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RUMEN PHYSIOLOGY

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Introduction

Ruminants are earth's dominant herbivores, due in part to the evolution within this group of a mechanism that utilizes microorganisms to digest plant components not susceptible to attack by mammalian enzymes. Cellulose is probably the most common organic chemical on earth, yet no mammal, and few if any animals above the level of the protozoa, secretes a cellulase, so none can digest it. Cellulose is a structural component of the plant needed to support the stems and leaves. Because the rumen ecosystem contains microorganisms that produce cellulase, which hydrolyzes cellulose, the ruminant animal can utilize cellulose as an energy source. The rumen is a complex anaerobic microbial ecosystem, with many microbes and reactions. It is peculiar that animal cellulases did not evolve and instead a symbiotic relationship developed between herbivorous animals, such as ruminants, and gastrointestinal tract microbes.

There are two basic theories why ruminants evolved 30 million years ago? The older theory is referred to as the "eat and run" theory that allowed ruminants to escape from their predators because they devoured their food quickly and masticated it later. The more recent theory was proposed by Van Soest (Nutritional Ecology of the Ruminant, 1994 Second Edition). He stated that pre-gastric digestion detoxified secondary plant substances, allowing pre-gastric fermenters greater latitude in dietary choice and adaptation.

Essential differences between digestive tracts of non-ruminants and ruminants are well recognized. For example, in man and pigs dietary protein that is consumed usually provides an accurate assessment of amino acid uptake from the small intestine. Unfortunately, this is not the case with ruminants. Ruminant digestive processes are different from non-ruminants in that pre-gastric modification of feed results in a completely different pattern of organic matter entering the intestines to that in feed. Ruminal microbes account for approximately 70% of digestion that takes place in the ruminant gastrointestinal tract. Intervention of microbes into digestive processes of ruminants causes substantial degradation of dietary protein and carbohydrates, and synthesis of microbial protein. Because these microbes can be both constructive and destructive in their action, providing nutrients to ruminants is more complex compared with non-ruminants.

Advantages of pre-gastric microbial fermentation:

- the ability to utilize cellulose as an energy source.
- with adequate energy in the form of ATP and a carbon skeleton, microbial protein can be synthesized from non-protein nitrogen (NPN).
- 10 to 15 % of dietary nitrogen is re-cycled across the rumen wall or via the saliva, increasing efficiency of nitrogen utilization.
- the ability of ruminal microbes to detoxify plant compounds.
- the ability of ruminal microbes to synthesize B-vitamins and the fat-soluble vitamin K resulting in reduction or elimination of animal's vitamin requirements.
- the efficiency in absorption of microbial end-products such as microbial protein and B-vitamins in the small intestine.

Disadvantages of pre-gastric microbial fermentation:

- fermentation of carbohydrates $\rightarrow$ heat and gases such as CO_2, CH_4 and H_2 can result in approximately 10-15% of energy intake being lost.
- amino acid flow to the small intestine may not be improved because high quality proteins can decrease in quality through fermentation.
- because ruminal microbes can alter drugs and antibiotics, they must be administered intravenously.

The Rumen Environment

The rumen:

- is a warm, anaerobic, chemically reducing environment rich in organic matter (OM) but often deficient in readily metabolizable compounds. Dry matter percentage of rumen contents varies with diet but can range from 7 to 15% with an average of 12.5% of ruminal net weight.
- is large, with contents comprising 7 to 15% of body weight.
- occupies 80 to 85% (total stomach volume) of the space filled by all sections of the stomach (3/4 of the space in the peritoneal cavity).

- has a volume of 4 to 10 liters in sheep and 100 to 150 liters in cattle.

- secretes no digestive juices, because it has no glandular cells among the epithelial cells of its mucous membrane.

- has good mixing of contents because of muscle contractions , rumination, insalivation and reduced particle size.

- is provided with a regular influx of fermentable feed providing continuous substrate to ruminal microbes. Substrate includes important microbial growth factors such as nitrogen, carbohydrate (energy in the form of ATP), branched-chain volatile fatty acids (VFA) and minerals such as sulfur.

It is important to note that end products and wastes from microbial fermentation do not accumulate in the rumen because they are removed by diffusion across the rumen wall and passage from the stomach. As a result, all factors relative to the microbial environment are regulated within narrow limits and the rumen functions as a continuous culture system in which substrate is constantly provided and end products removed.

Physical and Chemical Properties of the Reticulo-rumen

pH – can range from ~5.6 to 7.0 in ruminants, but in the dairy cow pH is usually kept below neutrality (5.8 to 6.5) by two mechanisms:

1. Continuous influx of saliva that acts to buffer acids during fermentation.

   Saliva:

   - is copious, usually one to two rumen volumes per day.

   - in cows is produced at 200 to 300 L/d or 50 to 75 gallons/day.

   - is composed mainly of a phosphate-bicarbonate buffer containing some urea and mucous but no enzymes.

2. Diffusion across the rumen wall:

   - the rumen wall is permeable to many solutes.

   - there is substantially more VFA that diffuse across the wall into the blood than pass onto the omasum.

Because fermentation acids are largely neutralized or removed, acidophilic lactic acid producing bacteria are not favored and a very mixed microbial population results. This population is subject to natural selection for maximum growth, i.e., for maximum yield of ATP. It can be shown that fermentation products allowing maximum ATP yield are acetic, propionic and butyric acids, $C0_2$ and CH_4. Other common fermentation products, such as lactic acid or succinic acid, ethanol or hydrogen give a lower yield of ATP and are not usually found in the rumen to a large extent.

Anaerobic – rumen microbes are predominantly obligate anaerobes and will tolerate some O_2 as long as fermentation in the rumen is active enough to facilitate disposal of O_2. A small fraction of the microbial population in the rumen is comprised of aerobic or facultative anaerobes.

- in the rumen, 0_2 enters with food or in swallowed air and is quickly removed by microbes resulting in a steady Eh (oxidation-reduction potential) ranging from -150 mv to -350 mv. The rumen is a highly reducing medium.

- high rate of 0_2 removal by microbes may protect anaerobic organisms, like protozoa, in their transfer between hosts.

- methane bacteria are the strictest anaerobes known, requiring Eh below -333 mv at pH 7.0 to initiate growth.

Temperature - is one of the most important variables affecting microbial growth. Effects of temperature are complex, because in addition to influencing rates of cellular reactions, temperature also affects the microbial environment.

- Temperature affects: pH, H_20 activity, ion activity, viscosity, hydration, toxic action of metabolic products and other unfavorable components of the medium (solubility of gases - ex. $C0_2$).

- Temperature in the rumen can range from 34 to as high as 41°C during active fermentation, with an average of 38 to 39C.

Gas Mixture - The principal gases in the rumen are CO_2 (65%) and CH_4 (27%), both of which are major end-products of microbial fermentation in the rumen. In addition to being produced directly by fermentation, CO_2 is also derived from bicarbonate, dietary carbonates, amino acids and other organic acids.

- The other gaseous end-product of fermentation is H_2; however it comprises only 0.2% of the gases produced in the rumen because it is used by methanogenic bacteria to reduce CO_2 to CH_4.

- Nitrogen comprises approximately 7% of the gas mixture in the rumen with a trace of H_2S and low transient quantities of O_2.

Oxygen - O_2 diffusion across the rumen wall has possibly caused the existence of a distinct population of adherent bacteria; 34 out of 164 strains of adherent bacteria are facultative anaerobes.

- These facultative anaerobic bacteria on the lumenal surface of the rumen provide sufficient O_2 "scavenging" activity to maintain anaerobic conditions for the obligate adherent anaerobes within the rumen.

Urea - Urea may enter the rumen as a feed supplement, in saliva and by diffusion across the rumen wall and its digestion by bacteria provide a source of NH_3 that is essential for bacterial protein production.

- Urease producing bacteria have been recovered from ruminal fluid but their numbers are so low and urease production is insufficient to account for observed rates of urea digestion in the rumen.

- It has been demonstrated that adherent bacteria have a much higher urease activity than that of the much larger number of bacteria in ruminal fluid and when epithelial cells are sloughed into the rumen, this results in high urease activity in fluid.

Therefore, it has been suggested that the bacterial subpopulation adherent to the rumen wall has the capacity to scavenge O_2 diffusing from the rumen wall and that it produces urease that digests urea that diffuses across the rumen wall or enters the rumen with food or saliva.

Ecological Niches - The rumen has various ecological niches to fill and from a microbiological standpoint, it may be considered as three interconnecting environments:

1. the liquid phase, free-living microbial groups in the rumen fluid comprise 25% of the microbial population.

2. the solid phase, microbial groups associated with food particles comprise 70% of the microbial population.

3. the rumen epithelium, microbial groups attach to animal cells such as the epithelial cells of the host and those that attach to protozoan cell surfaces comprise 5% of the microbial population.

The Rumen Microbial Population

The microbial population of the rumen is complex, containing many different types of interacting procaryotic organisms (those without a well-defined nuclear membrane, e.g., bacteria) and eucaryotic organisms (those with a well-defined nuclear membrane, e.g., fungi and protozoa) and based on the latest taxonomy, there are also archae.

- Bacteria – found at 10^{10} to 10^{11} per ml of rumen contents.

- Archae – an ancient bacteria that has no peptidoglycan (cell wall) that comprise approximately 3% of total microbial mass in the rumen.

- Ciliated protozoa – found at 10^{5} to 10^{6} per ml of rumen contents. Cilia are hair-like structures that aid in movement and engulfment of feed particles.

- Flagellated protozoa – found at 10^{3} to 10^{5} per ml of rumen contents. Flagella are thread-like appendages or extensions that have a whip-like motion that enables locomotion.

- Anaerobic fungi - previously thought to be flagellated protozoa, rather zoospore (early in life cycle with flagella) of Phycomycete. Depending on the genus, they have either single flagellum or multiple flagella.

- Bacteriophage – is a virus that infects bacteria and affects lysis of bacterial cells. There is increasing evidence that they may play a considerable role in determining the detailed composition of the bacterial population. A large diverse population of bacteriophage exists in the rumen and comprises about 5 x 10^{7} phages/ml of rumen fluid with more than 125 different morphological types.

Rumen ciliated protozoa are found at much lower numbers than bacteria, but they can account for half of the microbial mass in the rumen. Following are some general characteristics of rumen ciliated protozoa and bacteria:

Characteristics	Bacteria	Ciliated protozoa
Size (μm)	0.3 to 50	20 to 200
Numbers	10^{10} to 10^{11}	0 to 10^{6}
Net mass (mg/100 ml)	2,015	1,600
Percent of total mass	54	46
Doubling time	as little as 12 minutes	greater than 18 hours
Cell type	prokaryotic	eukaryotic

It is interesting to note that small bacteria account for approximately one half of the total biomass in a normal rumen. However, small bacteria account for a much greater share of the metabolic activity in the rumen because metabolic activity is generally related to organism size. In contrast, ciliated protozoa are much larger but fewer in numbers. Although ciliated protozoa represent ~46% of total biomass, this number exceeds their metabolic importance, which in terms of function in the rumen is not clearly understood. In fact, the rumen can function normally if devoid of ciliated protozoa but cannot function normally without bacteria.

Functions of Bacteria in the Rumen

Function – Substrate	Significance (predominant bacterial species)
Celluloytic – cellulose	Bacteria such as *Ruminococcus albus, Ruminococcus flavefaciens, Fibrobacter succinogenes* produce cellulase to digest cellulose which is important to ruminants that are grazing or fed forage in their diet. It should also be noted that cellulase of anaeorobic fungi is extremely active and can be effective in digestion of cellulose. Another key point is that fungi attach to the outer wall of plants and produce rhizoids that invade and penetrate the plant cell wall. Penetration of the rhizoidal system allows digestion from the inside-out. In contrast, bacteria and protozoa attach to the outside of the plant, release their enzymes on the surface and digest from the surface or outside-in.
Amylolytic –amylose (starch)	Bacteria such as *Ruminobacter amylophilus and Butyrivibrio fibrisolvens* produce amylase to digest starch. These bacteria are predominant in ruminants fed high starch rations, e.g. young growing beef cattle and high producing cows in early lactation.
Hemicellulolytic – hemicellulose	Bacteria such as *Butyrivibrio fibrisolvens* produce hemicellulase to digest hemicellulose in forages.
Pectinolytic – pectin	Bacteria such as *Butyrivibrio fibrisolven, Prevotella ruminicola and Lachnospira multiparous* produce pectinase to digest pectin which is important to ruminants that are fed sugar beet pulp, citrus pulp or other feeds high in pectins.

Function – Substrate	Significance (predominant bacterial species)
Proteolytic – protein	Bacteria such as *Ruminobacter amylophilus or Selenomonas ruminantium* produce protease and deaminase to digest protein. These bacteria hydrolyze protein to peptides and amino acids which are deaminated to ammonia (Figure 1). Nitrogenous sources derived from hydrolysis can be incorporated into bacterial protein.
Ureolytic – urea	Bacteria such as *Prevotella ruminicola* produce urease to digest urea, an NPN source, to ammonia which can be incorporated into bacterial protein.

The following schematic depicts nitrogen metabolism in the rumen and demonstrates how protein and NPN are broken down to nitrogenous products that can be incorporated into bacterial protein:

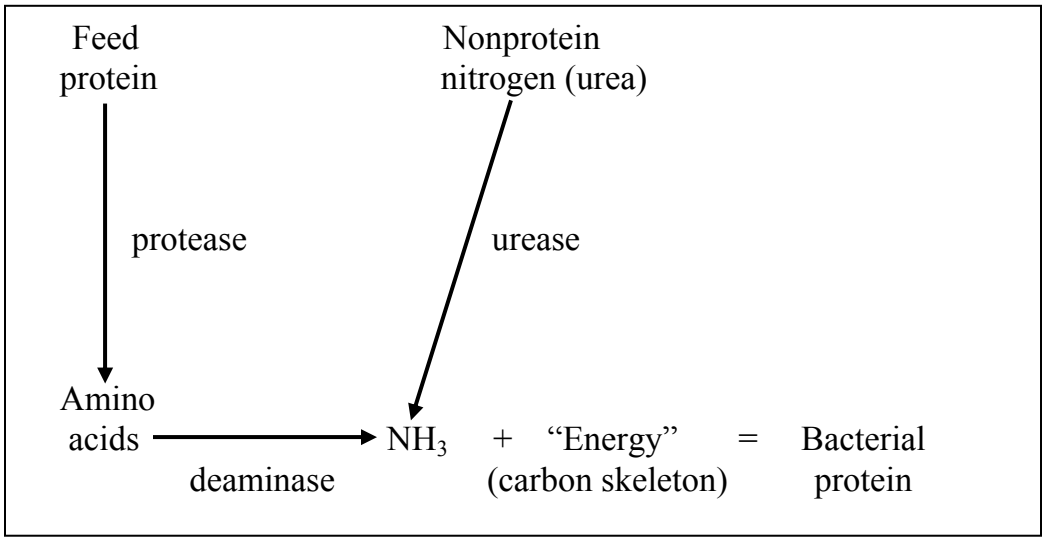

Figure 1. Nitrogen metabolism in the rumen.

Methanogenic – methane producer	Bacteria such as *Methanobrevibacter* utilize CO_2 and H_2 to synthesize methane.
Glucolytic – glucose	Bacteria such as *Lactobacillus acidophilus produce sucrase to ferment* sugars to produce VFA and ATP which is important when ruminants are fed molasses and other high sugar feeds.

Function – Substrate	Significance (predominant bacterial species)
Lipolytic – lipid	Bacteria such as *Anaerovibrio lipolytica* to produce lipase which aids in digestion of lipids in the ruminant diet.

Fermentation of Carbohydrate in the Rumen

Most carbohydrates consumed by ruminants are polymers of glucose present in the form of cellulose or starch. However, large amounts of hemicellulose and pectin may be present in some diets. Feeds like molasses and food processing by-products are high in mono- and disaccharides, but usually do not constitute a large portion of the diet. Consequently, for fermentation to occur, most carbohydrates must undergo hydrolysis in the rumen. Pyruvate is the intermediate through which all carbohydrates must pass before being converted to volatile fatty acids, carbon dioxide and methane (Figure 2). The proportion of end product depends on type of carbohydrate fermented, bacterial species involved and rumen environment during fermentation.

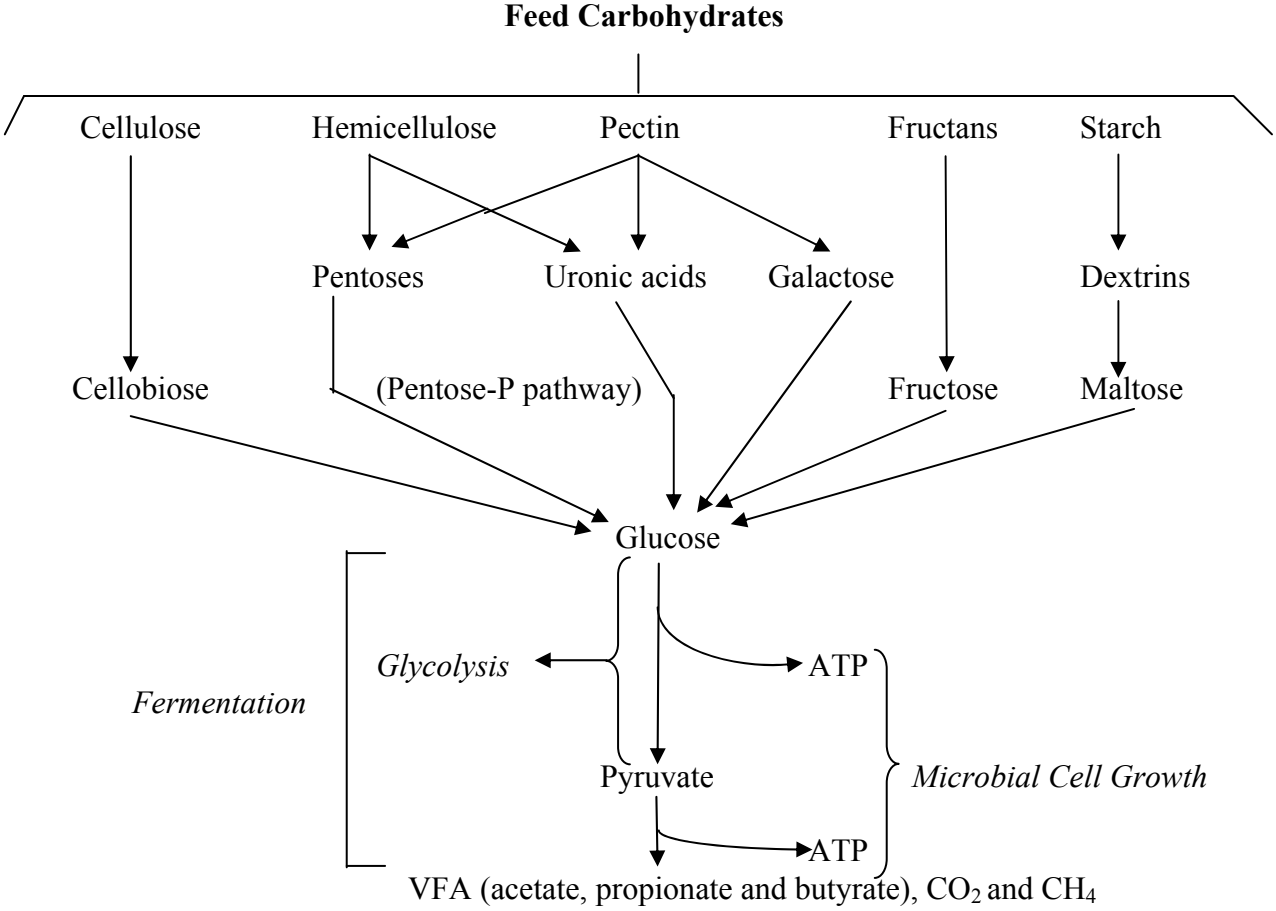

Figure 2. Overview of fermentation of feed carbohydrates in the rumen.

Factors Affecting Carbohydrate Fermentation and End-Product Formation

Diet plays a major role in determining population of ruminal microbes that will be present and fermentation products that will be produced. Diet affects substrates available for microbial growth.

- High-grain diets – an increase in grain feeding will decrease acetate production, increase propionate production and cause a small increase in butyrate production.

- Forage quality – feeding low quality forage results in greater acetate production compared with high quality forage.

- Forage processing – grinding, chopping and pelleting that result in reduced particle size increases propionate and decreases acetate production.

- Concentrate (grain) processing – such as steam flaking, increases propionate production.

- Feeding frequency and level of intake – both increase propionate production.

All of the above factors are related to rate of substrate availability to ruminal microbes and also effects of pH and liquid dilution rates, which have a major influence on microbial populations and VFA production. For example, carbohydrates in grain (starch and sugar) are more rapidly fermented compared with cellulose resulting in higher concentrations of total VFA in the rumen. In addition, with high-grain diets, finely processed forages, or very immature forages, rumination time and saliva flow decrease. These factors and rapid accumulation of VFA lead to a decrease in ruminal pH. Decrease in saliva flow results in decreases in buffering, liquid dilution rate and rumen volume. Decreases in ruminal pH and liquid dilution rate favor amylolytic bacteria that produce propionate and decreases cellulolytic and methanogenic bacteria that produce acetate and methane.

Volatile Fatty Acid Absorption and Metabolism

The importance of VFA as a source of energy for ruminants is well recognized. Nearly all VFA produced are absorbed in the rumen, reticulum and omasum with very little reaching the abomasum. Acetate and butyrate are classified as ketogenic VFA and propionate is gluconeogenic, being the only VFA to make a net contribution to glucose synthesis.

- Acetate – although a small amount of acetate absorbed through the rumen wall is converted to ketone bodies, most is carried to the liver unchanged. Most acetate is oxidized via the TCA cycle or used for fatty acid synthesis. Acetate is the main precursor for lipogenesis in ruminants and production of adequate concentrations of acetate in the rumen is essential to maintain adequate quantities of milk fat.

- Butyrate – is largely converted to ketones during absorption through the rumen epithelium resulting in very low levels of butyrate in the portal blood. Beta-hydroxybutyrate comprises more than 80% of ketones formed (besides acetoacetate and acetone), and is used for fatty acid synthesis in adipose and mammary gland tissue.

- Propionate – during absorption through the rumen epithelium, 2 to 5% of propionate is converted to lactic acid, with the remainder entering the blood as propionate. Propionate is the only VFA that makes a net contribution to glucose synthesis and is the most important precursor for gluconeogenesis.

Fermentation of Protein in the Rumen

Proteolysis in the Rumen

Ruminal degradation of protein from dietary feed ingredients is one of the most important factors influencing intestinal amino acid supply to ruminants. Proteolysis determines the availability of ammonia nitrogen, amino acids, peptides and branched-chain volatile fatty acids, which influence microbial growth rates in the rumen. Rate and extent of ruminal proteolysis not only affect microbial protein synthesis but also quantity and quality of undegraded dietary protein that reach the duodenum.

Dietary protein degradation in the rumen involves attachment of bacteria to feed particles, followed by activity of cell-bound microbial proteases. Approximately 70 to 80% of ruminal microorganisms attach to undigested feed particles in the rumen and 30 to 50% of the attached microbes have proteolytic activity. A large number of different microbial species form a consortium that attaches to a feed particle, acting symbiotically to degrade and ferment nutrients, including protein. Products resulting from this process are peptides and amino acids. Because the number of different bonds within a single protein is large, the synergistic action of different proteases is necessary for complete protein degradation. Rate and extent at which protein degradation occurs will depend on proteolytic activity of ruminal microflora and type of protein (susceptibility and accessibility of peptide bonds).

Peptides and amino acids resulting from extracellular rumen proteolytic activity are transported inside microbial cells (Figure 3). Peptides can be degraded further by peptidases into amino acids and the latter can be incorporated into microbial protein or further deaminated to VFA, CO_2 and ammonia. The fate of absorbed peptides and amino acids once inside the microbial cell will depend on availability of energy in the form of carbohydrates. If energy is available, amino acids will be transaminated or used directly for microbial protein synthesis. However, if energy is limiting, amino acids will be deaminated and their carbon skeleton fermented into VFA. Some ruminal bacteria lack mechanisms of amino acid transport from the cytoplasm to the extra-cellular environment and AA absorbed in excess must be excreted from the cytoplasm as ammonia.

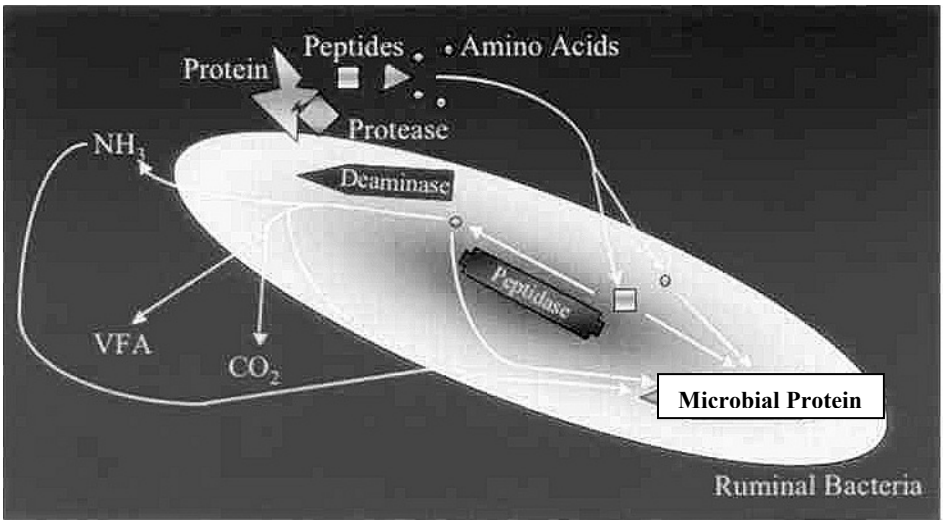

Figure 3. Proteolysis in the rumen and fate of fermentation end-products.

Factors that Affect Ruminal Degradable Protein (RDP)

- protein solubility – refers to the amount of protein that actually dissolves in ruminal fluid, which is distinct from being broken down by microbial activity. Protein solubility is a factor that can affect proteolysis but is not the same as RDP which is the proportion of protein that is hydrolyzed to amino acids or ammonia in the rumen.

- retention time in the rumen – the longer the retention time of protein in the rumen, the greater RDP due to increased exposure to microbial proteases.

- particle size – a decrease in particle size results in an increase in surface area of dietary protein and a greater RDP.

- ruminal pH – can affect RDP by altering microbial activity and changing protein solubility. Optimal pH occurs between 6 and 7 and a decrease in pH below 6.0 can result in a reduction in proteolysis.

- stage of plant growth – in immature plants, there is more NPN and less lignin bound N, therefore there is a greater RDP in immature plants.

Importance of RDP

Protein degradation is the largest variable in estimating the amount of protein supplied to the small intestine because it dictates N availability to microbes for growth and how much protein leaves the rumen undegraded. Ideally, degradation of protein should be optimized so that ***no*** excess protein is degraded in the rumen and N is not lost by NH_3 absorption from the rumen. This latter effect where excess NH_3 is absorbed across the rumen wall can be due to a

deficiency in fermentable carbohydrate (that provides energy and a carbon skeleton to ruminal microbes) or an excess of RDP.

Microbial Protein Synthesis

The ultimate goal of proper rumen nutrition is to maximize microbial growth and the amount of ruminal degraded protein that is captured into rumen microbial cells. Maximizing the capture of degradable N not only improves supply of AA to the small intestine, but also decreases N losses. The rumen is a complex environment inhabited by different microbial species, with each species having different nutrient requirements and metabolism. Therefore, considering the nutrient requirements of ruminal microorganisms is crucial to understanding N metabolism in the rumen as well as the factors (Figure 4) that may modify it.

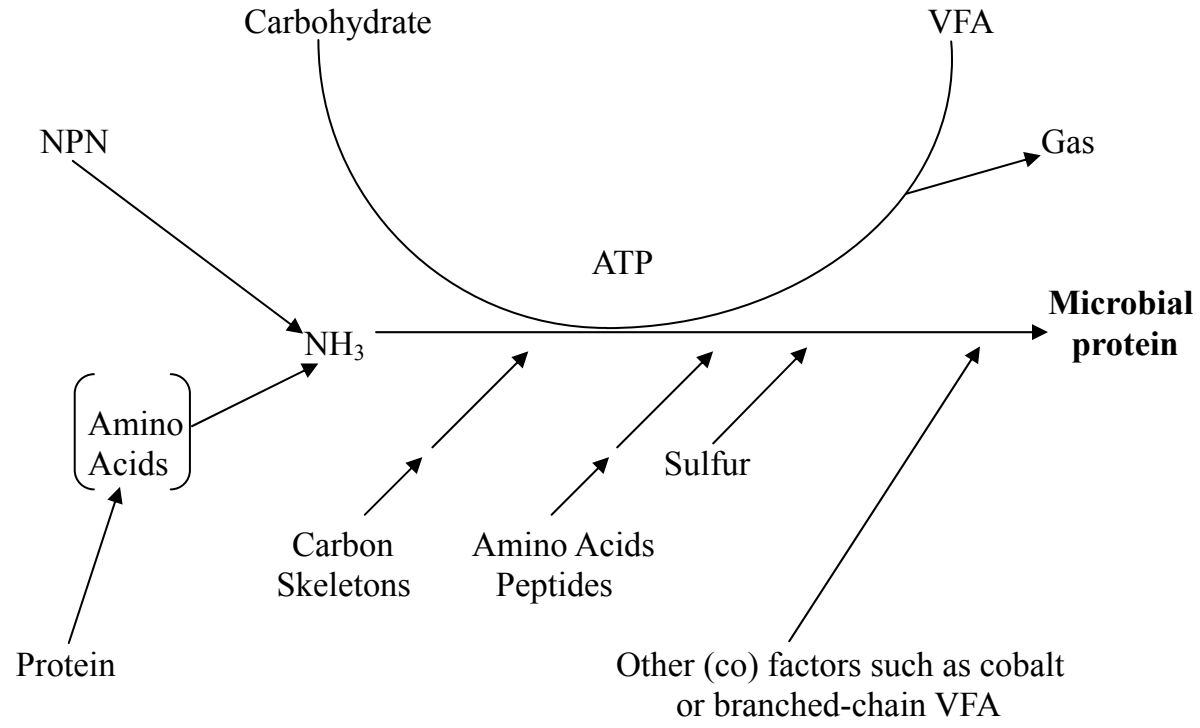

Figure 4. Microbial protein synthesis in the rumen.

Factors That Affect Ruminal Microbial Protein Synthesis

- available energy – is determined by fermentability of feed in the rumen which is affected by whether carbohydrate is structural or nonstructural. Starches and sugars are most rapidly fermented. Insufficient energy available to microbes may:

  1) reduce intestinal amino acid supply by reducing microbial protein synthesis

  2) increase absorption of NH_3 across the rumen wall resulting in an increase in stress on liver metabolism

- availability of N – dependent on rate of availability of N and the form of N; NH_3, amino acids or peptides.

- presence of micronutrients – sulfur requirements by ruminal microbes are associated with N for protein synthesis. It has been shown that a ratio of 10 parts of N to 1 part of sulfur allows microbes to achieve maximal growth. A few studies have shown an increase in microbial protein synthesis with supplemental cobalt while most other studies have shown no effect on protein synthesis.

- dilution rate – refers to the percentage of the rumen volume that is replaced per hour. In general, a faster dilution rate supports a more efficient bacterial population that grows faster resulting in greater microbial protein synthesis.

- branched chain VFA – are derived from deamination of branched-chain amino acids. For example:

  valine ⟶ iso-butyrate
  leucine ⟶ iso-valerate
  iso-leucine ⟶ 2-methylbutyrate

  The significance of branched-chain VFA is that certain bacterial species, such as the cellulolytic bacteria, have a great requirement for them which can impact microbial growth.

Importance of Microbial Protein

Contribution of microbial protein to intestinal amino acid supply is extremely important to dairy cattle and other ruminants because:

- Microbial contribution to total intestinal amino acid supply is quite high, usually within a range from 60 to 80% of total supply. The remainder of the supply is derived from ruminal undegraded (RUP) dietary protein and endogenous protein.

- Absorption from the small intestine of amino acids from microbial protein is consistent and ranges from 85 to 95% compared with amino acids from dietary RUP which averages 80% and can range from 30 to 90%.

- Microbial protein is extremely high in protein quality compared with animal products and dietary protein sources. The two-first limiting amino acids for ruminant production, lysine and methionine, are greater or similar in composition to that in wool, cattle tissue or milk (Table 1). Of greater significance is the comparison of essential amino acids between rumen microbes and dietary protein sources, which is much higher in microbial protein.

Table 1. Amino acid composition of proteins (expressed as a percentage of protein)

	Animal products				Dietary protein sources		
Amino acid	Wool	Cattle tissue	Milk	**Rumen microbes**	Alfalfa	Corn grain	Soybeans
Leucine	5.9	8.6	9.7	**9.4**	7.2	11.1	7.4
Lysine	3.6	6.0	8.1	**11.3**	7.2	2.5	6.3
Serine	9.0	3.9	5.6	**4.8**	3.9	3.9	3.9
Threonine	5.8	4.5	4.6	**6.4**	3.9	4.0	3.7
Alanine	2.9	4.0	4.9	**6.8**	4.6	5.1	4.8
Isoleucine	2.4	3.4	5.9	**7.3**	6.5	5.1	5.5
Valine	4.5	4.9	6.6	**7.2**	4.6	4.0	5.2
Methionine	.5	2.3	2.6	**2.6**	.7	2.0	1.3
Histidine	1.3	2.0	2.7	**2.2**	2.0	2.0	2.4
Tryptophan	.7	.7	1.4	**.6**	1.3	1.0	1.3

Rumen Physiology and Animal Health

Metabolic disorders such as acidosis and bloat are caused by changes that occur in the rumen environment and alteration of the rumen microbial population. Rumen acidosis is thought to be the most common digestive upset in dairy cattle. Rations that are low in fiber and high in rapidly fermentable carbohydrates have a greater tendency to be acidogenic. Rumen acidosis has been associated with low milk fat, erratic feed intake, diarrhea, laminitis, and liver abscesses, although the indistinguishable symptom of subacute rumen acidosis (SARA) is low rumen pH. Rumen acidosis is a function of fermentation end-product accumulation in the rumen, mainly VFA and lactic acid. Accumulation of end products is due to excessive

fermentation that produces large amounts of VFA, or an inadequate removal of these VFA from the rumen via absorption through the rumen wall, wash out from the rumen via passage rate, or neutralization with buffers or alkalinizers. Rumen acidosis is commonly classified as chronic when average rumen pH is about 5.6; acute when average rumen pH is about 5.2; and subacute (SARA) when average rumen pH is between 5.2 and 5.6. In addition to potential health issues that SARA may cause, SARA can also impact ruminal digestion of fiber and protein and may facilitate erratic feed intake and alter milk composition. There is a great deal of literature regarding acidosis and bloat; however one area that has not been addressed adequately is the effect of moldy feeds on rumen physiology and animal health.

Corn silage and high moisture corn are the fermented feeds most frequently used in beef and dairy operations in the US. A multitude of antiquality factors, including molds, may adversely affect the quality of fermented feeds. Molds identified in fermented feeds include: *Aspergillus* sp., *Cladiosporum* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp. Adverse effects of molds may occur either through their effects on nutrient quality or their production of mycotoxins. *Penicillium* sp. are common contaminants of fermented feeds and are known to produce several mycotoxins including patulin,(4-hydroxy-4H furo (3,2C)pyran- 2(6HO)-one).

Patulin contamination of silage has been associated with hemorrhagic disorders in English cattle and other patulin-related diseases have been reported in Japan, France, and Germany when cattle ingested moldy fermented feeds. Patulin is known to be toxic to a wide range of organisms including microbes, plants, and animals and has an antimicrobial effect on aerobic Gram positive and Gram negative bacteria and also affects anaerobic bacteria.

In collaboration with the Veterinary Diagnostics Laboratory at the University of Minnesota (Michael Murphy and Ofelia Tapia), we conducted research (Anim. Feed Sci. Technol. 97:239-246, 2002) to study the effects of patulin on rumen fermentation. Eight continuous culture fermenters (Figure 5) were used to study effects of different concentrations of patulin on rumen microbial fermentation. Two 1L fermenters were spiked with 0, 30, 60 or 90 ppm of patulin every 12 hours for three consecutive days.

Patulin adversely affected digestion, nitrogen metabolism and energy utilization in a dose-dependent manner. Apparent OM digestion and pH were similar among treatments (Table 2). However, true OM digestion, corrected for bacterial matter, decreased ($P < 0.05$) with addition of patulin. Similarly, digestion of ADF also decreased ($P < 0.01$) with addition of patulin. Total VFA production (m*M*) in fermenter effluent decreased ($P < 0.05$) from 180.1 to 119.5 with 90 ppm of patulin but was not affected by 30 and 60 ppm of toxin. Molar proportions of acetate decreased ($P < 0.01$) with patulin addition. This observation was consistent with low digestibilities of OM and ADF and may be the result of a modification of the cellulolytic bacterial population. Conversely, there was an increase ($P < 0.05$) in the molar proportion of butyrate in the fermenters treated with all levels of patulin. Molar proportions of propionate were not affected by any level of patulin.

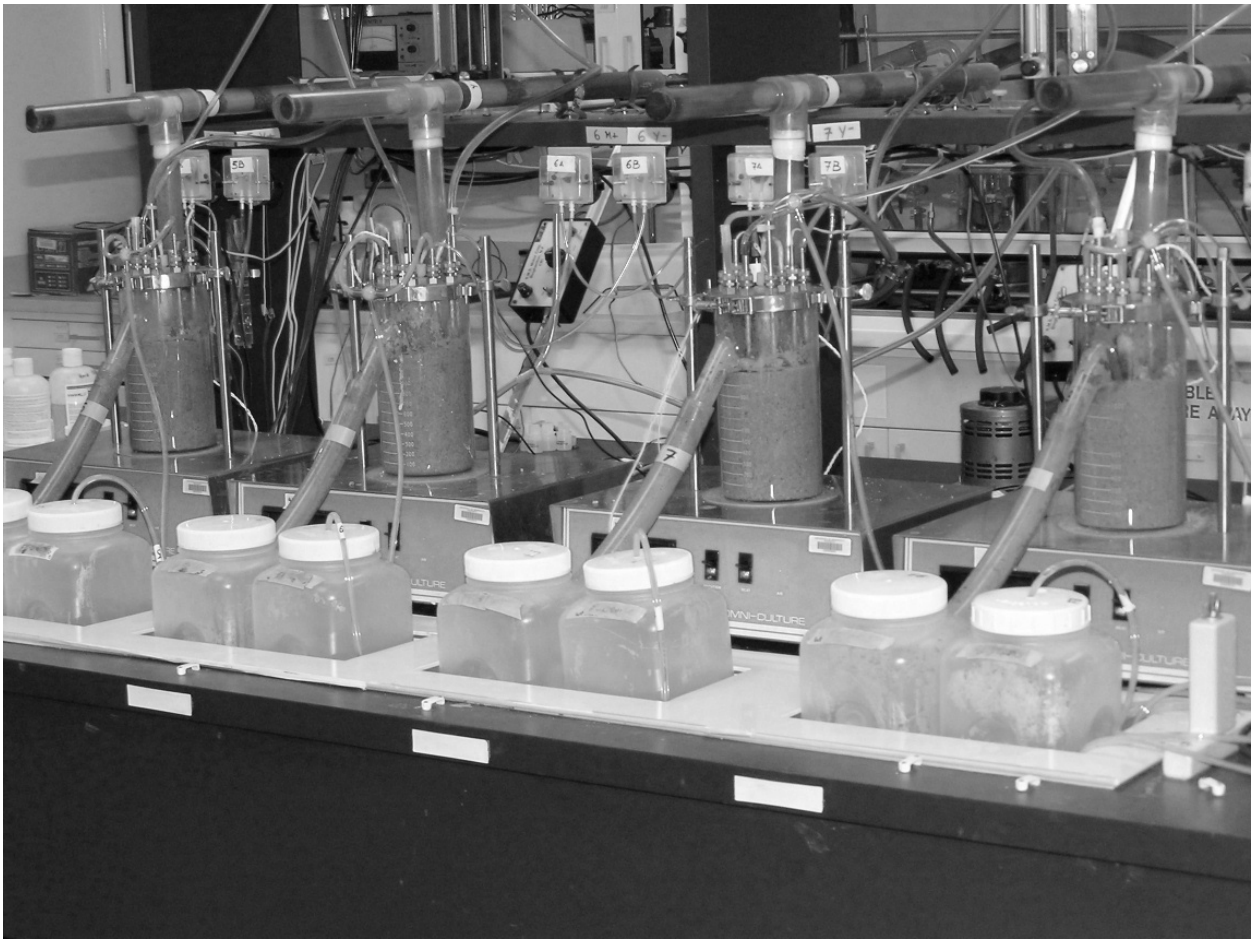

Figure 5. Continuous culture fermentation system at the University of Minnesota.

The highest addition of patulin (60 and 90 ppm) induced a reduction in branched-chain fatty acid (BCVFA) production ($P < 0.01$). Branched-chain amino acids are precursors to some BCVFA. As described earlier, some BCVFA are essential growth factors for certain ruminal bacteria, particularly the cellulolytic species. Low CP degradation (Table 3) may have led to low BCVFA concentrations and subsequent reductions in cellulolytic activity.

Effects of patulin on rumen nitrogen metabolism are shown in Table 3. Crude protein digestion and bacterial N flow were reduced by patulin treatment ($P < 0.05$ and $P < 0.01$, respectively)**.** The decrease in bacterial N flow correlated with the greater ($P < 0.05$) concentration of dietary N outflow observed in patulin treated fermenters. Ammonia N concentration in the 30 and 60 ppm fermenters decreased ($P < 0.05$) in comparison with the 90 ppm treatment, but not when compared to the control. Ammonia-N concentration in fermenter fluid depends on extent of protein degradation and rate of N uptake by the microbes. Crude protein degradation and bacterial N flow were lower in all patulin-treated fermenters. An equivalent decrease in both production and utilization of ammonia may

explain the absence of a change in NH_3-N concentration between the 30 and 60 ppm fermenters. Ammonia N concentrations in these fermenters were below 5 mg/dl, the concentration where maximal growth occurs. This possible limitation in available NH_3-N may be partially responsible for low bacterial N flow. The NH_3-N concentration increased ($P < 0.05$) with the 90 ppm treatment. The main cellulolytic bacterial species utilize ammonia as their main source of N for microbial protein synthesis. A decrease in this bacterial population or in its efficiency ($P = 0.07$) of N utilization (g of microbial nitrogen/ g of ruminal available nitrogen) could lead to an accumulation of NH_3-N in the effluent. It is also possible that the ability of ruminal bacteria to degrade urea provided by saliva might have exceeded the ability of bacteria to capture resulting ammonia into bacterial protein. Efficiency of bacterial synthesis expressed as grams of bacterial N per kg of OM truly digested, was affected ($P < 0.05$) by patulin treatment. Values ranged from 39.0 in fermenters without patulin to 19.7 g of N/kg of OM truly digested in fermenters treated with the highest concentration of toxin. Efficiency of bacterial synthesis depends on many dietary and ruminal factors as described earlier.

Table 2. Influence of patulin level on pH, digestion and VFA concentrations in continuous culture fermenters.

	Patulin level (ppm)				
Item	0	30	60	90	SE
pH	6.21	6.22	6.26	6.32	.02
Digestion, %					
Apparent OM	26.1	22.9	18.7	24.4	6.2
True OM[1]	43.0[a]	32.9[b]	29.0[b]	32.3[b]	4.1
ADF	28.7[a]	15.7[b]	16.9[b]	16.5[b]	5.1
Total VFA, mM	180.1[a]	153.5a[b]	159.9[ab]	119.5[b]	23.3
Individual VFA	----------mol/100 mol----------				
Acetate	63.6[a]	41.5[c]	44.7[c]	48.8[b]	4.3
Propionate	19.0	25.2	26.4	19.4	4.6
Butyrate	13.2[a]	25.8[b]	21.1[ab]	26.4[b]	5.0
Valerate	2.1[b]	4.6[a]	5.8[a]	4.8[a]	0.6
Branched-chain	2.1[b]	2.9[a]	1.0[c]	0.6[c]	0.5

[1] Corrected for contribution of bacterial OM in the effluent.
[a,b,c] Means within a row with uncommon superscripts differ ($P < .05$).

Table 3. Influence of patulin level on nitrogen metabolism in continuous culture fermenters.

	Patulin level (ppm)				
Item	0	30	60	90	SE
Ammonia N, mg/100 ml	6.0[b]	4.0[b]	4.3[b]	10.2[a]	1.3
CP degradation, %	64.5[a]	42.9[b]	40.9[b]	40.5[b]	8.8
Bacterial synthesis, g of N/kg of OMTD	39.0[a]	28.3[ab]	31.9[ab]	19.7[b]	7.5
N flow, g/d					
Total	2.02	1.98	2.03	1.93	0.17
Nonammonia	1.92	1.92	1.98	1.77	0.20
Bacterial	1.13[a]	0.65[b]	0.65[b]	0.44[b]	0.10
Dietary	0.79[a]	1.27[b]	1.32[b]	1.32[b]	0.19

[a,b] Means within a row with uncommon superscripts differ ($P < .05$).

Conclusions

The ruminal microbial population is an integrated system with numerous interrelationships. Alterations and modifications of the microbial population are likely a combination of various interactions. Therefore, changes in microbial fermentation and production of bacterial protein and bacterial end-products could be attributed to alterations of ruminal environment, nutrient source, concentration and metabolism, and other factors such as toxins in fermented feeds. These changes can impair fiber digestion, reduce feed intake and cause health disorders to dairy cattle resulting in a decrease in animal performance. Therefore, it is imperative to maintain a "normal" rumen in regard to bacterial population and function for sustaining a healthy animal.