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ULTRASTRUCTURAL UTILIZATION OF PLANTS BY HERBIVORES

L.H. Harbers

Animal Sciences & Industry
Kansas State University
Call Hall, Manhattan, Kansas 66506

Abstract

Study of the patterns of ruminal digestion of forages enhances the nutritional knowledge of how specific plant tissues are digested and adds its own dimension by characterizing the specific cells and the complex interactions of ruminal microflora with those tissues. A common pattern of digestibility exists for mono- and dicotyledon leaves: mesophyll and phloem are degraded readily, and sclerenchyma slowly, whereas cuticle and the remaining vascular tissues are rarely utilized. Digestion of stems is limited to parenchymal tissues in monocotyledons and to cortex and parenchyma in dicotyledons. Epidermal silica and cuticle are undigestible and restrict microbial entrance. Calcium oxalate crystals in legumes are utilized poorly by animals, suggesting the need for further attention to structure in feedstuff analyses. Future studies by animal scientists on plant utilization and by agronomists in genetics should include structural considerations along with the well recognized experimental procedures.

Introduction

Over the past century, animal nutritionists have relied almost exclusively on chemical analyses in their quest for improved feedstuff utilization and animal performance. Samples of forages, for example, are dried, ground through a 1-mm screen, and subjected to various physical, chemical, and biochemical analyses. While such tests have provided valuable data on total nutrient content, little information relative to nutrient location and specific tissue utilization has been obtained.

Attempts to use light microscopy to evaluate the digestive sequences and specific plant-tissue utilization have been impeded by lack of depth of field and resolution. The advent of the scanning electron microscope (SEM) greatly enhanced such studies because of the three dimensional perspective and the great depth of field at high magnifications. Research with the SEM on digestion of grasses, legumes, and silages is the subject of this review. Digested samples shown in photomicrographs were obtained using the nylon bag technique (14).

Grasses

Domestic ruminants generally graze on and consume hays from cool-season (C3) or temperate and warm-season or tropical (C4) grasses. The structural anatomy of leaves of these grasses differs (6,19); generally C3 grasses are better utilized than C4 species because of a greater percentage of mesophyllous tissues. Morphometric studies show that C3 grasses characteristically contain a higher percentage of mesophyllous tissues (52-64%) compared with C4 species (27-52%) (6). Leaf blades of tropical grasses average 22% less of the highly digestible tissues and 25% more of the tissues typically less digestible than do C3 grasses (6). Starch, a readily digestible carbohydrate, is present in the mesophyll of C3 species, but in C4 species it is localized within bundle sheath cells surrounding vascular bundles. In all monocotyledon leaves studied, mesophyll and phloem are degraded readily, while lignified tissues such as sclerenchyma and xylem usually are not degraded (6,20). Cuticle is ruptured following prolonged digestion but is not degraded (15,22).

The C3 grasses studied, including Bromus inermis Leyss. (bromegrass), Festuca arundinacea Schreb. (fescue), Poa pratensis L. (Kentucky bluegrass), and Dactylis glomerata L. (orchardgrass), show digestive patterns similar to those for C4 plants or
to each other (Fig. 1). Mesophyll and phloem are degraded rapidly (within 24 h fermentation) followed by slow digestion of bundle sheath cells and sclerenchyma (2, 6, 13, 22). Ruptured adaxial cuticle and intact abaxial cuticle remain after 72 h (22). Tissues from bromegrass appear to be degraded more rapidly than those from fescue; however, the extent of digestion is similar if the grass fragments remain in the rumen for 48 h (22). Recent studies with various C4, C3, and C4/C3 intermediates of Panicum species show that readily digestible mesophyllous tissues in leaves are degraded primarily by a diverse population of nonadhering bacteria, whereas parenchyma bundle sheaths, sclerenchyma, and epidermis require adhering encapsulated cocci and irregularly-shaped bacteria (9, 11). Complex interrelationships exist between fiber-digesting bacteria and forage-plant cell walls during digestion and that relationship is probably related to variation in cell wall nutrient availability (1). Stems from grass species vary in parenchymal lignification and, thus, digestion, suggesting the possibility for genetically produced plants with no or little parenchymal lignification (5). One species, Panicum antidotale (C4), shows no parenchymal lignification; the tissue is digested within 24 h.

Warm-season grasses studied by SEM initially involved Cynodon (bermudagrasses) and Andropogon (bluestems), but now include Panicum (13), Digitaria (Sorghum bicolor (L.) Stott., (10), Bothriochloa, and Eragrostis (4). These grasses contain much less of the readily available tissues (especially mesophyll), which partially accounts for their lower digestibilities compared with cool-season grasses (6). Mesophyllous tissues appear to be more compact and may have differential quantities and qualities of cell wall material (27). A typical example (Fig. 2) of a digested leaf shows the tissue remnants. Generally, lignified supportive cells (xylem, inner bundle sheath) are not degraded and stain positively with acid fluorogluconil, suggesting coniferaldehydinelignin. Slowly degradable cells such as sclerenchyma and outer bundle sheath are positive for chlorine-sulfite stain, and probably because of syringyl groups (8, 9). Horses also are able to digest the readily degradable tissues that ruminants digest but are unable to utilize these chlorine-sulfite-positive-cells that ruminants degrade slowly (24).

Starch is localized in bundle sheath cells of C4 grasses (19). Hydrolysis of these granules cannot occur until bundle sheath walls have been ruptured (3,7). Transmission electron microscopy studies reveal a thin, suberin layer in outer tangential and radial walls of bundle sheath cells of immature and mature leaves of indiangrass (Sorghastrum nutans (L.) Nash) and big bluestem (Andropogon gerardii Vitman), which resist degradation. Mature leaf blades subjected to 120 h incubation show only 25 to 50% of bundle sheath cells degraded. Rupture of the bundle sheath is dependent upon bundle size and stage of development (27). Bacterial entry may be less restrictive where the cell walls are contiguous to vascular tissues (no suberin layer). Masticated and exposed edges of vascular bundles may provide channels for bacterial distribution, at least in smaller bundles.

Silages

Storing freshly harvested plant material by ensiling in air-tight structures permit bacterial fermentation (mainly lactate formation) prior to utilization by ruminants. Leaf and stem tissues from corn (Zea mays L.) and sorghum (Sorghum bicolor (L.) Moench.) treated by this process have been studied by SEM (26).

Bacterial attachment is more prevalent on adaxial compared with abaxial surfaces of leaf tissues of both forages removed from silos. In corn silage, hydrolytic activity occurs in starch granules of corn silage deposited along vascular protrusions of adaxial leaf surfaces during the ensiling procedure. These cuticular areas appear to be vulnerable to bacterial degradation (Fig. 3). Sorghum leaf surfaces are planar, thus starch granules do not accumulate during ensiling. Sorghum seed is smaller than those of corn and generally escape fracture during harvesting. Internal leaf tissues remain intact, although numerous bacterial accumulation may be observed. Stem tissues remain intact. Corn stem parenchyma contains partially digested starch granules from ruptured corn kernels; sorghum stem parenchyma appears free of starch granules from seed as were the ensiled sorghum leaves.

Digestion of leaf tissues by rumen microorganisms shows that mesophyllous tissues are destroyed within 24 h in both corn and sorghum (Figs. 4, 5). Sorghum epidermis is thicker and more resistant to rupture than that of corn. Unreported research suggests that there may be differences between sorghum lines in the rate of leaf degradation by rumen microflora (Akin DE, personal communication).

Examination of ensiled stem tissues shows that rumen microorganisms preferentially digest thin parenchymal cells (Figs. 6, 7). Corn parenchyma is degraded within 48 h; however, sorghum stem parenchyma is degraded more slowly. Recent research in our laboratory suggests variation in digestion of stems between sorghum lines. Studies are currently underway to verify these findings and elucidate the reasons for resistance in some sorghum varieties.

Other structural inhibitors to digestion

Lignin, in combination with thick-walled cellular structures, forms major barriers to digestion of some internal tissues by microorganisms. The external leaf structures, silica and cuticle, provide a formidable barrier to microbial entrance, leaving only exposed edges and cuticle crushed from mastication as entrance sites (30).

Silica is deposited by a passive nonmetabolic mechanism (32) in grass epidermal cells (19), where it prevents microbial entrance (15). Grass cuticle is so resistant to microbial digestion that rumen fermentation has been used to isolate intact cuticle (31).

Typical X-ray dispersions of silica in adaxial and abaxial tissues of C3 (fescue) and C4 (bluestem) grasses are shown in Figs. 8 to 10. Silica in adaxial leaf surfaces of fescue (Fig. 8) is limited to cells contiguous to underlying vascular bundles, whereas abaxial cuticle contains silica in every cell (Fig. 9). Both surfaces show the characteristic fescuoid phytoliths. The nonsilicified cells of the adaxial cuticle are ruptured during fermentation (Fig. 1), probably by physical rupture, and so may aid in increased rate of passage of nonutilizable tissues by rapid particle reduction. Silica is present in each cell of adaxial and abaxial epidermis of bluestem (Fig. 10); dumbbell-shaped pani­coid phytoliths are present in parallel rows on both surfaces. Cuticular resistance in C4 plants is such that only a few epidermal cells are ruptured after underlying mesophyllous tissues are degraded (15).

Plants (fescue and bluestems) grown with and without silica (25) show that young grasses (30 days postemergence) with silica have cuticular barriers to digestion similar to those of naturally
Fig. 1. Cross-section of fescue (C₃) leaf blade digested 24 h by rumen microorganisms showing degraded mesophyll (M), ruptured cuticle (arrow), and intact sclerenchyma (S). Bar = 10 μm.

Fig. 2. Cross-section of 24 h digested bluestem (C₄) leaf blade with intact cuticle (C) and vascular tissue (V). Bar = 100 μm.

Fig. 3. Adaxial surface of ensiled corn leaf with starch grain (arrow) deposit near protrusion over vascular tissue (V). Bar = 100 μm.

Fig. 4. Adaxial view of ensiled corn leaf blade after 72 h ruminal digestion showing remnants of adaxial cuticle (C), vascular tissue (V), and epidermal cells (E) attached to abaxial cuticle. Bar = 300 μm.

Fig. 5. Section of sorghum leaf blade digested 12 h showing vascular bundles (V) and cuticle (C) intact. Bar = 100 μm.
Fig. 6. Columns of vascular bundles (V) minus parenchyma after 48 h ruminal digestion of ensiled corn stem. Bar = 500 μm.

Fig. 7. Exposed vascular tissue (V) and parenchyma (P) remaining on sorghum stem digested 48 h. Bar = 400 μm.

Fig. 8. X-ray dispersion of silica on adaxial surface of fescue leaf. Bar = 400 μm.

Fig. 9. X-ray dispersion of silica on abaxial surface of fescue leaf. Bar = 300 μm.

Fig. 10. X-ray dispersion of silica and panicoid phytoliths on surface of bluestem leaf blade. Bar = 30 μm.
Fig. 11. Abaxial surface of young fescue leaf blade grown without silica showing cuticular rupture following 12 h digestion. Bar = 500 μm.

Fig. 12. Digested alfalfa leaflet (24 h) with vascular tissue (V), cuticle (C), and abaxial macrohair (H) as only remnants. Bar = 100 μm.

Fig. 13. Cross-section of alfalfa stem showing cuticle (C), cortex (arrow), vascular tissue (V), and parenchyma (P). Bar = 300 μm.

Fig. 14. Digested alfalfa stem (48 h) showing remnants of vascular tissue. Bar = 300 μm.

Fig. 15. Calcium oxalate crystals (arrow) on vascular bundle remnants of digested alfalfa (24 h). Bar = 10 μm.
grown plants. When silica is absent, massive rupture of the cuticular layers is possible (Fig. 11), suggesting that silica is a major deterrent to microbial entrance in young plants. In older plants (60 days postemergence), the absence of silica has no influence on microbial penetration; other structures and components in the cuticle and epidermis resist rupture (25).

Siliceous deposits are found also in leaves of corn and sorghum. Sorghum leaf cuticle is resistant to microbial and physical rupture following enlisting and rumen fermentation (Fig. 5), but adaxial corn leaf cuticle is not as resistant (Fig. 4), suggesting that cuticular components other than silica differ among these forages (26).

Legumes

The dicotyledons have received less attention than monocotyledons because leaves of most of the former are readily digestible; their quality as feed is dependent upon the amount of highly lignified stem. The high digestibility of alfalfa does not agree with the supposition that lignin decreases digestibility, since legumes such as alfalfa generally contain more lignin than do most grasses. The idea was proposed more than 30 years ago that the location of lignin may have more effect on utilization than its percentage (17,29).

Studies on the rumen fermentation patterns of fresh, intact legume leaves show that microorganisms rapidly enter through stomata, penetrate intercellular spaces, separate plant cells, and penetrate the cell walls by general disorganization (16). SEM studies (14) on alfalfa hay leaves show that ruminal fermentation results in random sloughing of adaxial cuticle with rapid digestion of mesophyll. By 24 h the remaining leaf tissues consist of vascular bundles and abaxial cuticle with its macrohairs intact (Fig. 12). The indigestible abaxial tissues are also a feature of monocotyledons; however, legumes do not contain silica in epidermal tissues.

The classic dicotyledon structures (epidermis, cortex, and vascular tissues) are distinguished easily in stem cross-section (Fig. 13). Ruminal fermentation results in sloughing of the cuticle and hydrolysis of the dense cortex to sieve cells of the vascular tissue and simultaneous degradation of part of the pith parenchyma (Fig. 14). No additional digestion has been observed in either rumen fermentations or fecal remnants.

Studies with leaf and stem tissues of clovers (12) indicate that reduced digestibility with maturity is due to reduced degradation of stem interfacicular parenchyma. Differences in digestibility between arrowleaf clover (Trifolium vesiculosum Savi) and crimson clover (T. incarnatum L.) could not be explained by anatomical differences (12).

The SEM has been an especially useful tool in elucidating a nutritional problem associated with the feeding of alfalfa hay. Our dairy group reported approximately a twofold discrepancy in the requirement of calcium for milk production from that recommended by the National Research Council (NRC) when alfalfa-sorghum grain-soybean meal rations were fed (34). The NRC calcium requirement was derived from studies involving rations providing calcium from inorganic sources. The true digestibility (a combination of chemical and radio-calcium balance studies) of alfalfa calcium was found to average only two-thirds that of inorganic sources (21), but the work was done with steers.

Leguminous plants have 1 to 2% calcium. Thus, when NRC recommended allowances for total calcium are followed, rations with alfalfa as the only source of roughage require additional phosphorous, but not calcium. Lactating dairy cows fed alfalfa as the sole roughage source did not reduce milk production appreciably but were removed early from the herd compared to calcium-supplemented animals because of apparent skeletal problems (33).

Using a combination of secondary imagery and x-ray dispersion with the SEM in combination with Raman microprobe analysis (36), we were able to show that crystals surrounding vascular bundles in alfalfa were calcium oxalate (Fig. 15), a condition reported much earlier in clovers (18,28). These crystals remained in their sclerenchymatous sheaths in the rumen and were found as loose crystals in fecal remnants (36). Fecal residues from domestic and zoo ruminants, nonruminant herbivores, and birds fed alfalfa hay or dehydrated pellets showed crystals still intact or loose (23). Further studies on extractability in acid (35) and oxalate digestion (37), as well as chick growth studies (38) confirmed poor utilization of oxalate calcium in alfalfa.

Conclusions

Microbial digestion of plant tissues follows a remarkably similar hydrolytic pattern: nonlignified tissues are degraded readily by cellulase enzymes, cells containing syringyl-type lignin are degraded slowly by two general types of attached bacteria, and structural tissues containing coniferaldehyde-type lignin resist digestion. Cuticle acts as a barrier to bacterial entrance in most monocotyledons, and suberin surrounding C₄ bundle-sheath cells delays entrance into these starch-containing tissues. The relatively high concentration of calcium in dicotyledons (such as alfalfa) is not as available as generally believed because of insoluble calcium oxalate crystals attached to leaf vascular bundles.

References

Plant Digestion


Discussion with Reviewers

**D.E. Akin**: Could x-ray analysis of minerals and elements be used in re-defining NRC guidelines for animal feeding? **Author**: X-ray analysis for minerals in biological specimens is generally limited to qualitative types of experiments. The methodology is extremely useful in defining insoluble minerals such as the studies with calcium oxalate and silica. Future studies localizing partially available mineral complexes such as phytin phosphate and other suspected mineral complexes resulting in water insoluble molecules could best be studied by microscopic techniques but would have to be confirmed by quantitative animal performance for re-defining NRC guidelines.

**D.E. Akin**: You referred to the fact that absence of silica did not affect the resistance of cuticles in older plants, and that other compounds are possible factors. Do you think that various waxes, lipids, or suberin-like compounds may differentially influence cuticle breakdown in various plants? **Author**: Our investigations with corn and sorghum leaves as well as grasses grown with and without silica lead us to believe there are differences in cuticular composition. Literature on the chemical composition of leaves would certainly suggest differences.
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D.E. Akin: What do you feel will be the most important thrust for electron microscopy in future exploration of plant-microbe interactions?

Author: Specifically for the ruminant animal, additional research is needed to identify plant species and varieties containing readily digestible tissues and coordinating such findings with animal production information. Further studies are needed to identify and characterize bacteria and fungi that attack slowly degradable tissues. Such information would be of immediate benefit to animal scientists and agronomists and in future decisions by genetic engineers as to appropriate changes in both plants and bacteria to achieve optimal plant digestion by ruminants.

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