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Rumen digestive physiology and microbial ecology

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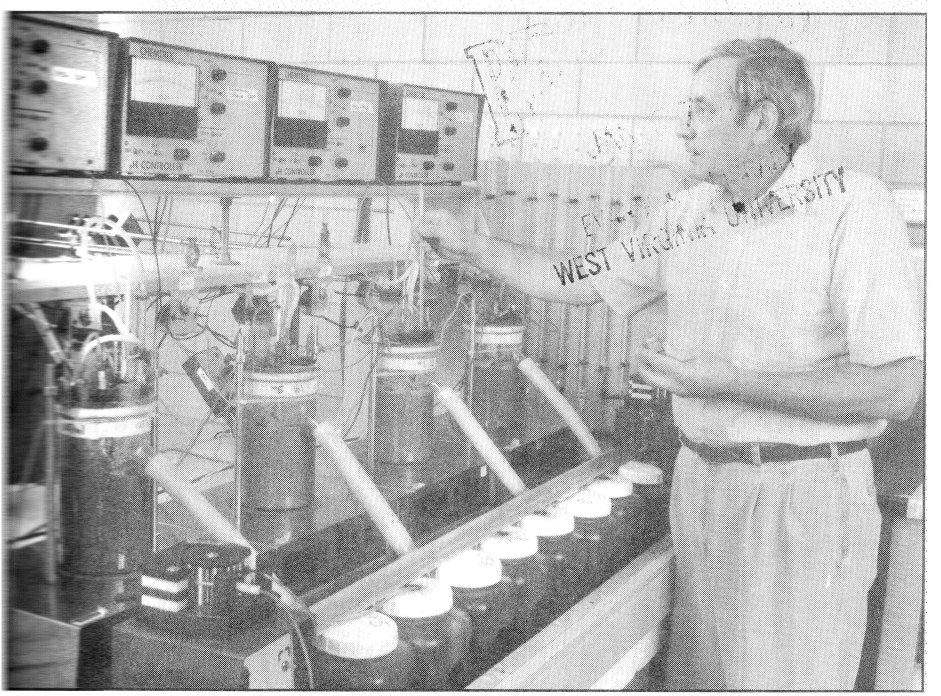

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Front Cover: Dr. William H. Hoover with the continuous culture system developed at WVU for studying rumen function.

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RUMEN CHARACTERISTICS

Rumen Conditions

Under normal circumstances, the rumen provides a suitable environment for the growth of numerous strictly anaerobic microbes, as well as some facultative anaerobes. The temperature usually is maintained within the range of 38–41C with 39 used as a common mean temperature. A mean redox potential of –350 mV, with fluctuations between –250 and –450 mV, reflects the strong reducing medium and the absence of oxygen. The rumen is usually well-buffered, due to the presence of organic acids produced from fermentation, the buffering capacity of various feeds (87) and the copious flow of saliva, in which the principal buffers are bicarbonates and phosphates. The pH ranges of these various buffers are in Table 1.

Table 1. Buffering Ranges of Substances in the Rumen.

Buffer	pH range
Fermentation acids	3.8–5.0
Forages	5.0–6.0
Bicarbonates	5.0–7.0
Phosphates	6.0–8.0

In addition, the presence of ammonia can act to prevent decreases in rumen pH, particularly when the concentration of NH_3 is 20 mg/dl or greater. Saliva production can be very high in a cow, reaching over 180 l/day. In spite of this potential to buffer rumen fermentation, rumen pH can range from around 7.0 on forage diets to as low as 4.6 when animals are fed high-grain diets. This is not only because of the production of acidic fermentation end-products, but also because certain feeds reduce saliva flow by failing to stimulate chewing and rumination. Compared to dry long hay, which stimulates the greatest extent of chewing and rumination, high moisture feeds such as pasture or silage can reduce the amount of saliva produced per kg dry matter by half, and grains or pelleted feeds can reduce flow to 20% of that on a long hay diet (15). The average percent composition of the gases in the rumen is shown in Table 2. Carbon dioxide and methane are the major gases

present. Since a large proportion of CO_2 is reduced to methane, the concentrations of these two gases become closer to equality with time after feeding. Oxygen varies from <.1% to slightly over .5% depending on the amount ingested in water and feed. The osmotic pressure of rumen contents ranges between 260 and 340 milli-osmoles (m OSM), with an average of about 280. High osmotic pressure is detrimental to rumen function, and rumen osmotic pressure in the range of 350–380 has been shown to stop rumination (97).

Table 2. Composition of Rumen Gases.

Component	Average percent
Hydrogen	.2
Oxygen	.5
Nitrogen	7.0
Methane	26.8
Carbon dioxide	65.5

Osmotic pressure affects not only water movement across the rumen epithelial tissue, but movement of volatile fatty acids (VFA) as well. As osmotic pressure increases, absorption of VFA from the rumen decreases (64). The magnitude of this effect for acetic acid is shown in Figure 1. Propionic and butyric acids are similarly affected. Feeding high levels of grains contributes to high osmotic pressure, as does the feeding of minerals. Consumption of 10 kg of a forage-grain diet containing 1.5% sodium bicarbonate, .5% salt and 1.0% dical can cause a transient increase of 75–80 m OSM in an 80 liter rumen. For this reason, care must be exercised to avoid the inclusion of excessive quantities of minerals in the diet.

Enzymatic hydrolysis of the feed components requires a liquid interface. The rumen has a high moisture content that remains fairly constant in the range of 85–90%. In addition, the moisture content increases from the top to the bottom of the rumen due to the stratification of the contents, thus providing a diverse habitat for a variety of organisms.

Absorption From the Rumen

Volatile fatty acids produced by the microbes are passively absorbed through the rumen wall. The rate of absorption is dependent on chain length, pH, concentration and, as indicated in the preceding section, osmolality. Between pH 4.5 and 6.5, rates of VFA absorption are butyrate > propionate > acetate; above 6.5, the rates are similar. Since the undissociated acid is more rapidly absorbed, as pH decreases, VFA absorption increases. The net absorption, which is the amount of VFA's that reach the blood, is dependent on both concentration in the rumen and the quantity metabolized by the cells

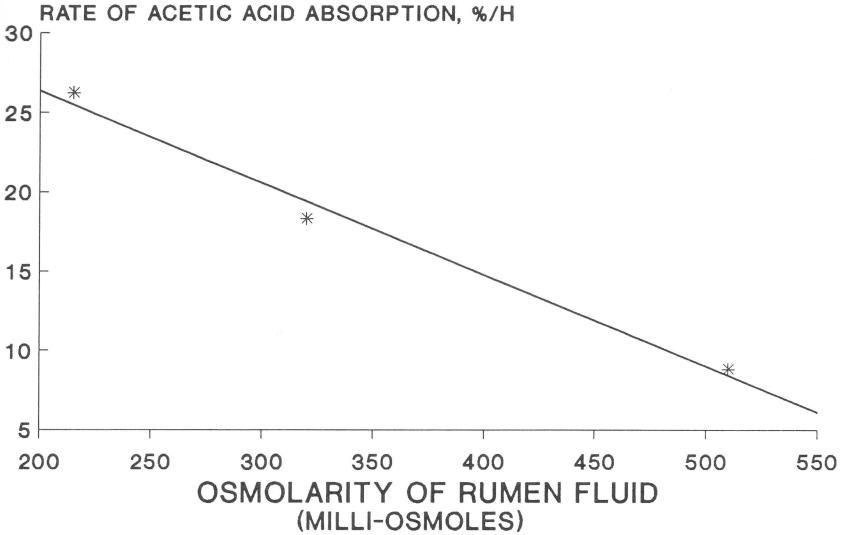


Figure 1. Effects of Osmotic Pressure on Movement of Acetic Acid Across the Rumen Epithelium (64).

of the rumen wall. The rates of metabolism by cells of the rumen wall are butyrate > propionate > acetate. Acetate is found in the greatest concentration in the rumen (ratios are usually 5:1.5:1 for acetate, propionate and butyrate, respectively). As a result of the higher concentration and lower rate of metabolism by the rumen wall, acetate enters the blood in the greatest quantity, followed by propionate. Because of the smaller amount in the rumen and extensive metabolism by the rumen wall, very little butyrate enters the blood. Lactate, when present, is absorbed directly through the rumen wall. In dairy cattle fed and managed well, lactic acid does not accumulate in the rumen contents to any large extent. It is found in highest concentration in the rumen of cattle placed suddenly on a high grain diet because sufficient numbers of lactate utilizing bacteria have not been cultured in the rumen in those circumstances. However, if gradual introduction of grain is practiced, the lactate-utilizing bacteria will develop and permit only a transient increase in lactic acid accumulation following ingestion of a diet high in readily fermentable carbohydrates (46). Low pH favors absorption of lactic acid, as well as production. Rumen microbes produce both L(+) and D(–) lactate in essentially equal amounts and both are readily absorbed. Compared to the L(+) form, the D(–) form is slowly metabolized by ruminant tissues such as the liver, and is a major cause of lactic acid toxicity. Glucose also can be absorbed through the rumen wall, but is usually present in nutritionally insignificant quantities. Ammonia is readily absorbed, with the rate being dependent on concentration and pH. Absorption is rapid at pH 6.5 and

higher, and declines to nearly 0 at pH 4.5. Amino acid absorption can take place, but concentrations of amino acids are maintained at low levels, and < 10% of total N absorption is usually as amino acids. Of the minerals, sodium and magnesium are actively absorbed through the rumen wall. Magnesium absorption is decreased by a Na:K ratio in the rumen of less than 5:1, and by high ammonia concentrations (50). Potassium and chlorine can pass through the rumen wall in either direction, depending on concentrations, other ion effects and osmolality. The rumen also is responsible for about one-third of total zinc absorption. The rumen wall is apparently impervious to calcium, phosphorus, copper, molybdenum and sulfur.

RUMEN MICROBES

The major microbial populations in the rumen are bacteria, protozoa and fungi. Of these, bacteria are found in the greatest numbers and diversity. So far, 22 genera and 63 species have been identified, with total concentrations of 10^9 to 10^{10} cells/g of rumen contents. Protozoa numbers can be as high as 10^5 to 10^6 cells/g contents, and are greatly affected by feeding practices. To date, six genera and 16 species have been identified. Protozoal numbers increase in proportion to the amount of grain fed unless the increase in acid production caused by high grain levels reduces rumen pH to 5.5 or below. In studies at Kansas State University (82) it was found that in feedlot cattle fed only 8–10% forage diets, 13% were defaunated while the remainder had protozoa concentrations ranging from 10^3 to 10^6/ml. The anaerobic fungi are the most recently recognized group of rumen microbes. These organisms were first identified in the rumen in 1975 by Orpin (63), who reported that certain flagellated cells thought to be protozoa were, in fact, zoospores of fungi. As of 1987, three genera and four species were known. Since then, additional genera have been discovered by Barr et al. (3) in 1989 and Breton et al. (8) in 1990, and other, unidentified fungi were reported in ruminants in Australia (65).

Microbial Compartmentalization

The microbes are not randomly distributed throughout the rumen, but tend to associate with various fractions of the rumen contents, or with the rumen wall itself. In adult ruminants, some bacteria and protozoa are found associated with the liquid phase of the rumen contents, some adhered to the solid digesta, and some associated with the rumen wall. The quantity and species of microbes in each of these pools are not static, but vary with time after feeding.

Bacteria. The compartmentation of the bacteria appears to be dependent largely on nutrient availability or specialized metabolic activity. The

liquid-associated, or non-adherent bacteria, consist of organisms found in the rumen fluid that feed on soluble carbohydrates and proteins. The solid-associated, or adherent bacteria, are bound to feed particles in order to digest the insoluble polysaccharides such as starch and fiber, as well as the less soluble proteins. An exchange of microbes between the solid- and liquid-associated compartments occurs immediately after feeding, wherein some of the solid-associated bacteria are displaced from the particulate matter and become part of the liquid-associated pool. These microbes appear to be attracted chemotactically by the presence of soluble nutrients in the rumen fluid as a result of feeding. In this manner, new feed particles are located and colonized (99).

In addition to the bacteria associated with the rumen contents, there also is a population found on the epithelial surface of the rumen. Although most of these "epimural" organisms are species that also are found in rumen contents, this population appears to contain higher levels of ureolytic, aerobic and facultative anaerobic microbes than does the rumen contents. The unique metabolic functions ascribed to the epimural microbes by Cheng and Costerton (13) are: 1. Utilization of oxygen that diffuses from the blood through the stratified squamous epithelial cells of the rumen, thus helping to maintain the anaerobic nature of the rumen contents; 2. Conversion of the urea that diffuses through the rumen wall, to ammonia. This is of significance in that few anaerobic microbes produce ureases; 3. Digestion of the sloughed epithelial cells of the rumen wall. These highly keratinized cells would be indigestible were it not for the microbial hydrolysis. It has been also observed, particularly on high concentrate diets, that some digestion of epithelial cells can occur before the cells are sloughed, resulting in damage to the underlying tissue. Jensen and co-workers (39) proposed that the subsequent bacterial invasion may be the source of liver abscesses in beef cattle. *Fusobacterium necrophorum*, an etiological agent in liver abscess, has been isolated from the epimural population.

Protozoa. The physical compartmentalization of protozoa is similar to that of bacteria, in that there are liquid-associated, solids-associated and epimural populations. In addition to the digestion of soluble and insoluble nutrients by liquid- and particulate-associated populations, respectively, the protozoa have a further motivation regarding adherence to feed particles and to the rumen wall. Because the time required for most species of protozoa to reproduce is longer than the turnover time of the rumen fluid, the protozoa must attach to large feed particles or to the walls of the reticulum and rumen in order to avoid wash-out. This has led to the use of the term "sequestered" in reference to the attached protozoa. With regard to digestion of soluble and insoluble nutrients and colonization of feed particles, however, the protozoal responses are similar to those described for bacteria. Upon feeding, the soluble nutrients leaching from the cut ends

of the plant materials chemotactically attract sequestered protozoa, which rapidly locate and colonize the new feed particles. Species of both holotrich and entodinimorph protozoa can ingest starch granules and small plant fragments, which they digest internally. In addition, there is evidence that organisms of both groups attach to large particles and hydrolyze fibrous polysaccharides via enzymes that are secreted into the surrounding medium or bound to the surface of the microbes (exoenzymes).

Fungi. The fungi appear also to be compartmented between attached and liquid-associated forms, but in a more complex manner because of the morphological types in the life cycle of these organisms. The vegetative stage of the fungi is a particulate-associated sporangium with an extensive rhizoid network that invades the plant material. The sporangia produce and release flagellated, motile zoospores that become fluid associated until attachment to a new feed particle. Like bacteria and protozoa, chemotaxis plays a role in the location and colonization of plant material by fungi. Zoosporogenesis is triggered by the presence of heme and other components in the newly ingested feed, and the presence of soluble nutrients serves to attract the newly released zoospore to the fresh feed particles (35).

Thus, each of the major microbial communities – bacteria, protozoa and fungi – maintain a pattern of feed colonization that allows them to respond to the types of feed and the variety of feeding cycles associated with various ruminants.

Establishment of Microbial Populations

Bacteria in the rumen contents. Bacterial colonization develops rapidly, with a very diverse population, soon after birth. By three days of age, anaerobic bacteria are present at concentrations of 10^9 colony-forming units (CFU) per g of contents. At this age, facultative anaerobes are found in moderate numbers, 10^7 to 10^8 CFU/g. These decline steadily to adult levels of about 10^5 to 10^6 CFU/g by 5 weeks of age. According to the work of Anderson et al. (2), other changes are:

	Colony Forming Units/grams	
Organisms	3 days	12 weeks*
Proteolytics	10^6–10^7	10^7–10^8
Amylolytics	10^8–10^9	10^9–10^{10}
Cellulolytics	10^2–10^3	$>10^7$
Methanogenics	10^1	10^8–10^9

* generally representative of adult populations

Ingestion of solid feed is necessary for the establishment of the adult microflora, and early weaning will cause microbes to reach adult proportions sooner. Early weaning from milk is not an absolute necessity, since microbes capable of hydrolyzing all feed components, including cellulose, are present

even in milk-fed calves. Transmission of these organisms to the calf is dependent on contact with adults, especially the mother, so as long as the calf remains in contact with other animals, ingestion of solid feed will eventually result in the appropriate colonization.

Bacteria of the rumen wall. As with the rumen contents, the rumen wall is colonized shortly after birth. A high proportion of the first microbes to become established, however, are not found in the adult. For example, up to one-third of the total epimural microbes may be aerobes during the first week of life, but represent less than 1% of the total by the third week. Mueller et al. (57) found that of 24 morphological types identified, only 7 persisted in the adult animal. The establishment of the epimural community is complex and involves successive appearance and disappearance of a number of species before the adult population is achieved, which is accomplished in 10–12 weeks.

Fungi. Following the bacteria, the next microbial community to become established is the fungi. These organisms appear in the rumen during the second week of life. Their appearance is not dependent on direct animal contact or the consumption of solid food, since fungi were found in the rumen of lambs isolated in pens approximately 6 feet from normal lambs. Feed consumption, particularly the type of feed, is critical to the development and maintenance of fungi. Fonty et al. (22) found that when concentrates were introduced into the rumen of 3-week-old lambs, the fungi disappeared, while they persisted in lambs fed alfalfa. In general, the fungal population in the adult is directly proportional to the fiber content of the diet.

Protozoa. The last microbial community to become established is the protozoa. These microbes are rarely found prior to 2 weeks, usually requiring 2–4 weeks for colonization. The most effective means of inoculation is direct mouth-to-mouth contact with other animals, or via feed recently contaminated by saliva from animals with established populations. Unlike the situation with fungi, young raised apart from other animals or from contaminated feed will not develop a protozoal population. The establishment of protozoa is dependent on rumen pH, in that moderately low pH (6.0 or lower) greatly inhibits protozoa development.

Interactions Among Microbes

Not all interactions among the rumen microbes are mutually beneficial. A prominent example is the predation, or engulfment, of the bacteria by both holotrich and entodiniomorph protozoa. In general, the rate of bacterial engulfment is proportional to the concentration of bacteria available. Coleman (16) reported that when bacterial concentrations were 10^9/ml, a value representative of that in rumen fluid, the average uptake for 18 entodiniomorph species was 493 bacteria/h/protozoa; when bacteria were at maximum density, the average uptake for the 18 species of protozoa was

3,739 bacteria/h/protozoa. Once engulfed, the bacteria are usually killed, but may not be digested by the protozoa. If not digested, the bacteria are eventually excreted. End-products of digestion are excreted and become available for use by the total microbial population. The overall effect of protozoal engulfment on bacterial numbers is considerable. It has been shown that removal of protozoa (defaunation) can result in a 2–4 fold increase in numbers of rumen bacteria. At first glance these data suggest that in normally faunated animals there may be a critical depletion of bacteria. In actuality, the numbers of bacteria in the presence of protozoa remain at levels of 10^9 cells/g, and in most in vivo situations represent 50–90% of the rumen biomass. The reduction in bacterial numbers is not, however, uniform across all species. Liquid-associated bacteria are engulfed to a greater extent than attached microbes, which means that, in general, more amylolytic than cellulolytic bacteria are engulfed. It is possible, therefore, that the extent of digestion of various carbohydrates differs between faunated and defaunated animals.

Engulfment of fungal zoospores by protozoa has been observed, suggesting a predator-prey relationship between protozoa and fungi similar to that between the protozoa and bacteria. Although decreases in numbers of zoospores may range from 27% (58) to 5-fold (6), the effects on rumen fermentation have not been determined.

A sizable number of bacteria, which may account for 1–10% of the total population (7), are attached to the surface of the protozoa. While this may be the safest location for highly sought-after species, the advantages of this association are not known for most of the bacteria and protozoa involved. A beneficial relationship for one group of attached bacteria has been proposed. Proportionately high numbers of methanogenic bacteria have been found attached to the entodiniomorph population. This appears to be an association beneficial not only to the methanogenic bacteria, but to other rumen microbes as well as to the protozoa. During metabolism, protozoa produce large quantities of hydrogen, which would be very detrimental to the metabolism of most rumen microbes if it escaped into the rumen. The methanogens require hydrogen to produce methane, and apparently take up the hydrogen by direct cell-to-cell contact with the protozoa. This prevents the appearance of free hydrogen in the rumen fluid and efficiently provides the methanogens with needed substrate.

DIGESTION OF FEED COMPONENTS

Carbohydrates

The composition of the carbohydrates found in a mixed forage-grain diet is shown in Table 3. Both the simple and complex carbohydrates are fermented by rumen microbes.

Table 3. Carbohydrate Content and Composition of Feeds. (87)

Carbohydrate	% of dry matter in Grasses	Alfalfa	Grains	Major components
Sugars	10	5–15	negl.	glucose, sucrose
Fructosans	1–25	0	0	fructose
Starch	0	1–7	80	glucose
Pectin	1–2	5–10	negl.	galacturonic acid
Cellulose	20–40	20–35	2–5	glucose
Hemicellulose	15–25	8–10	7–15	xylose

Traditionally, the utilization of complex carbohydrates was viewed as a function of substrate-specific organisms whose survival was dependent on the presence of a particular polysaccharide. Using cellulose as an example, cellulolytic organisms were thought to hydrolyze cellulose extracellularly to glucose or cellobiose, then absorb these components and ferment them to volatile fatty acids. In this situation, non-cellulolytic microbes would not have access to the glucose (or cellobiose) that resulted from the initial hydrolysis of the polysaccharide. However, there is a growing body of evidence suggesting that plant polysaccharides are degraded by extracellular enzyme complexes produced by the rumen bacteria and fungi, which results in the appearance of oligosaccharides in the surrounding medium. Such has been found for each of the major complex carbohydrates: cellulose, hemicellulose, pectin and starch. The result is a pool of soluble oligosaccharides of various carbohydrates in the fermentation medium that provides a source of nutrients for organisms that are both capable and incapable of hydrolysis of the intact polysaccharide. This relationship is depicted in Figure 2. The utilization of the carbohydrates in the oligosaccharide pool by microbes other than those producing these intermediates is called cross-feeding. The participation of protozoa in this scheme is less clear and may be more complex.

While some of the polysaccharide degrading enzymes associated with protozoa may have originated from engulfed bacteria, it has been determined that enzymes capable of hydrolyzing starch, hemicellulose and pectin are produced by both entodiniomorph and holotrich protozoa, and cellulose-degrading enzymes have been found in entodiniomorphs. Although the initial hydrolysis can be extracellular and contribute to cross-feeding, the final hydrolysis follows engulfment of the plant particulate matter. It is possible, however, that hydrolytic products from the digestion of hemicellulose and pectin may be excreted and become available to other microbes, since neither genus appears capable of metabolizing galacturonic acids or pentoses.

Although cross-feeding permits the maintenance of substantial numbers of microbes that are not able to hydrolyze a given polysaccharide, there is

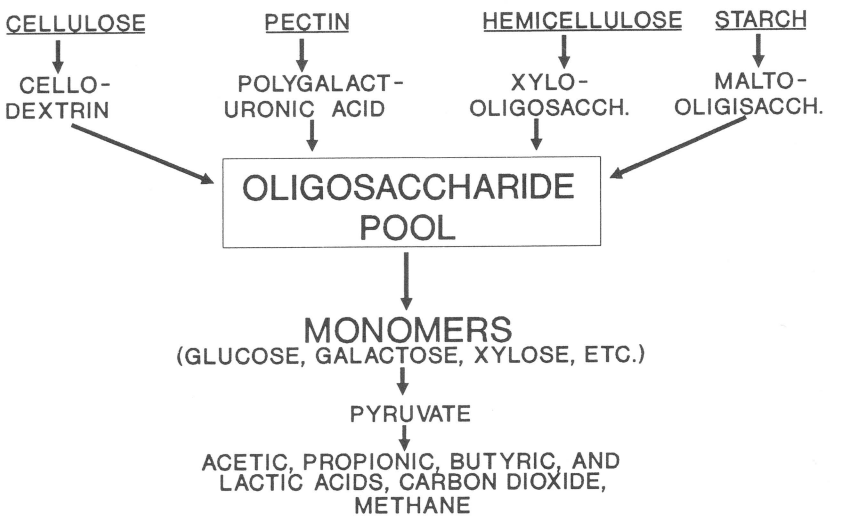
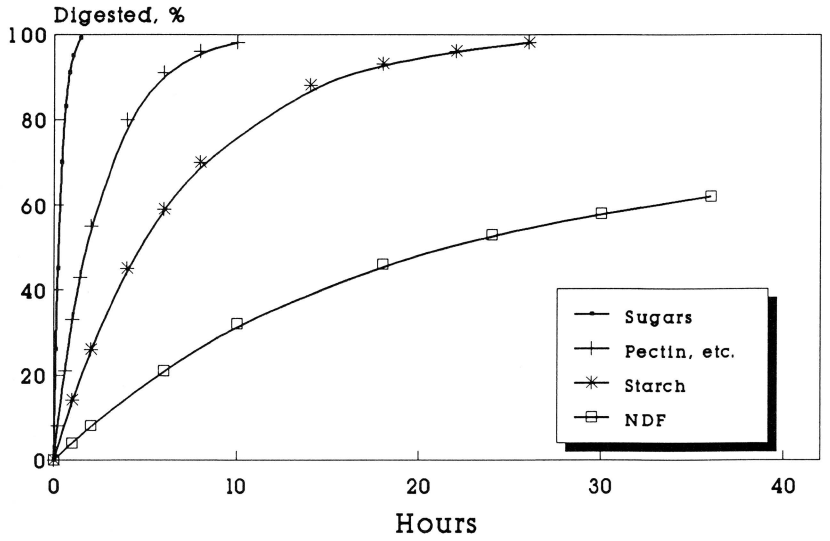


Figure 2. Hydrolysis and Fermentation of Complex Carbohydrates by Rumen Microbes.

still a sequential nature to the fermentation of various carbohydrates. The rates of hydrolysis of the carbohydrates dictate the extent to which each will be digested in the rumen, and the rate is, in large measure, dependent on ease of solubilization. Figure 3 shows the relative times required for the digestion of several carbohydrates. Sugars and pectins are very soluble and most rapidly fermented, followed by starches and fiber. The chemical structure of the polysaccharides also affects the rates of degradation. For example, oat, tapioca and paselli starches are more rapidly degraded than is maize starch; in addition, treatments such as steaming or popping can double the digestion rate. For the less soluble polysaccharides, the extent of lignification, acetylation and phenolic esterification are negatively related to digestion.

Relative rates of carbohydrate digestion are altered by feed ingestion, due to changes in predominant microbial species and in enzyme activities as well. Feeding causes sequential changes in both the relative numbers of free and particulate-associated microbes and in the activities of the polysaccharide degrading enzymes. There is a decrease in the number of non-adherent bacteria and protozoa immediately after feeding. This is followed by a rapid increase in numbers of sugar, starch and pectin digesters between 4 and 16 h post-feeding, and a slower increase in cell wall digesters during the same period.



ADAPTED FROM: Sniffen and Robinson (76)

Figure 3. Rates of Digestion of Carbohydrates in the Rumen.

The specific activities of polysaccharide-degrading enzymes differ between adherent and non-adherent microbes (98), and exhibit postprandial changes (99). The activities of glycoside hydrolases in the fluid-associated microbes are highest 1–2 h post-feeding, while in the adherent population, activities of hydrolases of α-linkages and galactosidases remain low throughout the 24 h post-feeding period. Glycoside hydrolases in the adherent population that are involved in cell wall digestion increase markedly after 12 h. Activities of the polysaccharide-degrading enzymes remain low but essentially constant over time in the fluid-associated population, but increase markedly between 8 and 12 h in the adherent microbes.

Protein

The major microbial communities in the rumen (bacteria, protozoa and fungi) all have been shown to hydrolyze protein and use the products as N sources for growth.

Bacteria, in particular, have been shown to have high proteolytic activities. Proteolytic enzymes are constitutive and, although serine, cysteine and metallo-proteinases all are present, cysteine proteinases appear to be most prevalent in mixed populations. While most bacterial proteases remain bound to the cell, there are some species in which the enzymes are liberated into the rumen fluid. For many proteins, the extent of proteolysis is propor-

tional to solubility. However, the presence of disulfide bridges can limit the hydrolysis of soluble proteins such as albumens and gamma globulins.

Studies with faunated and defaunated ruminants have shown that when protozoa are present, protein digestion is increased compared to digestion by bacteria alone, particularly if the dietary protein is of low degradability. Although some species of protozoa produce extracellular proteolytic enzymes, most of the proteolysis is intracellular and follows ingestion of the protein. Both holotrichs and entodiniomorphs not only ingest and degrade soluble proteins, but also digest the proteins in particulate matter. Bacteria supply a large part of the particulate protein ingested, but feed proteins are actively engulfed as well. This explains why protozoa increase the degradability of the less soluble feed proteins. For this reason, the presence of protozoa has a negative effect on protein availability to cattle in situations where the ration is high in by-pass protein. Protozoal proteases are primarily of the cysteine types, as are the bacterial proteases.

The anaerobic ruminal fungus *Neocallimastix frontalis* produces metalloproteinase(s) that become extracellular as growth increases (91). Proteases from this organism hydrolyzed casein and the protein in a grass-fishmeal substrate with a specific activity equal to that of rumen bacteria (94). Information was not located concerning the end-products of the fungal proteases.

The combined proteolytic activities of protozoa, fungi and bacteria result in rapid degradation of most feed proteins in the rumen. As degradation proceeds, peptides appear in the rumen contents. Although proteolysis rates exceed the rate of peptide hydrolysis, allowing for the peptide build-up in the rumen, the rate of peptide hydrolysis is rapid, resulting in the appearance of free amino acids and ammonia within minutes after feeding. Peak ammonia production is about 1 h post-feeding. Thus, the major sources required for microbial growth, including peptide-bound amino acids, free amino acids and ammonia, all become available at high concentrations within 1–2 h post-feeding, then decline in concentration until the next feeding.

Lipids

The lipids in forages are primarily galacto-glycerides, which are combinations of one or two galactose residues and one to two fatty acids attached to glycerol. In forages, the principal fatty acid is linolenic (C 18:3). In grains, the major fats are triglycerides, containing a high proportion of linoleic acid (C 18:2).

Both bacteria and protozoa produce extracellular lipases that hydrolyze glycerides, producing glycerol, galactose and fatty acids. Approximately one-third of the lipolytic activity in rumen contents is thought to be due to protozoal lipases. The glycerol and galactose are rapidly taken up and fermented, and the fatty acids adhere to feed particles and to the rumen

microbes. Some triglycerides are taken up by the microbes, but this appears to be <20% for most fats (4). The next steps in the metabolism remain unclear. It is known that most of the free fatty acids are biohydrogenated by exoenzymes while attached to the feed particles. The free linolenic and linoleic are rapidly hydrogenated to trans-11 octadecenoic acid (C 18:1, 11) and finally to stearic acid (C 18:0) by rumen bacteria. A free carboxyl group is a prerequisite for biohydrogenation, so the fatty acids of glycerides that escape lipolysis remain unsaturated.

The adherent and non-adherent bacteria differ in the extent to which they metabolize lipids. The adherent microbes contain greater amounts of total fat than the non-adherent bacteria, and are responsible for a higher proportion of biohydrogenation (4). Both populations of microbes take up fatty acids. With the exception of palmitic acid (C 16:0), which is incorporated into the polar lipids (primarily glycero phospholipids) of both groups, most fatty acids are stored in lipid droplets in the cells. With regard to the latter, the adherent bacteria were reported to take up and store 4.2, 8.6 and 30.5 times more of the C 18:0, C 18:1,9 and C 18:2,9,12 fatty acids, respectively, than did the non-adherent microbes (4). Once taken up and stored, the unsaturated fats appear to be protected from biohydrogenation.

Long chain free fatty acids, especially if polyunsaturated, are toxic to many rumen microbes. Cellulolytic and methanogenic bacteria are particularly susceptible, as are protozoa. At moderate to high levels of intake of unprotected fats, particularly if high in unsaturates, fiber digestion and methane production are depressed and protozoa numbers decrease.

FACTORS AFFECTING MICROBIAL GROWTH AND YIELD

Nutrients

From the preceding section it can be surmised that, after feeding, the rumen contents contain peptides of various sizes, amino acids and ammonia as well as carbohydrate polymers and monomers. In addition, the rumen will contain variable levels of other essential nutrients such as lipids, iso-acids, vitamins and minerals. These substrates vary with time after feeding and with the sources and quantities of feeds available. The challenge in feeding cattle is to provide feeds with rapid, intermediate and slow degradabilities in order to maintain constant supply of the nutrients required to optimize microbial growth. Although the major nutrients utilized and required by many microbial species have been identified, the types, amounts and combinations required to optimize total microbial growth have not been established. Rather than identify nutrients required by individual microbial species, an

attempt will be made to describe the nutrient requirements of the mixed microbial population.

Nitrogen. Most species of rumen bacteria can use ammonia for growth. After many attempts to determine the optimum level of ruminal ammonia needed, it must be concluded that there is no one level that is optimum for all feeding situations. One factor affecting this is the discovery that many microbes that use ammonia also can use – and some species prefer – amino acids or peptides. Further, large peptides are taken up more rapidly than most amino acids or small peptides, and are used more efficiently for microbial growth. From studies on bacterial metabolism of peptides of alanine, up to 5 residues in length, Wallace et al. (93) and Wallace and McKain (92) found more rapid rates of metabolism for the longer peptides. The large peptides apparently were further hydrolyzed by a dipeptidase closely associated with the bacterial cell wall. The resulting dipeptides were slowly metabolized, and thus accumulated in the extracellular fluid. Although all peptides may not be metabolized in the same manner as alanine peptides, either slow uptake of dipeptides or poor to no growth on dipeptides compared to larger peptides has been reported. The maximum peptide length assimilated by microbes and the length taken up most rapidly may differ. Uptake of peptides with 18 amino acids has been reported and peptides of > 10,000 molecular weight (MW) were found to enhance microbial growth on cellulose when compared to peptides of < 1500 MW. Current data indicate that peptides taken up most rapidly by bacteria contain between 3 and 7 amino acids.

In addition to chain length, other physical properties of peptides affect their metabolism. Chen et al. (12) reported that hydrophobic peptides, determined as those soluble in 90% isopropyl alcohol, were hydrolyzed more slowly than were hydrophilic peptides. Since the hydrophobic peptides contain higher proportions of the branched chain amino acids leucine and valine, as well as the phenolic amino acids, this could have implications for microbial metabolism in addition to their use by the microbes as a source of protein.

Compared to urea, amino acids improve microbial efficiency, measured as grams microbial nitrogen synthesized per kg carbohydrate digested. Complete mixtures of amino acids plus peptides stimulate total microbial growth to a greater extent than either alone. It has been shown that individual species of bacteria, as well as mixed organisms, reduce ammonia use in the presence of peptide sources, which may help explain the variability in observed ammonia requirements. In this regard, 16 studies of ammonia needed to maximize microbial growth or nutrient digestion were summarized (33). When the natural crude protein of the diets was >6%, growth or digestion was optimized when ammonia levels averaged 6.2 mg/dl. When protein levels were <6%, 21.4 mg/dl ammonia was needed (33). Protozoa

do not use ammonia, but directly assimilate amino acids produced during peptide and protein hydrolysis, particularly when digesting rumen bacteria. Protozoa also have been shown to take up dipeptides more rapidly than bacteria and to excrete most of the amino acids following hydrolysis.

Little is published on the N sources required by rumen fungi. In studies of the *Neocallimastix* species, amino acids supported more growth than did ammonia and could be directly incorporated into protein (35).

It appears, therefore, that in the rumen supplied with degradable protein sources and thus having N sources in addition to ammonia, the biomass probably derives a large portion of the required N from peptides and amino acids. Studies in vivo (60) and in vitro (71), both using ^{15}N, support this concept. In sheep fed alfalfa, as little as 30% and as high as 80% of microbial N was derived from rumen fluid ammonia; in incubations of mixed bacteria, 66% of cell N was obtained directly from casein without going through the ammonia pool.

Since it has been shown that amino acids, peptides and mixtures of amino acids and peptides enhance microbial growth over that on NH_3-N alone, maximum microbial yield from the rumen apparently is dependent on the availability of organic forms of N. Similar results were obtained when carbohydrate digestion was the criterion used to evaluate the efficacy of N sources. Complete proteins of high degradability – such as casein or soybean meal – or large polypeptides were found to double the digestion of starch and increase the digestion of cellulose by several fold compared to digestion on urea or amino acids alone (37, 81).

Carbohydrates. Although three rumen microbial species that require amino acids as energy sources have been identified (11), most rumen microbes depend on carbohydrates as sources of energy.

Strobel and Russell (78) determined the efficiency of microbial dry matter production (Y_{ATP}) from five carbohydrate sources following 10 h of incubation in pH-controlled batch cultures of mixed rumen microbes. At pH 6.7, Y_{ATP} was similar for starch, sucrose, cellobiose, xylan and pectin. Other short-term studies using cultures of rumen contents (49) demonstrated similar rates of VFA production from glucose, xylose, maltose and saccharose. These studies indicate that, following uptake and hydrolysis of the oligosaccharide that result from the initial digestion of polysaccharides, hexoses and pentoses are readily fermented and support microbial growth with similar efficiencies. Total cell protein yield per unit of time may not be the same for all oligosaccharides, however. For example, in the work of Strobel and Russell (78), cellobiose produced the highest yield while xylan produced the lowest. The latter result was caused by the slower rate of xylan hydrolysis. In other studies, the hydrolysis of xylans, the major component of hemicellulose, has been observed to be less than that of cellulose. In reports reviewed by Glenn and Canale (24) the ruminal digestion of hemicellulose

and xylose was lower than that of cellulose and glucose for both grasses and legumes, which agrees with the overall lower digestion coefficient for hemicellulose than for cellulose reported by Hespell (31).

Although starch hydrolysis rates differ with source and processing (18, 80), for most situations with lactating cattle, the extent of rumen fermentation of the diet initially will be dependent on the quantity of rapidly hydrolyzed carbohydrates, primarily the sugars, pectins and starches. Subsequently, energy availability will be regulated by the quantity and composition of the more refractive dietary cell wall components. Since rates of fiber digestion are known to vary depending on the sources, the total energy available from the fiber component will be affected by diet composition.

Iso-acids. It is likely that the amino acid requirement of some bacteria, particularly cellulolytic species, is associated with the requirements for branched-chain fatty acids such as isobutyric, isovaleric and 2-methylbutyric. These result from the deamination of valine, leucine and isoleucine, respectively. These acids, along with valeric, have been shown to be required in small amounts for growth of cellulolytic microbes in a number of studies, and addition of valeric and iso-acids to cultures of rumen microbes has been shown to improve cellulose digestion. The addition of iso-acids was found to increase cell wall or cellulose digestion 1.5-2 fold in vitro (5, 25, 47) and 1.13 fold in vivo (85).

As products of the deamination of amino acids, iso-acids can be produced in rumen contents from two sources. Miura et al. (53) demonstrated that sufficient iso-acids for the growth of the cellulolytic microbe *Ruminococcus albus* were provided from the growth and lysis of other microbes. Deamination of amino acids of ruminally degradable protein also provides iso-acids. It can be theorized that an insufficiency of iso-acids may occur when the solids retention time of rumen contents is short enough to cause decreased ruminal degradation of both dietary and microbial protein. In the work of Miura et al. (53), 36 h were required for bacterial lysis to increase iso-acid levels. In comparison, mean rumen retention times of 15–25 h have been reported in cattle fed forages and grains at high levels. Under such conditions, available protein could be further reduced by the feeding of protein of low rumen degradibility. The latter situation could reduce fiber digestion by not only limiting iso-acids but also ammonia levels as well. This was demonstrated in the continuous culture studies of Varga et al. (90). In this work, diets formulated with either normal or formaldehyde-treated soybean meals were supplied to fermentations in which the solids retention time was set at 16 h. Digestion of organic matter and neutral detergent fiber (NDF) were decreased in the diet with the treated soybean meal. Separate infusions of either urea or an iso-acid mixture partially restored organic matter and NDF digestion, while infusion of urea and iso-acids combined restored organic matter and NDF digestion to the levels obtained when

normal soybean meal was fed. The results of this study may explain the observed efficacy of iso-acids in improving performance of cattle fed diets containing either urea or having low levels of degradable protein.

Rumen pH

When sources of readily fermentable carbohydrates (RFC) are added to forage diets, fiber digestion has been shown to be depressed. As little as 10–15% added RFC can impair fiber digestion, but severe depressions are usually associated with RFC or grain levels of 30% or more of dry matter intake (10, 68, 83, 84).

Of the several theories advanced to explain the depressing effects of RFC on fiber digestion, the following have received most attention: a preference by rumen microbes for RFC rather than fiber components; a decrease in ruminal pH caused by rapid RFC fermentation with a resulting depression in fiber degradation; and competition for essential nutrients resulting in preferential proliferation of RFC-digesting microbes.

An interrelationship among the first two of these theories was suggested by the work of Mould and Orskov (55). They proposed that added starch reduced fiber digestion through a series of events involving carbohydrate preference, reduced rumen pH and decreases in numbers of cellulolytic organisms. A moderately reduced pH, to about 6.2, was found to exacerbate a depression in fiber digestion brought about by added starch; a more severe pH decrease, to <6.0, caused a reduction in cellulolytic microbes and severely limited fiber digestion. The initial reduction in fiber digestion, which was not pH related, was referred to as the "carbohydrate effect," and suggests that readily digested carbohydrates can inhibit cellulose digestion. This concept remains controversial; however, it is supported by the work of Smith et al. (75). Using a dialysis technique, they found that when the dialyzing medium contained cellobiose or glucose, cellulose digestion in the adjacent compartment, which contained a cell-free preparation of cellulases from *Ruminococcus albus*, was inhibited. In contrast, Hiltner and Dehority (32) found that cellobiose and cellulose were fermented simultaneously by each of three rumen microbial strains until sufficient cellobiose was fermented to reduce pH, at which time cellulose digestion decreased. No direct inhibition of cellulose digestion by cellobiose was observed. Since "cellulase" is a multienzyme system (23, 27, 36, 43), it is likely that many studies are not readily comparable because of differences in methods of extraction. This may explain some of the discrepancies in studies with cell-free preparations, which contain only part of the complex, compared to studies with intact cells.

Mould et al. (56) described the effects of pH as bi-phasic, whereby reduction in pH from 6.8 to about 6.0 results in moderate depressions in fermentation, while a decrease in pH below 6.0 causes severe depressions. Digestibility depressions due to pH below 6.0 are not equal for all nutrients.

Fibrolytic and proteolytic activities are most severely depressed, while fermentation of starches and sugars remains at a high level, even at pH 5.0 (34, 73).

The depression in fiber digestion caused by a pH reduction from 6.8 to 6.0 is not readily explained. The activity of isolated fibrolytic enzymes remains high in this pH range, and decreases in numbers of cellulolytic microbes have not been consistently associated with small decreases in pH.

Results of some studies suggest that attachment may be involved in the depression of fiber digestion associated with moderate decreases in pH. An apparent interaction between pH and attachment was shown in the studies of Smith et al. (75), where a decrease in the amount of cellulolytic enzyme adsorbed to a cellulose substrate was associated with low pH. Cheng et al. (14) also reported that low ruminal pH appeared to prevent a tight attachment of bacteria to plant cell walls, resulting in no overt fiber digestion.

The work of Shriver (73) supports this concept. In this study, continuous cultures of rumen contents given a 65% concentrate:35% forage diet were conducted at pH 5.8, 6.2, 6.6 and 7.0. At pH 5.8, the quantity of microbes attached to fiber particles was reduced by 43% compared to the average for pH 6.2 through 7.0. The NDF digestibility at pH 5.8 was 8.1%, compared to an average of 32.5% at the higher pH levels.

Decreases below pH 6.0 cause a precipitous loss of fibrolytic activity, usually with complete cessation of fiber digestion between 4.5 and 5.0. Effects of pH <6.0 were shown to be related to reductions in growth of several species of rumen bacteria in vitro (70), to washout of cellulolytic bacteria from continuous cultures (69) and to decreases in numbers of cellulolytic microbes in vitro and in vivo.

It thus appears that reductions in ruminal pH to the 5.8 to 6.2 range that are cyclic and of short duration will cause a moderate, transient depression in fiber digestion that may be alleviated by controlling pH through buffering or feeding strategy, resulting in only the marginal reductions in ruminal organic matter digestion that are associated with the presence of readily fermentable carbohydrates. Further pH reduction for longer periods will cause a washout of rumen microbial species associated with fiber digestion and result in a severe reduction in fiber and organic matter digestion, as well as decreased microbial dry matter production. Figure 4 depicts these relationships as determined in continuous culture (19,34,73).

Note that between pH 6.0–5.5, NDF digestion decreased from about 50% to 20%, with no perceptible decrease in microbial growth. Between pH 5.5 and 4.5, severe decreases in NDF digestion are associated with a moderate reduction in microbial growth.

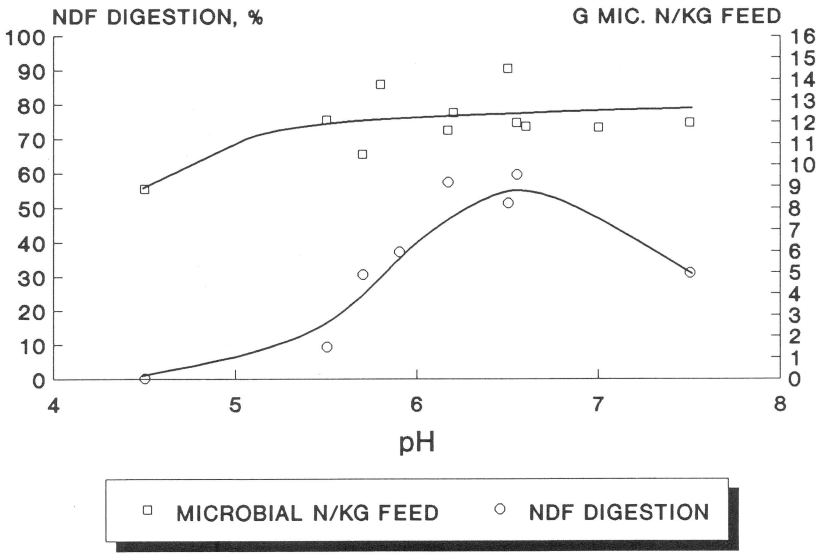


Figure 4. Effects of pH on NDF Digestion and Microbial Nitrogen Produced per Kg Feed Supplied to Continuous Cultures of Rumen Contents (19, 34, 73).

Rumen Turnover

The rumen is a continuous fermentation system that is provided with nutrients (feeds), buffers (salivary and other salts) and fluids (water and saliva) on both a continuous and an intermittent basis. The outflow of the undigested residues is proportional to the input; as intake increases, the rate of rumen turnover increases.

Turnover is conventionally expressed as the portion of the rumen contents that leaves the rumen per hour. For example, assume a cow has in her rumen a total of 100 kg of digesta containing 12% dry matter. If dry matter flow to the abomasum is 14.4 kg/24 h the calculations are:

Total rumen dry matter = 100 × .12 = 12 kg

Flow to duodenum/h = 14.4 ÷ 24 = .60 kg

Rumen dry matter turnover rate = .60 ÷ 12 × 100 = 5.0 %/h

Liquids and solids turn over at different, but usually related, rates. Liquid flow rates, as proportions of the total liquid volume, were found to turn over at rates that increased from <8 to 13.5%/hr as dry matter intake went from 5 to 21 kg/day (67). At the same time, solids turnover increased from 3 to 5%/hr due to the increased intake. In other studies, values of 17%/hr for liquids (89) and as high as 7.0%/hr for concentrates (17) were reported. In a typical ration of a dairy cow consuming >20 kg dry matter/day,

representative rumen digesta passage rates would be 15%/hr for liquids, 6%/hr for grains and 4.5%/hr for forages. The rates would all decrease with a lower level of intake.

Turnover rates greatly influence microbial survival and yield. For example, for liquid-associated microbes to remain in the rumen in large numbers, it is necessary that they reproduce at a rate equal to the liquid dilution rate, otherwise they will be washed from the rumen. The reciprocal of the dilution rate is the average retention time, in hours, of rumen digesta. Thus, using the example of a turnover rate of 15%/hr, the average time a liquid-associated microbe would be in the rumen is $1 \div .15 = 6.67$ h. Organisms that cannot reproduce every 6.67 h will be washed from the rumen if there is no way to increase the residence time. For slow-to-reproduce microbes such as protozoa, fungi and fiber digesters, attachment to feed particles or to the rumen wall is, therefore, necessary for their survival in the rumen.

Another important rumen characteristic associated with turnover rate is microbial yield, where microbial yield is defined as the quantity of microbial mass flowing from the rumen per day. A further, and important, refinement of the expression of microbial yield, which also is affected by turnover rate, is the efficiency of microbial yield. This is usually expressed as the grams microbial protein (or nitrogen) produced per kg of organic matter (OM) digested in the rumen. Both aspects of microbial production have applied significance. Microbial yield is important because this is an index of the amount of microbial protein available to the cow per day. Since, under proper feeding, microbial protein can supply well over 50% of the requirements of a lactating cow, the practical importance of maximizing microbial yield is obvious. Microbial efficiency is important because it is part of the calculation of microbial yield, where: microbial yield, (g of microbial N/day) = microbial efficiency (g microbial N/kg digested organic matter) × kg OM digested in the rumen per day. Intake, rumen turnover and microbial efficiency are all positively related, in that increases in intake cause turnover rate to become greater, which causes an increase in the efficiency of microbial growth. These interrelationships are illustrated in the following example taken from a study of rumen microbial growth in continuous cultures. In this study, liquid and solids flow rates were adjusted to be representative of that found in dairy cows in the transition from moderate to high intakes. As turnover rates increased, greater quantities of organic matter were provided to the fermenters. Intake and microbial responses are shown in Table 4.

These data show that as turnover rate increased, the microbial efficiency (measured as microbial dry matter produced per kg of organic matter fermented) became greater. This happened because the nutrients needed for maintenance were greatly reduced. The maintenance requirement is the nutrients needed to maintain the microbial population in the fermenter (or rumen) and is a function of the microbial mass present. As turnover in-

creases, the population in the fermenter actually decreases while the microbial mass flowing from the fermenter, the microbial yield, increases greatly.

Table 4. Effects of Turnover Rate Changes on Microbial Growth and Efficiency in Continuous Culture. (73)

	Turnover rates, %/h					
	L*	S†	L	S	L	S
	8.0	3.8	12.0	4.5	16.0	5.6
Parameter						
Organic matter intake, g/d	70		81		100	
Organic matter digested, g/d	42.8		50.5		57.9	
Total digesta flow from fermenters, kg/d	2.47		3.67		4.88	
Microbial dry matter flow from fermenters, g/d	11.1		15.5		19.5	
Microbial efficiency, g MIC DM/kg OM dig.	260		308		336	

* Liquid turnover rate.
† Solid turnover rate.

Because of the rapid rumen turnover rates commonly found in cattle with high dry matter intakes, such as lactating dairy cattle, high microbial efficiencies are expected. If, however, an imbalance in the nutrients available to the rumen microbes occurs, the microbial efficiency can be impaired. This is particularly evident if ruminally available nitrogen or carbohydrate sources are inadequate.

Protozoa Numbers

It was mentioned previously that protozoa are predators of fungal zoospores and bacteria (58). Compared to the defaunated state, bacterial numbers are reduced in the presence of protozoa. The decrease in total bacteria is often characterized by a relatively greater loss of amylolytic compared to cellulolytic bacteria. Jouany et al. (40) summarized a number of studies and concluded that, in general, the presence of protozoa resulted in two positive effects on the fermentation of carbohydrates. First, in most studies, cell wall digestion was enhanced when protozoa were present. Second, in cattle fed diets with appreciable grain levels, the protozoa serve as a buffer against an abrupt decrease in ruminal pH and excessive lactic acid production by bacteria, following ingestion of a meal high in concentrates. This results from the rapid assimilation of sugars and starches by protozoa, which are then stored in the protozoa as amylopectin. The stored carbohydrate is then slowly catabolized to volatile fatty acids by the protozoa.

Protozoa also have negative effects on rumen function. Because of their sequestration on large feed particles and on the rumen wall, the flow of protozoa from the rumen is less than would be predicted from their concentration in the rumen and rate of digesta flow. Therefore, although protozoa can represent 50% of the biomass in the rumen, they contribute 20% or less to the microbial protein flowing to the duodenum. In addition, the predation on bacteria causes recycling of bacterial protein in the rumen. Protozoa engulf and kill large quantities of bacteria, assimilating much of the protein from these organisms. Since most of the protozoa remain in the rumen until they lyse, microbial flow from the rumen also is reduced. In vivo measurements summarized by Jouany et al. (41) indicate that defaunation resulted in a 36% increase in g microbial N flowing from the rumen per kg organic matter fermented. An additional negative aspect of protozoa on digestive function that is important for lactating cattle is their engulfment and digestion of particulate feed protein. This permits protozoa to assimilate proteins of low rumen degradability which have been added to the diet as sources of by-pass protein.

The overall effects of the presence or absence of protozoa in dairy cattle are not well characterized because of the difficulty in rendering lactating cattle protozoa-free. Yang and Varga (101), using chemical defaunation techniques, reported that defaunation resulted in a reduction in milk production but the effects of the defaunating agent on other microbes was not determined.

Lipids

Although data on lipid metabolism by rumen fungi are lacking, protozoa, and particularly bacteria, metabolize lipids. While low levels of dietary fat (5% or less) do not negatively affect microbial growth, higher quantities of certain fats are inhibitory towards some microbes. Added fats have been shown to be particularly toxic to cellulolytic and methanogenic bacteria and to protozoa.

The mechanism of the toxicity remains obscure. A popular view is that the lipids coat feed particles, preventing attachment by the microbes. The result is a marked depression in nutrient digestion, particularly fiber. There are a number of observations that call this theory into question. First, the toxicity is greater towards Gram positive than Gram negative bacteria (59). Second, polyunsaturated fatty acids are more toxic than saturated fats, although both can be toxic, and third, cis forms are more toxic than trans forms (48). If the toxicity was due to a simple physical coating, both saturated and unsaturated fats should have similar effects. Additionally, inclusion of 7.9% soybean oil in the diet of lactating cows was not found to affect the quantity of attached microbes in studies by Legay-Carmier and Bauchart (45).

A second theory developed to explain the toxicity is based on the observation that calcium, when added to fat-containing diets, restored nutrient digestibility. It was proposed that the reaction of fatty acids with divalent cations (calcium) to form soaps limited the availability of calcium to the microbes, causing the toxicity. Activity was restored by supplementing the diet with excess calcium. It has been shown, however, that saturated fats form more stable soaps more readily than unsaturated fats (38, 79). It would follow that saturated fats should bind cations more effectively than unsaturated fats, and be more toxic, while the opposite is true.

Although the mechanism of the toxicity is not clear, it has been shown that if the fatty acids are provided as Ca soaps, the inhibitory effect on nutrient digestion is greatly decreased. This is true for both saturated and unsaturated fatty acids under most conditions in the rumen. In high-grain diets that reduce rumen pH, there is a danger that the soaps will dissociate, particularly soaps of unsaturated fatty acids, permitting free fatty acid formation and expression of toxicity. Sukhija and Palmquist (79) demonstrated that between pH 6.5 and 5.5, dissociation of soaps of soybean oil (containing high levels of polyunsaturates) increased from about 10% to >40%; over the same pH range, dissociation of soaps of saturated fatty acids remained at less than 10%. The association of pH and toxicity of soaps of saturated and unsaturated fatty acids was demonstrated in the studies of Webster (96). When continuous cultures of rumen microbes were maintained at different pH levels, effects of inclusion of soaps of unsaturated and saturated fats on fiber digestion differed. Results are in Table 5.

Table 5. Neutral Detergent Fiber Digestion of Diets With Added Soaps and at Different Fermentation pH.* (96).

	Level (% of DM) and type of Ca soap added		
Fermentation pH	0 — —	6 Highly saturated	6 Highly unsaturated
	digestion, %		
6.5	54	57	51
6.0	51	51	44
5.5	32	36	22

* Diets contained 50% forage and 50% concentrates.

These data show that as pH decreased, the fiber digestion for the control and the diet with 6% Ca soaps of saturated fatty acids did not differ greatly. While NDF digestion of the diet with 6% added Ca soaps of unsaturated fatty acids remained high at pH 6.5, digestion was markedly reduced at pH 6.0 and 5.5, compared to the other diets. This apparently reflects the greater dissociation of the unsaturated soaps at low pH.

The use of calcium to form soaps of fatty acids greatly reduces the toxicity of fats. In addition, use of primarily saturated fats, such as tallow, also is less toxic to rumen microbes, especially if extra dietary calcium is provided. If soaps with a high proportion (50% or so) of unsaturated fatty acids are fed, dissociation at low rumen pH can allow expression of toxicity.

Plant Components

Plants produce a number of compounds that provide, among other things, a degree of protection against microbial invasion. Included in these protective compounds are lignin, the phenyl-propanoids associated with lignin structure, tannins, cutin and silica. These components have been shown to negatively affect the growth and/or metabolic activity of rumen microbes (87). The presence of the phenyl-propanoid units, p-coumaric and ferulic acids, or their complexes with hemicellulose and cellulose, also reduce ruminal digestion (9, 28, 29). Jung and Fahey (42) found that the decline in fiber digestion in mature alfalfa was associated with increased lignin, while digestibility depressions in tall fescue were associated with increased plant levels of p-coumaric and ferulic acids. Moreover, Akin (1) demonstrated that purified p-coumaric and, to a lesser extent, ferulic acid depressed growth rates of microbes grown in a broth media and reduced the degradation of filter paper in vitro.

Tannins also have been shown to decrease microbial metabolism (44), inhibit enzymes, including cellulases (26, 52), and depress dry matter digestion in sorghum grain (72) and in forages (20, 74).

Silica is reported to have a negative effect on digestibility of grasses (88), causing a 3% decrease in in vitro digestible dry matter per unit of increase in silica, with most of the decrease in digestion of cell wall carbohydrate. A reciprocal relationship between silica and lignin content of forages has also been noted, and digestibility depressions were found to be more closely associated with the sum of lignin and silica than with either single component (86).

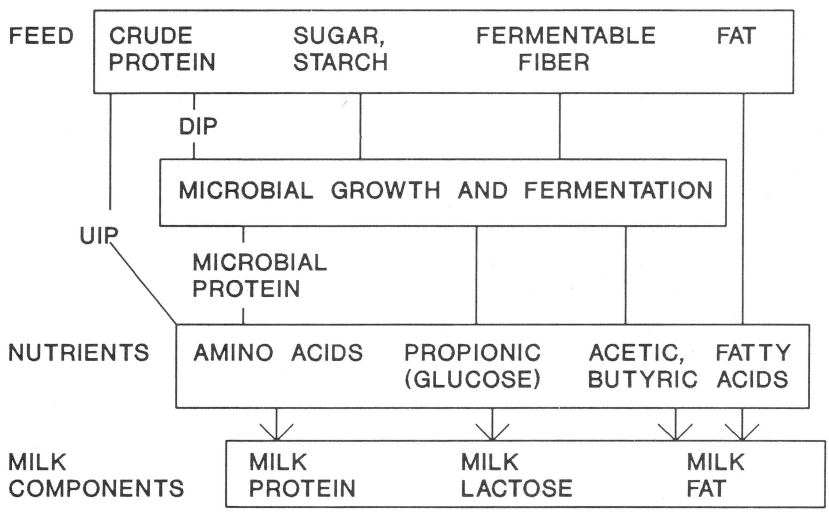
Cutin content has been shown to vary markedly among fiber sources, with seed hulls having particularly high levels (87). Monson et al. (54) reported that intact cuticle could cause a 6 to 48 h lag time in the initiation of digestion of plant particles, which was found to vary with plant species and genotype. They reported that no digestion took place as long as the cuticle remained intact. This emphasizes the importance of the interaction between chemical components and physical treatment of the plant on digestion. Disruption of cuticular material by mastication and rumination was a major factor associated with the rate of particle size reduction in the studies of Pond et al. (66).

These observations suggest that plant components can alter cell wall degradation through physical barriers including lignin, cutin and silica, and

that these may be expressed as a lag time phenomenon. The chemical effects of phenyl-propanoids and tannins on depressing microbial degradation occur through formation of a complex with the substrate in the case of phenyl-propanoids (28), or by attachment to enzymes as suggested for both phenyl-propanoids (1, 42) and tannins (102). These responses could be expressed both as lag time and as a decrease in rate of overall fermentation.

MAXIMIZING NUTRIENT FLOW FROM THE RUMEN

Figure 5, adapted from Oldham and Emmans (62), depicts the relationships among feed components, rumen fermentation and nutrients available for lactation. An important principle illustrated here is that a large proportion of the nutrients used by the ruminant is the end-product of rumen fermentation, primarily volatile fatty acids and microbial protein. This un-



ADAPTED FROM: Oldham and Emmans (62)

Figure 5. Feed, Nutrient Flow from the Rumen, and Milk Production.

derscores the need to manage the rumen so that microbial growth and fermentation will be optimized.

If total daily flow of microbial protein to the duodenum is used as the criterion of optimum fermentation, there are some feeding and management factors that can be identified as affecting this operation. As has been mentioned previously, microbial protein flow per day (microbial yield) is a function of the diet digestibility and the microbial efficiency, which is the microbial protein produced per unit of diet digested.

Formulae commonly used are:

1. G microbial protein/day = kg OM ruminally fermented x g microbial protein/kg OM ruminally fermented
2. Bacterial crude protein, g/d = 6.25 x (–30.93 + 11.45 NE_L)

Where: OM = organic matter
NE_L = net energy for lactation

Formula 2 is used by the National Research Council (61) to calculate bacterial protein flow in lactating cows. Both formulae emphasize the importance of ruminally digestible energy as a factor controlling microbial yield. Mean rumen retention times in lactating cattle often are in the range of 15 to 25 hours, which can limit digestion. On the other hand, rapid turnover rates can result in a high microbial efficiency by reducing the maintenance requirements of the microbes. A high microbial efficiency, however, can be accomplished only if the required nutrients are available on a continuous basis. It can be seen in Figure 5 that the macro-nutrients required by microbes can be identified as specific components of the organic matter and consist of ruminally available carbohydrates and protein.

Carbohydrate Requirements

With very few exceptions, the rumen microbes use only carbohydrates for energy, and growth will be proportional to the amount of carbohydrate fermented. The use of OM digested or NE_L to estimate energy available, and subsequently microbial yield, can be misleading, since both protein and fat contribute to OM digested and NE_L, but add little to the energy available for microbial growth. The more appropriate expression for calculation of the microbial protein yield is, therefore:

3. G microbial protein/day = kg total CHO digested/day in the rumen x g microbial protein/kg CHO digested

where kg total carbohydrate digested/day is used in place of organic matter digested or NE_L. In Figure 5, carbohydrate sources include the fermentable portion of the fiber and the sugars and starches. In practice, the analytical techniques used to determine carbohydrates do not provide a clear delineation of the various components in a forage-grain ration. Commonly, NDF is used to quantitate the total structural or cell wall carbohydrates, which include cellulose, hemicellulose and lignin. Sugars and starches are not individually determined, but are included, along with pectins, β-glucans, gums and other components, in a fraction referred to as the non-structural carbohydrates (NSC). The latter fraction is calculated by difference following determination of the crude protein, fat, ash and NDF.

Since digestibility of NSC in the rumen is considerably higher than that of NDF (Table 6), it follows that the amount of total carbohydrate digested per day is positively related to the proportion of NSC in the diet.

Studies to date indicate that diets containing 36-40% NSC and 28–32% NDF will provide acceptable quantities of total digestible carbohydrate. These values can vary due to differences among sources of both NSC and NDF. The ranges in digestion given in Table 6 reveal extensive variability in digestion of both fractions, due primarily to differences in rates of ruminal degradation among sources. It may be both nutritionally and physiologically sound to obtain a greater proportion of the ruminally available carbohydrate from the forage portion of the diet, so that reliance on grains can be reduced. At the same time, synchronization of rates of digestion among the sources of NSC and NDF should be evaluated with regard to providing a continuous supply of available carbohydrates between meals.

Table 6. Ruminal NDF and NSC Digestion by Lactating Cattle.*

Carbohydrate fraction	Average percent digested	Range
Starches and sugars (NSC)	67.7	46.6–87.4
NDF	43.6	11.4–62.8

* From: 21, 30, 51, 77, 95, 100.

A crude control of total digestible carbohydrate intake can be accomplished by adjusting the forage-grain ratio within the limits of appropriate rumen physiological function. When using the forage-grain ratio, the NDF and NSC levels still should be regulated, especially if by-products are used in the diet. These products may contain high levels of NDF, yet will be considered as grains. This can result in a diet with a low NSC high NDF content, even though the diet appears to have an appropriate forage-grain ratio.

Protein Requirements

Although energy, ie., carbohydrates, is usually considered to be the most limiting nutrient for maximum microbial growth, on high grain diets, ruminally available protein is often more limiting than available energy. In a summary of several continuous culture studies (35), it was found that increasing degradable protein had a small positive influence on carbohydrate digestion (Figure 6A) while markedly affecting microbial efficiency (Figure 6B). As with carbohydrates, there is very limited information on the importance of sources of protein required to optimize microbial growth. This includes both the requirements for NPN, amino acids and peptides as well as appropriate rates of protein degradability needed to synchronize protein and carbohydrate availability.

In in vivo studies, microbial efficiency was highest when degradable intake protein (DIP), as a percent of total ration dry matter, was in the range

of 10–13% (35). Degradable protein in this range was found to be very beneficial to rumen fermentation. While the greatest improvement was in microbial efficiency, carobhydrate digestion was enhanced as well (Table 7). At the high level of DIP, the improved carbohydrate digestion and microbial efficiency resulted in the greatest total microbial yield, and probably the highest flow of other nutrients, such as VFA, as well.

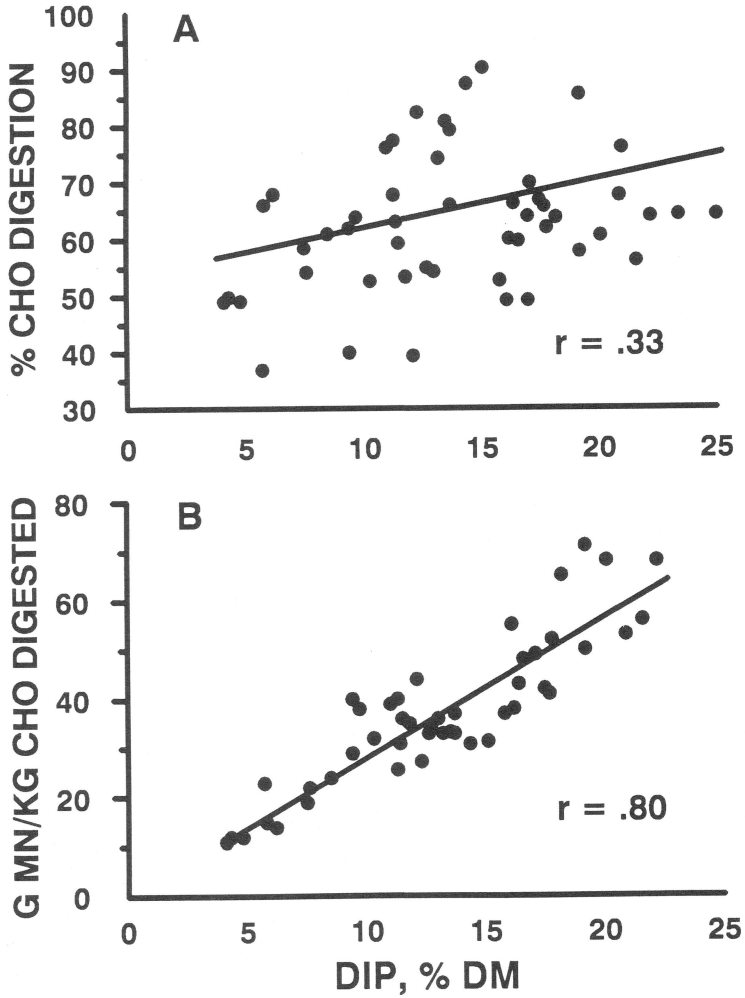


Figure 6. (A) Carbohydrate (CHO) Digestion and (B) Microbial Efficiency (G Microbial N/Kg Carbohydrate Digested) in Response to Degradable Intake Protein (DIP) Level in Continuous Culture (35).

Table 7. Average Rumen Carbohydrate (CHO) and Nitrogen Metabolism Values for Lactating Cows, Ranked by DIP. (35)

			Means			
	DIP*, % of DM		NSC,	CHO dig.,		Microbial N,
N	Range	Avg.	% CHO†	kg/d	YCHO‡	g/d
4	10-13	12.3	56	7.9	44.5	334
7	7-10	9.3	57	6.6	41.3	269
4	<7	6.3	59	5.1	29.8	152

* Degradable intake protein.

† Nonstructural carbohydrate as percent of total carbohydrate.

‡ Grams microbial N/kg total carbohydrate digested.

SUMMARY

The rumen is a dynamic, continuous fermentation compartment that provides a suitable environment for a variety of species of anaerobic bacteria, protozoa and fungi. These microorganisms have a complex series of interactions with the feeds supplied to the host, with some using particulate matter as both sources of nutrients and sites of sequestration to avoid being washed from the rumen by the rapid flow of fluids. Because of the ability to use soluble nutrients and to reproduce rapidly, other microbes associate primarily with the liquid phase of the rumen contents. Due to the metabolic activity of all microbial populations, feeds are converted to microbial matter and fermentation end products, which serve as nutrients for the ruminant. Optimum feed utilization by ruminants is dependent on achieving maximum rumen fermentation and flow of microbial protein to the duodenum. At this time, it is clear that the major nutrients required by the microbial populations include both fibrous and nonfibrous sources of carbohydrates and nitrogen in the form of ammonia, amino acids and peptides. Despite five decades of research, the exact quantities and sources of these nutrients that will result in optimum rumen fermentation rates and microbial yields are only partially known. However, tentative guidelines can be recommended, based on recent studies of lactating cows. For production level in excess of 60 lb/day, neutral detergent fiber should be limited to 32% or less and non-structural carbohydrates (NSC) should be 35% or more of diet dry matter. This will help assure the proper balance of rapidly and slowly degradable carbohydrates. For diets containing significant levels (20% or more) of bran, midds, soyhulls or beet pulp, NSC should be limited to about 30% of DM. This is because the fiber in these sources is rapidly fermented and can increase the risk of rumen acidosis. Under these conditions, buffering and use of a total mixed ration are recommended. Protein must be provided in adequate amounts and in the proper forms to synchronize with the available carbohydrates. For high producers, it appears necessary to increase both the total crude protein

and degradable intake protein (DIP) over that recommended by the National Research Council (NRC). For example, if the NRC recommendation for total crude protein is 17%, it is suggested this value be divided by .94 (17 ÷ .94 = 18). Of the total crude protein determined in this manner, 12% should be degradable and the remainder should be from by-pass sources. To assure that there is an adequate supply of rapidly available protein to match the rapidly available carbohydrate in the NSC fraction, about half (40–50%) of the DIP should be soluble protein.

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