Chapter 3
Ruminal Fermentation

Fredric N. Owens and Mehmet Basalan

Classification of Animals by Foods or Feeds Consumed

Carnivores, Omnivores, and Herbivores

Diet selection by animals is dictated largely by capacity of the digestive tract and the ability of enzymes available within their digestive tract to digest or ferment the primary nutrients in a diet (carbohydrates, protein, lipid) to simpler compounds that can be absorbed and metabolized by mammalian tissues. Lacking amylases that attack starch in grains, starch must be gelatinized to be well used by carnivores (cats, dogs). The small capacity of the digestive tract of carnivores, of young animals, and omnivores (poultry, swine, humans) limits their ability to consume bulky, fibrous feeds. In contrast to carnivores and most omnivores, adult herbivores have a digestive tract large enough to maintain fibrous feeds for a time period sufficiently long for anaerobic fiber-digesting microbes resident within their digestive tract to ferment cell walls (hemicellulose and cellulose) to volatile fatty acids (VFA, primarily acetic, propionic, and butyric acids). These products in turn are absorbed and metabolized by the host animal. This capacity allows herbivorous animals to thrive when their diet contains forages and feeds that are bulky and rich in fiber and water.

F.N. Owens (✉)
Professor Emeritus, Oklahoma State University, Stillwater, OK, USA
e-mail: owens.fred@gmail.com

M. Basalan
Kirikkale University, Kirikkale, Turkey

© Springer International Publishing Switzerland 2016
D.D. Millen et al. (eds.), *Rumenology*, DOI 10.1007/978-3-319-30533-2_3

Non-ruminant Herbivores Versus Ruminants

Non-ruminant herbivores (horses, rabbits, hares) have an enlarged cecum and colon where anaerobic microbes ferment cell walls to VFA. Because these organs for fermentation are located posterior to the small intestine of non-ruminant herbivores, the microbes involved with post-gastric fermentation excrete most microbial products that could have nutritional value to the host animal (protein, vitamins, phosphorus, sulfur, ammonia) in feces. Nevertheless, post-gastric fermentation allows VFA derived from fermentation to be absorbed, so post gastric fermentation enhances energy recovery from fermentation of nutrients that escape intestinal digestion.

In ruminants, fermentation occurs in the reticulo-rumen, a site anterior to gastric pouch (the abomasum) and the small intestine. Most of VFA are absorbed through the ruminal or omasal wall. In addition, the microbial mass (containing protein, vitamins, phosphorus, sulfur) together with dietary feed components that resist or escape ruminal fermentation become available for digestion and absorption when passed to the abomasum (true stomach) and intestines. Because the site of fermentation is located ahead of the small intestine, ruminants make efficient use of protein and other nutrients synthesized by microbes in the rumen. In addition, ruminants recycle certain essential nutrients (N, S, P) to the rumen that, together with salivary buffers, provide nutrients and maintain growth conditions conducive to growth and activity of anaerobic microbes. In addition to fermenting fibrous components not digested by mammalian enzymes, ruminal microbes will detoxify many substances prevalent in certain plants and herbs that can prove toxic for non-ruminants. As with non-ruminant herbivores, capacity to graze allows all herbivores to both harvest forages from plants found in areas inaccessible for mechanical harvest and to obtain energy and protein from fiber-rich byproducts inedible by omnivores. Through using such products and grazing forage, herbivores increase the supply of calories available for human consumption.

Ruminants are adapted to a wide variety of environmental and dietary conditions. They thrive when fed diets are high or low in moisture content. They thrive when diets are rich in fiber or rich in starch. Thus, surplus grains as well as numerous byproducts of food, feed, and fuel production and products of agricultural and industrial manufacturing that are undesirable or inedible by humans or other animal species can be converted by ruminants into foods valued in the human diet. Milk and meat derived from ruminants provide high quality protein and possess flavors and textures preferred by humans. The capacity of ruminants to convert fiber-rich, undesired, or surplus feeds into desired food products while forming additional products (wool, mohair, leather, manure) prized by humans worldwide depends largely upon the rumen fermentation process. This chapter is an attempt to outline normal rumen function and dysfunction, to discuss potential methods for altering rumen function, and to outline some of the research methods employed to increase our understanding about and control of fermentation within the rumen.

Fermentation

Fermentation is defined as an anaerobic (without using oxygen) cellular process whereby organic foods are converted to simpler compounds and energy is released. Fermentation is common among many microbial species and even by mammalian muscle tissues when muscles act anaerobically. During fermentation, acid fermentation end-products accumulate over time. Through inhibiting continued microbial metabolism, acid accumulation gradually slows and stops fermentation stabilizing the product (i.e., silage or pickles). But when bases or buffers are present or added to neutralize these acids or when acids are removed by being absorbed as from the rumen, or when basic compounds are released during fermentation (as from proteolysis) to neutralize acid, fermentation can continue and persist. Feeds or foods can be preserved when stored anaerobically in the presence of acids that are weak (as from fermentation) or strong acids or under basic conditions (as with added ammonia or strong bases). Either strong acids or basis will minimize bacterial activity so that fermentation will be minimal or nonexistent.

Extent of Fermentation

When not halted by accumulation of acid or base, microbial fermentation will persist. If allowed to continue for days or weeks, most of the carbon and hydrogen from organic compounds (except for lignin and other polyphenols) will be converted to methane and carbon dioxide, the nitrogen present will be released as ammonia, and sulfur will be released as hydrogen sulfide. Thereby, fermentation allows the organic wastes within biogas (methane) fermenters or at sewage plants to be fully degraded. Released methane can be combusted to release energy. In contrast to such extensive fermentation, fermentation within the rumen differs: time is limited and end-products (VFA) are continuously removed. This allows most of the energy in end-products to be used by the host animal. Fiber, defined as any product that cannot be digested by mammalian enzymes, and other organic compounds are fermented by microbes in the rumen to yield products useful for mammalian tissues with limited loss of energy as methane and heat.

Types of Fermentation

Fermentation processes used industrially are classified into two types—batch systems or continuous flow systems and into two classes—open or closed depending on whether microbes from the environment are allowed to enter the fermentation vessel.

Batch Fermentation Systems

Typically industrial batch fermentation vessels are closed and substrates often are sterilized to avoid epiphytic microbes. This allows fermentation to proceed with selected microbes to yield enzymes or other products. When desired products have accumulated, the entire mass is harvested and the products of interest are isolated and marketed. To release glucose from starch from grain for generation of ethanol, batches of ground starch first are hydrolyzed and the released sugars are fermented with yeast or enzymes. Numerous organic compounds (nutrients, enzymes, hormones) of industrial and nutritional interest are generated through batch fermentation systems.

Silage formation is a batch fermentation system that often relies on the epiphytic microbial population though specific inoculants often are added to speed or direct fermentation to specific products (primarily lactic and acetic). These acidify moist forages or grains to preserve the mass for later use. Similarly, organic or inorganic acids can be added to preserve feeds or foods (pickling). The accumulation of acid end-products inhibits further fermentation and preserves the ensiled mass so long as oxygen is excluded. Upon re-exposure to oxygen, microbes will catabolize the acids, generate heat, and begin to oxidize the mass.

Often fermentation will be incomplete. If the amount of carbohydrate is insufficient to produce enough acid to inhibit microbes or if the substrate is strongly buffered, microbial degradation will continue yielding various spoilage products (ammonia, butyric acid) that reflect substantial loss of nutrients.

Fermentation of silage is classified as homolactic or heterolactic depending on the products generated. With homolactic fermentation by lactobacilli, D+ and L-lactic acid and its derivatives are the sole organic products. Other microbial species can form a wide variety of other products (ethanol, VFA) from lactate. In the rumen fermentation is largely heterolactic with numerous intermediate products (succinic acid, malic acid, hydrogen, ethanol, as well as lactate) being formed. These compounds typically are reduced further by fermenting microbes or by other microbes so that the primary products of ruminal fermentation are VFA, carbon dioxide, reduced (hydrogenated) fatty acids, and other products that were used as electron or hydrogen (NADH) acceptors (e.g., methane, nitrite or ammonia, and hydrogen sulfide), and the synthesized microbial mass. The mass of microbes generated during fermentation generally is limited by (1) the supply of energy (ATP) derived from fermentation or by (2) availability of other required nutrients (e.g., ammonia).

Many microbial species require only energy plus a source of carbon and a source of nitrogen plus trace amounts of minerals. From these compounds alone, many microbial species will thrive by synthesizing all needed organic substances for growth and reproduction. This process often is called "de novo" or "from nothing." In contrast, other microbial species require pre-formed organic substances that vary in complexity (specific amino acids or fatty acids, vitamins) for growth. These microbes thrive by assembling compounds they synthesize with other nutrients obtained from their environment or diet into microbial organic matter. For example, when grown in pure culture (absence of other microbial species), most species of ruminal bacteria that digest cellulose require a source of branched-chain fatty acids

or amino acids for growth. However, supplementing the ruminant's diet with these nutrients is not necessary because these same compounds are produced and released by other bacterial species within the rumen so the inherent supply is adequate.

Continuous Flow Systems

When feed material is provided to a fermentation vessel at frequent intervals and output is continuous or semi-continuous, the system is classified as being continuous. Most waste digestion systems and the rumen are semi-continuous due to frequent addition of substrates and open so that fermentation is performed by both epiphytic (environmental) present within the feeds provided as well as endophytic (digestive tract) microbes inherent within the digestive tract. Products may be continuously removed from fermentation vessels at specific points or removed selectively. Unless readily stirred, continuous fermentation systems develop multiple strata or layers that will differ in chemical composition. Within the rumen, entwined forages form a floating mat or raft that is situated partially submerged atop the liquid mass. Slow but continuous mixing and churning, as occurs within the rumen, inoculates freshly consumed feeds and forages, maintains close contact between microbes and substrates, exposes acids to the rumen wall for removal by absorption, and allows fermentation gases to be cleared. Ethanol generation from sugar cane and biogas (methane) generators typically employ continuous flow fermentation systems.

Fermentation Within the Gastro-Intestinal Tract

Microbes (bacteria, protozoa, yeast, fungi) within the digestive tract are responsible for fermentation. These microbes, whether epiphytic or endophytic relate to other organisms or animals in either a mutualistic (sharing), a synergistic (beneficial to host) or a parasitic (harmful to the host) fashion. All microbes have preferred conditions for maximum growth being mesophylic (body temperature) or thermophylic (high temperature), with preferred or required substrates, and typically yielding specific end-products from a given substrate. Specificity of substrates and products and microscopy were used extensively in the past to identify and classify microbes that often revealed substrates and products as well as metabolic pathways. Today, microbial classification generally is based on genetics instead.

Being largely anaerobic, the complete digestive tract of mammals and other fiber-digesting organisms like termites is an active site of fermentation. Without microbes, fibrous compounds and lignin from plants would accumulate within the environment. (Perhaps coal and petroleum reserves developed at an age before or at a faster rate than microbial degradation could ferment those substances further.) Acidity within the stomach and addition of antibiotics or other modifiers can help inhibit or control the fermentation process, but because the GI tract is an open system, the amount and extent of control imposed by the host is

limited. Fortunately, fierce competition with microbes already present in the rumen and in the digestive tract of non-ruminants typically prevents invasion by epiphytic or pathogenic microbes. Anaerobic bacteria line the digestive tract of all animals (except within gnotobiotic animals delivered by Caesarian section and maintained under sterile conditions). Fermenting microbes are particularly active at locations within the digestive tract where they have an appropriate supply of nutrients, favorable growth conditions, and retention of digesta within an organ (e.g., cecum, large intestine, rumen) is sufficiently long for fermentation to continue so that microbes can multiply faster than they are washed along. With fermentation being a reductive process, organic products within the rumen are reduced to organic acids (VFA), carbon dioxide, and methane. Some products are absorbed (VFA, CO_2), others are eructed or belched (methane and CO_2), others are passed to the small intestine (microbial mass and remaining carbohydrates, protein, lipids, ash) for digestion, and the remainder is excreted in feces.

Energy (ATP; NADH; NADPH) that is released during fermentation or metabolism is available for use by microbes for their growth and multiplication. Compared with respiration where oxygen or other electron acceptors (sulfur, metals) are freely and readily generate ATP from NADH through the electron transport chain, fermentation is anaerobic and the amount of other electron acceptors limited. Consequently, the quantity of energy released during fermentation is quite limited compared with respiration in the presence of oxygen or other electron acceptors (nitrate, sulfate). With aerobic respiration, carbon dioxide and water are the major products. But during fermentation, carbon dioxide, methane, and a very limited amount of water is produced. Products of fermentation will differ with the substrate being used, the organisms or tissue involved, synergistic metabolism among multiple microbial species, and conditions of fermentation. Indeed, within the rumen, fermentation products can differ drastically with ruminal pH conditions likely due largely to shifts in microbial species (Owens and Goetsch 1988; RAGFAR 2007).

Characteristics of Fermentation Within the Rumen

Within non-ruminant animals, gastric acidity (HCl) hinders the entry of non-spore forming microbes that are sensitive to acid. With ruminants, any microbes in the diet meet an acid barrier only when leaving, not when entering, the rumen. Being an open system, the rumen continuously receives microbes with feed and water being consumed. As a result, the microbial population in the digestive tract might be considered "uncontrolled." Uncontrolled fermentation is evident considering various maladies characteristic of ruminants due to accumulation of specific fermentation products (lactic acid causing acidosis; gases causing bloat; nitrate and sulfide toxicoses).

Compounds enter the rumen largely from two sources—when swallowed (feed plus saliva) and by diffusion through the rumen wall. Feed components carry

not only organic solids and liquids but also water and a limited amount of oxygen. Feed, saliva, and ruminated (re-chewed ruminal contents) products enter the reticulo-rumen through the esophagus. Exchange across the stratified ruminal wall depends on the relative osmolality of the rumen versus blood. Certain blood components (urea, bicarbonate, a limited amount of water and oxygen) may enter the rumen through diffusion from blood. Urea enters the rumen both via saliva and by diffusion through the rumen wall. When hydrolyzed, urea yields ammonia, a source of N for ruminal microbes plus carbon dioxide. The ruminal entry rate of urea by diffusion is enhanced by hydrolysis to ammonia by ureolytic bacteria imbedded within the rumen wall. Diffusion of the released ammonia, being basic, into the rumen mass is accelerated by a low ruminal pH. Likewise, bicarbonate enters the rumen both via saliva and due to exchange with VFA during absorption (VFA ions being exchanged for bicarbonate). Note that ruminal pH conditions can alter extent of nutrient recycling into the rumen through the stratified epithelium of the rumen wall.

Removal of products of fermentation from the rumen follows three routes—eructation, diffusion into the blood or the lymphatic stream, and flushing of liquefied digesta (chyme) containing particulate matter to the omasum. Part of the carbon dioxide produced will diffuse from the rumen into the blood stream. But most carbon dioxide, almost all of the methane (due to its low solubility in blood), and most hydrogen sulfide is removed from the rumen in the form of a gas by eructation (belching). Organic acids (VFA, lactate-that is not considered to be volatile like most other short chain fatty acids) are removed from the rumen largely through the ruminal epithelium by passive or attenuated absorption. Other absorbed compounds (ammonia, ionized minerals) leave the rumen via blood or the lymphatic stream depending on their solubility. Microbial products and undigested feed components that are sufficiently dense and small in size are flushed with liquid digesta from the rumen to the omasum via the reticulo-omasal orifice.

The Rumen—An Ideal Media for Anaerobic Microbes?

Conditions maintained within the rumen by the animal are similar to those of an ideal commercial fermentation vessel (Table 3.1).

Characteristics of Ruminal Microbes

Facultative bacteria and aerobic yeasts readily consume oxygen that enters the rumen with feed or water or diffuses into the rumen from blood. This helps to maintain strict anaerobic conditions within the reticulo-rumen preferred by ruminal microbes. With this semi-continuous fermentation system, microbial strains are selected or evolve to thrive best given the substrate and environmental conditions provided. Specific factors inherent to an individual strain of ruminal microbes can provide a selective

Table 3.1 Ruminal characteristics conducive to microbial growth

1.	Fresh substrate and water provided on a regular and frequent basis
2.	Supplemental nutrients (urea, sulfur, phosphorus) recycled via saliva or by diffusion through the rumen wall
3.	Particle size reduction through mastication and rumination
4.	Sufficient water to maintain a fluid or liquid mass
5.	Continuous mixing and churning of ingesta with rumen contents by strong muscular contractions
6.	Stratification of ruminal contents with a floating raft for prolonged retention time of slowly fermented, longer, and buoyant particles
7.	Retention time of particles that is sufficiently long for microbial growth and replication
8.	Temperature of 38–42 °C ideal for growth of mesophylic microbes
9.	pH between 5.5 and 7.0 due to bicarbonate input via saliva and bicarbonate exchange with absorbed ionized acids
10.	Osmolality maintained through exchange with blood fluids
11.	Removal of end products by eructation, attenuated diffusion into blood, and flushing to the omasum

advantage over other ruminal strains or over epiphytic microbes that enter the rumen with feed and water as outlined by Russell (1984) and shown in Table 3.2.

Those microbial species that multiply faster than being washed from the rumen or dying will increase in number; conversely, microbes that fail to multiply faster than they are lost from the rumen population will decrease in population. As a consequence, competition among ruminal microbes is vigorous, continuous, and cutthroat. Although genetically altered "superbugs" might be developed, it is unlikely that ruminal inoculation would prove successful in altering the ruminal population unless the organism (a) can utilize some unused or underused substrate (e.g., lignin, biuret, oxalate) found in the rumen, (b) can tolerate or degrade a compound that inhibits growth of competing ruminal microbes (e.g., mimosine), (c) produces some compound that inhibits growth of competitors (e.g., bacteriocins, methyl-glyoxal), or (d) operates synergistically with some other ruminal microbes to increase growth efficiency. Despite this vigorous competition among microbial strains, the ruminal population remains sufficiently diverse so that the population can shift readily when the diet is changed. Culture studies indicate that microbial shifts to a new substrate are completed within a few days if energy intake is stable. But when energy intake by an animal fluctuates, as often occurs when diet composition is changed, microbial populations can fluctuate wildly until substrate supply and ruminal conditions become stabilized. Consequently, limiting or restricting the supply of feed for ruminants to avoid overconsumption of a new diet (e.g., one that is rich in concentrate) helps to reduce the wide fluctuations in end-products that can induce acidosis. In order to enhance steady state conditions in the rumen, many feedlots establish and adhere to a very regimented feeding schedule so that each meal is delivered to a given pen of cattle at precisely the same time each day.

Though the ruminal population within any individual animal is quite diverse, each individual animal appears to maintain a very specific mix of microbes according to

Table 3.2 Factors providing advantages to specific microbial strains found within the rumen

1. Ability to ferment those substrates present in feeds consumed
2. High affinity for and, in some cases, ability to attach to available substrates
3. Capacity to metabolize diverse types of substrates
4. High yield of ATP from fermented feeds
5. A maintenance energy requirement that is low
6. Rapid replication (capacity to multiply faster than being flushed from the rumen)
7. Capacity to survive fluctuations in temperature, pH, and osmolality
8. Ability to store energy to remain viable between meals
9. Low metabolic cost for ammonia and substrate uptake
10. Capacity to attach to the rumen wall to use diffusing substrates (urea, oxygen)
11. Association with floating particles to avoid washout (most evident with protozoa)

population studies. This mixture of microbes will be characteristically different from that present in the rumen of other cows fed the same diet. Following ruminal evacuation and transfer of ruminal contents, the ruminal population will return to the population characteristic of the individual cow within several days (Weimer et al. 2010). This indicates that in addition to substrate supply, other factors (e.g., water intake, rate of eating, extent of chewing and rumination, saliva flow, ruminal pH, ruminal mixing and stratification, ruminal retention times for liquids and particulates, VFA concentrations and absorption, reticulo-omasal opening times) that are characteristic of an individual animal must play a role in regulating the microfloral population within that individual. Similarly, within a set of cattle, only a few animals will possess certain bacterial strains (e.g., Megaesphaera). And rumen fluid from only certain individual animals and not others within a set of steers similar in genetics and nutritional background will readily and consistently produce lactic acid when provided with glucose or starch. Explanations that can explain these animal-to-animal differences in the presence and activity of ruminal microbes remain unknown.

The population and distribution among various classes of bacteria will shift and change depending on the availability of, not if energy and essential nutrients. Ultimately, the microbial population will increase so that all of the readily available energy will be fermented within the available time. This concept that nutrients are fully fermented is based on the microbiological principle that whenever the supply of energy for microbial growth is increased, the microbial population capable of fermenting that energy automatically will increase to use that energy. Consequently, no available energy should ever exist within the rumen except when microbes encounter (1) deficiencies or excesses of of specific nutrients, (2) antimicrobial compounds or drugs, (3) time is insufficient for fermentation, (4) substrates are inaccessible due to presence of some physical barrier, or (5) following diet engorgement.

Though one might expect the population of cellulose digesting bacteria in the rumen to be lower when forage-poor (concentrate-rich) diets are fed, culture counts indicate that the population of cellulose fermenting bacteria remains relatively stable regardless of diet. This presumably reflects the capacity for cellulolytic microbes to use certain substrates besides cellulose as source of energy. In contrast, the population of microbes capable of fermenting starch increases markedly when diets rich in starch are fed; this in turn decreases the relative prevalence of cellulose-digesting bacteria even though the absolute population of cellulose-digesting bacteria remains stable.

Types of Ruminal Microbes

Specific microbes within the rumen differ in class (bacteria, archaea, protozoa, fungi, yeasts), population, the classes of compounds they ferment, and the products that they produce (Hungate 1966). Some organisms cultured from the rumen are opportunists that are consumed coincidentally with feed or water. However, the majority of rumen organisms multiply within the rumen and, so long as they multiply more rapidly than they are flushed from the rumen, become inherent residents. Billions of bacteria are found within each ml of ruminal contents though the relative population size of various species will vary depending on available substrates and ruminal conditions. Only about half of the bacterial species found in the rumen have been cultured, classified, and identified.

Concentrations of culturable microbes per ml of ruminal fluid can vary daily and with time of the day depending on the supply of available nutrients and dilution by consumed water and saliva. Within the rumen, certain strains of bacteria (facultatives that use diffusing oxygen; ureolytics that cleave diffusing urea) line the ruminal epithelium. But most bacteria are either free floating in the ruminal liquid phase or can be found attached to particles. Attached bacteria can prove difficult to dislodge and count. Though attachment of microbes to fiber is required for fiber digestion, even cellulose digesting bacteria must detach and float freely in order to colonize freshly consumed feeds. This delay for colonization can explain why nutrient fermentation or gas production exhibits a "time lag" before reaching a maximum rate after a new feed is added.

Microbes involved with degradation of feed components and catabolism of monomers (sugars, amino acids, fatty acids) have received the greatest research attention. Compared with aerobic organisms, anaerobic ruminal microbes require critical culture conditions. Although some ruminal microbes are facultative and can survive with oxygen present, most are strict anaerobes and cannot survive in the presence of oxygen. Facultative microbes readily remove oxygen consumed with feed and water and the small amount of oxygen that diffuses into the rumen from the blood stream to maintain anaerobic conditions within the rumen. Presence of oxygen would permit oxidative respiration that would increase ATP yield from substrates and thereby increase the yield of microbial mass, a change that might prove useful under certain conditions. However, oxidative respiration within the rumen would deprive the animal of energy. If substrates were fully oxidized during respiration, not merely partly oxidized to yield the VFA that provide the ruminant with energy, supply of energy for the host animal would be

reduced. To maintain a balance between the supply of energy and the supply of protein for the host, some researchers have theorized that ruminal outflow of liquid and rumen turnover might be regulated based on its protein content. This is supported by the finding that protein content of duodenal chyme across a wide variety of forage and concentrate diets is surprisingly constant. Perhaps this concept could be employed to relieve partially the bulk fill limits to feed intake and increase ruminant productivity under certain conditions.

Traditionally, bacteria were classified based on substrates used and their fermentation products. Today with accessibility of genetic identification, genetics rather than metabolism are used for classification. Genetic typing links bacterial types based on genetic relationships and evolutionary background and can help identify species that possess certain useful genes that may be expressed. However, genetic information alone fails to provide information about degree that specific genes are used and active metabolism of a species. Certain bacterial strains with similar metabolism often are revealed to not be related genetically whereas other strains closely related genetically will differ markedly in their substrate preference and end products. Though a bacterium must possess a specific gene if it is to perform certain reactions or digest a specific compound, gene presence alone fails to reflect activity or metabolism of the microbes being characterized or even viability. Indeed, mRNA prevalence rather than DNA should more closely reflect metabolic activity.

When isolated in pure culture, a given bacterial strain often requires a specific nutrient for growth (e.g., most cellulose digesting bacteria require branched chain fatty acids or amino acids) that yield these acids and many will produce compounds (e.g., succinate, ethanol) not found within the rumen where mixed cultures exist. Cross-feeding among mixed cultures, where one species will produce or metabolize products of other species results in and readily explains differences apparent between results of pure culture studies and ruminal metabolism. Indeed, synergism among bacterial strains and synergism between protozoa and bacteria typically increases extent of ruminal digestion and alters the ratios of end-products (especially methane and VFA) of fermentation. Synergism between the numerous bacterial classes and strains within the rumen often renders data related to specific requirements for and products of a microbial strain when grown in isolation completely invalid and inapplicable under ruminal conditions where substrates and products represent the composite metabolism of a diverse mixture of bacterial strains. Various classes and strains of ruminal microbes have been outlined in Chap. 2 of this book.

Ruminal Fermentation

Physical aspects and microbial types found within the rumen are described in Chap. 2 of this book. This chapter will cover the degradation of feedstuffs as well as formation of specific products during the ruminal fermentation process. Within the rumen, substrates typically are degraded to monomers that subsequently are fermented or metabolized quite rapidly to carbon dioxide and methane. An overview of this process is presented in Table 3.3 and Fig. 3.1.

Table 3.3 Substrates and products of ruminal fermentation

Feed component	Polymer	Monomer(s)	Products of fermentation
Carbohydrates			
Starch	Amylose	Maltose, Glucose	VFA, esp. propionate
	Amylopectin		
Neutral detergent fiber			
Cellulose	B1,4-glucosan	Cellobiose, Glucose	VFA
Hemicellulose	Pentosan	Pentose sugars	VFA, esp. acetate
Crude fat	Triglycerides	Fatty acids	Saturated fatty acids
	Phospholipids	Glycerol	Propionate, P
	Galactosides	Galactose	VFA
Sugars			VFA
Crude protein			VFA
True protein	Protein	Amino acids	Ammonia & VFA
Non-protein N	–	Urea	Ammonia and CO_2
DNA, RNA	Nucleic acids	Purines, pyrimidines	Ammonia and CO_2
Crude ash	–	Minerals	Reduced minerals

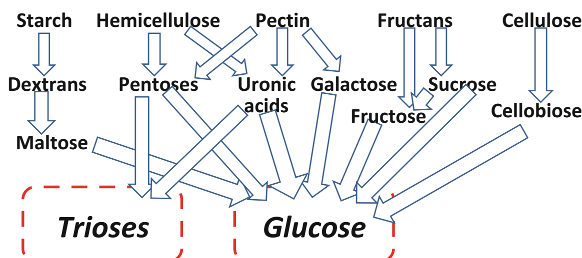

Fig. 3.1 Ruminal conversion of polymers to simple sugars for fermentation

Limits to Ruminal Digestion

Most feedstuffs are composed of a mixture of chemically diverse components physically encapsulated within plant or animal cell walls. Before internal components can be degraded, physical structures or barriers designed to protect plant or animal tissues from microbial or insect attack must be breached or fractured so that microbes and enzymes have access to components internal to the seed or tissue. External or encasing barriers include the pericarp of grains and oilseeds and the primary and secondary cell walls of plants and animal tissues. Within cereal grains, additional physical barriers (e.g., protein-coated or encapsulated starch granules) and hydrophobic regions (e.g., starch granules imbedded in prolamin) provide additional protection from enzymatic digestion. Likewise, within plant cell walls, the more digestible fiber components (e.g., hemicelluloses) usually are cross-linked with indigestible lignin that in turn serves to guard the fiber complex from attack.

Feed Processing

Physical barriers are breached to varying degrees by mechanical processing of feedstuffs before a feedstuff is fed to animals. Feed processing methods can vary from simple methods of comminution to reduce the particle size (grinding), crushing (rolling) that fractures particles with or without added moisture or heat (steam rolling, flaking, extrusion), microbial acidification that softens particles and structures (fermentation), or application of chemicals, enzymes, or microbes (base, acid, or enzyme treatment or inoculation). Because ruminants chew food being consumed, no grinding method will precisely match the particle exposure and sizes entering the rumen.

A reduction in particle size increases the rate of fermentation primarily through exposing a greater surface area for bacterial attachment for digestion. Mean particle size and its distribution differ among various processing methods (rolling versus grinding). Slicing or abrasion through a hammer mill results in a much wider diversity in particle size than crushing between rollers of a roller mill. Rollers result in both fewer fine particles and fewer coarse particles than grinding. Thus, rolling is preferred for producing a milled product of consistent size. The shear forces generated by rolls set at differential speeds will shred the edges of particles exposing more surface area for microbial attachment and fermentation. Screening diets or processed feeds through multiple sieves that differ in hole size (e.g., Penn State Separator; Z-box) gives an indication of particle size of an ingredient or a mixed diet (length being estimated by horizontal shaking; diameter by vertical shaking). Wet filtration can differentiate particles by density. Sieving methods usually are employed to appraise “effective fiber,” an estimate of the capacity of forages to stimulate rumination and saliva input, and be responsible for bulkiness that can limit feed intake. Through comparing the particle size of screened feeds and orts one can quantify the degree that an individual animal or a group of animals is sorting their feed.

Geometric mean diameter is commonly used as an index of particle size and potential for digestion. Coarser particles of a feed typically are less rapidly fermented and, when not retained for a sufficient time in the rumen, will be less extensively fermented than finer feed particles. However, geometric mean diameter provides only a single index of the total spectrum of particle sizes, not the relative prevalence of the coarse and fine particles. If only the coarser particles resist fermentation and digestion, then some measurement of particles above a specific size or geometric mean diameter would seem preferable for predicting rate of digestion. One method for calculating the amount of surface area exposed from sieved samples was outlined by Baker and Herman (2002).

Chewing to Reduce Particle Size of Feeds

Feeds consumed by ruminants are chewed to reduce particle size and increase the surface area available for microbial or enzymatic attachment or attack. The amount of time that ruminants will chew their feed prior to swallowing appears directly related

to the amount of time an animal needs to produce sufficient saliva so that moistened feed can be swallowed. As a result, adult ruminants with larger more developed and active salivary glands produce copious amounts of saliva so they consume their diet rapidly and swallow their feed with considerably less chewing than young ruminants. Consequently, the need for and benefit from processing grain and forage is greater for adult ruminants than young, growing animals. Similarly, moist feeds generally are consumed more rapidly with less extensive chewing than dry, coarse feedstuffs. In contrast, high moisture feeds and forages can be readily swallowed with little chewing. Because they have smaller mouths and consume smaller bites and they spend more time chewing, the extent and effectiveness of chewing prior to swallowing, is greater for smaller ruminant species (sheep, goats) than larger species. Hence, the digestibility response to feed processing generally is less for the young and for smaller ruminant species than for adults and for larger ruminant species.

Rumination and Particle Retention

During rumination, moistened partially fermented feed particles are re-chewed. Because ruminated particles are moist, particle size reduction is more extensive than with dry particles. The circular jaw motions during rumination allow molars to grind and pulverize particles via both shear and crush forces. Churning of ruminal contents by the extensive ruminal muscles also reduces particle size by abrasion among feed particles and by contact with the rumen wall.

The duration of time animals spend ruminating is proportional to the quantity of fiber floating in the ruminal raft. This is because the rumination reflex is stimulated by sensory (scratch) receptors in the cardia area of the rumen. Dry, fibrous particles that enter the rumen being buoyant will float and become entangled in the floating fibrous mat in the rumen. In contrast, dense particles, intact grains and concentrates can readily sink in rumen fluid and often are flushed from the rumen to the omasum rapidly. Within the rumen, particles near the omasal orifice will be swept from the rumen with fluid whenever the omasal orifice is open. Because most particles leaving the rumen and found in feces are smaller than 1.14 mm, omasal filtration in the past was proposed to be one method by which the rumen selectively retained larger feed components for prolonged fermentation. However, whole grains (>6 mm geometric mean diameter) often are found in feces of cattle fed grain and concentrate particles can be recovered from the abomasum during or immediately after a meal. This presumably reflects the fact that ruminal location, not omasal filtration, dictates the likelihood that a specific feed particle will leave the rumen. Location of particles within the rumen varies with density or buoyancy of the particle, its size, and its entanglement with the ruminal mat. The optimal specific gravity of particles for ruminal exit is between 1.0 and 1.2; particles with greater density (e.g., metal, sand) will settle and be retained longer in the ventral rumen while lighter, more buoyant particles float in the rumen and be retained longer. Continual mixing and churning of ruminal contents pumps liquid digesta containing particles from the reticulum over

the ruminal mat allowing digesta to percolate through the mat. Size and shape of the particle as well as composition and thickness of the ruminal mat size dictate the degree that particles are caught in the mat and selectively retained within the rumen. Though forage concentration in the diet and other forage characteristics associated with particle size and forage type will alter the thickness and porosity of the raft, these factors have received limited research attention. Ruminal stratification along the horizontal plane with the ruminal mat that is present when coarse fibrous materials are fed is widely recognized. However, when ruminants are fed concentrate-rich diets or only finely ground and dense fiber, the rumen may have no ruminal raft.

In addition, to horizontal stratification, vertical stratification within the rumen also seems evident. Undigested particles leave the rumen by passing through the omasal orifice. That orifice is located near the junction of the reticulum and rumen in close proximity to the point that the esophagus enters the rumen. (See discussion of the cardia and the closure of the esophageal groove in Chap. 1 of this book.) Being near the point where feed, water, and remasticated boluses enter the rumen, digesta near the cardia has higher moisture content than other portions of the rumen, likely due to incomplete ruminal mixing of salivary fluids and drinking water from this area with the full contents of the rumen. Rapid omasal exit of fluid from this area shortens the ruminal retention time for such fluids as well as any dense, fine particles found within this region of the rumen. A shortened ruminal retention time for consumed water than for total water within the rumen also may be the result of horizontal and vertical stratification of ruminal contents. How diet and degree of ruminal fill physically alter stratification and retention time need further research attention.

Digestion and Fermentation of Various Feed Components

Most components of feeds and forages exist as polymers (Table 3.3). In contrast, microbial fermentation involves degradation of monomers. The extent to which polymers are degraded to monomers within the rumen differs with substrate type. Before being fully fermented, carbohydrates must be hydrolyzed to glucose or trioses that subsequently are fermented; intact proteins are hydrolyzed to amino acids that are rapidly deaminated forming ammonia and linear or branched chain VFA; nucleic acid polymers are hydrolyzed to purines, pyrimidines and phosphorus; and accessible lipids are partially hydrolyzed to free fatty acids and glycerol within the rumen. Extent of degradation of a polymer also differs with the time for and accessibility of components for microbial or enzymatic attack. Once released, monomers or dimers are rapidly fermented if microbes can generate ATP from their fermentation. (Lack of ATP yield can explain why triglycerides composed of saturated lipids are not readily or rapidly hydrolyzed.) In discussion below various factors that influence the rate and extent of degradation of polymers will be outlined and fermentation of monomers to various products will be discussed. Monomer degradation always occurs internal to microbial cells, but much of the degradation of polymers occurs externally. However, this distinction is not universal. For example,

protozoa act as micro-ruminants continually engulfing and digesting both small feed particles and bacteria. And certain polymers (proteins, soluble starch) may be internalized intact before being degraded by certain species of ruminal bacteria. In contrast, coarser feed components (e.g., fiber), due to their immense size relative to bacteria, are attacked by enzymes external to but yet associated with bacteria. The shortened polymers or dimers when released are immediately internalized by adjacent bacteria, cleaved, and fermented.

Degradation of Polymers

Food components (e.g., sugars, amino acids, some polymers) that are soluble in rumen fluid as well as the soluble cell contents released when cell walls are breached are readily and rapidly attacked. True digestion of the non-starch cell contents in the total digestive tract is very high (98 %). Relative to true digestion, apparent digestion values always are lower because some fecal material originates from the animal's body, not just the diet. So fecal matter includes not only the residue remaining from undigested feed but also metabolic matter from the animal due to incomplete re-digestion and resorption of secreted enzymes and lipids as well as sloughed intestinal cells that are abraded by passing digesta. Extent of degradation of polymers to monomers both in the rumen by microbial digestion and in the intestines by secreted enzymes will vary among polymer types as well as characteristics and accessibility of the polymer. Because microbes and enzymes are transported in an aqueous media, any components that inhibit water access (hydrophobicity) hinder both the rate and extent of digestion.

Starch Hydrolysis

Of the dietary starch consumed, from 40 % to 90 % typically disappears before leaving the rumen. Rate of degradation of starch will vary with microbial accessibility of the starch granules (faster with finely ground grains), degree to which fed starch is embedded within a hydrophobic protein matrix (slower from more vitreous or flint corn or sorghum grains than from softer grains; slower for corn and sorghum than for other cereal grains), the degree of degradation of the prolamins that encapsulate starch granules (faster with fermented grain or silage), and time for ruminal fermentation (less with shorter ruminal retention time). High feed intake of diets rich in NDF, through reducing ruminal retention time of particles, reduces the extent of ruminal digestion of both concentrate and forage particles. Electron microscope photos from McAllister et al. (1994) clearly illustrate that ruminal bacteria bore holes into starch granules to hydrolyze starch. Rate of degradation also differs with starch structure. Amylopectin, with a branched structure exposes more non-reducing terminal glucose molecules for endoglucanase attack than the more linear amylose. Hence, amylopectin is more

rapidly and extensively fermented than amylose. Extent of ruminal starch digestion is considerably greater for small grains (oats, barley, rye) than for grains that have a larger fraction of starch in the vitreous or flint form (sorghum grain, dent or flint corn). Starch gelatinization (i.e., disruption of starch granules through heat and steam processing of grains) increases the amount of starch exposed for digestion and increases rate and extent of starch digestion, especially for grains with a high proportion of vitreous starch. However, during the flaking process, surface smearing of the starch with melted prolamin may retard microbial access to some of the starch present. With ensiled high moisture grain or corn silage, the fermentation process disrupts surface proteins and decreases hydrophobicity so rate and extent of starch digestion within the rumen are increased. Increased exposure for attack by ruminal bacteria can explain the greater ruminal starch digestion of steam flaked and high moisture maize than dry rolled maize (84 % and 78 % versus 68 % of dietary starch averaged across published trials with feedlot cattle). In contrast, with lactating cows, ruminal starch digestion with these same processed grains has been reported to be 57, 87, and 52, all being lower due to shorter retention time for ruminal digestion combined with the time lag involved with moistening of consumed dry feeds.

Within the intestine, hydrolysis of starch by intestinal beta amylase yields the dimer maltose that subsequently is hydrolyzed to glucose by maltase. In contrast, degradation of starch within the rumen may not involve maltase but instead appears to be driven primarily by endoglucoamylases (that cleave glucose links within the chain) and exoglucoamylases (that cleave glucose from the reducing ends) yielding glucose directly, not with a maltose intermediate.

Both bacteria and protozoa possess amylases. Because protozoa engulf small feed particles, finding amylase internal in protozoa that hydrolyze starch is not surprising, but amylase also has been located within ruminal bacteria. Nevertheless, photomicrographs indicate that digestion of starch granules usually involves external or surface-bound amylases. By increasing the concentration of free amylase in rumen fluid, microbial lysis has been proposed to increase the rate of glucose release and thereby might increase the likelihood of ruminal lactic acidosis. That will be discussed further in Chap. 5 of this book.

Cell Wall Degradation

Plant cell walls are the least extensively digested organic components found in feeds. Chemical links between hemicellulose and lignin through ferulate ester and ferulate ether bonds both physically and chemically restrict microbial access to cell wall components and retard or inhibit cell wall digestion. Although free hemicellulose is fermented more rapidly than cellulose in vitro, the extent of degradation of these two components of NDF from grasses in the total digestive tract in vivo typically is quite similar in magnitude. Presumably, this reflects steric interference with attack of closely adjacent structures. Treatment with base (sodium hydroxide; calcium oxide) cleaves some of the linkages between hemicelluloses and lignin and deacetylates lignin. Likewise, grasses or legumes

with lower lignin content (e.g., brown midrib mutants) have an increased rate of digestion of NDF and contain less indigestible NDF. Pectin, more prevalent in legume than grass cell walls, is fermented by pectinases within the rumen even though pectin appears to resist degradation during fermentation of ensiled legumes. Lignin, a polyphenol and not truly a carbohydrate, resists anaerobic digestion but, as mentioned earlier, can be degraded aerobically. Lignin can be attacked or solubilized to a limited degree by ruminal fungi presumably through the action of peroxidases, but lignin is not digested by bacterial or protozoal enzymes. Lignin is not uniformly distributed within all plant tissues; more lignified fragments or tissues are more resistant to attack. Because lignin is not extensively fermented or digested, virtually all dietary lignin is excreted in feces. Hence, lignin can be used as an indigestible marker internal to feeds to calculate the digestibility of other feed components by difference. When all feces are collected, lignin also can be used as an indicator of feed intake of grazing ruminants if lignin intake is quantified through analysis of a representative sample of clipped forage samples or of esophageal samples. Differences in its size of lignin polymers and reduction in size can complicate plant analysis and lead to incomplete recovery of lignin from feces.

Degradation of the pentosans in pectin and hemicelluloses, like degradation of the hexosan cellulose, appears to involve complexes of multiple enzymes generally classed as hemicellulases and beta-glucanases. Like cellulases, these enzymes presumably are surface bound or associated with the glycocalyx surface films of specific bacterial strains. The ratios of specific pentose sugars in hemicellulose (arabinose and xylose), whether through steric hindrance or cross linking, can alter the susceptibility of hemicellulose to hydrolysis. Likewise, cellulose exists in both an amorphous and a crystalline type with various cellulolytic bacteria differing in their capacity for fermenting the more resistant crystalline cellulose. Pentosans appear to be degraded directly to component monomer pentose sugars whereas cellobiose, 1,6-beta linked glucose, is an intermediate in cellulose degradation. Furthermore, pentosans appear to be largely converted metabolically to hexose and triose through the pentose cycle before being fermented.

Bacteria must attach to cell walls before digestion can begin. Attachment may be limited because both feed particles and bacteria appear to be negatively charged. Based on in vitro studies, additions of bicarbonate, biotin, and trace minerals appear to accelerate bacterial attachment to feed particles. Methods to accelerate the rate of attachment of ruminal microbes to fiber deserve further research attention because reducing the time lag before fermentation begins allows rate and extent of ruminal fiber digestion to be enhanced. Fractures of the cell wall or pores in cell tissues help to accelerate cell wall hydrolysis. Because the cellulolytic microbes within the rumen associated with plant tissues typically are found internal to plant tissues, they are envisioned to digest fiber from the inside outward. Such an internal location also helps protect bacteria from protozoal predation. Alternatively, their internal location helps to prevent bacteria from being dislodged during sample preparation for microscopic examination.

Lipid Hydrolysis

Lipids isolated from oilseeds and animals are primarily tri-acylglycerides while lipids of growing plants and forages include substantial amounts of phospholipids and galactolipids. Triglycerides are hydrolyzed to a varying degree within the rumen. Lipids within oilseeds are hydrolyzed only partially to diglycerides and monoglycerides while triglycerides added to the diet as an oil appear to be completely hydrolyzed to glycerol and free fatty acids within the rumen. Heat treatment of oilseeds reduces the extent of lipid degradation within the rumen, likely due to some alteration of proteins associated with the seed, not due to some direct impact on the oil. Ruminal lipolysis appears more extensive when oils are dosed more frequently; this reflects the concept that hydrolysis by ruminal bacteria or protozoa is less aggressive for lipids than of carbohydrates. Released long-chained fatty acids that are saturated are largely inert within the rumen. Compounds (amino acids; vitamins) can be coated or complexed with lipid; being protected from microbial attack, such compounds are marketed widely as "ruminal escape" compounds or complexes. In contrast with the largely insoluble long chain fatty acids, medium chain length fatty acids can prove toxic to some species of ruminal bacteria. The ruminal population of protozoa can be decimated (partially or completely defaunated) through feeding proper dosages of lauric and myristic acids.

Although free fatty acids released during lipolysis that are saturated are not degraded further within the rumen, most unsaturated fatty acids are fully or partially saturated by ruminal bacteria and protozoa. Hydrogenation of unsaturated fatty acids is one process by which ruminal microbes dispose of excess hydrogen. Disposal of reducing equivalents is a persistent bottleneck for anaerobic microbes. Besides biohydrogenation, other methods that ruminal microbes employ for hydrogen disposal include methane formation from carbon dioxide and sulfate or nitrate reduction to hydrogen sulfide and to nitrite or ammonia. These processes are retarded slightly when unsaturated fatty acids or triglycerides are included in a ruminant's diet. The quantitative significance of biohydrogenation of unsaturated fatty acids as a method for hydrogen disposal appears minor compared to these other reactions likely is due to the limited supply of unsaturated lipids in the typical diet and the limited number of sites to hydrogenate.

During microbial biohydrogenation of linoleic and linolenic acids in the rumen, byproducts including conjugated trans-fatty acids (conjugated linoleic acid; CLA) are formed that are absorbed and may be further metabolized and deposited or secreted by ruminants. Being a general class of fatty acids, conjugated fatty acids or their precursors of multiple types exist that differ in the location of their double bond, the structure of that double bond (cis or trans), and the number of double bonds. These factors alter biological activity. Certain CLA possess anti-cancer activity; others depress fat synthesis by the mammary gland and reduce the percentage of fat in secreted milk. Industrially, trans-fatty acids are produced when unsaturated plant oils are hydrogenated. Hydrogenation decreases fluidity and increases shelf life of food products. However, industrially produced trans-fatty acids have adverse effects on cardiovascular health. In contrast, the trans-fatty acid

CLAs found in milk and meat from ruminants, being potent anticancer compounds, are highly desirable and healthful food components.

The fatty acid composition of deposited triglycerides in all species of animals reflects the composition of those fatty acids synthesized by the body when merged with those fatty acids absorbed from the small intestine. As intake of fat increases, biosynthesis by the liver in non-ruminant species or by depot tissues of animals decreases so that synthesized fatty acids comprise a smaller portion of the lipid deposited. Including unsaturated fats in diets for swine and poultry can result in "soft" or "oily" fat. This is not the case for ruminants because of extensive biohydrogenation of dietary fats by microbes in the rumen. This in turn means that the fatty acids present in depot fat or milk fat from ruminants is more saturated than fat from poultry or swine. Because intake of large amounts of fat containing saturated fatty acids has been correlated with certain cardiovascular diseases, intake of fat from ruminant animals as well as certain tropical (e.g., palm and coconut) oils, that are even more saturated than fat from ruminants, should be limited according to certain health professionals.

Nearly half of the fatty acids in milk or meat from ruminants are mono- or polyunsaturated fatty acids. Nevertheless, increasing the concentration of unsaturated and omega-3 fatty acids in the human diet even further has been suggested to have health benefits for some individuals. To increase the concentration of unsaturated or selected fatty acids in ruminant tissues, the postruminal supply of unsaturated fatty acids can be increased or the prevalence of desaturases could be increased. Supply can be increased by feeding coated lipids or heat-treating oilseeds. However decreasing the saturation of ruminant fats simultaneously increases its potential for oxidative rancidity that in turn shortens the shelf-life of milk, milk products, and meat. Increasing the dietary supply of certain antioxidants (e.g., vitamin E) that are deposited in tissues or excreted with milk helps to retard oxidative rancidity and lengthen the shelf life of ruminant products.

Net energy content per unit of weight is greater for lipids than for protein or carbohydrate. Consequently, increasing the concentration of dietary lipid to as much as 6–8 % of feed dry matter will increase net energy content of the diet. This in turn often decreases feed (but not energy) intake. Concurrently, adding fat to the diet often increases carcass value by increasing the dressing percentage (ratio of carcass to live weight) and may increase deposition of intramuscular fat to enhance meat flavor. Dietary lipid concentrations above 8 % often result in decreased fat digestibility, perhaps due to insufficient saponification or lack of lipase in the small intestine. Supplemental lipids often decrease digestibility of fiber, as well. Whether this decrease is due to lipid physically coating fiber particles or antimicrobial activity of fatty acids is uncertain. High intakes of fat from certain unsaturated oilseeds (sunflower, cottonseed) and from fish oils occasionally have had adverse effects on milk and on the flavor of cooked or re-heated meat, probably associated with oxidative rancidity.

The amount of fat included in diets formulated on a least-cost basis will vary with the relative cost of net energy from fat versus other sources of energy. When cost per unit of net energy is lower for vegetable or animal fat than other sources of energy including fat in a diet will reduce cost of gain. In addition, when dietary

bulkiness and rumen fill limits energy intake, as early in lactation of high producing dairy cows, added fat, through increasing energy intake, often increases the level of milk production. Addition of various liquids (fat, molasses, water, distillers' solubles) or of moist feed (wet byproducts, silages) to a diet often is desired to reduce separation of fine particles from the fibrous components of the diet. By preventing diet sorting by livestock and avoiding segregation of diet components during mixing or in the feed manger, the incidence of metabolic disorders (bloat, acidosis) usually is reduced. Diet sorting, though difficult to detect with group-fed ruminants, can lead to imbalanced diets whereby some animals in a pen will select a high concentrate diet leaving others with the residual fiber or vice versa. Diet sorting also can be reduced by decreasing the supply of excess feed, reducing the diversity in particle size of the various feed ingredients, or by providing adequate bunk space so that all animals within a pen can eat simultaneously. To reduce heat stress of high producing lactating cows, fat often is added to the diet. Because fat is not fermented as extensively is carbohydrates in the rumen, heat from ruminal fermentation is reduced and the heat increment during metabolism also is lower for fat than for carbohydrates because fatty acids can be deposited directly avoiding the need for fatty acid synthesis from carbohydrate that generates heat.

For high producing lactating cows, including oilseeds (soybeans, cottonseed) as a source of dietary fat in the diets helps to increase energy density of the diet and feed intake early in lactation when heat production is high and bulk fill may limit feed intake. Instead of oilseeds, most lipid included in feedlot diets is supplemented as vegetable oil, tallow of various grades, or blends of vegetable and animal fats. Antioxidants (e.g., ethoxyquin) usually are included in such fat sources to avoid oxidative rancidity that can reduce feed intake due to altered odors and flavors of feeds. Byproducts of ethanol production also contain substantial amounts of fat; this can explain partially why such products appear to have more net energy per unit of weight than the grains used to produce ethanol. Further discussion of ruminal digestion and metabolism of fat is available from Palmquist and Jenkins (1980), Bauchart (1993), and Bauman and Lock (2006), and in Chap. 4 of this book.

Protein Hydrolysis

Crude protein, by definition, is nitrogen content of a sample multiplied by 100/16 = 6.25 (based on the fact that peptide-linked amino acids chains contain about 16 % N). Because N is used as the index of protein content of a food or feed mixture, crude protein includes numerous compounds such as non-protein N (urea, biuret, ammonium salts, amino acids, and purines), as well as the peptide-linked amino acids of true protein. True proteins in turn differ in amino acid content, structure, and composition. Structure and amino acid composition in turn alter solubility of a protein in various solvents, one standard method used to classify proteins. Among various protein types, proteins more soluble in water or salt solutions (albumens and globulins) are more extensively degraded by ruminal bacteria than less soluble protein types (glutamines, glutelins). This supports the

general concept that compounds that are soluble in ruminal fluid are more likely to be attacked and degraded by ruminal bacteria. Because protein solubility also varies with pH of the solvent, differences in ruminal pH, through altering protein solubility, also alters the extent of ruminal degradation of a protein sources. Strangely, soy proteins generally are more soluble at a neutral pH whereas the proteins from corn grain tend to be more soluble at a lower pH. In contrast to bacteria that digest soluble protein and attach to and attack feed particles from the outside, protozoa engulf feed particles (as well as bacteria) and readily digest all protein types. Predation of bacteria by protozoa leads to intra-ruminal degradation of bacterial protein, a very inefficient process energetically. As a result, ruminal defaunation (reduction or obliteration of protozoa through dietary manipulation or additives) often improves energetic efficiency and reduces the amount of dietary true protein that needs to be supplemented. The population of protozoa within the rumen typically is lower for ruminants fed concentrate-rich than forage-rich diets. This difference should reduce the amount of true protein degraded in the rumen, increase the supply of both dietary and microbial protein that reaches the small intestine, and decrease the need for dietary protein. Nevertheless, the crude protein content of diets fed to feedlot cattle has tended to increase in recent years, particularly when the grain being fed is extensively processed (e.g., steam flaked). Much of this added protein is urea that is degraded to ammonia within the rumen. Precisely why the added ammonia might be beneficial is uncertain. Certainly, additional ammonia is needed when grains are processed to meet the increased need of ruminal bacteria for growth with more extensive ruminal fermentation of starch. In addition, ammonia will serve as a base to neutralize ruminal acids and increase urinary output. This in turn will increase water intake and ruminal fluid turnover. Synthesis of urea also may prove beneficial for maintaining a proper blood acid-base balance. Dietary urea also appears to reduce meal size and increase meal frequency, factors that may increase mastication time and production of saliva, and extent of ruminal and total tract digestion.

Cyclic proteins (e.g., ovalbumin) with no exposed terminal amino acids are not degraded by bacteria. Likewise, processing methods that block terminal amino acids, that amalgamate proteins to reduce solubility, or that encapsulate protein or amino acids can retard degradation of dietary proteins. Surprisingly, proteins with a longer chain length often are more rapidly and extensively degraded than shorter peptides, perhaps due to more rapid or efficient uptake of longer amino acid chains by certain species of bacteria. Strangely, many of the actively proteolytic bacterial species in the rumen cannot use amino acids for growth. This indicates that some proteolytic species must be using the liberated amino acids as an energy source for generating ATP for microbial growth and multiplication. However, the quantitative importance of protein as an energy source for growth of ruminal microbes appears limited. Some models developed to predict microbial yield from ruminal fermentation are based only on carbohydrate and ignore any contribution of ATP from degraded protein or fat.

Like proteolytic enzymes of mammals, the proteolytic enzymes of ruminal microbes are of two general types: those that attack amino acids at the end of a protein chain (exopeptidases) and those that cleave peptide linkages between

specific amino acids or amino acid types (endopeptidases). With mammals, the activity of specific endopeptidases can be inhibited by providing peptides or peptide analogs that cannot be hydrolyzed. Such inhibitors have failed to reduce proteolytic activity within the rumen. Some researchers have interpreted this to mean that ruminal microbes possess diverse types of proteases and thereby cannot be inhibited. However, any inhibitor that is not internalized by microbes would not be located at the primary site of proteolysis.

Diets for ruminants often contain non-protein nitrogen (NPN) products as a source of ammonia for bacterial growth. Whether derived from the diet or from recycling to the rumen via saliva or diffusion from blood through the rumen wall, urea is hydrolyzed rapidly to ammonia and carbon dioxide by high populations of ureolytic bacteria in the rumen. Ruminal entry of urea from blood is enhanced by facultative ureolytic bacteria associated with the ruminal wall that hydrolyze urea to ammonia near the rumen wall; released ammonia is rapidly ionized and removed into the rumen because ruminal pH is always lower than the pK (9.3) of ammonia.

Rate of urea hydrolysis within the rumen is reduced by adaptation of ruminants to diets containing urea. Adaptation of animals to urea diets results in a reduced meal size but an increased meal frequency, changes should help reduce the incidence of ammonia intoxication. Urea hydrolysis also is subject to end-product inhibition. Consequently, most cases of urea (ammonia) intoxication involve animals that are not adapted to diets containing urea or animals gaining access to a diet that contains an excessive amount of urea. With abrupt intake of urea and hydrolysis to ammonia, ruminal ammonia concentrations can exceed 100 mg/dl. When combined with a high ruminal pH that speeds ammonia absorption, ammonia toxicity (often erroneously called urea toxicity) can occur. Ruminal fluid has limited base buffering capacity above a pH of 6.9, so ammonia, acting as a weak base (pK = 9.3), readily elevates ruminal pH. This ruminal pH increase is relevant because ruminal absorption always is greater for non-ionized compounds (ammonia, free fatty acids) than for their ionized counterparts (ammonium, ionized fatty acids). Ammonia will be absorbed more rapidly than the ammonium ion. (Conversely, as ruminal pH drops, more fatty acids are protonated and thereby are more rapidly absorbed from the rumen.) Ammonia intoxication can be avoided by dosing animals with an acids (e.g., vinegar) that, by reducing the amount of protonated ammonia, will reduce ruminal absorption. The liver readily and actively detoxifies any ammonia in blood to urea and prevents its accumulation. Presumably, ammonia uptake by the lymphatic system, by circumventing liver detoxification, is responsible for elevated blood ammonia levels that cause recombency and death associated with ammonia (urea) toxicity.

To retard rate of ammonia release within the rumen and avoid ammonia toxicity, modified NPN sources and slow-release encapsulated urea often are substituted for urea in the diet. Like urea, uric acid from poultry waste also is degraded to ammonia that can result in ammonia intoxication, but other NPN sources or complexes (biuret, triuret, cyanuric acid) are slowly degraded by ruminal microbes. Microbes usually adapt to such products after several days or weeks. Coated urea

products, though useful to prevent ammonia toxicity, have proven to be no more useful than urea as a source of ammonia for bacterial growth or for ruminant production. Urea recycling to the rumen is extensive when a diet contains an adequate concentration of either digested protein or urea. Slowing or attenuating the ammonia release rate of NPN compounds in the rumen to match the rate of carbohydrate degradation, though an intriguing theory supported by lab experiments and batch culture fermentation studies, is not supported by trials with animals. This likely is due to the high extent and efficiency of recycling of urea to the rumen. Under field conditions, high meal frequency (often 6–12 meals) and continuous digestion of a large mass of digesta in the rumen accumulated from previous meals helps to avoid asynchrony between carbohydrate and protein availability for ruminal microbes (Fig. 3.2). Through gradually releasing its ammonia, encapsulated urea also may help buffer ruminal pH postprandially. Thereby, attenuated ammonia release may inhibit decreases in ruminal pH that inhibit activity of cellulose digesting ruminal bacteria.

Similar to proteins, peptides are degraded to amino acids when incubated with ruminal fluid. Calculated as crude protein minus ammonia and precipitable protein, peptides differ in amino acid composition. Even though concentrations of free amino acids usually are too low to be detected in rumen contents, peptides often reach detectable concentrations in rumen fluid indicating that peptides are less rapidly degraded than amino acids. Though peptides appear to be required for growth of certain isolated strains of ruminal bacteria, the background concentrations of peptides in rumen fluid when combined with cross feeding among bacterial species appear to supply more peptides than needed to meet nutrient requirements of the mixed microbial cultures found in the rumen. Nucleic acids from RNA and DNA in the diet or synthesized by microbes within the rumen, if free, are degraded to purines and pyrimidines within the rumen. Nucleic acids also can be re-used for nucleic acid synthesis by ruminal microbes. A schematic of ruminal N interchange was developed by Satter (1978) as shown in Fig. 3.3 and a summary of nitrogen metabolism of ruminants was compiled by Owens and Bergen (1983).

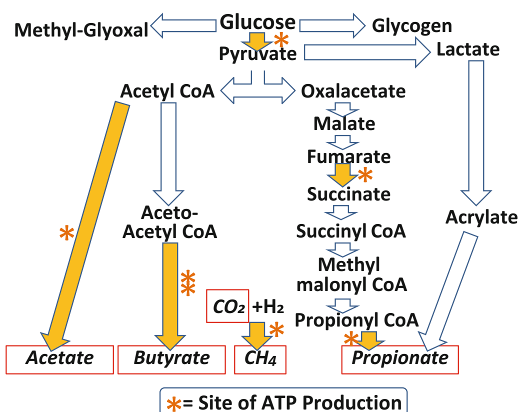

Fig. 3.2 Ruminal conversion of available sugars to fermentation products. See Baldwin and Allison (1983) and Wolin (1960) for further details

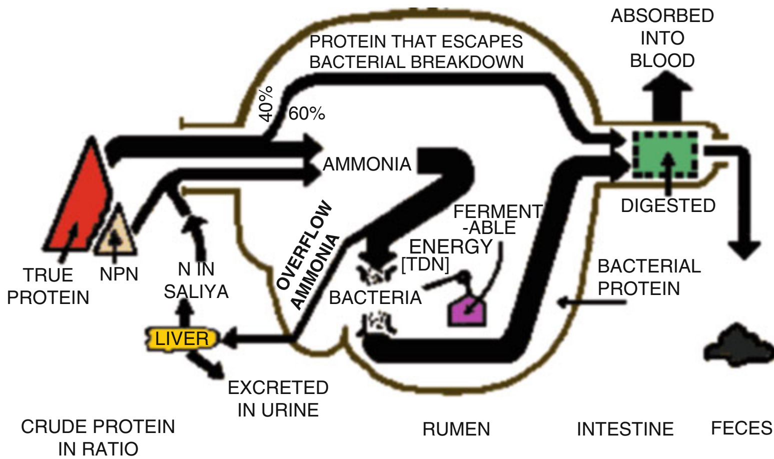

Fig. 3.3 Schematic summary of nitrogen utilization by the ruminant. Source: Satter 1978. Minnesota nutrition conference proceedings

Minerals

Availability of monovalent and divalent cations to microbes within the rumen differs with their solubility; soluble cations are readily available while insoluble cations and anions (e.g., defluorinated phosphate; sulfides; some carbonates) are not. However, minerals that can be solubilized by acid in the abomasum and absorbed in the intestines (e.g., defluorinated phosphates) can be used when they are recycled to the rumen via saliva. Similarly, RNAse produced in high concentrations by the ruminant pancreas, degrades microbial nucleic acid polymers. These phosphates may comprise as much as 4 % of bacterial dry matter. Liberated phosphorus is recycled to the rumen. Within the rumen, soluble sulfates from water or consumed feed are partially reduced to sulfide within the rumen for disposal of excess hydrogen. These sulfides also react with various divalent cations (e.g., copper, zinc) forming sulfides that are insoluble and thereby are not available for microbes in the rumen and are not absorbed from the intestine. In a similar fashion, selenate and selenite are partially reduced to selenide within the rumen. These also complex with cations reducing the availability of those cations. When present at high concentrations and when ruminal pH is low, hydrogen sulfide gas is released in the rumen. When inspired into the lungs during eructation of gases, hydrogen sulfide damages lung tissues and together with absorbed sulfate can cause polioencephalomalacia and possibly other persistent lung problems including emphysema.

Mineral supplements provided to ruminants typically are provided as inorganic salts, but minerals chelated with amino acids or protein also are marketed widely. Chelation with amino acids or protein may increase bioavailability of certain minerals through avoiding the formation of insoluble salts; chelation also may help to

avoid antimicrobial effects of certain cations in the rumen. However health, nutritional or performance benefits of chelated or protein bound minerals over inorganic forms of the same mineral have seldom been demonstrated. Acid conditions of the abomasum and upper small intestine appears sufficient to solubilize most mineral salts although certain insoluble salts (sulfides, some carbonates) may not become soluble and thus are not absorbed. For optimal cellulose digestion, supplementation with certain minerals (e.g., cobalt to speed bacterial attachment) may prove beneficial. Because ionophores stress sensitive microbes by increasing their energy requirements by causing influx of sodium (or calcium), higher ruminal concentrations of certain cations may increase their effectiveness.

Monomer Fermentation

Polymer degradation yields a mixture of monomers (amino acids, glucose, monovalent cations, some divalent cations). Although all of these monomers can be absorbed passively through the ruminal wall, the ruminal concentrations of amino acids and glucose found in the rumen usually are so low that direct absorption is quantitatively insignificant. Such low ruminal concentrations reflect rapid catabolism of these compounds by ruminal microbes. In contrast, ammonia, sodium, and potassium are readily absorbed through the rumen wall. As with certain polymers, degradation of certain monomers can be an adaptive response of the ruminal population. Thus, in vitro findings may not prove applicable in vivo when ruminal microbes are not adapted to the novel substrate being tested (e.g., amino acid derivatives). Ruminal microbes exhibit an immense ability to adapt to and degrade any organic substance that can be reduced. Note that fully saturated compounds (e.g., fatty acids, polyethylene glycol) and insoluble substances (e.g., protein complexes, plastics) generally resist ruminal fermentation. This permits such compounds to be used as coating materials to enhance ruminal escape of dietary substances of interest.

Glucose

Stepwise conversion of glucose to VFA is illustrated in Fig. 3.2. Once liberated within the rumen, glucose normally is catabolized very rapidly. Some bacterial species accumulate glucose, polymerize it to bacterial glycogen, particularly when those microbes are facing a shortage of some nutrient (e.g., ammonia deficiency) that limits microbial growth. When bacteria containing glycogen or when protozoa containing engulfed starch particles are flushed from the rumen, these stored polymers serve to supplement other polysaccharides that escape ruminal digestion. Thereby, microbial polymers of glucose serve as a source of glucose for the host ruminant to metabolize. Glycogen storage by bacteria is considered to be an energetically inefficiency process. By sequestering glucose, glycogen synthesis uses ATP and glycogen storage reduces the supply of energy immediately available for

growth of a bacterial species. As a result, bacterial yield and yield of microbial protein for the animal is reduced. A second more sinister pathway some ruminal bacteria employ when supplied with an excess of glucose is synthesis of methyl glyoxal, a compound that is toxic to most bacteria (Russell 1998). Appearance of methyl glyoxal in the rumen often precedes ruminal stagnation and ruminal acidosis.

Although the concentration of free glucose in the rumen of productive ruminants usually is very low, often too low to be detected, concentrations can increase to more than 100 mg/dl following grain engorgement. When ruminal glucose concentrations become abnormally high, certain bacterial strains (e.g., Streptococcus bovis) that are inefficient at competing with other rumen microbes under normal conditions, will grow rapidly and produce a copious amount of lactic acid. This can result in subclinical or clinical ruminal acidosis. The elevated ruminal osmolality associated with the high concentrations of free glucose, volatile fatty acids and glucose in the rumen, draws liquids into the rumen. Dehydration of the ruminal epithelium and high localized acid concentrations can damage ruminal tissues resulting in erosion of the stratified epithelium of the rumen, damage the rumen wall, and result in sepsis that can lead to liver abscesses.

When glucose is incubated with ruminal bacteria being grown in pure culture, a wide diversity of fermentation products (succinate, malate, hydrogen gas, ethanol, methanol) often are produced by individual strains (Hungate 1966). But when incubated with mixed cultures or with the complete spectrum of ruminal microbes, the diversity of end-products is markedly reduced due to cross-feeding among bacterial species. Normal end-products of glucose fermentation in the rumen are four major volatile fatty acids (VFA), acetate (2 carbons), propionate (3 carbons), butyrate (4 carbons), valerate (5 carbons) in addition to carbon dioxide and methane.

When starch-rich feedstuffs with very rapid fermentation rates are fed or following engorgement of starch-rich feeds, ruminal acidosis can result. Accumulation of acids, often but not always including lactic acid, reduces ruminal pH, hinders activity of acid-sensitive ruminal microbes (this includes all cellulose digesting bacteria), and halts ruminal motility. When ruminal pH falls below about 5.5, the condition is classified as subacute acidosis; a ruminal pH below 5.0 is diagnosed as acute acidosis and often proves fatal. More detailed discussion and summaries relating to the causes, treatment, and prevention of ruminal acidosis can be found in various publications (Owens et al. 1998; Schwartzkopf-Genswein et al. 2003; Oetzel 2007; RAGFAR 2007) and in Chap. 5 of this book.

Fermentation Balance Based on VFA Production from Glucose

Because most of the energy available for ruminal microbes from diets fed to ruminants is derived from carbohydrate, ruminal conversion of glucose, pentoses, and trioses to volatile fatty acids is the primary source of energy (ATP) for ruminal microbes (Fig. 3.2). If one ignores the carbohydrate converted to microbial organic matter, one can calculate a “fermentation balance” that fully describes the conversion

Table 3.4 Fermentation balance based on VFA produced from glucose

	Fermentation products		
	Acetate	Propionate	Butyrate
Molar ratio of products	A	P	B
Glucose used, moles	0.5A	0.5P	B
Gas yield, moles	A	0	2B
Carbon dioxide, moles	.5A	.25P	1.5B
Methane yield, moles	0.5A	−.25P	0.5B
NADH produced, moles	2A	−1P	2B
ATP yield, moles	2.5A	2.75P	3.5B
Potential microbial yield at 10 g/mole ATP	25A	27.5P	35B
Glucose energy used, mcal	0.3365A	0.3365B	.673B
Energy in VFA, mcal	.2094A	.3672P	.5243B
Methane energy lost, mcal	.1054A	−.0527P	.1054B
ATP energy, mcal	.0175A	0.01925P	.0245B
Heat + ATP, energy	.0217A	.022P	.0433B
Heat loss, mcal	.0042A	.00275P	.0188B

of glucose to VFA (Wolin 1960), the amounts of gases (carbon dioxide plus methane), and the presumed yield of ATP that is available for microbial growth (Table 3.4). Based on these balance equations and the relative oxidation state of the substrates and products, glucose fermentation must yield a specified mixture of VFA and gases. The VFA ratio in turn will vary with substrate type, substrate concentration, and fermentation conditions, particularly pH. The ratios of the VFA produced stoichiometrically dictates the specific amount and the composition of the gases released, energy retention in the fermentation products, and the yield of ATP available for microbial growth. These relationships are illustrated in Table 3.4. By inserting mole ratios of the end products of fermentation that are observed, i.e., acetate, propionate, and butyrate, for A, P and B into each of the formulas of Table 3.4, glucose use and energy yields can be calculated per mole of glucose fermented. Note that these are ratios of VFA produced. The exact ratio of VFA in a sample of ruminal fluid can differ slightly from true ratios produced if and when the rates of ruminal absorption of various VFA differ (Firkins et al. 2006). Consequently, considering VFA concentrations to represent relative VFA production rates can prove erroneous though the magnitude of error generally is reasonably small. To calculate total yield of products daily from a specified diet, one also must determine the total amount of carbohydrate that has been fermented within the rumen or the total amounts of VFAs formed. This can be calculated from disappearance of carbohydrate from the rumen (intake of carbohydrate minus outflow—at the abomasum or duodenum—of carbohydrate).

Though a mixture of VFA is formed during fermentation of glucose, the composition of end-products dictates the amount of hexose that has been fermented, the energetic efficiency of fermentation, and the amount of ATP

available for generating microbial organic matter. The amount of ATP generated, that varies with the VFA ratios formed, must not be confused with the efficiency with which that ATP is used by microbes for growth (Yatp or grams of microbial cells per mole of available ATP).

As shown in Table 3.4, each butyrate (4 carbon atoms) mole formed requires one mole (180 g) of glucose (6 carbon atoms) or 162 g of polymerized glucose as cellulose or starch considering the water involved with hydrolysis. In contrast to only 1 mole of butyrate, 2 moles of acetate (2 carbon atoms) and of propionate (3 carbon atoms) can be formed from a single mole of glucose. All the residual carbon from fermentation of glucose must be lost as gas (carbon dioxide plus methane). Consequently, for each mole of butyrate produced, 2 moles of gas are released. Furthermore, with formation of acetate and butyrate, reducing equivalents (NADH) are formed that must be handled. This excess hydrogen is used primarily to convert carbon dioxide to methane. Four moles of reducing equivalent are used for each mole of methane formed from carbon dioxide. During production of propionate, no gas is generated but unlike acetate and butyrate, some of the excess hydrogen generated during their production is used to form propionate. Consequently, the amount of methane formed from carbon dioxide during fermentation depends on the amount of excess hydrogen (NADH) produced during fermentation of glucose to acetate or butyrate. Other hydrogen acceptors (sulfur, nitrate, unsaturated fatty acids) also can serve as an alternative hydrogen sink and reduce methane production slightly. In essence, the greater the ratio of acetate and butyrate to propionate, the higher the total yield of gas and the higher the proportion of methane within that gas (Fig. 3.4).

Amount of ATP generated differs depending on the ratios among the VFA produced (Table 3.4). Amounts of ATP formed during production of acetate, propionate, and butyrate, after considering the ATP yield from conversion of carbon dioxide to methane using the NADH generated, will be 2.5, 2.75, and 3.5 moles, respectively. On the basis of glucose, for each mole (180 g) of glucose or 162 g of

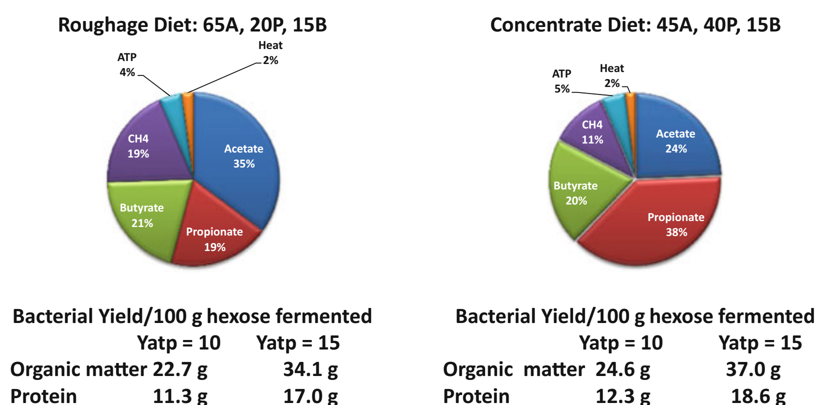

Fig. 3.4 Fate of fermented energy with roughage and concentrate diets and bacterial yields with Yatp or 10 or 15 g organic matter synthesized per mole of hexose fermented

hexosan fermented, considering the different yields of VFA from glucose, will release 5, 5.5, or 3.5 moles of ATP when the fermentation end product is acetate, propionate, or butyrate, respectively. Alternatively, on the basis of gas production, for each mole of gas released, production of acetate and butyrate yields 2.5 and 1.75 moles of ATP, respectively, but with propionate production, because no gas is released, ATP yield per unit of gas is infinite. Consequently, gas production alone, though suitable as a general index of extent of fermentation and energy value of various feedstuffs, provides an incomplete picture of the amount of ATP generated or the amount of microbial mass formed. Through simultaneously monitoring VFA concentrations and gas yields, potential yield of microbial mass can be estimated thanks to these stoichiometric relationships.

Growth of microbes within the rumen typically is limited by supply of energy (ATP). Carbohydrate conversion to the VFA ratios found during fermentation of fiber and of concentrate feeds should yield 4–4.6 moles of ATP per mole of hexose fermented. With de novo growth (being supplied with only hexose and ammonia), potential maximum cell yield or Yatp calculates to be between 20 to 29 g dry cells per mole of ATP; when supplied with pre-formed organic substances, Yatp should average about 20 % greater or about 29 (Hespell and Bryant 1979). These estimates are based on the calculated requirements for ATP for synthesis or assembly of all polymers found in various strains of bacteria and will differ with chemical composition of the bacteria.

Protein synthesis represents the major expenditure of ATP by bacteria, but lipid biosynthesis also is expensive. The fact that measured Yatp usually is 10–15g or dry cells per mole of ATP, considerably less than the theoretical amount required for assembly of components indicates that a substantial portion of the ATP used by bacteria is expended for functions other than growth (maintenance and replacement of lysed cells). Measured Yatp always is lower than maximum Yatp simply because bacteria, like all organisms, must expend energy for maintenance. Yatp also can differ when bacterial cells differ in chemical composition; strains richer in protein and lipid require more ATP for cell assembly and thereby have a lower Yatp (less cell yield per mole of ATP). In addition, specific nutrient deficiencies that increase energy requirements for bacterial maintenance (e.g., lower pH, slower passage rates, greater protozoal predation, low ammonia concentrations whereby more energy is required for uptake) or conditions that increase lipid, protein, or carbohydrate content of the mixed bacterial population within the rumen all can decrease microbial yield through lowering the Yatp. Bergen (1977) and Dewhurst et al. (2000) have outlined the numerous factors that can alter Yatp as well as laboratory methods for measuring the yield of microbial protein derived from ruminal fermentation.

Generally, growing bacteria synthesize only 10–15 g of bacterial dry mass from each mole of ATP (Yatp = 10–15). This is only 35–50 % of the theoretical maximum Yatp estimates of 20–29 above. This means that between 22 and 42 g of bacteria could be formed from every mole of glucose (180 g) fermented if Yatp is 9–15 and ATP yield is 4.5–5. This equals a bacterial mass yield of equal to 22–42 % of the

organic matter fermented from glucose depending on the specific VFA being produced and the Yatp.

The fraction of glucose energy that is retained within the VFA and becomes available for absorption and metabolism by the animals is equal to 62 % for acetate, 78 % for butyrate, but 109 % for propionate. The value for propionate exceeds 100 % due to transfer of hydrogen to propionate from acetate and butyrate. Methane loss per mole of VFA formed is similar for butyrate and acetate, but per mole of glucose fermented is less per mole of acetate than with acetate than per mole of butyrate produced (16 % versus 31 %). Methane loss would be negative if only propionate were produced! Because high concentrate diets yield a higher proportion of propionate during fermentation, increasing the concentrate level in a ration generally reduces methane production (Fig. 3.4). Other dietary alterations that can decrease methane yield include providing additives that alter the ratio of VFA (e.g., ionophores, malate), management to increase ruminal escape of carbohydrate or decrease the extent of ruminal digestion (e.g., high feed intakes, supplemental tannins), specific compounds that inhibit growth or specific steps in metabolism of methane-producing archea within the rumen, and diet components that serve as hydrogen sinks in the rumen (nitrate, sulfate, unsaturated fatty acids). Note that some of these alterations conserve energy for the animal and thereby should increase efficiency of ruminant production while other alterations do not.

After one subtracts the energy in VFA and methane from the gross energy originally present in glucose, the remainder must be released as either heat or ATP. Some of the energy converted to ATP also is released as heat during microbial metabolism through some energy is retained within polymers synthesized by microbes. Unfortunately, most of the energy used for polymer synthesis by microbes is lost before being of use to the animal due to polymer hydrolysis within the digestive tract prior to absorption by the animal. The minimum heat lost during glucose fermentation as a fraction of gross energy content of glucose based on these relationships will range from 0.8 % for propionate to 2.7 % for butyrate.

The microbial mass and thereby the amount of microbial protein that becomes available for the animal depends on (1) supply of nutrients for microbes (typically limited by energy—ATP—or ammonia), (2) efficacy with which energy from ATP is converted to microbial organic matter and protein in the rumen versus being used for microbial maintenance functions (to maintain cell functions and survive between meals) and (3) degree to which microbial cells lyse or are cannibalized by other microbes within the rumen versus being flushed to the abomasum. The latter two combined represent the overall Yatp within the rumen. Efficiency of microbial protein synthesis, like efficiency of animal growth, is greater when microbes are flushed through the rumen rapidly (harvested younger) so more of the available ATP is used for growth and not for specific maintenance functions (maintaining osmotic pressure, internal turnover of compounds). By increasing the proportion of ATP used for growth rather than maintenance, faster turnover of ruminal microbes causes microbial efficiency (Yatp) to increase and, if extent of fermentation remains constant, the size of the microbial population and microbial yield will increase. Yield of microbial protein for the animal in turn is the multiple of the microbial population and the outflow (or dilution

rate) of microbes from the rumen. By increasing outflow rate, efficiency of production of microbial mass will be increased. But microbial efficiency (Yatp) must not be confused with microbial yield. Whether the total yield of microbial mass is or is not increased as microbial efficiency (Yatp) increases depends on the degree that faster ruminal outflow reduces the quantity of organic matter digested (and yield of ATP) within the rumen. An increase in outflow from the rumen, though it always increases efficiency of microbial growth, may or may not increase ruminal output of microbes. Numerous additional factors that can alter microbial protein yield in the rumen were outlined by Firkins et al. (2006).

Ruminal microbes exist in multiple pools within the rumen. Some strains are attached to or embedded within the ruminal wall, others are attached to either grain or forage particles, while others freely float within the liquids within the rumen. With formation of a rumen mat, forage particles are retained for a longer time (have a lower dilution rate) within the rumen than liquids. In contrast, fine and dense feed particles are readily flushed with fluids from the rumen. Dilution rate of ruminal fluids always is greater than dilution rate of particles from the rumen, particularly when a ruminal raft exists. Liquid dilution rate increases when fluid input from saliva and possibly from drinking water increases. Consequently, an increase in rumination time, through increasing salivary input, generally increases efficiency of microbial growth, particularly of free floating microbes, and particle size reduction will speed passage of particles. As liquid dilution rate increases, particle dilution rate also increases because particles near the reticulo-omasal orifice are swept from the rumen by liquids.

Although bacteria attached to feed particles might be expected to be the primary type found in the rumen, even cellulolytic bacteria must detach and flow with liquids to colonize new feed particles. Most measurements indicate that between 40 % and 60 % of ruminal bacteria are associated with particles in the rumen with the remainder being associated with the liquid fraction of rumen contents. Because ruminal dilution rate always is lower for particles than for rumen liquids, one would expect that greater attachment to particles with higher forage diets should decrease efficiency of converting fermented organic matter to microbial protein. Yet, estimates of microbial organic matter per unit of organic matter truly fermented (organic matter apparently fermented minus microbial organic matter) indicate the opposite. True microbial efficiency always appears greater when diets contain more forage. Though no explanation for this dilemma is obvious, this could reflect an accelerated liquid turnover with forage than with concentrate diets, reduced protozoal predation of attached than of free bacteria, a greater maintenance cost for free floating than attached bacteria due to a lower pH that increases the energy requirement of microbes or the inconsistency in supply of available energy from concentrate during the time interval between meals.

A "time lag" is evident between the time that forage particles are introduced into the rumen or added to an in vitro flask and the time that fermentation of the added forage begins. Factors that could speed attachment of microbes deserve further research attention. Lasting several hours with some forages, this lag markedly shortens the time available for digestion. In turn this increases ruminal fiber fill and, given a limited time for digestion, decreases the extent that organic matter is digested in the rumen.

Amino Acids

All amino acids that are not linked or guarded from attack are extensively degraded in the rumen to ammonia, carbon dioxide, VFA, and branch chain fatty acids. Degradation of amino acids can proceed either through decarboxylation yielding the amine plus carbon dioxide or by oxidative deamination yielding carbon chains that subsequently will be catabolized to VFA. Decarboxylation appears more prevalent with concentrate diets. Certain decarboxylated amino acids (e.g., histamine, tyramine) when absorbed can have adverse effects on feed intake and on metabolism of the host animal (e.g., laminitis). Oxidative deamination is more prevalent with forage-based diets. This difference presumably is due to higher prevalence of microbes involved with decarboxylation (e.g., Allisonella histaminiformans) with ruminal conditions and a low rumen pH from feeding of concentrate diets.

When grown in pure culture, some ruminal bacteria including most that digest cellulose require a source of branch chain fatty acids (isobutyrate, isovalerate, 2-methylbutyrate) or of their respective amino acids precursors (valine, leucine, and isoleucine) for growth. Theoretically, the supply of these amino acids might be inadequate with diets very low in true protein or in these specific amino acids. Indeed, concentrations of these branch-chained fatty acids within the rumen normally are low with low protein diets. However, supplementation with these compounds typically has not improved productivity or microbial cell yields from the rumen indicating that the ruminal supply must have been adequate, presumably from release of these fatty acids during ruminal degradation of protein of dietary and microbial origin.

As much as 40 % of the ruminal degradation of hydrolyzed protein has been attributed to several species of hyper-ammonia producing bacteria (HAP) that catabolize amino acids or peptides as a source of energy. Through degrading dietary protein, peptides, and amino acids, the HAP reduce the supply of protein for the host animal. Many of these strains are inhibited by ionophores. This may explain why ionophores may reduce a ruminant's need for dietary protein.

Though most ruminal bacteria can use ammonia as their sole source of nitrogen, results of some laboratory studies indicate that supplying amino acids in addition to urea can increase microbial organic matter yield. This may reflect a reduced need of ATP for amino acid biosynthesis (and a higher Yatp) of bacteria that can incorporate amino acids from the media. In vitro laboratory studies have indicated that strains of ruminal bacteria that typically digest structural carbohydrates are unable to use amino acids but instead thrive when will use and thrive when ammonia serves as their sole source of N for growth. In contrast, providing peptides in vitro has increased growth rates of bacteria that digest nonstructural carbohydrate. This resulted in the proposal that two-thirds of the N for bacteria digesting cell contents should be provided by intact protein or peptides in diet formulation programs (Russell et al. 1992). This also has led to the suggestion that source of ruminally degraded N (true protein versus NPN) as well as its level can alter microbial yields. In contrast, subsequent continuous culture and in vivo trials have found little if any improvement in digestibility or in bacterial growth or efficiency from providing additional peptides to rumen fluid when

the ammonia supply was adequate except at very rapid bacterial growth rates. Consequently, the precise conditions remain unclear whether or when peptides might limit digestion or bacterial growth in the rumen if the supply of ammonia is adequate.

The response of cattle to source of supplemental N is opposite from the suggestion that intact protein should prove most beneficial with diets rich in non-structural carbohydrate. Instead, production responses to intact protein sources over NPN are common for cattle fed forage-based diets but rare among cattle fed concentrate diets. Indeed, for cattle fed grain-based diets, urea can readily and fully displace the need for supplemental protein. Reasons for this discrepancy between in vitro and in vivo responses remain unclear though "energy spilling metabolic reactions," increased lysis and protozoal predation leading to greater turnover of bacterial constituents, and differences in glycogen synthesis may be involved.

Altering the Products of Ruminal Fermentation

For growing-finishing cattle, a high ratio of propionate to other VFA, often achieved with feeding of starch-rich diets, is considered desirable energetically probably due to a reduced ruminal loss of energy as methane that often exceeds 6 % of digested energy from a diet. In contrast, a high propionate to acetate ratio for lactating cows is considered undesirable because it is associated with an increased retention of energy by body tissues leaving less energy for milk production. Likewise, milk fat concentration can be depressed by elevated propionate production. Diets that yield higher propionate to acetate ratios also are more likely to lead to metabolic problems (subclinical or clinical acidosis). These factors as well as attempts to decrease ruminal release of methane as a greenhouse gas have led to various approaches to alter the end-products of ruminal fermentation.

Ratios of VFA produced within the rumen can be altered by adjusting the composition of the diet, level of feed intake, and through manipulation of the types of microbes or their activity within the rumen. Increased knowledge of the degree to which specific ruminal microbes possess desired or undesired characteristics can be examined through genetic profiling techniques as described by Firkins (2010). Specific methods that might be used to control or alter ruminal populations include (1) administering specific chemical compounds that inhibit undesired microbial strains (selective antibiotics or ionophores, plant extracts, essential oils, fatty acids, bacteriophages, bacteriocins), (2) increasing the populations of species desired in the rumen through administration of probiotics, antibiotics, oligosaccharides, direct fed bacteria, yeasts, or fungal products, or (3) enhancing the availability of nutrients either within feeds (enzyme or microbial inoculants for feed) or within the rumen (specific fed enzymes, inoculation with fibrolytic or toxin-degrading microbes). These approaches have been described by Nagaraja (2012), and more information can be found in Chap. 6 of this book.

Lab Procedures to Appraise Ruminal Fermentation of Feeds and Forages

Ruminal Digestion Measurements

In vivo measurements of energetics, digestion, and performance are expensive, time-consuming, logistically complex, require access to multiple animals, and utilize a large amount of test material. As a result, laboratory methods are used extensively by researchers as a proxy for in vitro trials. The source of inocula preferably is ruminal liquor obtained from animals previously adapted to a diet similar to that being tested by in vitro procedures. When cannulated animals are not available as donors, rumen fluid from slaughter plants or fecal matter has been used as a substitute for fresh rumen fluid. Attempts to freeze rumen fluid for later use generally have not proven successful.

Results based on in vitro disappearance may differ from in vivo measurements for multiple reasons.

(a) Particle size distribution. Obtaining a distribution in particle size of a feed to match that of particles entering or retained within the rumen is difficult. The physical characteristics of esophageal samples are almost impossible to simulate through typical mechanical rolling or grinding procedures.
(b) Particle retention. Batch or in situ methods retain all feed particles for the full duration of the fermentation. In contrast, feedstuff components in vivo are segregated by density and particle size into multiple fractions that are selectively retained for various lengths of time. Combining an average rate of digestion with an average rate of passage to calculate the proportion of a feed component should be fermented in the rumen overlooks these physical characteristics that result in selective retention and passage of different fractions and components.
(c) Product accumulation and nutrient additions. With in vitro procedures, pH declines as acids accumulate, but within the rumen the fatty acid produced are absorbed. Buffers, minerals, and urea are continuously added from saliva and exchange through the rumen wall in vivo, activities difficult to simulate in vitro.
(d) Adaptation to substrates. Within a short term batch incubation system, unlike the rumen, the time available for microbes to adjust or adapt to a specific substrate is very limited. Addition of new feeds, chemicals, or compounds may temporarily shock fermentation within the rumen, but within several days the ruminal population will shift to accommodate a substrate change.
(e) Recovery of undigested products. Though called "digestion" in vitro or in situ measurements are estimates of "disappearance" of the test material. Following fermentation, material typically is recovered by filtration. Compounds that disappear are assumed to have been digested. Yet, during fermentation, cell contents and minerals become soluble and size of particles often is reduced to the point that it may not be recovered by filtration or retained within a Dacron bag. In situ bags with a larger pore size will permit more particles to escape through the pores. Because finely ground or pulverized feeds have more small particles, disappear-

ance and presumed digestibility will be greater, so care must be exercised when interpreting in situ disappearance values. In contrast with excessive loss from Dacron bags, microbes that attach tightly to or are located internal to feed components will not be fully dislodged during filtration or pepsin digestion. Consequently, disappearance of materials in vitro may not truly equate with ruminal digestion of that material. Although a "blank" fermentation in which rumen fluid is incubated without addition of substrate often is employed in an attempt to quantify the amount of undigested residue that is present with the inoculated rumen fluid or has entered a Dacron bag, death and lysis of microbes during incubation without a substrate over time may lead to an overestimates of digestibility.

Despite these shortcomings, comparative results still can prove useful to compare feeds or grain processing methods or to screen samples or compounds for more detailed in vivo testing. In vivo results may or may not match with in vitro results. Unfortunately, confirmation of results based on two or more laboratory procedures often is considered to be "verification" of a specific concept or observation. Any in vitro or in situ finding, despite its consistency, must be appraised through in vivo testing to assess its validity, veracity, and applicability in vivo. Usefulness and limitations of various in vitro and in situ procedures were outlined by Owens and Goetsch (1988).

Batch Methods

In vitro procedures. The most common method for estimating ruminal digestion, the Tilley and Terry (1963) procedure, involves incubation of a feedstuff or compound with buffered rumen fluid in a sealed, anaerobic vessel maintained at 39 °C. To remove microbes generated during incubation and attached to the residue, digestion of the residue with a pepsin-HCl solution usually follows ruminal incubation of the test material. The amount of a material that remains in the vessel following an extended incubation time period (12, 24, 48 h) and pepsin incubation is considered to be not digested. Extent of digestion is calculated by difference from that present in the vessel at the start of the fermentation period. Digestion is calculated as input minus residue divided by input. Residues typically are recovered by filtration. As a result, any compounds that become soluble and "disappear" are classified as being digested. Care must be used to maintain viability of rumen samples used for inoculation and to provide adequate conditions for ruminal microbes (sufficient ammonia and minerals, buffering to avoid an acid accumulation that reduces pH). To adjust for differences in rumen fluid and in incubation conditions, disappearance often is adjusted by comparing values within a run against values for "standard" samples known to have a high and a low in vivo digestibility.

In situ procedures. Disappearance of feed components from porous Dacron bags incubated in the rumen of fistulated animals is another common method for estimating ruminal digestion. Pore size of the Dacron bags is critical because if pore size too small (under 20 μm), entry of protozoa is reduced whereas if pore

size is too large (over 50 μm), particles small in size can pass through without being digested (wash-out) or after being partially digested. Again, standards of known in vivo ruminal digestibility should be used to adjust for differences among in situ runs due to variability in ruminal conditions among animals and among days within an animal, both of which alter in situ disappearance. Washout of small particles placed into bags often can be estimated by measuring disappearance of dry matter from bags rinsed with water, but because particle size also decreases during fermentation, initial washout may underestimate subsequent loss of small particles from bags. In comparative studies, some research has indicated that washed particles from forages have an in vitro digestion rate similar to those particles retained within the bags, but this concept needs further study for feeds or diets that contain particles that differ in both particle size and digestibility; compared with dried forages, dried grains typically pulverize more readily when ground. With some feedstuffs (e.g., oats grain) and oil-rich feeds, films may form that will clog pores and inhibit water movement through the bag that is essential both to inoculate the feed and remove products of digestion.

Yield of Specific Products or Substrate Disappearance

End-products of digestion include released gases (methane and carbon dioxide), volatile fatty acids, and microbial components. Through measuring yields of these products, both rate and extent of digestion can be monitored over time.

In Vitro Gas Production

Carbon dioxide and methane production curves over time can be separated mathematically so that rate and extent of digestion of multiple "pools" can be quantified. Through measuring various nitrogen containing products as well, protein yield (the sum of microbial protein and undegraded feed proteins) and of undigested cell contents also can be calculated. One advantage of gas production measurements is that rates as well as extent of digestion can be evaluated continuously over time. The gas production procedure has proven very useful to evaluate energy availability of novel feedstuffs in developing countries. Although such batch fermentation do not directly match the continuous fermentation conditions within the rumen, the size of various "pools" and their relative rates of gas production when matched with in vivo estimates of production can help guide nutritionists in making appropriate diet modifications to improve productivity. Limitations as noted above include making appropriate correction for products included with the rumen fluid as an inoculum, auto-degradation of microbes with long-term incubations, retention of all particles for the full incubation period, release of carbon dioxide from the buffered incubation media during fermentation, and lack of potential for microbial adaptation to substrates being fermented.

Rusitec

Through maintaining rumen fluid over time and repeatedly adding and removing Dacron bags containing various substrates, disappearance of individual feed components can be estimated. Maintaining and operating this semi-continuous culture system can become quite complex and time consuming but in contrast with other incubation systems, this procedure allows time for microbes to adapt to specific feeds when normal microbial activity is retained.

Continuous Flow Fermenters

Fermenters equipped with automated feeding systems, designed to recover both liquid and particulate effluent separately, and equipped to monitor and control pH and other parameters have been engineered in an attempt to simulate ruminal fermentation of specific feeds and to test feed additives. As with the Rusitec system, devices are complex and often fail to maintain typical conditions found with fresh ruminal contents (pH, gas production rates and ratios, in vitro digestion rates, protozoal numbers and activities) for more than 1 week.

In Vivo Measurements

The live ruminant animal is the gold standard for quantifying rate and extent of digestion and absorption, products of metabolism, and energetic efficiency. Often smaller ruminants (sheep, goats) are used as proxies for larger ruminants to reduce cost and the amount of space and feedstuff needed, but species differences must be borne in mind when extrapolating among different ruminant species and even within a species (lactating dairy cows versus non-lactating pregnant heifers versus finishing feedlot steers). Through collecting urine and feces, apparent digestion and retention of nutrients can be calculated. By using indigestible markers inherent in diet components or added to a diet, digestion and retention can be calculated so that total excreta need not be collected. Similarly, metabolism chambers that collect expired gases can quantify whole animal metabolism. Live animals often are surgically equipped with portals to monitor intake, digestion, and metabolism. With esophageal fistulas, composition of forage selected by grazing animals, effects of rumination on regurgitated digesta, and the eructation complex can be studied. Ruminal cannulas provide a window that allows research scientists to monitor the populations and activity of ruminal microbes, characteristics and products of fermentation (pH, temperature, passage), and ruminal motility. Cannulas in the abomasum, the small intestine, and the large intestine permit study of the site and extent of nutrient digestion and passage. Isolated ruminal sacs and tissue culture methods allow study of absorption of specific nutrients, and catheters at specific locations within the digestive tract or isolated tissues (mammary gland; hind limb; fat tail; isolated organs or tissues) yield data regarding net absorption of specific nutrients

and tissue metabolism. Devices to continuously monitor important ruminal factors (e.g., pH, temperature, motility, ammonia) provide a record of ruminal conditions for researchers and can help detect ruminal problems of individual animals within a producing herd that need additional nutritional or medical attention.

Epilogue

Our current scientific understanding of ruminant metabolism, production, and health and practical production practices is based largely on results of in vitro and in vivo measurements. Future improvements in productivity and health of ruminants are dependent on deepening our understanding of ruminant activity, digestion, and metabolism. Past efforts have been largely based on chemical and microbiological measurements with the more difficult physical measurements often being ignored. Targeted application of science and technology in ruminant production will help to reduce the environmental footprint of ruminants, unique animals that both harvest and efficiently convert unused and underutilized feeds and wastes into healthful and desirable products that enhance human life worldwide.

References

Baldwin RL, Allison MJ. Rumen metabolism. J Anim Sci. 1983;57:461–77.

Baker S, Herman T. Evaluating particle size. Kansas State University Publication MF-2051; 2002. http://www.ksre.ksu.edu/library/grsci2/mf2051.pdf.

Bauchart D. Lipid absorption and transport in ruminants. J Dairy Sci. 1993;76:3864–81.

Bauman DE, Lock AL. Concepts in lipid digestion and metabolism in dairy cows. In: Proc. tri-state dairy nutr. conf, p. 1–14.

Bergen WG. Factors affecting growth yields of micro-organisms in the rumen. Trop Anim Prod. 1977;4:1.

Dewhurst RJ, Davies DR, Merry RJ. Microbial protein supply from the rumen. Anim Feed Sci Technol. 2000;85:1–21.

Firkins JL. Reconsidering rumen microbial consortia to enhance feed efficiency and reduce environmental impact of ruminant livestock production systems. R Bras Zootec. 2010;39:445–57.

Firkins JL, Hristov AN, Hall MB, Varga GA, St-Pierre NR. Integration of ruminal metabolism in dairy cattle. J Dairy Sci. 2006;89:E31–51.

Hespell RB, Bryant MP. Efficiency of rumen microbial growth: Influence of some theoretical and experimental factors on YATP. J Anim Sci. 1979;49:1640–59.

Hungate RE. The rumen and its microbes. New York: Academic; 1966.

McAllister TA, Bae HD, Jones GA, Cheng KJ. Microbial attachment and feed digestion in the rumen. J Anim Sci. 1994;72:3004–18.

Nagaraja TG. A microbiologist's view on improving nutrient utilization in ruminants. In: Florida nutrition conference; 2012. p. 135–60. http://dairy.ifas.ufl.edu/rns/2012/11NagarajaRNS2012.pdf.

Oetzel GR. Subacute ruminal acidosis in dairy herds: physiology, pathophysiology, milk fat responses, and nutritional management. In: Proc. Amer. assn. bovine practitioners 40th annual conference, September 17, 2007, Vancouver, BC, Canada. http://www.vetmed.wisc.edu/dms/fapm/fapmtools/2nutr/sara1aabp.pdf.

Owens FN, Bergen WG. Nitrogen metabolism of ruminant animals: historical perspective, current understanding, and future implications. J Anim Sci. 1983;57:498–518.

Owens FN, Goetsch AL. Ruminal fermentation. In: Church DC, editor. The ruminant animal. Digestive physiology and nutrition. Englewood Cliffs, NJ: Prentice-Hall; 1988. p. 145–71.
Owens FN, Secrist DS, Hill WJ, Gill DR. Acidosis in cattle: a review. J Anim Sci. 1998;76:275–86.
Palmquist DL, Jenkins TC. Fat in lactation rations. A review. J Dairy Sci. 1980;63:l.
RAGFAR (Reference Advisory Group on Fermentative Acidosis of Ruminants). Ruminal acidosis—aetiopathogenesis, prevention and treatment; 2007, 56p. http://www.dairyaustralia.com.au/Animals-feed-and-environment/Feeding-and-nutrition/~/media/Documents/Animals-feed-and-environment/Feeding%20cows/Nutrition%20management%20latest/A%20Review%20of%20Ruminal%20Acidosis%20-%20July%202007.ashx.
Russell JB. Factors influencing competition and composition of the ruminal bacterial flora. In: Gilchrist FMC, Mackie RI, editors. Herbivore nutrition in the subtropics and tropics. Craighall: Science Press; 1984. p. 313–45.
Russell JB. Strategies that ruminal bacteria use to handle excess carbohydrate. J Anim Sci. 1998;76:1955–63.
Russell JB, O'Connor JD, Fox DG, Van Soest PJ, Sniffen CJ. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. J Anim Sci. 1992;70:3551–61.
Satter LD. Feeding the dairy herd. In: Minnesota nutrition conference; 1978.
Schwartzkopf-Genswein KS, Beauchemin KA, Gibb DJ, Crews DH, Jr, Hickman DD, Streeter M, McAllister TA. Effect of bunk management on feeding behavior, ruminal acidosis and performance of feedlot cattle: a review. J Anim Sci. 2003;81(E.Suppl.2):E149–158.
Tilley JMA, Terry RA. A two-stage technique for the in vitro digestion of forage crops. J Br Grassl Soc. 1963;18:104–11.
Weimer PJ, Stevenson DM, Mantovani HC, Man SL. Host specificity of the ruminal bacterial community in the dairy cow following near-total exchange of ruminal contents. J Dairy Sci. 2010;93:5902–12.
Wolin MJ. A theoretical rumen fermentation balance. J Dairy Sci. 1960;43:1452–9.

Additional Readings

Church DC. The ruminant animal, digestive physiology and nutrition. Prospect Heights, IL: Waveland Press; 1993. 564p.
Ischer V, Heinrichs J, Varga G. From feed to milk. Understanding rumen function. Extension Circular 422. 1996. Penn. State Univ. Available at: http://pubs.cas.psu.edu/freepubs/pdfs/ec422.pdf.
MicrobeWiki, 2012. At: http://microbewiki.kenyon.edu/index.php/Bovine_Rumen.
Stern MD. Rumen physiology. In: Minnesota dairy health conference, 2011. https://conservancy.umn.edu/bitstream/118922/1/Stern.pdf.
University of Minnesota Extension. Rumen anatomy and physiology, 2008. WW-00469. http://www.extension.umn.edu/distribution/livestocksystems/components/di0469-02.html.
Van Soest PJ. Nutritional ecology of the ruminant. 2nd ed. Ithaca, NY: Cornell University Press; 1994. 528 pp.