A REVIEW OF THE MICROBIAL DIGESTION OF FEED PARTICLES IN THE RUMEN

T. A. McAllister[1], H. D. Bae, L. J. Yanke, K.-J. Cheng and J. K. Ha[2]

Agriculture and Agri-Food Canada, Research Centre, P.O. Box 3000 Main
Lethbridge, Alberta, Canada T1J 4B1

Summary

Microbial digestion of feed in the rumen involves a sequential attack culminating in the formation of fermentation products and microbial cells that can be utilized by the host animal. Most feeds are protected by a cuticular layer which is in effect a microbial barrier that must be penetrated or circumvented for digestion to proceed. Microorganisms gain access to digestible inner plant tissues through damage to the cuticle, or via natural cell openings (e.g., stomata) and commence digestion from within the feed particles. Primary colonizing bacteria adhere to specific substrates, divide to form sister cells and the resultant microcolonies release soluble substrates which attract additional microorganisms to the digestion site. These newly attracted microorganisms associate with primary colonizers to form complex multi-species consortia. Within the consortia, microorganisms combine their metabolic activities to produce the diversity of enzymes required to digest complex substrates (e.g., cellulose, starch, protein) which comprise plant tissues. Feed characteristics that inhibit the microbial processes of penetration, colonization and consortia formation can have a profound effect on the rate and extent of feed digestion in the rumen. Strategies such as feed processing or plant breeding which are aimed at manipulating feed digestion must be based on an understanding of these basic microbial processes and their concerted roles in feed digestion in the rumen.
(Key Words: Rumen Bacteria, Feed Digestion, Colonization, Cellulose, Starch)

Introduction

Ruminants are among the most widely distributed groups of mammals on Earth, having adapted to arctic, temperate and tropical environments. This global distribution is possible because of the unique ability of ruminants to digest a wide range of vegetation. The rumen, and its distinct microflora, enables ruminants to derive energy from a wide variety of fibrous feedstuffs.

To be useful as feed, plants must be resistant to microbial attack while growing in the field, but susceptible to penetration, colonization and digestion by microorganisms within the rumen (Kennedy, 1990; Cheng et al., 1991a). Protective barriers (e.g., waxy cuticle, husk) and compounds (e.g., condensed tannins, phenolic acids) that defend against microbial attack (Forsberg and Cheng, 1992) are necessary for the survival of the plant in its natural environment, but impede the digestion of plant material within the rumen. Readily digestible proteins and carbohydrates (e.g., sugars, starch) are often located within internal compartments (e.g., endosperm in cereal grain) and are thereby protected from digestion by ruminal microorganisms (Wilson, 1990; Cheng et al., 1991a). Ruminal microorganisms must circumvent these physical defences through less resistant plant structures such as stomata (Cheng et al., 1991a; Cheng et al., 1983/84), or through disruptions in the protective barriers due to chewing or mechanical processing (Beauchemin et al., 1993).

Once access is gained, rumen bacteria attach to inner tissues, start to form biofilms (Costerton et al., 1981; 1987), and initiate digestion (Kudo et al., 1987; McAllister et al., 1990c). These initial colonizers release digestion products which in turn attract additional bacteria to the site of digestion and a complex and varied consortium develops which digests internal plant tissues. Thus, digestion of feeds in the rumen proceeds from within and the extent to which ruminal microorganisms can access internal tissues often dictates both the rate and the degree to which a particular feed is digested and the extent of microbial protein synthesis in the rumen (Cheng et al., 1991a; Beauchemin et al., 1993).

[1]Address reprint requests to Dr. T. A. McAllister, Agriculture and Agri-Food Canada, Research Centre, P.O. Box 3000 Main, Lethbridge, Alberta, Canada T1J 4B1.
[2]Seoul National University, Suweon 441-744, Korea.
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Microbial Penetration and Feed Processing

The extent and rate to which rumen microorganisms penetrate and digest nutrient-rich intracellular components is determined by the chemical composition and arrangement of protective tissues within the plant. The cuticular surface layer in forages and the pericarp in cereal grains are formidable barriers to penetration by microorganisms (Akin, 1989, McAllister et al., 1990c). In forages, the resistance of the cuticle to rupture can be enhanced by the presence of silica (Harbers et al., 1981). Using energy-dispersive x-ray analysis (EDX), we have found that 98% of the surface of rice straw is covered by silica (Our unpublished data). Consequently, when observed by scanning electron microscopy, the surface of rice straw is seen to be virtually undamaged after 24 h of incubation in rumen (figure 1A). The few ruminal bacteria which colonize cuticular surfaces, are unable to penetrate this resistant structure. Rather, bacterial digestion occurs only in areas where this resistant layer has been fractured (figure 1B), allowing ruminal bacteria access to the tissue underlying the cuticle (figure 1C). When the cuticle is removed, bacteria preferentially colonize the areas between cellulose fibers (figure 1D). We have observed that the cuticle of rice straw remains as a barrier to microbial penetration even after it is treated with 3% ammonium hydroxide, 3% sodium hydroxide or 3% hydrogen peroxide (Our unpublished data). These chemical treatments may improve digestibility by reducing internal obstacles to microbial degradation. Thus, access of bacteria to more digestible inner tissues requires disruption of the cuticle by grinding, chopping or chewing.

Since disruption of resistant protective barriers is essential for effective microbial penetration and digestion of feed, mechanical processing can be used as a means of manipulating the rate and extent of feed digestion in the rumen (Fahey et al., 1993). Ruminants can effectively digest whole corn, because the kernel pericarp is extensively damaged by chewing during eating and rumination (Beauchemin et al., 1993). In contrast, barley and wheat kernels are not severely damaged by mastication during eating and rumination and consequently these grains must be rolled or ground for efficient digestion in the rumen. Thus, the degree of physical processing required to facilitate the penetration and digestion of feed by ruminal microorganisms depends on the extent to which it is damaged by mastication. Care must be taken to ensure that feeds (e.g., barley, wheat) that are rapidly fermented are not over processed. In the last 15 years, the occurrence of bloat in Canadian feedlot cattle has been substantially reduced by replacing finely ground barley in feedlot diets with coarsely rolled barley, in which the pericarp is just cracked sufficiently to allow microorganisms to gain access to the endosperm (Cheng and Hironaka, 1973).

Rumen bacteria readily penetrate the damaged areas of alfalfa and red clover cells, and the subsequent rapid release of soluble material (e.g., proteins, chloroplast fragments) contributes to the development of pasture bloat (Cheng et al., 1980; Fay et al., 1981). Bloat-safe legumes such as sainfoin and birdsfoot trefoil contain condensed tannins which slow the rate of microbial attachment and penetration (Bae et al., 1993) and retard the release of soluble materials. Agriculture Canada has nearly completed a plant breeding program selecting alfalfa for a slow initial rate of ruminal digestion, a process which resulted in thickened cellulosic cell walls (Goplen et al., 1993). While this selection was partially successful, the reduction in the initial digestion rates of the selected alfalfa tended to be greater during dry periods (15%) than during wet periods (4-6%, Goplen et al., 1993).

Association of Microorganisms and Feed

Ruminal microorganisms adhere to the rumen wall (Cheng and Costerton., 1980) and are present in rumen fluid, but by far the greatest proportion of the population (75%) is associated with partly digested feed particles (Forsberg and Lam, 1977). Several researchers have demonstrated that attachment of ruminal microorganisms to their substrate is a prequisite for the digestion of feed particles in the rumen (Miron et al., 1990; Miron, 1991; McAllister et al., 1994). The degree of colinization and mode of attack are specific to each microbial species (Cheng et al., 1983/84; Kudo et al., 1987). Cellulolytic bacteria adhere to substrates by means of their extracellular glycocalyx and possibly by protuberant structures called cellulosomes (Miron et al., 1989; Costerton et al., 1992). Some cellulolytic species adhere intimately to the plant cell wall (e.g., *Fibrobacter succinogenes*; figure 2A), while others perform their digestive role at a distance

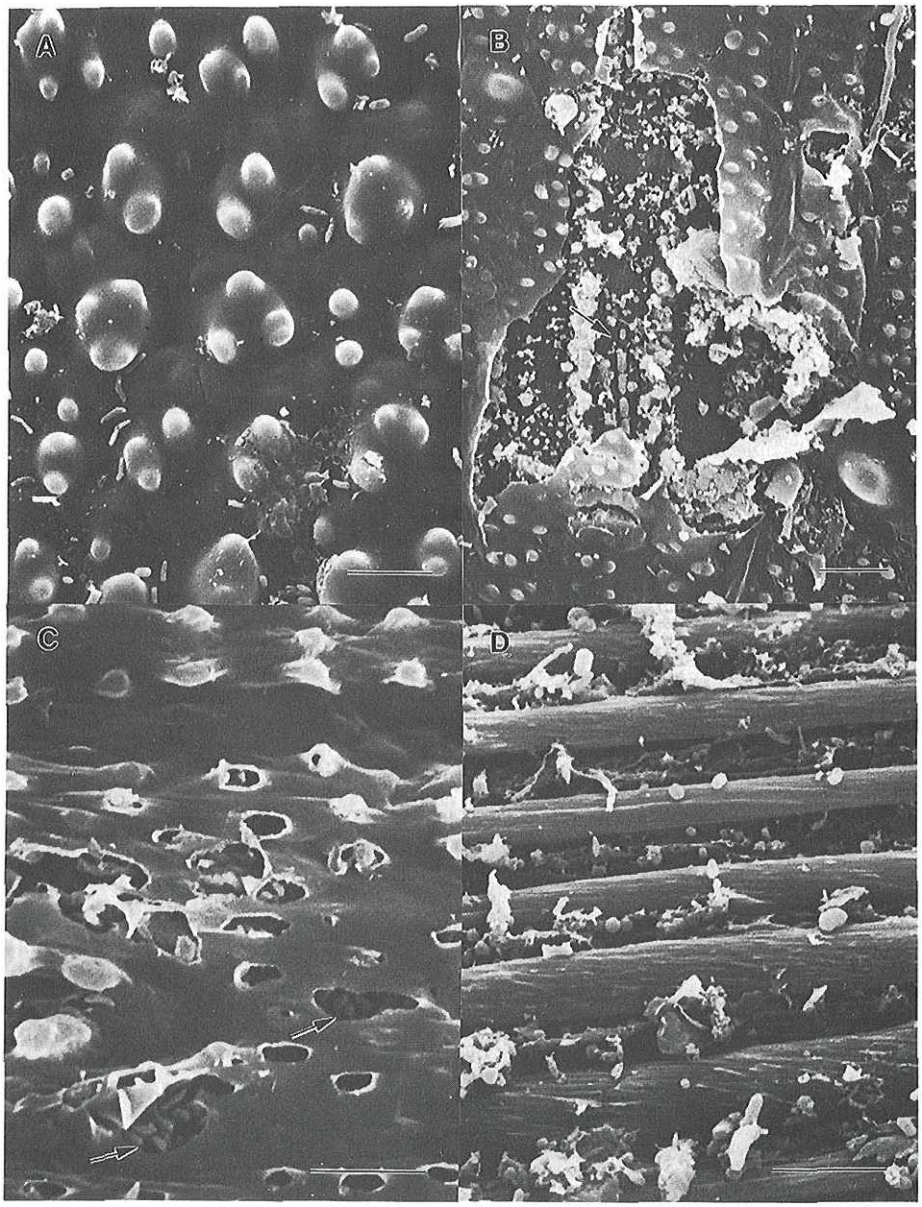

Figure 1. (A) Scanning electron micrograph of the cuticular surface of rice straw after 24 h of incubation in rumen. Note that the cuticular surface is intact and colonized only by a limited number of bacteria which do not penetrate this resistant surface. Bar = 5 μm.
(B) Scanning electron micrograph of the cuticular surface of rice straw after 48 h of incubation in the rumen. Note that bacteria (arrow) preferentially colonize the disrupted region of the cuticle. Bar = 10 μm.
(C) Scanning electron micrograph of rice straw incubated for 48 h with the cellulolytic bacterium *Fibrobacter succinogenes* S85. Note that bacteria (arrow) have gained access to underlying tissues through damaged areas of the cuticle. Bar = 5 μm.
(D) Scanning electron micrograph of rice straw incubated in the rumen for 48 h in which the cuticle has been removed to expose underlying cellulose fibers. Note that numerous bacteria colonize the regions between the cellulose fibers. Bar = 5 μm.
(All figures from our unpublished data).

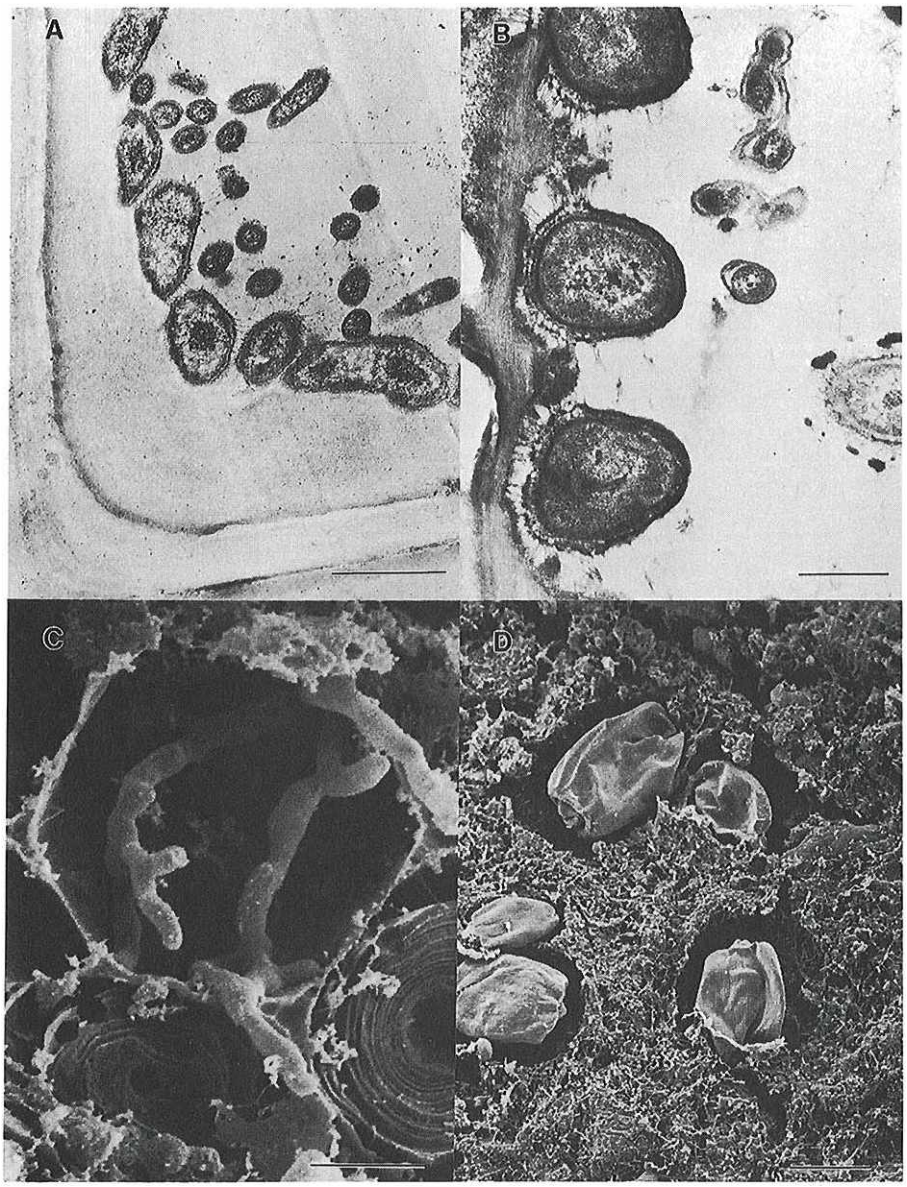

Figure 2. (A) Transmission electron micrograph of *Fibrobacter succinogenes* attached to the surface of a plant cell wall. Note that these bacteria conform to the cell wall surface. Bar = 2 μm.(Costerton et al., 1985: Copyright 1985, Plenum Publishing).

(B) Transmission electron micrograph of *Ruminococcus* spp. attached to the surface of a plant cell wall. Note that these organisms adhere to the cell wall by their glycocalyces and perform digestion at a distance from the surface of the cell wall. Bar = 0.5 μm. (Cheng et al., 1991a: Copyright 1991, Academic Press).

(C) Scanning electron micrograph of a rumen fungus grown on corn for 96 h. Note that rhizoids have penetrated the protein matrix and have completely digested the contained starch granule. Surrounding starch granules show digestion by extracellular amylases. Bar = 5 μm. (From McAllister et al., 1993a: Copyright 1993 Canadian Journal of Microbiology).

(D) Scanning electron micrograph of ciliate protozoa associated with the surface of the endosperm of corn (Our unpublished data). These microorganisms engulf starch granules which lie on the surface of endosperm cells. Bar = 30 μm.

from the cell wall surface (e.g., *Ruminococcus* spp.; figure 2B). Ruminal fungi produce hyphae which attach to their substrate and penetrate recalcitrant plant structures (figure 2C). Protozoa associate closely with the surface of cereal grains and engulf starch granules. (figure 2D, Bonhomme, 1990).

Rumen microorganisms do not randomly colonize feed particles. Rather, they adhere selectively to those substrates which they are most adept at digesting. Even in co-culture, there is little competition among cellulolytic bacteria for adhesion sites on barley straw (Bhat et al., 1990). Cellulolytic bacteria also differ in their biochemistry of adhesion. For example, 3-phenylpropanoic acid improves the adhesion of *Ruminococcus albus* to cellulose, but has no effect on the adhesion of *Ruminococcus flavefaciens* or *Butyrivibric fibrisolvens* (Stack and Cotta, 1986). The specificity of particular groups of ruminal bacteria for their substrates is so pronounced that it can be used to isolate specific rapidly-adhering subpopulations of bacteria from cattle fed mixtures of cereal grain and forages (Minato and Suto, 1978, 1979).

The close association between digestive bacteria and feed particles concentrates digestive enzymes close to their specific substrates (Chesson and Forsberg, 1988; Pell and Schofield, 1993). Enzymes that are freely released into the ruminal fluid are quickly inactivated by proteolytic enzymes produced by other ruminal microorganisms. Digestive enzymes are often enclosed within protective vesicles and can continue to associate with and digest substrates even when living bacterial cells are no longer present on the surface of feed particles (Forsberg et al., 1981; Lamed et al., 1987). For some substrates (e.g., crystalline cellulose) close association appears to be a perquisite for digestion. This is evidenced by methylcellulose, which causes the detachment of both cellulolytic bacteria and fungi from filter paper and prevents the digestion of cellulose (figure 3, Kudo et al., 1987; Cheng et al., 1991b).

After colonization, the rate of digestion is determined by the extent to which other cooperative bacteria integrate into the microbial consortium. Bacteria within the consortium produce specific enzymes which combine to degrade chemically complex plant tissues. Natural compounds which impede the microbial processes of association and adhesion (e.g., condensed tannins, figure 3, Bae et al., 1993; silica, Harbers and Thouvenelle; 1980; or terpenes, Demeyer 1981) can have a profound effect on the microbial digestion of plant tissues in the rumen.

Digestion from Within Concept

Microbial digestion of sectioned fresh forage stems proceeds from the cut ends inward to the internal tissues of the mesophyll and phloem. Digestion of these internal components is rapid, but slows as bacteria are confronted by the more resistant external plant structures of the parenchyma bundle sheath, sclerenchyma and cuticle. Even after long periods of exposure to ruminal microorganisms, the protective cuticular surface of some plants remains virtually unaltered by digestion (Cheng et al., 1980; Akin, 1986; McAllister et al., 1990c). In fact, the husks of cereal grains often appear in the feces and their intact nature has led to the assumption that cereal kernels have passed through the digestive tract without being digested. However, if the cuticular tissue is sectioned and examined, it is often found that the endosperm has been completely digested.

Once the pericarp of cereal grain is cracked the protein matrix and the endosperm cell walls dictate the rate at which bacteria gain access to readily digestible starch granules. Many of the bacteria capable of digesting starch are unable to digest the endosperm cell wall. Fibrolytic bacteria must first penetrate the cell wall so that amylolytic bacteria can gain access to the interior of the endosperm cell. Within the endosperm cell, starch granules are surrounded by a protein matrix which must be digested to allow amylolytic attack of starch granules. In corn, the protein matrix is extremely resistant to invasion and rumen fungi appear to be the only microorganisms capable of penetrating this structure (McAllister et al., 1990c; McAllister et al., 1993a). Conversely, the protein matrix in barley and wheat is readily penetrated by a variety of proteolytic bacteria and digestion proceeds rapidly (McAllister et al., 1990c). The properties of the protein matrices control the rate of digestion, and account for the ruminal escape of 40% of the starch in corn, whereas less than 10% of the starch in barley or wheat reaches the small intestine (Orskov, 1986).

Digestive Consortia

Although many microbial species are capable

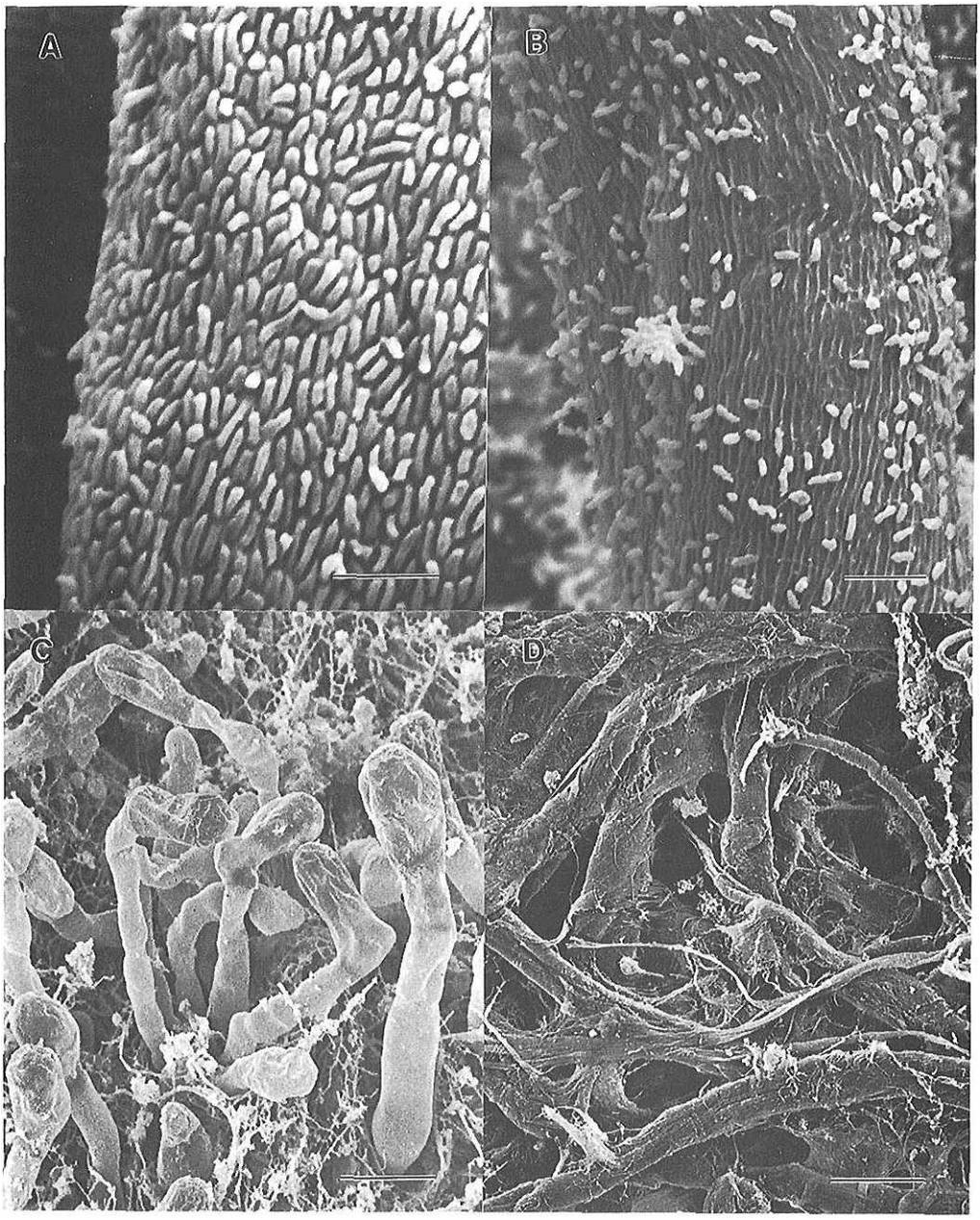

Figure 3. (A) Scanning electron micrograph showing the alignment and adhesion, upon a cellulose fiber, of cellulolytic cells *Fibrobacter succinogenes* S85. Bar = 3 μm. (Our unpublished data).
(B) Scanning electron micrograph showing the pitted and partly digested surface of a cellulose fiber from which most *Fibrobacter succinogenes* S85 cells have been detached by exposure to 400 μg mL^{-1} of sainfoin concensed tannin. Bar = 3 μm. (Our unpublished data).
(C) *Scanning electron micrograph* of the surface of cellulosic filter paper showing the active cellulolytic attack of rumen fungi on this fibrous substrate. Bar = 30 μm. (Cheng et al. 1991b, Copyright Canadian Journal of Microbiology).
(D) Scanning electron micrograph of the surface of filter paper incubated with rumen fungi in the presence of 0.1% methylcellulose. Note that attachment has not occurred and there is no evidence of digestion. Bar = 30 μm. (Cheng et al., 1991b, Copyright Canadian Journal of Microbiology).

of singularly colonizing and digesting feed *in vitro*, the rate and extent of digestion is never as great as that observed in the natural rumen ecosystem. Direct observations of partly digested plant materials show adjacent bacteria (e.g., *Treponema bryantii, Butyrivibrio fibrisolvens*) which support primary cellulolytic (e.g., *F. succinogenes*) and amylolytic (e.g., *Ruminobacter amylophilus*) organisms in the digestion of fiber and starch (figure 4), respectively (Dinsdale et al., 1978; McAllister et al., 1990c). If these supportive organisms (e.g., *Treponema*, methanogenic bacteria) are grown in co-culture with primary cellulolytic microorganisms, the overall rate of cellulose digestion is dramatically increased (Kudo et al., 1986; Teunissen et al., 1992). In the rumen, multi-species consortia of bacteria develop, and the by-products of one species (e.g., succinate) become the substrates for other bacterial species (Dehority, 1993) so that end-product inhibition of digestion is avoided (Wolin, 1990).

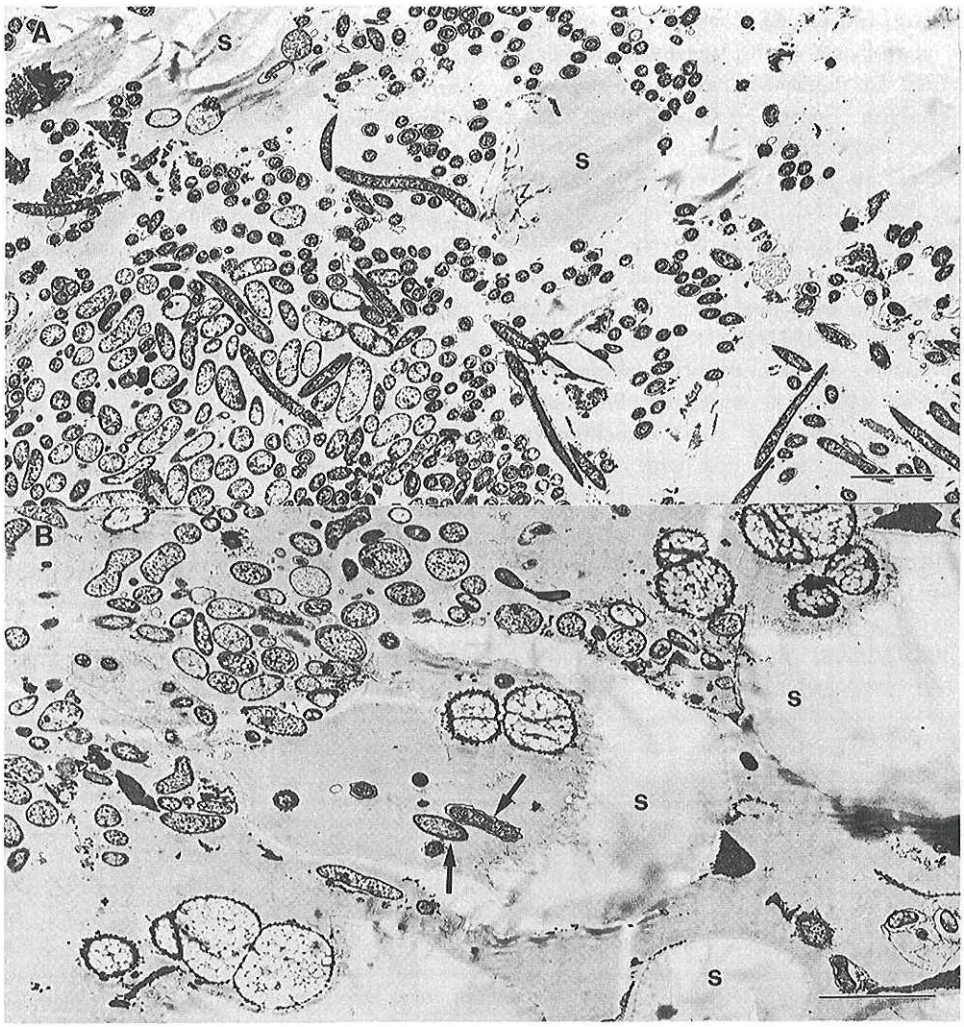

Figure 4. Transmission electron micrograph of the microbial consortia involved in the digestion of (A) barley and (B) corn starch. Note the presence of numerous bacterial morphotypes in both preparat ons. A long thin Gram-negative, rod-shaped bacterium is associated with barley starch granules (S), whereas arge Sarcina-like cells appear to be involved in the digestion of corn starch granules. A small number of Gram-negative rods (arrows) also occupied the pits in corn starch granules. Bars in figures A and B indicate 3 μm. (Figures from McAl ister et al., 1990b, Copyright Animal Feed Science and Technology).

Importance of Adaptation

Diet is the major factor which influences the nature of the rumen environment. Factors such as the degree of feed processing, the presence of feed additives and the ratio of cereal grain to forage in the diet all affect the numbers, proportions and digestive activity of ruminal microorganisms. Microorganisms cultured in the laboratory on artificial substrates invariably lose some of their digestive capabilities (Cheng et al., 1991c). When mixtures of defined "laboratory adapted" microorganisms are introduced concurrently into the rumen of gnotobiotic lambs, the diversified microflora required to digest complex plant cell walls does not establish (Fonty and Gouet, 1989; Cheng and Stewart, unpublished data). Rather, the persistence of an active cellulolytic population depends on the sequential introduction of "adapted microorganisms" which progressively alter the rumen environment and enables cellulolytic microorganisms to establish and function within the rumen (Cheng et al., 1991a).

In the rumen, adaptations occur within individual species or within an entire microbial consortium. We have observed that autochthonous bacterial populations associated with the rumen wall adapt with seasonal changes in the type of forage consumed by Svalbard reindeer (Cheng et al., 1993). In summer, nutrients are readily available and the rumen wall-associated microbial populations are varied and complex. Most bacterial cells within these populations are large and contain extensive deposits of glycogen. In contrast, during the winter, when the reindeer subsist exclusively on low-quality forage, only a limited number of small-volume bacterial cells are associated with the rumen wall. Adaptations in bacterial numbers and volume are responses by which the reindeer ruminal microflora persists in nutrient deficient environments. When nutrients once again become available, the microorganisms revert to normal growth in which bacterial populations flourish and cell volume increases (Morita, 1982).

Microorganisms that digest cell wall components are generally different from those that digest starch. Consequently, one might expect a substantial difference in the species of ruminal microorganisms in cattle fed cereal grains as compared to those fed forages. Although the dominant ruminal species change during the transition from forage to concentrate diets, the major species in the climax microbial populations are remarkably similar in cattle fed these two types of diets (Leedle et al., 1982). Even when steers are fed an alfalfa diet that contains no starch, 50% of the bacteria isolated from the rumen are amylolytic (table 1, McAllister et al., 1993b). The inherently stable nature of adapted microbial populations ensures that the host animal receives a continuous, uniform supply of volatile fatty acids and microbial protein for maintenance, growth and reproduction.

TABLE 1. PROTEOLYTIC OR AMYLOLYTIC ACTIVITY IN BACTERIA ISOLATED FROM THE RUMENS OF STEERS FED DIFFERENT DIETS

	Diet			
	Barley	Wheat	Corn	Alfalfa
Total number of isolates	65	83	108	70
Proteolytic[a]	35	76	73	89
Amylolytic[a]	39	84	85	50
Both positive[a]	15	68	63	42
Both negative[a]	40	8	4	8

[a] Expressed as a percentage of total isolates. (Reproduced from McAllister et al., 1993b; Copyright, Journal of Animal Science).

The development of a stable microflora upon transition from a forage to a cereal grain diet is not an immediate process. The numbers of bacteria which produce lactic acid (e.g., *Streptococcus bovis*, *Lactobacillus* sp.) increase with the introduction of cereal grains into the diet. Simultaneously, the numbers of bacteria which metabolize lactic acid increase, precluding the accumulation of lactic acid in the rumen. With time, the numbers of lactic acid producing bacteria decrease and the rumen

ecosystem develops a stable "climax" population in which *Prevotella ruminicola* (formerly *Bacteroides ruminicola,* Shah and Collins, 1990) and *B. fibrisolvens* are often numerically predominant (Van Gylswyk et al., 1992; Mackie and Gilchrist, 1979). However, if the transition from a forage to a cereal grain diet is too abrupt or if the particle size of the cereal grain is too small, the microbial population may become unstable. Under these conditions, lactic acid accumulates in the rumen and acid tolerant bacteria predominate. When rumen pH drops below 5.0, growth of lactic acid utilizing bacteria is inhibited, and the ruminant develops lactic acidosis. Additionally, the rumen contents may become viscous and a stable foam may form in the rumen. This foam prevents eructation, gas accumulates in the rumen and feedlot bloat ensues. These conditions are largely avoided if cereal grains are not overprocessed. Digestive disturbances are seldom encountered if cattle are fed coarsely processed cereal grains and if the microflora is given time to adapt to cereal grains over a 3- to 4-week period during which increasing amounts of cereal grain are substituted for forage (Elam, 1976).

Manipulation of Feed Digestion

A thorough understanding of the microbial processes of penetration, association, consortia formation and adaptation is essential if manipulation of the feed is to result in significant improvements in ruminant production. Chemical (e.g., hydrogen peroxide, ammonia, sodium hydroxide, Kerley et al., 1987; Mason et al., 1990) and biological treatment (e.g., white-rot fungi; Reid, 1989; Eriksson, 1990) of low-quality forages are prime examples of treatments which enhance these microbial processes. Conversely, methods which moderate these microbial processes can prevent the too-rapid digestion of barley and wheat and avoid the associated digestive disturbances such as lactic acidosis and bloat. Micronization (infra-red heat treatment) or treatment with formaldehyde substantially increase the resistance of the protein matrix in barley to microbial penetration and adhesion (McAllister et al., 1990a). Normally, the protein matrix in barley is rapidly digested, and the starch granules are readily exposed to the digestive enzymes of the microbial population (figure 5A). In micronized barley, ruminal bacteria gain access to underlying starch granules by digesting starch granules that lie on the surface of the endosperm cells, rather than by digesting the surrounding protein matrix. The altered protein matrix slows down the rate of barley digestion because it acts as a barrier to microbial penetration and delays adhesion and microbial consortium formation. After an extended period in the rumen (48 h) the starch granules are digested and the intact protein matrix is left behind (figure 5B).

The nutritional value of plants depends on the physiological status of the plant at harvest (e.g., maturity, percentage moisture) and on genetically determined characteristics. Plant breeding programs have focused largely on the improvement of yield and persistence of the stand and comparatively few studies have been directed at altering the nutritional utilization of plants (Wheeler and Corbett, 1989). Further characterization of natural plant compounds that alter the microbial processes of penetration, adhesion and consortia formation would facilitate the use of these compounds as selection criteria in plant breeding programs. For example, condensed tannins bind to proteins via hydrogen bonds. This binding is likely responsible for the bloat-safe properties of forages (e.g., sainfoin and birdsfoot trefoil) that contain condensed tannins (Waghorn, 1990). Protein is more resistant to microbial digestion when complexed with condensed tannins than when free, thus the flow of essential amino acids to the small intestine is often increased in ruminants fed forages containing condensed tannins (Waghorn et al., 1987). However, condensed tannins also form complexes with enzymes and interfere with microbial adhesion, thereby inhibiting the digestion of fiber by cellulolytic bacteria (Bae et al., 1993). The ideal legume would contain condensed tannins that have a minimal effect on cellulose digestion while maintaining a positive effect on ruminal protein digestion.

Genetic engineering may allow manipulation of feed digestion through alterations of both microbial, plant and animal genomes. Several researchers have proposed methods of genetically altering rumen bacteria as a means of increasing fiber digestion in the rumen and at least 45 different genes have been cloned from rumen bacteria (Forsberg et al., 1986; Russell and Wilson, 1988). These gene isolations have broadened our knowledge of the genetics of ruminal bacteria, but progress towards improving fiber digestion has been limited by an incomplete understanding of the

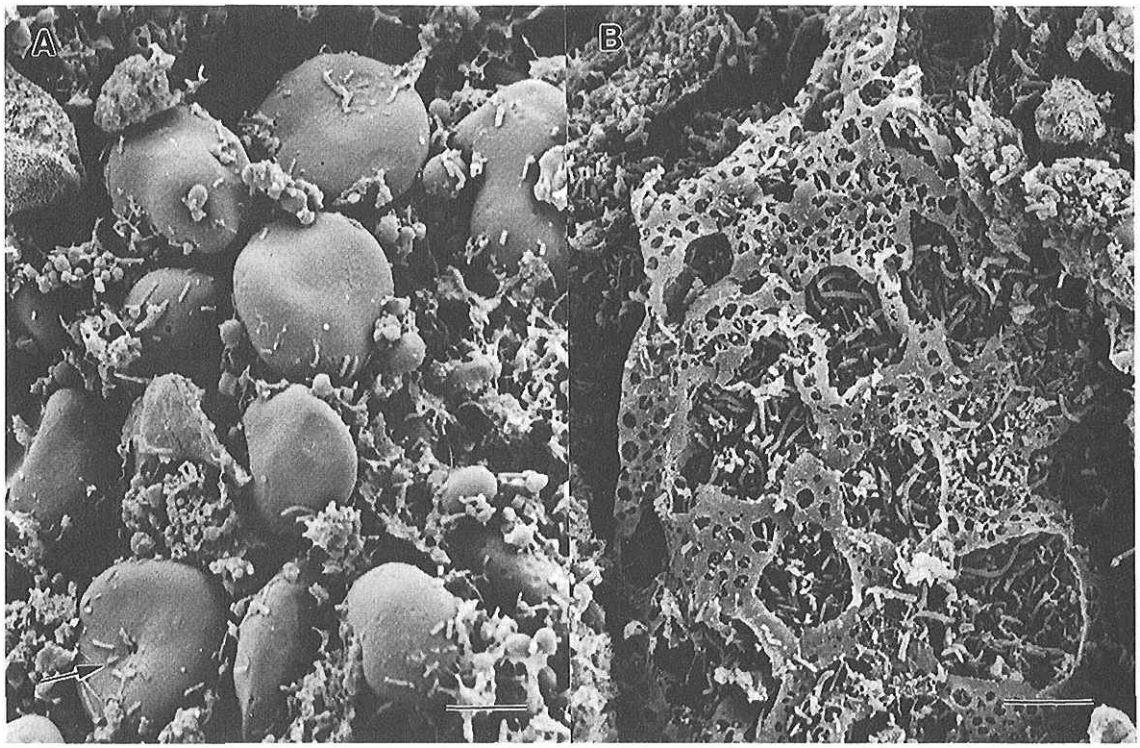

Figure 5. (A) Scanning electron micrograph of the surface of the endosperm of untreated barley after 48 h of incubation in the rumen. Note that bacteria (arrow) have gained access to and adhered to starch granules and that the protein matrix is heavily colonized. Bar = 10 μm.
(B) Scanning electron micrograph of the of micronized (infrared heat treatment) barley after 48 h of incubation in the rumen. Note that the protein matrix is resistant to digestion and has formed a cast indicating the previous location of starch granules which have been digested away. Bar = 10 μm. (Figures are our unpublished data).

molecular mechanisms of cell wall digestion (Forsberg and Cheng, 1992).

Genetic manipulation of ruminal microorganisms may also effect their ability to persist in the rumen. Introduction of a plasmid for antibiotic resistance into the proteolytic bacterium, *Prevotella ruminicola*, increased the doubling time of this microorganism by more than 30% over that of the original antibiotic-sensitive strain (Russell and Wilson, 1988). Such alterations in growth characteristics have a profound effect on a microorganism's ability to adapt and integrate into the consortia of microorganisms required for feed digestion. Even a genetically unaltered laboratory strain of *P. ruminicola* had a half-life of less than 30 minutes in the rumen (Attwood et al., 1988). Survivability in the rumen may depend on the species of recombinant bacteria introduced, since Flint et al. (1989) found that *Selenomonas ruminantium*, but not *Bacteriodes multiacidus* could be reestablished in the rumen.

The ecological complexities of the rumen could be avoided by introducing glucanase and xylanase genes from ruminal microorganisms directly into ruminants. Hall et al. (1990, 1992) were the first to examine the expression of fibrolytic genes in cultures of eukaryotic cells and successfully introduced and obtained expression of the endoglucanase E gene from *Clostridium thermocellum* in Chinese hamster ovary cells. These workers have since introduced the endoglucanase E gene into the pancreas of mice and the transgenic animals produce and secrete endoglucanase into the small intestine (Hall et al., 1993). Although considerable progress has been made in the development of techniques to introduce genes into animal cells (Ward et al., 1989; Forsberg et al., 1993; Pursel and Rexroad, 1993), the efficiency of transferring

genes into livestock (e.g., pigs, sheep, cattle) remains low. It will likely require at least another decade to overcome the technical obstacles that preclude the use of transgenic domestic animals for food production.

Conclusions

Research in the past decade has shown the importance of ecological factors in the process of feed digestion. Direct manipulation of the ruminal microbial systems is notoriously difficult because the feed dictates most ecological changes in the ruminal microflora. It is, therefore, logical that attempts to manipulate the ruminal microbial population should first be made through changes in the feed. In many ways our knowledge of microbial feed digestion is still rudimentary and the characterization of enzymes involved in feed digestion is lagging behind the rate at which genes are being cloned. Our lack of understanding of the overall constraints to microbial digestion of feeds impedes attempts to improve feed utilization via genetic engineering of ruminal microorganisms. Penetration, adhesion, adaptation and consortia formation are all essential processes in the digestion of feed by ruminal microorganisms. The success of future effforts to improve feed utilization by ruminants, whether through genetic, mechanical or chemical manipulations of the feed, or through genetic engineering of the digestive organisms involved, depends on a thorough understanding of each of these processes.

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