	72.05[n]	47.26[n]	36.99[n]	24.78	9.29[n]
	Bottom	Boot	64.68[p]	38.65[p]	31.99[p]	26.03	6.25[n]
		Heading	74.80[n]	50.72[n]	39.92[r]	24.08	7.81[n]
		Average	64.32	39.93	32.05	24.39	7.38
		SE	1.68	1.08	.67	1.81	.64

[a]Each value represents the mean for eight samples.
[b]Significant effect of subparts, maturity, portion and interaction of subparts*portion (P<.05).
[c]Means of leaf blades and leaf sheaths differ from means of stems (P<.05).
[d]Significant effect of subparts, maturity and interaction of subparts*maturity*portion (P<.05).
[e]Means of leaf blades differ from means of leaf sheaths (P<.05).
[f]DM basis.
[ghi]For leaf blades, means within columns with different superscripts differ (P<.05).
[jkl]For leaf sheaths, means within columns with different superscripts differ (P<.05).
[mnpr]For stems, means within columns with different superscripts differ (P<.05).

Leaf blades were lower (P<.05) in NDF, ADF and cellulose of top and bottom portions than leaf sheaths, especially in more mature samples. The values for ADF and cellulose in top were lower than in bottom portions of stem (P<.05) at early stages of development. Interactions (P<.05) of subpart*maturity*portion were seen for NDF, ADF, cellulose and lignin contents.

Jung and Vogel (1986) reported that stem showed comparatively more increase in cell wall and lignin content with increase in maturity than leaves. The rigidity of stem anatomy itself is probably responsible for more lignification than leaf blades or leaf sheaths (Pigden, 1953). Similar results were reported by Morrison (1980) for temperate grasses stems and leaves. He reported that increased lignin concentration was more marked in stem tissue than leaf tissue.

In the present study, no differences were found in lignin values for leaf blades at all stages of maturity and between top and bottom portions, but some values differed (P<.05) for leaf sheaths and stems in bottom portions of mature samples.

Changes in Hemicellulosic Monosaccharides Associated with Maturity of Barley. Among plant subparts, xylose values averaged over maturity were higher (P<.05) for stems and leaf

sheaths than leaf blades (Table 3). Arabinose content was lower ($P<.05$) in stems, compared to leaf blades and leaf sheaths.

With advancing maturity, percent xylose increased ($P<.05$) in all subparts. Within subparts, the effect of maturity on xylose values of leaf blades was not apparent in early stages of development. Compared to leaf blades, the effect of maturity ($P<.05$) on xylose appeared at early stages of development in leaf sheaths and stems. In contrast to xylose, arabinose content was decreased ($P<.05$) in stems and increased in leaf blades with maturation. A decreasing trend was also observed in arabinose content of leaf sheaths. Subparts*maturity interactions ($P<.05$) were observed for arabinose and xylose. Our results are in agreement with the earlier findings of Morrison (1980), who reported a positive effect of plant maturity on xylose content of temperate grass leaves and stems.

Morrison (1974) examined the hemicellulosic monosaccharides of ryegrass collected at different stages of maturity. He found an increase in xylose:arabinose ratio of linear xylan, whereas the composition of branched xylan remained unchanged with increasing maturity. The increase in the proportion of linear xylan to branched xylan was also

TABLE 3. EFFECT OF PLANT MATURITY ON NEUTRAL MONOSACCHARIDE COMPOSITION OF HEMICELLULOSE IN BARLEY BOTANICAL FRACTIONS[a] (MIDDLE PORTION)

Subparts	Stage of maturity	Arabinose[bc]	Xylose[bcd]	Mannose[e]	Glucose[bd]	Galactose[bcd]
		----------%[f]----------				
Leaf blades	Vegetative	3.41[g]	9.34[g]	.29[gh]	2.42[g]	.46[g]
	Boot	3.49[g]	9.68[g]	.00[g]	.95[h]	1.56[h]
	Heading	5.70[h]	15.47[h]	.55[h]	2.14[hg]	2.35[h]
	Average	4.20	11.50	.28	1.83	1.45
Leaf sheaths	Vegetative	4.93[i]	13.76[i]	.15[ij]	6.57[i]	.04[i]
	Boot	3.35[j]	17.67[j]	.00[i]	2.72[j]	.27[j]
	Heading	4.48[ij]	18.46[j]	.54[j]	.80[k]	.43[j]
	Average	4.25	14.96	.23	3.36	.56
Stems	Vegetative	4.14[l]	14.09[l]	.00	4.44[l]	.09[l]
	Boot	2.28[m]	19.34[m]	.00	2.02[m]	.25[lm]
	Heading	2.63[m]	18.47[m]	.00	.57[n]	.71[m]
	Average	2.68	17.30	.00	2.34	.35
	SE	.38	.55	.13	.36	.14

[a]Each value represents the mean for eight samples.
[b]Significant effect of subparts, maturity and interaction of subparts*maturity (P<.05).
[c]Means of leaf blades and leaf sheaths differ from means of stems (P<.05).
[d]Means of leaf blades differ from means of leaf sheaths (P<.05).
[e]Significant effect of subparts*maturity interaction (P<.05).
[f]Cell wall basis.
[gh]For leaf blades, means within columns with different superscripts differ (P<.05).
[ijk]For leaf sheaths, means within columns with different superscripts differ (P<.05).
[lmn]For stems, means within columns with different superscripts differ (P<.05).

reported in temperate grasses with plant maturation (Morrison, 1980).

No consistent effect of maturity was found in mannose and galactose values. The glucose values of leaf sheaths and stems decreased ($P<.05$) with advancing maturity, but no consistent effect was observed in leaf blades.

Results for monosaccharides composition of top and bottom plant portions (Table 4) averaged over maturity indicated high arabinose values in leaf blades and low in stems, but the opposite was seen for xylose. No consistent effect was observed on mannose and glucose content, however, a decreasing trend was noted in galactose with advancing maturity. Within subparts, percent arabinose of leaf blades was higher in bottom portions at all stages of maturity than top portions. In contrast to leaf blades, a negative effect ($P<.05$) of maturity on arabinose was noted in top stem samples than bottom stem samples. However, differences due to maturity were not found in arabinose values of top and bottom leaf sheaths. The xylose concentration increased ($P<.05$) with maturity for all the subparts, the increase being maximum in stem. The xylose values for top and bottom portions differed ($P<.05$) for all the three subparts, however, differences were not apparent at early stages of development. Interactions ($P<.05$) of subpart*maturity*portion were seen

TABLE 4. EFFECT OF PLANT MATURITY ON NEUTRAL MONOSACCHARIDE COMPOSITION OF HEMICELLULOSE IN TOP AND BOTTOM PORTIONS OF BARLEY[a]

Subparts	Portion	Stage of maturity	Arabinose[bcd]	Xylose[bcd]	Mannose[e]	Glucose[f]	Galactose[bcd]
			----------%[g]----------				
Leaf blades	Top	Boot	3.80[h]	11.17[h]	.94[h]	1.60[h]	1.53[h]
		Heading	4.23[h]	13.03[i]	.00[i]	3.53[i]	.84[i]
	Bottom	Boot	5.16[i]	12.34[hi]	.27[i]	1.17[h]	2.15[h]
		Heading	5.63[i]	14.09[i]	.29[i]	2.56[h]	2.44[i]
		Average	4.67	13.29	.34	1.99	1.81
Leaf sheaths	Top	Boot	3.47	13.62[j]	.76	2.72	.83[j]
		Heading	3.86	13.82[j]	.53	1.26	.00[k]
	Bottom	Boot	3.39	16.21[j]	.53	2.32	1.34[j]
		Heading	3.32	20.20[k]	.62	1.90	.81[j]
		Average	3.98	15.83	.50	1.95	.38
Stems	Top	Boot	3.66[l]	14.31[l]	.58	3.05	.29
		Heading	2.14[m]	19.26[m]	.00	2.19	.00
	Bottom	Boot	2.94[l]	15.96[l]	.33	2.54	.11
		Heading	2.18[l]	20.54[m]	.00	1.39	.25
		Average	2.47	17.45	.34	1.96	.27
		SE	.34	.87	.20	.39	.15

[a]Each value represents the mean for eight samples.
[b]Significant effect of subparts, portions and interactions of subparts*portions and subparts*portions*maturity (P<.05).
[c]Means of leaf blades and leaf sheaths differ from means of stems (P<.05).
[d]Means of leaf blades differ from means of leaf sheaths (P<.05).
[e]Significant effect of subparts*portions*maturity interaction (P<.05).
[f]Significant effect of portions (P<.05).
[g]Cell wall basis.
[hi]For leaf blades, means within columns with different superscripts differ (P<.05).
[jk]For leaf sheaths, means within columns with different superscripts differ (P<.05).
[lm]For stems, means within columns with different superscripts differ (P<.05).

for xylose, arabinose, mannose and galactose contents. A decreasing trend was noted for mannose, glucose and galactose with maturity for all subparts.

Changes in Alkali-labile Phenolic Monomers Associated with Maturity of Barley. Within botanical fractions, no consistent effect due to maturity was found on vanillin, vanillic acid and syringaldehyde content of leaf blades (Table 5). This may be due to the fact that P-OH benzaldehyde, vanillin and syringaldehyde are usually the end product of nitrobenzene oxidation of grass lignin (Higuchi et al., 1967). Generally, a decrease in P-OH benzaldehyde and vanillin and an increase in vanillic acid was found in leaf sheaths and stems. Subparts*maturity interactions ($P<.05$) were seen for all phenolic monomers.

The two major phenolic acids identified were p-coumaric and ferulic acids. In all three botanical fractions, the content of p-coumaric and ferulic acid tended to increase with plant maturity. However, the increase was more for p-coumaric acid relative to ferulic acid. Increases ($P<.05$) were found in stems and trends for increases were seen for other parts. Hartley (1972) reported increases in major alkali-labile phenolic monomers, p-coumaric and ferulic acids, of Italian ryegrass leaf blades and leaf sheaths with maturity, and the increase in p-coumaric acid was relatively

TABLE 5. EFFECT OF PLANT MATURITY ON ALKALI-LABILE PHENOLIC MONOMERS OF BARLEY BOTANICAL FRACTIONS[a] (MIDDLE PORTION)

Subparts	Stage of maturity	Alkali-labile phenolic monomers[b]						
		BAL[cdef]	VAN[cdef]	VA[cd]	CA[cef]	SYA[cdf]	FA[cde]	TPA[ce]
		mg·g^{-1} cell wall						
Leaf blades	Vegetative	2.68[g]	1.17[gh]	1.00[g]	0.78	0.00	1.19[g]	6.83
	Boot	1.35[h]	2.05[g]	0.00[h]	1.25	0.00	1.60[g]	6.25
	Heading	0.00	0.28[h]	0.75[gh]	1.59	0.00	3.45[h]	6.05
	Average	1.32	1.17	0.58	1.21	0.00	2.08	6.38
Leaf sheaths	Vegetative	0.12	1.91[i]	0.34[i]	1.28	0.37[i]	1.93	5.94
	Boot	0.00	0.29[j]	0.25[i]	1.78	0.11[j]	2.83	5.24
	Heading	0.00	0.36[j]	2.35[j]	2.20	0.00[j]	2.65	7.55
	Average	0.04	0.85	0.98	1.75	0.16	2.47	6.24
Stems	Vegetative	0.42	0.71	1.31	2.13[k]	0.00[k]	2.43[k]	7.00[k]
	Boot	0.00	0.49	1.25	3.18[k]	0.25[l]	2.62[k]	7.76[k]
	Heading	0.00	0.44	1.91	4.45[l]	0.00[k]	4.38[l]	11.15[l]
	Average	0.14	0.55	1.49	3.25	0.08	3.14	8.63
	SE	.14	.35	.24	.32	.03	.32	.78

[a]Each value represents the mean for four samples.
[b]BAL=p-hydroxybenzaldehyde, VAN= vanillin, VA= vanillic acid, CA=p-coumaric acid, SYA=syringaldehyde, FA=ferulic acid, TPA=sum of individual phenolics.
[c]Significant effect of subparts and maturity (P<.05).
[d]Significant effect of subparts*maturity interaction (P<.05).
[e]Means of leaf blades and leaf sheaths differ from means of stems (P<.05).
[f]Means of leaf blades differ from means of leaf sheaths (P<.05).
[gh]For leaf blades, means within columns with different superscripts differ (P<.05).
[ij]For leaf sheaths, means within columns with different superscripts differ (P<.05).
[kl]For stems, means within columns with different superscripts differ (P<.05).

more in leaf blades. Similar results were reported in timothy (Theander et al., 1981), tall fescue hay (Jung et al., 1984), smooth brome grass hay (Jung and Fahey, 1983) and Italian and perennial rye grasses (Hartley and Jones, 1977).

In the present study, the values of phenolic monomers averaged over maturity stages indicated significant differences ($P<.05$) between leaves and stems but not between leaf blades and leaf sheaths ($P>.05$). Highest values were found in stems and lowest values in leaf blades.

Mean values of alkali-labile phenolic monomers in different botanical fraction of top and bottom portions of barley showed no consistent effect of maturity on benzaldehyde, vanillin, vanillic acid and syringaldehyde (Table 6). Benzoic acid was not detected in any of three botanical fractions. Contrary to our results, Hartley and Jones (1977) reported p-OH benzoic and benzaldehyde in senescence leaf blades and leaf sheaths of Italian and perennial rye grasses. In the present study, within botanical fractions, stems tended to have higher p-coumaric acid and ferulic acid than leaf blades or leaf sheath, although differences due to maturity were not found. Within leaf blades, the ferulic acid content increased with maturity, although the increase was larger ($P<.05$) for bottom leaf blades.

TABLE 6. EFFECT OF PLANT MATURITY ON ALKALI-LABILE PHENOLIC MONOMERS OF BARLEY TOP AND BOTTOM BOTANICAL FRACTIONS[a]

Subparts	Portion	Stage of maturity	Alkali-labile phenolic monomers[b]						
			BAL[cdefg]	VAN[cdefg]	VA[d]	CA[defg]	SYA[d]	FA[cdef]	TPA[def]
			mg·g^{-1} cell wall						
Leaf blades	Top	Boot	0.71^{h}	0.88^{h}	1.03	0.24	0.00	1.65^{h}	4.52
		Heading	0.00^{i}	0.93^{h}	1.23	1.10	0.00	1.75^{h}	5.01
	Bottom	Boot	0.36^{j}	1.48^{i}	1.74	1.10	0.00	1.35^{h}	6.02
		Heading	0.00^{i}	0.64^{i}	1.52	1.30	0.00	3.71^{i}	7.15
		Average	.41	1.37	1.04	1.10	0.00	2.25	6.16
Leaf sheaths	Top	Boot	0.00	0.19	0.40^{k}	2.04	0.18	3.36	6.16
		Heading	0.00	0.22	2.08^{l}	2.47	0.00	2.89	7.66
	Bottom	Boot	0.00	0.34	0.95^{k}	2.61	0.00	2.31	6.21
		Heading	0.00	0.21	0.59^{k}	2.46	0.37	2.99	6.62
		Average	0.00	0.27	1.10	2.43	0.11	2.51	6.41
Stems	Top	Boot	0.00	0.54	1.37	2.88	0.00	2.66	7.44
		Heading	0.00	0.19	0.60	3.20	0.41^{m}	3.00	7.40
	Bottom	Boot	0.00	0.33	1.74	3.57	0.71^{n}	3.18	9.52
		Heading	0.00	0.00	0.77	3.95	0.00^{m}	2.96	7.68
		Average	0.00	0.33	1.27	3.04	0.23	2.80	7.66
		SE	.09	.14	.32	.34	.13	.33	.85

[a]Each value represents the mean for four samples.
[b]BAL=p-hydroxybenzaldehyde, VAN=vanillin, VA=vanillic acid, CA=p-coumaric acid, SYA=syringaldehyde, FA=ferulic acid, TPA=sum of individual phenolics.
[c]Significant effect of maturity and portions.
[d]Significant effect of subparts*maturity interaction (P<.05).
[e]Significant effect of subparts and interactions of subparts*maturity*portions (P<.05).
[f]Means of leaf blades and leaf sheaths differ from means of stems (P<.05).
[g]Means of leaf blades differ from means of leaf sheaths (P<.05).
[hij]For leaf blades, means within columns with different superscripts differ (P<.05).
[kl]For leaf sheaths, means within columns with different superscripts differ (P<.05).
[mn]For stems, means within columns with different superscripts differ (P<.05).

Changes in Rate and Extent of Dry Matter Digestion Associated with Maturity of Barley. No differences were found in the rate and extent of IVDMD of leaf blades with advancing maturity (Table 7). The effect of maturation was evident in IVDMD values of leaf sheaths and stems at all incubation periods. Stems showed a consistent decrease ($P<.05$) in IVDMD values with plant maturity. The differences in the extent of digestion indicated the poor nutritive value of stems lower than 50%, especially at the heading stage. A decrease in IVDMD values of timothy and orchard grass with maturity were reported by Kamstra et al. (1958). They found the negative effect of maturity on IVDMD could be removed by isolating cellulose, which indicated that the extent of digestion was related to cellulose availability to microbial attack, thus suggested a physical effect of maturity. Jones (1970) commented that the decrease in IVDMD with increasing maturity cannot be explained by simply relating to any particular structural constituent. In general, a linear model is suggested to predict the relationship between lignin and DMD. Jung and Vogel (1985) demonstrated a curvilinear model between lignin and forage digestibility, which suggests that the mechanism by which lignin inhibits forage digestion is complex, indicating interactions between different structural components.

TABLE 7. EFFECT OF PLANT MATURITY ON RATE AND EXTENT OF IN VITRO DRY MATTER DIGESTION IN BARLEY BOTANICAL FRACTIONS[a] (MIDDLE PORTION)

Subparts[bcd]	Stage of maturity	Time (Hours) 12	24	48	96
		----------%[e]----------			
Leaf blades	Vegetative	41.09	53.97	67.25	79.39[f]
	Boot	33.72	52.36	60.02	77.92[fg]
	Heading	41.54	59.26	60.86	75.01[g]
	Average	38.78	55.20	62.71	77.44
Leaf sheaths	Vegetative	48.92	69.57[h]	79.13[h]	80.13[h]
	Boot	27.32	40.11[i]	51.64[i]	68.51[i]
	Heading	25.65	39.88[i]	49.16[i]	64.96[i]
	Average	33.96	49.85	56.64	71.19
Stems	Vegetative	51.84[j]	72.53[j]	78.24[j]	84.67[j]
	Boot	34.38[jk]	50.23[j]	60.94[j]	74.39[k]
	Heading	25.99[k]	32.98[k]	34.49[k]	47.43[l]
	Average	37.40	51.92	57.89	68.83
	SE	1.12	2.13	1.08	2.01

[a]Each value represents the mean for twelve samples.
[b]Means of leaf blades and leaf sheaths differ from means of stems (P<.05).
[c]Means of leaf blades differ from means of leaf sheaths (P<.05).
[d]Significant effect of subparts, maturity and interaction of subparts*maturity (P<.05).
[e]In vitro DM digestibility.
[fg]For leaf blades, means within columns with different superscripts differ (P<.05).
[hi]For leaf sheaths, means within columns with different superscripts differ (P<.05).
[jkl]For stems, means within columns with different superscripts differ (P<.05).

Bittner (1983) suggested that xylose to arabinose ratio is a negative index of forage quality, with higher xylose to arabinose ratio indicative of less digestible forage. The increasing association of lignin and xylose (Jones, 1970; Morrison, 1980) resulted in lower IVDMD values of maturing grasses. Burritt et al. (1984) found a strong negative correlation (r = -.85) between IVDMD and xylose. Waite et al. (1964) observed that digestibility of xylans decreased more substantially during plant growth than other cell-wall constituents. In present study trends in composition and digestibility of barley subparts in all the three portions were as expected; increasing cell wall, xylose and p-coumaric acid concentrations and decreasing digestibility with plant maturation. Despite expected trends, our data demonstrated no strong relationships between IVDMD and lignin, xylose and (or) p-coumaric acid, although low but significant correlation values were observed between IVDMD and lignin, p-coumaric acid and (or) xylose (r = -.61; -.41 and -.5, respectively).

Among subparts, highest IVDMD values were found in leaf blades and lowest in stems at heading (Table 8). For leaf blades, lower IVDMD values ($P<.05$) were noted for heading stage, compared to boot stage. In contrast to leaf blades, leaf sheaths and stems showed differences in top and bottom

TABLE 8. EFFECT OF PLANT MATURITY ON RATE AND EXTENT OF IN VITRO DRY MATTER DIGESTION IN BARLEY TOP AND BOTTOM PORTIONS[a]

Subparts[bc]	Portion	Stage of maturity	Time (Hours) 12	24	48	96
			----------%[de]----------			
Leaf blades	Top	Boot	29.56^{f}	51.90^{f}	60.77^{f}	76.87
		Heading	29.74^{f}	52.42^{f}	62.59^{f}	75.69
	Bottom	Boot	33.90^{f}	56.09^{g}	58.14^{f}	75.42
		Heading	24.12^{g}	39.40^{h}	47.13^{g}	69.39
		Average	29.33	49.95	57.16	74.34
Leaf sheaths	Top	Boot	33.87^{i}	52.38^{i}	66.77^{i}	75.40^{i}
		Heading	35.67^{i}	50.37^{i}	45.30^{j}	63.40^{j}
	Bottom	Boot	31.23^{i}	46.68^{j}	54.99^{k}	66.84^{k}
		Heading	24.96^{j}	32.17^{k}	36.46^{l}	59.10^{l}
		Average	31.43	45.40	50.88	66.19
Stems	Top	Boot	40.44^{m}	53.38^{m}	69.09^{m}	85.88^{m}
		Heading	28.26^{n}	37.13^{n}	36.04^{np}	47.43^{np}
	Bottom	Boot	30.64^{n}	38.82^{n}	44.47^{n}	62.90^{n}
		Heading	24.31^{n}	32.17^{n}	33.74^{p}	40.84^{p}
		Average	30.91	40.38	45.84	59.26
	SE		1.34	2.19	1.34	3.24

[a]Each value represents the mean for twelve samples.
[b]Means of leaf blades and leaf sheaths differ from means of stems ($P<.05$).
[c]Means of leaf sheaths differ from means of leaf blades ($P<.05$).
[d]In vitro DM digestibility.
[e]Significant effect of subparts, portions, maturity and interactions of subparts*portions*maturity, subparts*maturity*time and portions*maturity*time ($P<.05$).
[fgh]For leaf blades, means within columns with different superscripts differ ($P<.05$).
[ijkl]For leaf sheaths, means within columns with different superscripts differ ($P<.05$).
[mnp]For stems, means within columns with different superscripts differ ($P<.05$).

portions at early stages of development. Results suggested that leaf blades were digested at a faster rate compared to leaf sheaths and stems and digestibility of sheaths was intermediate.

Changes in Cell Wall Constituents Associated with Maturity of Millet. The NDF, ADF and lignin values for three botanical fractions from the middle portion of millet plants averaged over stages indicated higher ($P<.05$) values for stems than leaf blades and (or) sheaths (Table 9). Higher ($P<.05$) values for NDF, ADF, cellulose and lignin and lower value for hemicellulose were found in leaf sheaths than leaf blades. Within subparts, the effects of maturity were more apparent for leaf blades and leaf sheaths than stems. Neutral detergent fiber, ADF and cellulose for leaf blades and leaf sheaths increased ($P<.05$) between vegetative and boot stages. However, no differences were found for hemicellulose and lignin in leaf blades with plant maturity. Stems showed higher ($P<.05$) NDF values and increasing trend for ADF, hemicellulose and lignin at heading stage than at boot stage. Interactions for subparts*maturity were seen for NDF and ADF contents. Burton et al. (1964) reported that young leaf blades collected from top portion of Gahi-1 pearl millet and Georgia 337 sudan grass culms, at heading stage, contained

TABLE 9. EFFECT OF PLANT MATURITY ON CELL WALL CONSTITUENTS OF MILLET BOTANICAL FRACTIONS[a] (MIDDLE PORTION)

Subparts	Stages of maturity	NDF[bcd]	ADF[bcd]	Cellulose[bd]	Hemicellulose[b]	Lignin[b]
		----------%[e]----------				
Leaf blades	Vegetative	54.89[f]	26.58[f]	20.95[f]	28.31	4.70
	Boot	61.54[g]	32.54[g]	25.56[g]	29.00	6.41
	Heading	64.71[g]	33.22[g]	26.04[g]	31.49	6.73
	Average	60.38	30.77	24.18	29.60	5.94
Leaf sheaths	Vegetative	53.17[h]	27.98[h]	26.84[h]	25.19[h]	4.12[h]
	Boot	71.07[i]	41.94[i]	33.35[i]	29.13[hi]	7.40[i]
	Heading	76.16[j]	42.90[i]	33.33[i]	33.26[i]	8.51[i]
	Average	66.80	37.17	31.17	29.19	6.68
Stems	Vegetative	ND[k]	ND[k]	ND[k]	ND[k]	ND[k]
	Boot	70.13[l]	44.75	36.63	25.38	7.74
	Heading	75.01[m]	46.42	36.62	28.59	9.67
	Average	72.57	45.58	36.63	26.98	8.71
	SE	1.24	.97	.55	1.49	.66

[a]Each value represents the mean for eight samples.
[b]Significant effect of subparts and maturity (P<.05).
[c]Significant effect of subparts*maturity interaction (P<.05).
[d]Means of leaf blades differ from means of leaf sheaths (P<.05).
[e]DM basis.
[fg]For leaf blades, means within columns with different superscripts differ (P<.05).
[hij]For leaf sheaths, means within columns with different superscripts differ (P<.05).
[k]Not determined due to insufficient sample.
[lm]For stems, means within columns with different superscripts differ (P<.05).

less lignin than older leaves (6.6 vs 7.7%) taken from the same culms.

Lower (P<.05) values of NDF, ADF, cellulose and lignin and higher for hemicellulose were found in top portion, compared to bottom portion at all stages of maturity (Table 10). Within plant portions, the effect of maturity was evident (P<.05) only in top portion for NDF, ADF and cellulose.

Changes in Hemicellulose Monosaccharides Associated with Maturity of Millet. Averaged over three stages, trends for higher values for xylose and for lower values for arabinose and glucose were found in stems than leaf sheaths and leaf blades (Table 11). Within plant subparts, no effect (P>.05) of maturity was found in arabinose content. The concentration of xylose, however, was increased (P<.05) in leaf sheaths and stems with advancing maturity. No relationship was found between maturity and variation in galactose and mannose content of leaf blades, leaf sheaths and stem. Glucose concentrations within plant parts did not follow a consistent pattern. Subparts*maturity interaction (P<.05) were seen for xylose and glucose.

Differences (P>.05) associated with plant maturity were not found in monosaccharide composition of hemicellulosic fractions isolated from top and bottom portions of millet plants (Table 12). These results indicated that differences

TABLE 10. EFFECT OF PLANT MATURITY ON CELL WALL CONSTITUENTS OF MILLET TOP AND BOTTOM PORTIONS[a]

Portions	Stage of maturity	NDF[bc]	ADF[bc]	Cellulose[bc]	Hemicellulose	Lignin[b]
		----------%[d]----------				
Top	Boot	62.43[e]	33.38[e]	26.53[e]	29.06	6.30[e]
	Heading	67.12[f]	38.76[f]	30.82[f]	28.36	7.13[e]
	Average	64.78	36.07	28.67	28.71	6.71
Bottom	Boot	70.43[f]	45.27[g]	35.50[g]	25.17	9.26[f]
	Heading	68.63[f]	42.81[g]	33.76[g]	25.83	9.00[f]
	Average	69.53	44.04	34.63	25.50	9.13
	SE	1.05	.50	.49	1.35	.19

[a]Each value represents the mean for eight samples.
[b]Significant effect of portions (P<.05).
[c]Significant effect of portions*maturity interaction (P<.05).
[d]DM basis.
[efg]Means within columns with different superscripts differ (P<.05).

TABLE 11. EFFECT OF PLANT MATURITY ON NEUTRAL MONOSACCHARIDE COMPOSITION OF HEMICELLULOSE IN MILLET BOTANICAL FRACTIONS[a] (MIDDLE PORTION)

Subparts	Stage of maturity	Arabinose[c]	Xylose[cd]	Mannose[c]	Glucose[cd]	Galactose[c]
		----------%[e]----------				
Leaf blades	Vegetative	3.43	15.58	.79	6.79[f]	.73
	Boot	4.08	15.61	.23	2.32[g]	.48
	Heading	4.77	15.58	.29	4.11[g]	.71
	Average	4.09	15.59	.44	5.41	.64
Leaf sheaths	Vegetative	4.68	15.91[h]	.00	6.97[h]	.24
	Boot	5.04	18.17[hi]	.00	6.62[hi]	.35
	Heading	5.17	21.89[i]	.18	4.95[i]	.37
	Average	4.96	18.65	.06	6.17	.32
Stems	Vegetative	ND[j]	ND[j]	ND[j]	ND[j]	ND[j]
	Boot	2.34	18.15[k]	.31	3.27	.22
	Heading	2.83	23.08[l]	.80	3.10	.09
	Average	2.58	20.61	.56	3.18	.16
	SE	.75	1.02	.15	.47	.11

[a]Each value represents the mean for eight samples.
[b]Means of leaf blades differ from means of leaf sheaths (P<.05).
[c]Significant effect of subparts (P<.05).
[d]Significant effect of maturity and interaction of subparts*maturity (P<.05).
[e]Cell wall basis.
[fg]For leaf blades, means within columns with different superscripts differ (P<.05).
[hi]For leaf sheaths, means within columns with different superscripts differ (P<.05).
[j]Not determined due to insufficient sample.
[kl]For stems, means within columns with different superscripts differ (P<.05).

TABLE 12. EFFECT OF PLANT MATURITY ON NEUTRAL MONOSACCHARIDE COMPOSITION OF HEMICELLULOSE IN TOP AND BOTTOM PORTIONS OF MILLET[a]

Portions	Stage of maturity	Arab-inose	Xylose	Man-nose	Glu-cose[b]	Gala-ctose
		----------%[c]----------				
Top	Boot	3.73	21.38	0.12	5.19	0.49
	Heading	3.95	20.37	0.17	3.93	0.43
	Average	3.84	20.87	0.18	4.56	0.46
Bottom	Boot	3.24	18.46	0.79	2.89	0.33
	Heading	3.73	21.10	0.71	2.45	0.42
	Average	3.48	19.78	0.74	2.67	0.37
	SE	.55	1.29	.28	.37	.17

[a]Each value represents the mean for four samples.
[b]Significant effect of portions ($P<.05$).
[c]Cell wall basis.

observed in the values of two main hemicellulosic neutral sugars, arabinose and xylose, associated with plant subparts (as shown in Table 11) were over-shadowed when plant portions as a whole were analyzed.

Changes in Alkali-labile Phenolic Monomers Associated with Maturity of Millet. Mean values of alkali-labile phenolic monomers for plant subparts averaged over maturity indicated generally higher values for total phenolic acids, p-coumaric acid, ferulic acid and vanillin in stems followed by leaf sheaths (Table 13). Higher concentrations of total phenolic acids in stem samples attributed to the higher values of p-coumaric and ferulic acids, compared to leaves. Differences ($P<.05$) were observed between leaf blades and leaf sheaths. Similar results were reported by Hartley and Jones (1978), who found highest values of p-coumaric and ferulic acids in stems and lowest values in leaf blades of normal and mutant Zea mays. In the present study, stems and leaf blades showed highest values of p-coumaric and ferulic acid at heading stage, but differences were usually not significant ($P>.05$). Within leaf sheaths, generally, the content of major phenolic acids, p-coumaric and ferulic acids, decreased with advancing maturity, although differences were not always significant. Our results are in agreement with Chaves et al. (1982) who found a positive effect of maturity

TABLE 13. EFFECT OF PLANT MATURITY ON ALKALI-LABILE PHENOLIC MONOMERS OF MILLET BOTANICAL FRACTIONS[a] (MIDDLE PORTION)

Subparts	Stage of maturity	Alkali-labile phenolic monomers[b]							
		BAL[cde]	BA[e]	VAN[cde]	VA[e]	CA[cde]	SYA[cde]	FA[e]	TPA[cd]
		mg·g^{-1} cell wall							
Leaf blades	Vegetative	0.00[f]	0.27[f]	0.26[f]	2.82[f]	2.37	0.00[f]	2.14	7.86
	Boot	0.03[f]	0.00[f]	1.40[g]	1.49[g]	2.95	0.36[g]	2.31	8.53
	Heading	0.49[g]	0.00[g]	0.68[fg]	0.74[g]	3.74	0.29[g]	3.51	9.45
	Average	0.17	0.09	0.78	1.68	3.02	0.22	2.65	8.61
Leaf sheaths	Vegetative	0.00	0.13[h]	0.22	1.38	5.27[h]	0.23	4.59[h]	11.81[h]
	Boot	0.00	0.00[i]	0.00	1.54	4.58[i]	0.00	2.58[i]	8.71[hi]
	Heading	0.08	0.21[h]	0.59	1.96	3.36[i]	0.00	1.54[i]	7.74[i]
	Average	0.03	0.12	0.27	1.63	4.40	0.08	2.91	9.42
Stems	Vegetative	ND[j]	ND[j]	ND[j]	ND[j]	ND[j]	ND[j]	ND[j]	ND[j]
	Boot	0.00	0.00	0.83	1.15	7.07[k]	0.00	2.11[k]	11.16[k]
	Heading	0.00	0.00	1.24	0.78	8.97[l]	0.00	4.68[l]	15.66[l]
	Average	0.00	0.00	1.04	0.97	8.02	0.00	3.40	13.42
	SE	.04	.03	.23	.22	.42	.05	.42	.91

[a]Each value represents the mean for four samples.
[b]BAL=p-hydroxybenzoic acid, BA=benzoic acid, VAN=vanillin, VA=vanillic acid, CA=p-coumaric acid, SYA=syringaldehyde, FA=ferulic acid, TPA=total phenolics.
[c]Means of leaf blades differ from means of leaf sheaths (P<.05).
[d]Significant effect of subparts and maturity (P<.05).
[e]Significant effect of subparts*maturity interaction (P<.05).
[fg]For leaf blades, means within columns with different superscripts differ (P<.05).
[hi]For leaf sheaths, means within columns with different superscripts differ (P<.05).
[j]Not determined due to insufficient sample.
[kl]For stems, means within columns with different superscripts differ (P<.05).

on ferulic acid and p-coumaric acid values of digitgrass. The percentage of p-coumaric acid doubled from 2 to 4 wk and then increased at a slower rate. It appears from our findings and also from previous work (Hartley and Jones, 1977; Chaves et al., 1982) that tropical grasses contain higher levels of p-coumaric acid and lower levels of ferulic acid than temperate grasses. No consistent effect due to maturity was found on benzoic acid, vanillic acid, p-OH benzaldehyde, vanillin and syringaldehyde values.

Mean values of alkali-labile phenolic monomers, averaged over maturity, indicated higher values ($P<.05$) of p-coumaric in bottom portions than top portions (Table 14). Within plant portions, mean values of all phenolic monomers were not consistently affected by maturity. Lower levels of ferulic acid was found at heading stage in the top portion, and higher levels in the bottom portion, compared to boot stage (maturity x portion interaction, $P<.05$). The effects were reflected in low total phenolic acids. A decreasing trend in the values of vanillin and vanillic acids were found with advancing maturity, although not statistically differed. Benzoic acid and p-hydroxy benzaldehyde were found only in small quantities (<.5 mg/g cell wall) in immature plants.

Changes in Rate and Extent of Dry Matter Digestion Associated with Maturity of Millet. Among subparts small and

TABLE 14. EFFECT OF PLANT MATURITY ON ALKALI-LABILE PHENOLIC MONOMERS OF MILLET TOP AND BOTTOM PORTIONS[a]

Portion	Stage of maturity	Alkali-labile phenolic monomers[b]							
		BAL	BA	VAN	VA	CA[c]	SYA	FA[d]	TPA
		mg·g^{-1} cell wall							
Top	Boot	0.30	0.09	0.97	2.04	3.86[e]	0.00	3.93[e]	11.18[e]
	Heading	0.00	0.00	0.31	2.40	3.72[e]	0.00	1.60[f]	8.01[f]
	Average	0.15	0.05	0.64	2.22	3.79	0.00	2.77	9.60
Bottom	Boot	0.00	0.00	0.57	1.63	5.73[f]	0.00	2.17[g]	9.74[e]
	Heading	0.00	0.00	0.36	0.93	5.42[f]	0.00	3.55[h]	10.26[e]
	Average	0.00	0.00	0.47	1.28	5.60	0.00	2.87	10.01
	SE	.06	.05	.13	.31	.14	.00	.09	.26

[a]Each value represents the mean for four samples.
[b]BAL=p-hydroxybenzaldehyde, BA=benzoic acid, VAN=vanillin, VA=vanillic acid, CA=p-coumaric acid, SYA=syringaldehyde, FA=ferulic acid, TPA=sum of individual phenolics.
[c]Significant effect of portions ($P<.05$).
[d]Significant effect of maturity*portions interaction ($P<.05$).
[efgh]Means within columns with different superscripts differ ($P<.05$).

inconsistent differences were found in the rate and extent of DM digestion (Table 15). At all times of incubation, generally, plant parts at heading stage showed lower values ($P<.05$) than vegetative and boot stage. The decrease in IVDMD values parallel increases in lignin, hemicellulose, xylose and p-coumaric acid content. Philips et al. (1954) suggested that decreases in IVDMD and subsequently in nutritive value with advancing maturity are, in general, due to increases in fiber and lignin content. Wedig et al. (1986) indicated that high hemicellulose and lignin contents in mature grasses appear to depress IVDMD, primarily due to covalent bonding between lignin and hemicellulose. Data of the present study indicated that a generalized statement is not always correct, since a nonsignificant increase in lignin and hemicellulose is insufficient to explain decrease ($P<.05$) in IVDMD of stem. No relationship was found between IVDMD and lignin. Jung and Vogel (1986) found a curvilinear relationship between IVDMD and lignin, which suggests interactions between different structural components. Bittner (1983) suggested that xylose is the most resistant to hydrolysis among all chemical components of hemicellulose. In the present study, increases ($P<.05$) in xylose and p-coumaric acid of stem samples with increasing maturity partly explain the lower IVDMD values, although no strong relation-

TABLE 15. EFFECT OF PLANT MATURITY ON RATE AND EXTENT OF IN VITRO DRY MATTER DIGESTION IN MILLET BOTANICAL FRACTIONS[a] (MIDDLE PORTION)

Subparts	Stage of maturity	Time (Hours) 12	24	48	96
		----------%[bc]----------			
Leaf blades	Vegetative	36.97[d]	47.68	57.89[d]	67.64
	Boot	34.43[d]	44.79	48.43[e]	63.57
	Heading	26.29[e]	44.93	49.94[e]	66.30
	Average	32.56	45.80	52.08	65.83
Leaf sheaths	Vegetative	38.01[f]	46.92[f]	56.59[f]	73.77
	Boot	36.26[f]	51.49[g]	51.53[f]	69.34
	Heading	32.97[g]	47.99[f]	46.24[g]	68.78
	Average	35.75	48.80	51.45	70.63
Stems	Vegetative	ND[h]	ND[h]	ND[h]	ND[h]
	Boot	32.33	45.34[i]	49.30	74.30[i]
	Heading	31.11	45.17[j]	37.32	63.74[j]
	Average	31.72	45.26	43.31	69.02
	SE	2.52	2.52	2.52	2.52

[a]Each value represents the mean for twelve samples.
[b]In vitro DM digestibility.
[c]Significant effect of maturity and interaction of subparts*maturity*time (P<.05).
[de]For leaf blades, means within columns with different superscripts differ (P<.05).
[fg]For leaf sheaths, means within columns with different superscripts differ (P<.05).
[h]Not determined due to insufficient sample.
[ij]For stems, means within columns with different superscripts differ (P<.05).

ships were found between IVDMD and xylose and (or) p-coumaric acid (r = -.45; -.28, respectively). Negative correlations have been reported between IVDMD and xylose (Burritt et al., 1984) and (or) p-coumaric acid (Chaves et al., 1982; Hartley and Jones, 1978). However, in the present study, no differences in IVDMD values were found between vegetative and boot stages. The IVDMD values at 12, 24, 48 and 96 h of whole top and bottom portions indicated differences between plant portions with maturity (Table 16). At all incubation periods, higher rate of IVDMD was found for top portions, compared to bottom portions. However, at same maturity stages, no differences (P>.05) were found between top and bottom portions in the extent of IVDMD at 96 h.

Changes in Cell Wall Constituents Associated with Maturity of Berseem Clover. The cell wall constituents for three botanical fractions averaged over maturity indicated higher ADF (P<.05), cellulose (P<.05) and lignin (P>.05) but lower hemicellulose (P<.05) for stems and petioles than leaflets (Table 17). No differences (P>.05) were noted between petioles and stems for cell wall constituents. However, slightly higher average values for lignin (P>.05) were found in stems, than petioles. Our results are in agreement with Kamstra et al. (1958), who reported that cellulose and lignin content of alfalfa increased (P<.05) in early stages of de-

TABLE 16. EFFECT OF PLANT MATURITY ON RATE AND EXTENT OF IN VITRO DRY MATTER DIGESTION IN MILLET TOP AND BOTTOM PORTIONS[a]

Portion	Stage of maturity	Time (Hours) 12	24	48	96
		---------- %[bc] ----------			
Top	Boot	37.45[d]	56.50[d]	64.52[d]	76.57[d]
	Heading	29.28[e]	44.29[e]	45.47[e]	65.35[e]
	Average	33.36	50.39	55.00	70.96
Bottom	Boot	28.59[e]	46.40[e]	40.97[ef]	78.16[d]
	Heading	32.03[e]	44.70[e]	37.71[f]	64.64[e]
	Average	30.31	45.55	39.34	71.40
	SE	1.61	1.61	1.61	1.61

[a]Each value represents the mean for twelve samples.
[b]In vitro DM digestibility.
[c]Significant effect of portions, maturity and interaction of portion*maturity (P<.05).
[def]For top and bottom portions, means within columns with different superscripts differ (P<.05).

TABLE 17. EFFECT OF PLANT MATURITY ON CELL WALL CONSTITUENTS OF BERSEEM BOTANICAL FRACTIONS[a] (MIDDLE PORTION)

Subparts	Stage of maturity	NDF	ADF[bcd]	Cellu-lose[bcde]	Hemice-llulose[bcd]	Lignin[bcd]
		----------%[f]----------				
Leaflets	Vegetative	48.54	14.82	11.13	33.73	4.68
	Early bloom	47.08	16.13	10.77	30.95	6.81
	Average	47.81	15.47	10.95	32.34	5.75
Petioles	Vegetative	47.67	31.23	26.66[g]	16.45	8.36
	Early bloom	47.30	28.71	22.39[h]	18.59	8.57
	Average	47.48	29.97	24.52	17.52	8.46
Stems	Vegetative	46.11	27.70	23.58[i]	18.41	8.90
	Early bloom	42.64	28.37	18.67[j]	14.27	9.19
	Average	44.37	28.04	21.13	16.34	9.05
	SE	2.60	1.25	.63	2.75	.79

[a]Each value represents the mean for eight samples.
[b]Significant effect of subparts (P<.05).
[c]Means of leaflets and petioles differ from means of stems (P<.05).
[d]Means of leaflets differ from means of petioles (P<.05).
[e]Significant effect of maturity and interaction of subparts*maturity (P<.05).
[f]DM basis.
[gh]For petioles, means within columns with different superscripts differ (P<.05).
[ij]For stems, means within columns with different superscripts differ (P<.05).

velopment (pre- vs early bloom), but did not differ much in later stages of development (early- vs full bloom). Interaction for subparts*maturity was seen for cellulose. Within plant part, usually, stage of maturity had no effect on cell-wall constituents.

Averaged over maturity, lower values of ADF, cellulose and lignin were recorded for the top than bottom portion (Table 18). Hemicellulose content was higher in top portion, but NDF values remained unchanged. Both portions showed increases ($P<.05$) in NDF and ADF, with advancing maturity.

Changes in Hemicellulosic Monosaccharides Associated with Maturity of Berseem Clover. Average values for xylose and glucose, for all the botanical fractions, increased with plant maturity (Table 19). Stems had higher values for xylose,arabinose and galactose, compared to leaflets and petioles. Between portions the content of xylose was higher ($P<.05$) and arabinose tended to be lower in bottom portions, compared to top portion (Table 20). No differences ($P>.05$) were found in content of the other sugars, when top and bottom portions were compared at the same or different stages.

Changes in Alkali-labile Phenolic Monomers Associated with Maturity of Berseem Clover. Vanillin, vanillic acid and syringaldehyde were the only phenolic monomers quantified in berseem leaflets, petioles and stems at vegetative and early

TABLE 18. EFFECT OF PLANT MATURITY ON CELL WALL CONSTITUENTS OF TOP AND BOTTOM PORTIONS OF BERSEEM[a]

Portions	Stage of maturity	NDF[b]	ADF[b]	Cellulose[b]	Hemicellulose[c]	Lignin[c]
		----------%[d]----------				
Top	Early bloom	45.67[e]	24.58[e]	17.28[e]	21.27[e]	8.19[e]
	Late bloom	57.51[f]	40.28[f]	24.45[ef]	17.22[e]	14.78[f]
	Average	51.60	32.43	20.89	19.46	11.49
Bottom	Early bloom	48.22[e]	35.25[g]	22.73[ef]	12.96[f]	12.31[f]
	Late bloom	58.76[f]	46.25[h]	29.87[f]	12.51[f]	14.97[f]
	Average	53.49	40.75	26.30	12.74	13.64
	SE	2.87	2.79	.85	.56	1.22

[a]Each value represents mean for eight samples.
[b]Significant effect of portions and interaction of maturity*portions ($P<.05$).
[c]Significant effect of portions ($P<.05$).
[d]DM basis.
[efgh]Means within columns with different superscripts differ ($P<.05$).

TABLE 19. EFFECT OF PLANT MATURITY ON NEUTRAL MONOSACCHARIDE COMPOSITION OF HEMICELLULOSE OF BERSEEM BOTANICAL FRACTIONS[a] (MIDDLE PORTION)

Subparts	Stage of maturity	Arabinose[bcd]	Xylose[bcdef]	Mannose	Glucose[e]	Galactose[bcdf]
		----------%[g]----------				
Leaflets	Vegetative	1.07	1.75	.30	2.77	1.13
	Early bloom	.70	1.55	.00	3.60	.52
	Average	.87	1.65	.15	3.19	.83
Petioles	Vegetative	2.39	4.56	.37	1.55[h]	1.66
	Early bloom	2.76	5.51	1.07	3.24[i]	1.83
	Average	2.58	5.03	.72	2.39	1.75
Stems	Vegetative	2.50	5.38j	.08	1.99[j]	1.59[j]
	Early bloom	3.29	8.31k	.24	3.54[k]	2.43[k]
	Average	2.90	6.85	.16	2.76	2.01
	SE	.29	.51	.38	.40	.14

[a]Each value represents the mean for eight samples.
[b]Significant effect of subparts (P<.05).
[c]Means of leaflets and petioles differ from means of stems (P<.05).
[d]Means of leaflets differ from means of petioles (P<.05).
[e]Significant effect of maturity (P<.05).
[f]Significant effect of subparts*maturity interaction (P<.05).
[g]Cell wall basis.
[hi]For petioles, means within columns with different superscripts differ (P<.05).
[jk]For stems, means within columns with different superscripts differ (P<.05).

TABLE 20. EFFECT OF PLANT MATURITY ON NEUTRAL MONOSACCHARIDE COMPOSITION OF HEMICELLULOSE IN TOP AND BOTTOM PORTIONS OF BERSEEM[a]

Portion	Stage of maturity	Arab-inose	Xylose[b]	Man-nose	Glu-cose	Galac-tose
		----------%[c]----------				
Top	Early bloom	2.25	3.90[d]	.25	2.10	1.46
	Full bloom	1.93	7.69[ef]	.56	2.70	1.73
	Average	2.09	5.80	.41	2.40	1.60
Bottom	Early bloom	1.79	6.93[e]	.42	3.12	1.82
	Full bloom	1.51	8.42[f]	.45	2.60	1.36
	Average	1.65	7.68	.44	2.86	1.59
	SE	.16	.34	.17	.52	.23

[a]Each value represents the mean for eight samples.
[b]Significant effect of portions and interaction of portions* maturity ($P<.05$).
[c]Cell wall basis.
[def]Means within columns with different superscripts differ ($P<.05$).

bloom stage (Table 21). P-coumaric and ferulic acids, the major alkali-labile phenolic monomers in grasses, were too low for detection. These findings are in agreement with those of Hartley and Jones (1977), who reported only small quantities of p-coumaric and ferulic acids (<.1 mg/g cell wall) in red clover leaflets, petioles and stems harvested before and after flowering. Lower values of alkali-labile phenolic monomers suggest that phenolic esters, which respond to alkaline treatment, especially in grasses, are present only in traces in legumes. In the present study, slightly higher values of vanillic acid, vanillin and syringaldehyde, which were detected in stems and petioles at vegetative stage than at heading stage might have arisen from non-lignified cell walls and are also an indication of lignin precursors. Decreases in the content of lignin precursors with increasing maturity are parallel with increase in lignin content.

Generally, mean values of vanillin and vanillic acid were higher in immature berseem, compared to mature berseem (Table 22). Syringaldehyde was not detected in top and bottom portions at all stages of maturity. No differences were found between top and bottom portions at two stages of maturity, although slightly higher values were found in bottom portions. Our results agree with the findings of Jung et al. (1984), who found no differences in the phenolic acid content

TABLE 21. EFFECT OF PLANT MATURITY ON ALKALI-LABILE PHENOLIC MONOMERS OF BERSEEM BOTANICAL FRACTIONS[a] (MIDDLE PORTION)

Subparts	Stage of maturity	Alkali-labile phenolic monomers[b]			
		VAN[c]	VA[cdef]	SYA[def]	TPA[cdef]
		mg·g^{-1} cell wall			
Leaflets	Vegetative	1.05	0.55[g]	0.00	1.59[g]
	Early bloom	2.26	1.05[h]	0.00	3.31[h]
	Average	1.65	0.80	0.00	2.46
Petioles	Vegetative	2.24	0.72	2.40[i]	5.56[i]
	Early bloom	1.59	0.46	0.00[j]	2.05[j]
	Average	1.91	0.59	1.20	3.81
Stems	Vegetative	2.87	0.95	3.31	7.13[k]
	Early bloom	1.23	1.41	1.99	4.63[l]
	Average	2.05	1.18	2.65	5.88
	SE	.40	.42	.45	.71

[a]Each value represents the means for four samples.
[b]VAN=vanillin, VA=vanillic acid, SYA=syringaldehyde, TPA=sum of individual phenolics.
[c]Significant effect of subparts*maturity interaction (P<.05).
[d]Significant effect of subparts (P<.05).
[e]Means of leaflets and petioles differ from means of stems (P<.05).
[f]Means of leaflets differ from means of petioles (P<.05).
[gh]For leaflets, means within columns with different superscripts differ (P<.05).
[ij]For petioles, means within columns with different superscripts differ (P<.05).
[kl]For stems, means within columns with different superscripts differ (P<.05).

TABLE 22. EFFECT OF PLANT MATURITY ON ALKALI-LABILE PHENOLIC MONOMERS IN TOP AND BOTTOM PORTIONS OF BERSEEM[a]

Portion	Stage of maturity	Alkali-labile phenolic monomers[b]		
		VAN	VA	TPA
		----------mg·g^{-1} cell wall----------		
Top	Early bloom	2.05^{c}	0.74^{c}	2.79^{c}
	Full bloom	1.01^{d}	0.94^{c}	1.95^{d}
	Average	1.53	0.84	2.15
Bottom	Early bloom	2.45^{c}	1.94^{d}	4.39^{e}
	Full Bloom	1.59^{d}	0.62^{c}	2.21^{cd}
	Average	2.02	1.28	3.30
	SE	.24	.03	.21

[a]Each value represents the mean for four samples.
[b]VAN=vanillin, VA=vanillic acid, TPA=sum of individual phenolics.
[cde]Means within columns with different superscripts differ (P<.05).

of immature (bud stage) and mature (full bloom) lucerne hays. However, contrary to our results, they also found lower levels of p-coumaric and ferulic acids in both lucerne hays (.57 to .63 and .98 to 1.04 mg/g of DM, respectively). Jung et al. (1983) reported lower levels of vanillin, vanillic acid, p-coumaric and ferulic acid (.004, .015, .064 and .013 mg/g cell wall, respectively) in alkaline extracts of soybean stover. Results from the present study indicate that plant maturation affects legumes differently than grasses.

Changes in Rate and Extent of Dry Matter Digestion Associated with Maturity of Berseem Clover. Mean IVDMD values for leaflets, petioles and stems averaged over stages did not show much differences up to 48 h of incubation (Table 23). However, the values for 96 h digestion were lower ($P<.05$) for leaflets, compared to petioles and stems. At early bloom stage, leaflets showed lower IVDMD values for all incubation periods than petioles and stems. Differences due to maturity were not observed in the IVDMD values of top and bottom portions, when incubated for 12, 24, 48 and 96 h (Table 24). Within plant portions, IVDMD values for 12 and 24 h did not differ much for both portions. However, lower IVDMD values were recorded for 48 and 96 h at full bloom stage, compared to early bloom. Our results agree with the findings of Kamstra et al. (1958), who reported decrease in IVDMD with

TABLE 23. EFFECT OF PLANT MATURITY ON RATE AND EXTENT OF IN VITRO DRY MATTER DIGESTION IN BERSEEM BOTANICAL FRACTIONS[a]

Subparts	Stage of maturity	Time (Hours) 12	24	48	96
		----------%[de]----------			
Leaflets	Vegetative	37.35f	40.96	44.50	65.78
	Early bloom	28.57g	35.48	43.94	61.92
	Average	28.96	38.22	44.20	63.85
Petioles	Vegetative	30.49[h]	38.00[h]	45.08[h]	74.13
	Early bloom	32.19[i]	45.42[i]	52.61[i]	73.65
	Average	31.34	41.71	48.85	73.89
Stems	Vegetative	31.77[j]	35.22[j]	45.48	77.92
	Early bloom	35.55[k]	42.89[k]	40.04	74.65
	Average	33.66	39.06	42.76	76.29
	SE	.83	.98	1.01	1.21

[a]Each value represents the mean for eight samples.
[b]Means of leaflets and petioles differ from means of stems (P<.05).
[c]Means of leaflets differ from means of petioles (P<.05).
[d]In vitro DM digestibility.
[e]Significant effect of subparts and interactions of subparts*maturity and subparts*maturity*time (P<.05).
[fg]For leaflets, means within columns with different superscripts differ (P<.05).
[hi]For petioles, means within columns with different superscripts differ (P<.05).
[jk]For stems, means within columns with different superscripts differ (P<.05).

TABLE 24. EFEECT OF PLANT MATURITY ON RATE AND EXTENT OF IN VITRO DRY MATTER DIGESTION IN BERSEEM TOP AND BOTTOM PORTIONS[a]

Portion	Stage of maturity	Time (Hours) 12	24	48	96
		---------- %[bc] ----------			
Top	Early bloom	29.44	37.93	48.86[d]	70.07[d]
	Full bloom	26.39	35.60	36.13[e]	57.47[e]
	Average	27.92	36.45	42.50	63.77
Bottom	Early bloom	20.21	40.15	52.78[d]	73.68[d]
	Full bloom	24.84	35.06	38.22[e]	55.91[e]
	Average	22.53	37.61	45.50	64.80
	SE	.12	.10	1.12	2.10

[a]Each value represents the mean for eight samples.
[b]In vitro DM digestibility.
[c]Significant effect of maturity (P<.05).
[de]Means within columns with different superscripts differ (P<.05).

increasing maturity. In the present study, no relationship was found between IVDMD and xylose, p-coumaric acid and (or) lignin.

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CHAPTER V

EFFECTS OF CHEMICAL TREATMENT ON CHEMICAL COMPOSITION AND IN VITRO DRY MATTER DIGESTIBILITY OF BARLEY AND MILLET STRAW

ABSTRACT

An experiment was conducted to determine the effects of chemical treatments on cell wall constituents, hemicellulosic monosaccharides, alkali-labile phenolic monomers and in vitro dry matter digestibility (IVDMD) of barley and millet straw. Five treatments were used for each straw: straw with no added water (control), straw with 40% added H_2O (H_2O), straw treated with NH_4OH (NH_3), urea (urea), or alkaline (pH 11.5) solution of H_2O_2 (H_2O_2). Ammoniation by urea and NH_4OH increased ($P<.05$) the CP content of barley straw. No differences were observed in the ash content of untreated and treated barley straw. Hemicellulose and NDF were slightly decreased ($P>.05$) in ammoniated straw compared to untreated straw, whereas hemicellulose concentration was reduced ($P<.05$) in alkaline H_2O_2-treated straw. The content of ADF, cellulose and lignin were increased ($P<.05$) in alkaline H_2O_2-treated barley straw, compared to untreated and NH_3 and urea-treated straw. No differences were observed in neutral sugar composition of hemicellulosic fractions between treated and (or) untreated barley straw. Total phenolic acids were

reduced (P<.05) in treated, compared to untreated straw. Among chemical treatments, maximum decreases (P<.05) in p-coumaric acid and ferulic acid contents were observed in urea-treated straw. In vitro DM digestibility was increased in all the treatments. The highest increase was observed in barley straw treated with alkaline H_2O_2.

Urea- and NH_3-treated millet straw showed an increase (P<.05) in CP, compared to untreated. No differences were observed in cell wall constituents of millet straw treated with urea and NH_3, compared to untreated. However, millet straw treated with alkaline H_2O_2 showed higher (P<.05) NDF, ADF, cellulose and lignin than untreated millet straw. Urea-treated millet straw showed maximum decrease in arabinose and xylose content, compared to untreated. No effect was observed in arabinose values of alkaline H_2O_2-treated straw but xylose was reduced. (P<.05). Total phenolic acids were not affected (P>.05) in NH_3-treated millet straw. However, the values decreased in alkaline H_2O_2-treated straw. The effect was pronounced for ferulic acid content but not for p-coumaric. The IVDMD increased (P<.05) for all chemical treatments, with higher increase for NH_3-treated straw, than alkaline H_2O_2 treatment. Results of this experiment indicate that all chemical treatments improved the nutritive value of straws. Results from alkaline

H_2O_2-treated straws suggested that the levels of H_2O_2 used had little effect.

(Key Words: Straw, Chemicals, Hemicellulosic Sugars, Phenolics, IVDMD).

INTRODUCTION

Cereal straws and other fibrous agricultural residues contain more than 80% cell wall constituents, which represent a potential source of energy for ruminants (Lewis et al., 1987). However, crop residues are low in protein, soluble carbohydrates, minerals and vitamins (Staniforth, 1978). Availability of nutrients is generally limited by the low voluntary intake of crop residues (Klopfenstein, 1978), the chemical association between lignin and cell wall polysaccharides via ester and ether linkages (Hartley and Jones, 1978; Scalbert et al., 1985) and physical limitation (bulkiness, surface density and particle size) of cell wall components to microbial fermentation (Fan et al., 1980).

Numerous technologies have been used, including chemical, physical, physico-chemical and biological treatment to upgrade the feeding value of agricultural residues as ruminant feed. Chemical treatment of crop residues by various alkalis, such as NaOH (Klopfenstein and Woods, 1970; Ololade et al., 1970), NH_4OH (Waiss et al., 1972; Hartley and Jones, 1978), and urea (Orskov et al., 1983; Hadjipanayiotou,

1982) has shown improvement in digestibility and voluntary intake.

Chemical treatment of barley and millet straw with urea, NH_4OH and alkaline H_2O_2 has not been extensively investigated. The experiment was conducted to determine the effect of chemical treatments on 1) alkali-labile phenolic monomers, 2) hemicellulosic monosaccharide components and 3) in vitro DM digestibility.

MATERIALS AND METHODS

Barley (Hordeum vulgare, L., winter variety) and millet (Pannisetum americanum, L. dwarf variety) straws were obtained from the experimental plots described earlier (Chapter II). Four 19 x 11 m plots for each forage were allowed to form seed and mature. At maturity, plants were cut 7.08 cm above the ground with a Gravely mower[5]. The straw was collected and kept separate for the four replicates.

Barley seeds were separated by a small thresher and straw was collected in bags. The millet plant heads were cut 5.08 to 7.08 cm below the head base. Straw was then spread on wooden floor for 3 wk, to complete drying.

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[5] Gravely, Inc., Clemmons, North Carolina.

Both straws were later chopped in a hammer mill through a 2.5 cm screen. The following treatments were used for each kind of straw: i) control; straw; ii) treated with 40% moisture; iii) urea, NH_4OH or H_2O_2 (at pH of 11.5).

Urea Treatment. The chopped straws were treated in 1 kg batches. Each batch was sprayed with urea solution to raise the moisture level to 40% and give an estimated 3% NH_3, DM basis. The straw was mixed thoroughly by turning while being sprayed. The wet treated straws were then packed in double polyethylene bags. After pressing the straw bags to expel air, the bags were tied and kept for 4 wk at room temperature (30 to 35 C). Each replicate was treated in duplicate, thus eight bags were prepared for each treatment.

Ammonia Treatment. Samples (1 kg) of chopped barley and millet straw were placed in double polyethylene bags and water was sprayed to raise moisture level to 40%. Straw was mixed thoroughly and aqueous ammonia (NH_4OH, 25 to 30% NH_3) was then sprayed on the straw in the bags at a level that resulted in 3.0% added NH_3, DM basis. Samples were mixed quickly by turning and rolling the bags and the bags were tied. Each replicate was treated in duplicate. The treated straw samples were then kept at room temperature (30 to 35 C) for 4 wk.

Alkaline Hydrogen Peroxide Treatment. Chopped straw (1 kg), 500 ml of 35% H_2O_2 and 50 liters of H_2O were placed in a 140-liter plastic container. Sodium hydroxide (approximately 450 ml of a 50% solution) was added until pH reached 11.5. The mixture was then thoroughly mixed by a wooden stirrer and allowed to stand overnight. After 16 h, the mixture was filtered through metallic screen and resoaked in water for 15 min. The washing procedure (soaking and filtration) was repeated thrice. The solids were squeezed to remove excess water.

At the end of reaction period samples were frozen for later analysis. Samples for control, urea and NH_4OH-treated straw were taken after 4 wk. Sample for untreated straw was taken at time of treatment. Samples for all the treatments were dried at 60 C till constant weight. Dried samples were ground in Wiley mill through 1 mm screen and stored for laboratory analysis.

Crude protein was determined on fresh samples by the macro-Kjeldahl procedure (AOAC, 1984). Dry matter contents of treated and untreated straw samples were determined by drying approximately 2 g of straw in aluminum pans for 24 h at 100 C.

Dried samples were analyzed for NDF (Van Soest and Wine, 1967), ADF (Goering and Van Soest, 1970), lignin and

cellulose (Van Soest and Wine, 1968). Hemicellulose was calculated as the difference between NDF and ADF.

Determination of Hemicellulosic Sugars. The monosaccharide composition of hemicellulose in acid hydrolysate of plant cell walls (NDF, Van Soest and Wine, 1967; no Na_2SO_3 and decahydronaphthalene were used) were determined by gas chromatography according to the method of McGinnis (1982), as described in Chapter IV.

Standard sugar solution was prepared by adding arabinose and xylose, 50 mg each, in 100 ml of 1 N H_2SO_4 Thus, the running standard solution contained .5 mg/ml of each sugar. Mannose was used as internal standard in GC reagent (.2 g/20 ml).

Determination of Alkali Labile Phenolic Monomers. The cell wall bound phenolic acids and aldehydes of forage cell walls were extracted according to the method of Ford and Hartley (1988). Phenolic methyl esters were prepared and determined according to the method described in Chapter IV.

In Vitro Dry Matter Digestibility. Digestibility of control and treated dried ground straw were determined after 48 h of incubation with ruminal fluid using the Tilley and Terry (1963) procedure as described in Chapter IV.

Statistical Analyses. The data were treated by analysis of variance by general linear model procedure of SAS (1982).

Block and treatments were included in the model. Differences of treatment means were tested by Tukey's HSD procedure.

RESULTS AND DISCUSSION

Physical Characteristics of Barley Straw. Straw treated with urea appeared light brown in color, slightly coarse and had stronger NH_3 smell than straw treated with NH_4OH. The alkaline-H_2O_2-treated barley straw appeared softer and fluffier in texture, and was pale white with no odor.

Changes in Cell Wall Constituents of Barley Straw. Ammoniation by urea and aqueous NH_4OH increases the CP content of barley straw by 9.44 and 7.56 percentage units, respectively (Table 25). No differences ($P>0.05$) were observed in ash content of untreated and treated straw. Hemicellulose of NH_3-treated straw decreased by 5.9 percentage units, compared to the control. No other differences were observed for the urea and NH_3 treatment. Donelley et al. (1974) reported that hemicellulose of corn cobs was solubilized by dilute alkali and Waller (1976) suggested that the reduction in total cell wall constituents of alkali-treated crop residues was due to hemicellulose solubilization. Solaiman et al. (1979) reported a decrease of 8.6 and 42.7% in the values of NDF and hemicellulose of NH_4OH-treated wheat straw. Moore (1987) found no differences in any fiber constituents when orchard grass hay was treated with aqueous NH_3 at 15 or 30%

TABLE 25. CHEMICAL COMPOSITION AND IN VITRO DRY MATTER DIGESTIBILITY OF UNTREATED AND TREATED BARLEY STRAW[a]

Item	Treatment					SE
	None	Water	Urea	NH_4OH	Alkaline H_2O_2	
	---------- %[b] ----------					
Crude protein	3.92[c]	3.94[c]	13.36[d]	11.46[e]	ND[f]	.33
Ash	2.70	2.77	2.55	2.45	2.57	.13
NDF[g]	89.28[cd]	86.47[de]	85.73[e]	83.09[e]	90.52[c]	.87
ADF[h]	59.57[c]	59.08[c]	60.07[c]	59.22[c]	68.92[d]	.95
Hemicellulose	29.68[c]	27.37[cd]	25.66[cd]	23.82[de]	21.60[e]	.84
Cellulose	46.83[cd]	46.16[cd]	45.14[d]	46.00[cd]	50.07[c]	.97
Lignin	11.60[c]	12.53[c]	13.29[c]	12.81[c]	18.82[d]	.65
IVDMD	27.70[c]	28.24[c]	34.79[d]	45.40[e]	38.71[d]	2.52

[a]Each value represents the mean for eight samples.
[b]DM basis.
[cde]Means for treatments within rows with different superscripts differ (P<.05).
[f]ND=Not determined due to excessive alkali.
[g]Neutral detergent fiber.
[h]Acid detergent fiber.

moisture levels in open or closed system. However, lower values were recorded for NDF, hemicellulose and cellulose in ammonia-treated straw. Woolford and Tetlow (1985) also reported a decrease in NDF of ammoniated perennial ryegrass. Grothier et al. (1985) reported that treatment of coastal bermuda grass hay with ammonia at a rate of 3.0% decreased NDF and hemicellulose by 8.7 and 9.9%, respectively.

The content of ADF and lignin were increased by 9.3 and 7.2 percentage units, respectively, in barley straw treated with alkaline H_2O_2. No change was observed in NDF of alkaline H_2O_2-treated straw, compared to untreated straw. The hemicellulose content was decreased by 8.1 percentage units, compared to untreated straw. The lower hemicellulose concentrations of alkaline H_2O_2-treated straw indicate partial solubilization of hemicellulose. The increase in permanganate lignin in washed substrate may be due to the removal of soluble components during washing. Higher ADF and lignin values indicated that the low levels of H_2O_2 probably were insufficient to cleave lignin or lignin-like molecules. Lewis et al. (1987) reported that filtering and washing the alkaline H_2O_2-treated wheat straw either pre- or post-neutralization with 6 N H_3PO_4, removed reactant chemicals and solubilized compounds. This resulted in increased concentration of NDF, ADF, cellulose and lignin in treated straw,

compared to untreated straw. However, they observed no change in NDF, ADF and cellulose values of wheat straw treated with 0, 5.0, 7.5 or 10.0% H_2O_2, DM basis, at a pH of 11.5, maintained by the addition of NaOH. A significant decrease in ADL values was reported for straw treated with H_2O_2 at or above 7.5%, DM basis.

All the chemical treatments showed increases ($P<.05$) in IVDMD values, ranging from 7 to 18 units, compared with the control (Table 25). Maximum IVDMD was obtained with aqueous NH_3 treatment followed by alkaline H_2O_2 and urea treatments. Among chemical treatments, no differences ($P>.05$) were found between urea and alkaline H_2O_2 treatments. The values for NH_3-treated straw were quite high and differed ($P<.05$) from urea and alkaline H_2O_2-treated straw. The improvement in IVDMD values from alkaline treatment probably resulted from the saponification action of alkali on the ester linkages of lignocellulosic compounds (Buettner et al., 1984) and (or) ruptured ether linkages (Lau and Van Soest, 1980) and maybe the swelling effect on cellulose (Tarkow and Feist, 1969).

Results in present study agree with the findings of Wanapat et al. (1986), Hadjipanayiotou (1982) and Hartley and Jones (1978). They reported increases in IVDMD of barley straw by 9 to 11 units for urea-treated straw and 14 to 17 units for NH_4OH-treated straw. Percent increase in IVDMD of

alkaline H_2O_2-treated straw was, however, lower than reported by Lewis et al. (1987) who reported almost doubled values for wheat straw treated with 1% solution of H_2O_2 at pH 11.5, post washed and neutralized with 6 M H_3PO_4, compared to untreated straw. They reported that hydrated treated straws were more degradable, compared to dry treated straw. Fan et al. (1980) suggested that drying may allow for reformation of intermolecular hydrogen bonding of native cellulose, resulting in a more condensed form with less susceptibility to degradation. Lower responses to DMD in the present study are probably due to low levels of H_2O_2 used for treatment.

Neutral Sugar Composition of Hemicellulosic Fraction in Barley Straw. Analysis of hemicellulosic fractions, NDF hydrolysate, of treated and untreated barley straw did not reveal any major changes in the concentration of arabinose (Table 26). Xylose concentration tended to be lower ($P>.05$) in treated barley straw. These findings are consistent with the earlier reports of Graham and Aman (1983/84) and Lindberg et al. (1984). They found no significant change in the content or relative proportion of neutral sugar residues for ammoniated straw, compared to untreated straw.

Alkali-labile Phenolic Acids of Barley Straw. Ferulic, p-coumaric and vanillic acids represented the majority of the phenolic compounds in barley straw (Table 27). Other

TABLE 26. NEUTRAL SUGAR COMPOSITION OF HEMICELLULOSE FRACTIONS FROM CELL WALLS OF TREATED AND UNTREATED BARLEY STRAW[a]

Treatment	Arabinose	Xylose	Xylose: Arabinose
	----------%[b]----------		
None (control)	2.57	16.24	6.32
Water	1.86	13.17	7.18
Urea	2.21	14.85	6.77
Ammonium Hydroxide	2.29	14.55	6.37
Alkaline H_2O_2	2.27	13.61	6.10
SE	.19	.85	.42

[a]Each value represents the mean for eight samples.
[b]Cell wall basis.

Table 27. EFFECT OF CHEMICALS ON ALKALI-LABILE PHENOLIC ACIDS AND ALDEHYDES CONTENT OF BARLEY STRAW[a]

Item	Treatment					SE
	None	Water	Urea	NH_4OH	Alkaline H_2O_2	
	----------$mg \cdot g^{-1}$ cell wall----------					
P-hydroxybenzaldehyde	0.15	0.08	0.41	0.00	0.02	.12
Vanillin	0.62[b]	0.30[bcd]	0.52[bc]	0.00[d]	0.07[cd]	.10
Vanillic acid	3.52[bc]	2.27[b]	1.40[cd]	0.58[d]	0.66[c]	.27
cis-p-Coumaric acid	0.04	0.04	0.00	0.02	0.05	.02
trans-p-Coumaric acid	4.73[b]	4.57[b]	2.16[d]	3.61[bc]	3.08[cd]	.26
Total p-coumaric acid[e]	4.77[b]	4.61[b]	2.16[d]	3.63[bc]	3.13[cd]	.26
cis-Ferulic acid	0.00	0.03	0.02	0.05	0.18	.03
trans-Ferulic acid	2.19[b]	2.02[b]	0.45[c]	1.63[b]	1.61[c]	.12
Total ferulic acid[f]	2.19[b]	2.05[b]	0.47[c]	1.68[b]	1.62[b]	.13
Total phenolic acids[g]	10.94[b]	9.29[b]	4.95[c]	6.56[c]	5.50[c]	.30
Ratio p-CA:FA	2.16[b]	2.26[b]	4.79[b]	2.16[b]	1.98[b]	.29

[a]Each value represents the mean for eight samples.
[bcd]Means for treatments within rows with different superscripts differ (P<.05).
[e]Calculated by summation of cis- and trans- p-coumaric acids.
[f]Calculated by summation of cis- and trans- ferulic acid.
[g]Calculated by summation of individual phenolic acids.

phenolic acids and aldehyde detected were vanillin and p-OH benzaldehyde. Syringaldehyde and p-OH benzoic acids were not detected in treated or untreated straw. Results indicated that all chemical treatments reduced the alkali-labile phenolic acids. Decreases ($P<.05$) were observed for vanillin and vanillic acid in NH_3 and alkaline H_2O_2-treated straw. Barley straw treated with urea showed a large reduction in ferulic acid content, compared to control. The content of p-coumaric acid was decreased ($P<.05$) in urea- and alkaline H_2O_2-treated straw, compared to untreated straw. Values for urea- and alkaline H_2O_2-treated straw were decreased by 2.61 and 1.64 percentage units, respectively. These results are in general agreement with the earlier findings of Hartley and Jones (1978) and Graham and Aman (1984) for ammoniated- and alkali-treated barley straw. They observed a greater loss of ferulic acid than p-coumaric acid from the plant cell walls, but in the present study the decrease in p-coumaric acid was higher. Hartley and Jones (1978) suggested that removal of phenolic acids depends upon the strength of alkali. Ester bonds are generally found to be associated with the linkages between lignin and polysaccharides (Allinson, 1969). Sawai et al. (1983) reported that digestion of orchardgrass NDF and ADF was inhibited when p-coumaric acid and ferulic acid were esterified to fiber

fractions. Infrared absorbance spectral characteristics of NH_3-treated and untreated tall fescue hays indicated that ammoniation significantly reduced the number of ester bonds, presumably resulting from breakage of ester bonds through ammonolysis (Buettner et al., 1982). Hartley and Jones (1977) concluded that removal of alkali-labile phenolic acid caused an increased degradability of carbohydrates of the plant cell walls by ruminal carbohydrases.

Physical Characteristics of Millet Straw. The urea-treated millet straw appeared light brown, and had a strong NH_3 smell, whereas, NH_3-treated straw appeared dark brown in color and had mild NH_3 smell. Similar observations were made by Sundstol et al. (1978) for wheat straw treated with anhydrous ammonia. Klopfenstein (1978) suggested that alkaline reactions solubilizes hemicellulose and cleave the linkages between cell wall constituents and lignin. Gould (1985) suggested that solubilization of hemicelluloses allows hydration of the cellulose chain and, therefore, a more amorphous state is achieved. Scalbert et al. (1985) suggested that alkali not only cleave the linkages between substituted arabinose, uronic acid and acetyl groups of hemicelluloses, but also cleave the ester and ether linkages of bifunctional phenolic acids at pH 11.5 and 12.5. The browning of ammoniated straw was presumed to be associated

with chemical reactions (Maillard) involving oxidation and condensation of aldehydic groups of sugars with the nitrogenous bases (Schuerch and Davidson, 1971).

The alkaline H_2O_2-treated millet straw appeared softer and fluffier in texture, compared to ammoniated straw, and was white in color, with no odor. Gould (1984) suggested that dual action of alkaline H_2O_2, coupling hydrolytic and oxidative dissociation, changes the cell wall integrity partly by hemicellulose solubilization and partly by delignification or formation of other lignin-like molecules. Gould (1985) illustrated a mechanism that explains a dual function of alkali in H_2O_2 treatment. At pH 11.5 to 12.5, H_2O_2 dissociates into its active intermediates, OH^- and O_2^-, which are then able to penetrate native crystalline structure of cell wall complexes and solubilizes C-C double bonds of the phenolic constituents of lignin.

Changes in Cell Wall Constituents of Millet Straw. Treatment with urea and NH_3 increased ($P<.05$) the CP percentage from 9.0 to 20.3 and 17.1%, respectively (Table 28). No differences ($P>.05$) were observed in CP content between NH_3- and urea-treated straw. The increases in CP agree with the values reported by Solaiman et al. (1979) and Obamehinti (1987). In the present study, urea-treated straw showed numerically higher ($P>.05$) CP content than NH_3-treated straw,

Table 28. CHEMICAL COMPOSITION AND IN VITRO DRY MATTER DIGESTIBILITY OF UNTREATED AND TREATED MILLET STRAW[a]

Item	Treatment					SE
	None	Water	Urea	NH_4OH	Alkaline H_2O_2	
	---------- %[b] ----------					
Crude protein	9.01[c]	9.68[c]	20.25[d]	17.13[d]	ND[f]	1.08
Ash	4.86	5.32	4.92	4.83	4.56	.23
NDF[g]	79.02[c]	76.46[c]	76.73[c]	75.31[c]	88.48[d]	.67
ADF[h]	51.23[c]	47.37[c]	47.97[c]	49.90[c]	62.05[d]	1.39
Hemicellulose	27.79	29.09	28.86	25.42	26.43	1.82
Cellulose	40.71[c]	38.94[c]	39.81[c]	41.14[c]	50.75[d]	.80
Lignin	9.47[c]	9.99[c]	9.24[c]	8.55[c]	14.72[d]	.74
IVDMD	35.73[c]	37.17[c]	52.01[d]	51.03[d]	44.74[e]	2.19

[a]Each value represents the means for eight samples.
[b]DM basis.
[cde]Means for treatments within rows with different superscripts differ (P<.05).
[f]Not determined due to excessive alkali.
[g]Neutral detergent fiber.
[h]Acid detergent fiber.

indicating a higher retention of N, which probably was undissociated urea. No differences ($P>.05$) were observed in ash values for treated and untreated millet straw.

The NDF, ADF, hemicellulose, cellulose and lignin values were not different ($P>.05$) in urea- and NH_3-treated straw, compared to untreated straw. However, slightly lower values for NDF and hemicellulose were noted for ammoniated straw, probably due to solubilization of hemicellulosic sugars (Donelley et al., 1974 and Waller, 1976). The straw subjected to alkaline H_2O_2 treatment showed increases ($P<.05$) in NDF, ADF, cellulose and lignin, compared to untreated straw. The hemicellulose value was not affected by alkaline H_2O_2-treatment.

All the treatments increased ($P<.05$) the IVDMD, compared to untreated straw (Table 28). The maximum IVDMD was obtained for straw treated with urea and NH_4OH. Straw treated with alkaline H_2O_2 showed an increase of 9 percentage units, compared to untreated straw, whereas urea and NH_4OH treatment increased the IVDMD by 15.3 to 16.8 units, respectively. The lower levels of H_2O_2 (.25%) used in the present study is probably responsible for poor response, compared to the other alkaline treatments. The responses to urea and NH_3 treatments agree with the earlier findings of Oji et al. (1972) and Obamehinti (1987) for corn stover. However, they re-

ported slightly better results for NH_4OH treatment than urea treatment.

Neutral Sugar Composition of Hemicellulosic Fraction in Millet Straw. No differences ($P>.05$) were found in the values of arabinose and xylose in untreated straw with or without water (Table 29). The arabinose content was lower ($P<.05$) in urea-treated straw, however, straw treated with NH_3 and alkaline H_2O_2 showed no differences, compared to untreated straw. Decreases ($P<.05$) were noted for xylose in urea and alkaline H_2O_2-treated straw, but an increase was recorded for NH_3 treatment. Ratio of xylose:arabinose indicated no differences between urea and NH_3-treated straw. The trends for ratio of xylose/arabinose in alkaline H_2O_2-treated straw reflected the decrease in xylose values compared to urea treatment. Morrison (1974) reported that xylans were associated closely with lignin in lignin-carbohydrate complexes. Burritt et al. (1984) established a significant correlation ($r = -.85$, $P<.05$) between percent xylose and IVDMD. Lower values of xylose in our study, especially in urea- and alkaline H_2O_2-treated straw along with an increase in IVDMD suggest a positive effect of treatment in improving the nutritive value of straw. Contradictory results were reported by Graham and Aman (1984), who found no differences ($P>.05$) for untreated or NH_3-treated barley

Table 29. NEUTRAL SUGAR COMPOSITION OF HEMICELLULOSE FRACTIONS FROM CELL WALLS OF TREATED AND UNTREATED MILLET STRAW[a]

Treatment	Arabinose	Xylose	Xylose: Arabinose
	----------%[b]----------		
None (control)	2.81[c]	16.50[c]	5.92[cd]
Water	2.60[c]	15.67[c]	6.02[cd]
Urea	1.85[d]	13.66[d]	7.43[c]
Ammonium hydroxide	2.79[c]	18.84[e]	6.81[c]
Alkaline H_2O_2	2.42[cd]	12.67[d]	5.24[d]
SE	.14	.35	.34

[a]Each value represents the means for eight samples.
[b]Cell wall basis.
[cde]Means for treatments within rows with different superscripts differ ($P<.05$).

straw, although slightly higher ($P>.05$) values were reported for NH_3-treated straw.

Alkali-labile Phenolic Monomers of Millet Straw. Ferulic, p-coumaric and vanillic acids were the major phenolic acids detected in alkaline NDF-hydrolysate of untreated and treated millet straw (Table 30). The highest concentrations were for p-coumaric acid, followed by ferulic and vanillic acids. Vanillin, p-hydroxybenzaldehyde and p-hydroxybenzoic acid were not detected in untreated or treated straw. The cis-isomers of ferulic and p-coumaric acids were detected in untreated and treated straw. Generally, chemical treatments reduced the phenolic acid, but differences were not always significant. Variations in the values suggested differential responses. The largest decrease in total phenolic acids was for the alkaline H_2O_2 treatment. The decrease in total phenolic acid reflected mainly decreased ($P<.05$) p-coumaric and ferulic acid content. Straw treated with urea and NH_3 showed lower values of p-coumaric acid, compared to untreated straw. Differences were significant ($P<.05$) in NH_3-treated straw but not in urea-treated straw, compared to untreated straw. No differences ($P>.05$) were found in ferulic acid and p-coumaric acid content between urea and NH_3-treated straw. Chemical data suggests that responses of millet straw to ammoniation either by urea or

TABLE 30. EFFECT OF CHEMICALS ON ALKALI-LABILE PHENOLIC ACIDS AND ALDEHYDES CONTENT OF MILLET STRAW[a]

Item	Treatment					SE
	None	Water	Urea	NH_4OH	Alkaline H_2O_2	
	$mg \cdot g^{-1}$ cell wall					
Vanillic acid	1.07	1.28	0.38	0.41	0.96	.22
cis-p-Coumaric acid	0.16^{b}	0.15^{b}	0.00^{b}	0.08^{b}	0.00^{b}	.04
trans-p-Coumaric acid	8.53^{b}	8.46^{b}	7.66^{bc}	6.57^{c}	4.88^{d}	.37
Total p-coumaric acid[e]	8.68^{b}	8.61^{b}	7.66^{bc}	6.65^{c}	4.88^{d}	.37
Syringaldehyde	0.23^{b}	0.00^{c}	0.65^{b}	0.50^{bc}	0.00^{c}	.13
cis-Ferulic acid	0.19^{b}	0.04^{b}	0.63^{c}	0.20^{b}	0.00^{b}	.10
trans-Ferulic acid	3.28^{b}	3.35^{b}	2.63^{b}	3.40^{b}	1.32^{c}	.28
Total ferulic acid[f]	3.47^{b}	3.39^{b}	3.26^{b}	3.59^{b}	1.32^{c}	.33
Total phenolic acids[g]	13.44^{b}	13.28^{b}	11.95^{b}	11.14^{b}	7.30^{c}	.47
Ratio P-CA:FA	2.56^{b}	2.55^{b}	2.55^{b}	1.87^{b}	3.83^{c}	.26

[a]Each value represents the mean for eight samples.
[bcd]Means for treatments within rows with different superscripts differ ($P<.05$).
[e]Calculated by summation of cis- and trans- p-coumaric acids.
[f]Calculated by summation of cis- and trans- ferulic acids.
[g]Calculated by summation of individual phenolic acids.

NH_3, in terms of alkali labile phenolic acid, differ from barley straw.

Compared to untreated straw, higher values of DMD and lower values of total phenolic acids, especially ferulic and p-coumaric acids, for treated barley and millet straw suggest that removal of alkali-labile phenolic acids causes an increase in the degradability of plant cell wall, probably by cleaving the ester linkages between phenolic monomers of lignin and cell wall carbohydrates (Buettner et al., 1982) and (or) by increasing the accessability of carbohydrases to cell wall carbohydrates (Hartley and Jones, 1978).

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CHAPTER VI

GENERAL DISCUSSION

Quality of forages, generally declines with advancing maturity due to increases in structural carbohydrates and lignin, and to increased association between different cell wall components with correspondingly decrease in soluble carbohydrates and protein. Van Soest (1982) reported variability associated with plant maturity not only among different types of plants but also within the same plant and (or) plant parts.

Results from the present study for temperate and tropical grasses indicate higher values of NDF, ADF, cellulose and lignin and lower values of hemicellulose for stems followed by leaf sheaths and leaf blades. These results are in agreement with the findings of earlier authors for temperate and tropical grasses (Jones, 1970; Jung and Vogel, 1986; Burton et al., 1964). Contrary to our observations, Morrison (1980) reported that in grasses, high hemicellulose content was associated with high lignin.

In the present study, chemical composition of hemicellulosic sugars reveals a significant impact of plant maturity, particularly on xylose and arabinose content. A higher increase in xylose and decrease in arabinose was seen

in stems than leaf blades, whereas leaf sheath values were intermediate. Morrison (1974) has shown that plant maturity exerts a positive impact on xylose:arabinose ratio, particularly in linear xylan, compared to branched xylan.

In both grasses, cell walls hydrolyzed with alkali showed higher values for p-coumaric acid, followed by ferulic acid with advancing maturity, for all botanical fractions, compared to other phenolic acids. Increases ($P<.05$) were found in stems and trends for increase were seen for leaf blades in barley and millet. Several other authors (Hartley and Jones, 1978; Theander et al., 1981; Jung et al., 1984) have reported similar results for tropical and temperate grasses, while Hartley (1972) reported that increase in p-coumaric acid was relatively more in leaf blades of Italian rye grass than in leaf sheaths.

Greater in vitro digestion was observed for leaf blades, compared to leaf sheaths and stems of both grasses. Higher IVDMD values of leaf blades probably reflects their lower cell-wall constituents, xylose, xylose:arabinose ratio and alkali-labile phenolic monomers. Chaves et al. (1982) have shown strong negative correlation between IVDMD and p-coumaric acid, while Burritt et al. (1984) have shown strong negative correlation between IVDMD and xylose. Wedig et al. (1986) suggested that high hemicellulose and lignin contents

in mature grasses appear to depress DMD, primarily due to covalent bonding between lignin and hemicellulose.

In contrast to grasses, changes associated with plant maturity in cell wall constituents, and contents of neutral monosaccharides and alkali-labile phenolic monomers were not found in berseem clover. Regardless of maturity, highest values were recorded for stems and lowest for leaflets. Maturity did not affect DMD of berseem plant parts.

Results from SEM of barley plants indicated increased proportion of lignified parenchyma and sclerenchyma cells due to maturity in leaf sheaths and stem but not in leaf blades. In contrast to barley, leaf sheaths of millet showed little anatomical differences with increasing maturity. However, both leaf fractions of millet showed more lignification than barley leaf fractions, even at vegetative stage. Similar observations were noted by earlier workers for temperate (Akin et al., 1977) and tropical plants (Akin and Burdick, 1975).

Increased proportion of lignification in leaf sheaths and stems of barley plants and leaf sheaths of millet plants resulted in increased resistance to microbial digestion. Similar effects were observed by Akin et al. (1973), who incubated tall fescue leaves with ruminal fluid for different periods.

In contrast to lignified sclerenchyma and xylem in vascular bundles, the nonlignified mesophyll and phloem underwent rapid and extensive microbial digestion. The ease and extent of digestion in the present study agree with a general degradation pattern of grasses reported by Akin and Burdick (1975) that mesophyll and phloem degraded at a faster rate than sclerenchyma, bundle sheath and epidermal tissues, but mature xylem tissue remained undigested. Stafford (1963) pointed out that a decrease in digestibility with increasing maturity was related to a specific type of lignin, chlorine sulfite positive lignin, which is an indication of the presence of syringyl lignin. Transmission electron microscopic (TEM) studies of barley leaf blades and leaf sheaths supported our observations on SEM.

Cheng et al. (1984) reported that nutritive availability of forages depends upon the ability of microorganisms to colonize and digest plant cell walls. In the present TEM studies, no adhesion was noted between degraded parenchyma cells and bacteria, although microbes were present in close proximity to degraded cell walls. Akin et al. (1974) suggested that bacteria degraded the primary cell walls, apparently by extracellular enzymes and without any prior attachment. In the present study, rumen microbes of diverse morphologies seen in inter- and intra-cellular spaces ap-

peared to access through cut or damaged surfaces. The predominate bacteria resembles Ruminococcus flavefaciens and Bacteroides succinogenes, as reported by Akin et al. (1974) and Latham et al. (1978). The presence of these bacteria is an indication of diet fed to donor animal from which ruminal fluid was collected (Cheng et al., 1984).

In the present study, both grasses and legume showed similar digestion patterns. Higher resistance toward microbial attack was observed in stems than leaf blades/leaflets, whereas the degradation of leaf sheaths/petioles was intermediate. A basic difference was noted between grasses and legume in the mode of bacterial attack. The bacterial penetration of legume cell wall proceeded by general disorganization rather than by specific digestion pits, since the damaged and (or) cut areas of specimens and stomatal openings of leaflets were heavily colonized by bacteria. Chet et al. (1973) indicated that leaf leachates which escaped through stomatal openings exerted a chemotactic effect on Pseudomonas lachrymans.

Chemical data and observations obtained from SEM and TEM suggested that variation in degradability depends not only upon anatomical characteristics but also upon type and amount of lignin, ratio of xylose to arabinose, amount of phenolic

acids, as well as the nature of association between phenolic acids and lignin and (or) hemicellulosic sugars.

Crop residues represent a major source of underutilized resource. Voluntary intake and digestibility of these residues, which determine their feeding value to ruminants is generally low (Klopfenstein, 1978). The digestibility of these materials is limited by lignin, which encrusts cell wall carbohydrates and prevents the access of ruminal cellulolytic and hemicellulolytic microbes; chemical association between lignin and cell wall polysaccharides via ester and ether linkages, as well as the crystalline arrangement of native cellulose.

Results from present studies indicated that ammoniation of barley straw by urea and NH_3 decreased the NDF and hemicellulose content ($P>.05$), and treatment with alkaline-H_2O_2 decreased ($P<.05$) hemicellulose content of barley straw. Decreased NDF and hemicellulose content was also observed in treated millet straw, except that an increase in NDF content was observed in alkaline-H_2O_2-treated millet straw. Acid detergent fiber, cellulose and lignin were increased in alkaline-H_2O_2-treated millet and barley straws, compared to untreated and ammoniated straws. Contrary to our findings, Kerley et al. (1985) reported decline in acid detergent lignin (ADL) values of wheat straw treated

with alkaline-H_2O_2. Gould (1985) proposed a mechanism by which H_2O_2 at a pH of 11.5 cleave lignin and lignin-like molecules into simple soluble products which resulted in lower ADL values in treated products. In the present studies, high ADL values of alkaline-H_2O_2-treated straw indicate that levels of H_2O_2 (.25%, DM basis) employed may have been insufficient to cleave lignin bonds. Kerley et al. (1985) reported decline in ADL values when wheat straw was treated with higher levels of H_2O_2 (>1%, DM basis). Similar results were reported by Lewis et al. (1987) when wheat straw treated with H_2O_2 at 5, 7.5 and 10% (DM basis) levels. Lower ADL values were recorded in treated wheat straw, compared to untreated (11.4, 8.2, 7.5 and 11.6, respectively.

A decreasing trend was noted in contents of arabinose and xylose of treated than untreated straws. Lower values of arabinose and xylose were recorded for barley straw treated with alkaline-H_2O_2 and millet straw treated with urea than untreated or straw treated with other chemicals. Earlier reports also showed a neutral response in monosaccharide composition of hemicellulose towards alkaline treatments (Graham and Aman, 1983/84; Lindberg et al., 1984).

The detrimental effect of phenolic acids, especially guaiacyl- and syringyl-like compounds, on microorganisms has been reported (Jurd et al., 1971). Exposure of phenolics

apparently results in membrane damage, lysis of bacteria and release of cell contents. Harris and Hartley (1976) suggested that ferulic and p-coumaric acids, present in relatively high concentrations in forages, may be important in degradation of plant OM in the rumen, possibly by influencing the attachment of the fibrolytic microorganisms to fiber particles (Varel and Jung, 1986). Several workers have reported that p-coumaric and its aldehyde were the most toxic to rumen protozoa and bacteria involved in cellulolytic and xylanolytic activities (Akin, 1982; Chesson et al., 1982; Varel and Jung, 1986; Borneman et al., 1986). Since phenolic acids are bifunctional and form ether and ester linkages with cell wall components (Hartley, 1972; Scalbert et al., 1985), they act as cross linking moieties between lignin and hemicellulose. Harris and Hartley (1976) reported that major alkali-labile phenolic acids (p-coumaric and ferulic acids) are bound to unlignified as well as lignified cell walls in grasses.

Decreases in arabinose, ferulic acid and p-coumaric acid in treated barley and millet straw indicate that alkali had solubilize the substituted arabinose and the ester and ether bonds of bifunctional phenolic acids. Similar results were reported by Hartley and Jones (1978) and Graham and Aman

(1984), although they observed a greater loss of ferulic acid than p-coumaric acid.

Various chemical treatments employed in present study enhanced the IVDMD. It is apparent that treatment with NH_3 had a higher impact on degradability than urea and alkaline-H_2O_2. Improved digestibility of treated straw probably resulted from the saponification action of alkali on the ester and ether linkages of lignocellulosic compounds (Buettner et al., 1984). Tarkow and Feist (1989) suggested that alkaline treatment caused hydration of cellulose by which a more amorphous form is achieved with more susceptibility to microbial attack.

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