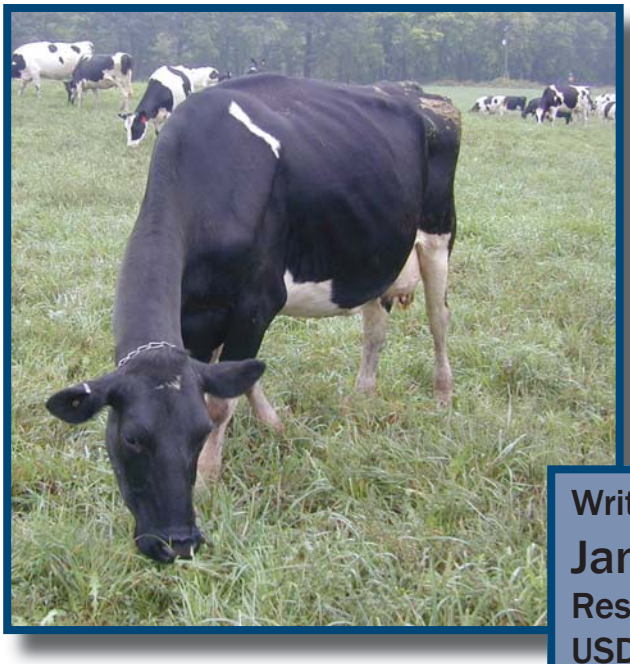
Ruminations

Questions & Answers

and other thoughts on the role of rumen microbes in dairy cattle



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and other thoughts on the role of rumen microbes in dairy cattle

by James B. Russell, PhD

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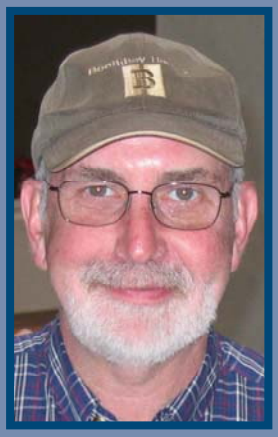

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It is based on a series of articles, written by James B. Russell, that originally ran in Farm Progress publications.

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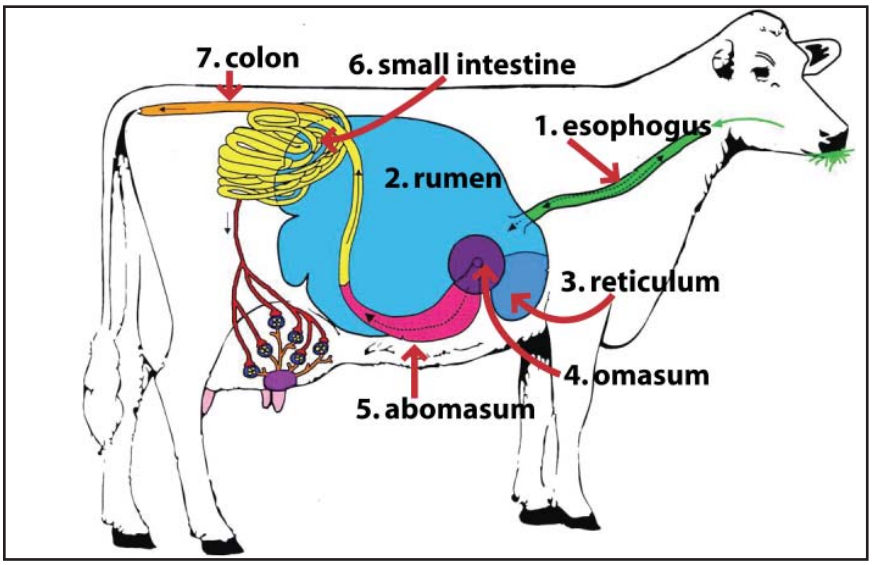
Q: What is a ruminant?

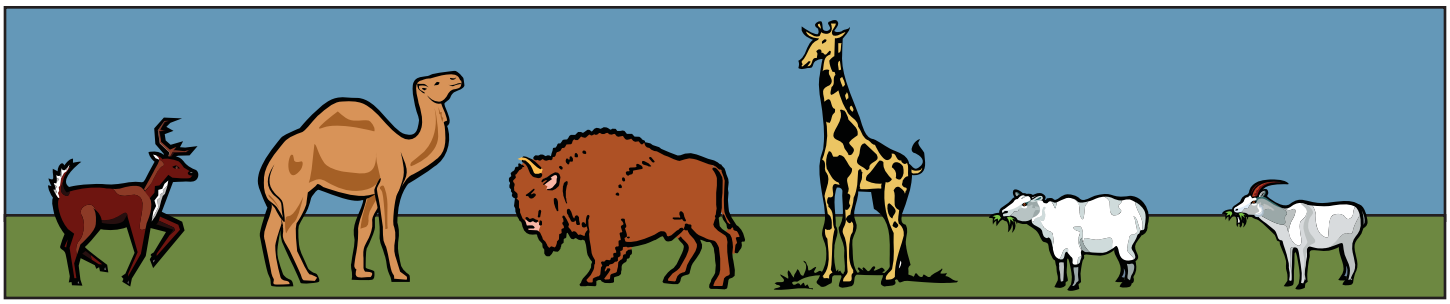
A: Man has long depended on ruminant animals for food. Prehistoric hunters often followed herds of grazing ruminants, and domestication of these herbivores allowed early man to more fully exploit the photosynthetic potential of the temperate grasslands. Domesticated ruminants were a symbol of economic status and well being, and ancient Jews were quick to recognize the comparative safety of ruminant meat. "Whatever divides a hoof, thus making split hoofs, and chews the cud, among the animals, that may you eat" (Leviticus 11:3). Aristotle, a scholar of many disciplines, described the four compartments of the ruminant stomach, but the role of microorganisms in the ruminant style of digestion was not recognized until 1884 when H. van Tappeiner added antiseptics to rumen fluid and demonstrated an inhibition of fiber digestion.

> "... four-footed, hoofed, even-toed, and cud chewing mammals ... which have a stomach consisting of four divisions or chambers."

Webster's dictionary describes ruminants as "any of a group of four footed, hoofed, even toed, and cud chewing mammals as the cattle, buffalo, bison, goat, deer, antelope, camel, giraffe, llama, etc., which have a stomach consisting of four divisions or chambers, the rumen, reticulum, omasum, and abomasum; the grass etc. that they eat is swallowed unchewed and passes into the rumen or reticulum from which it is regurgitated, chewed and mixed with saliva, again swallowed, and then passed through the reticulum and omasum into the abomasum where it is acted on by gastric juice." This definition is an anatomically detailed description, but it lacks any mention of microorganisms or fermentation.

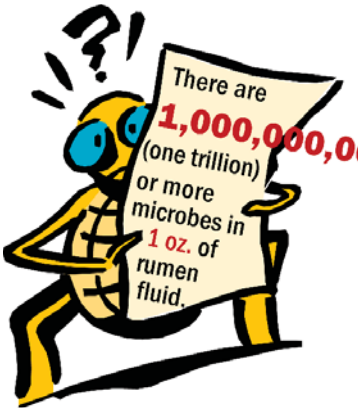


Modern agricultural practices have placed new constraints on domestic ruminants and rumen fermentation. Genetic improvements have produced animals that have much greater nutritional needs, and the increased size of farms has intensified the problem of manure disposal. Following is a series of articles on rumen fermentation. Each of these articles will focus on some aspect of rumen fermentation or a problem unique to ruminant animals. ❖



Q: Are rumens really full of bacteria and other bugs?

A: Yes, and we're glad they are. Rumen microbes could be considered a cow's best friend. Without microbes, a cow's digestive system would shut down and she would starve to death.



Cows and microbes actually have a mutually beneficial relationship. Microbes give the cow:

- labor to digest feed;
- a source of protein;
- a source of volatile fatty acids;
- the ability to digest forage.

On the other hand, cows provide microbes with:

- water;
- warmth;
- grinding (cud chewing or rumination) of feed;
- anaerobic (no oxygen) conditions.

There are three main groups of rumen microbes:

- ***Bacteria*** carry out most of the digestion of sugars, starch, fiber, and protein for the cow.
- ***Protozoa*** swallow and digest bacteria, starch granules, and some fiber.
- ***Fungi*** make up only a small fraction of the rumen microbial population, but they appear to be important particularly if the forage quality is poor. They are though to open plant fibers to make them more easily digested by the bacteria.

Almost all rumen microbes are anaerobic; they will only grow in an oxygen-free environment. New microbes are constantly being produced in the rumen (under adequate conditions) while old ones are passed on down through the cow's digestive tract. One type of rumen bacteria can double its population in 24 minutes!

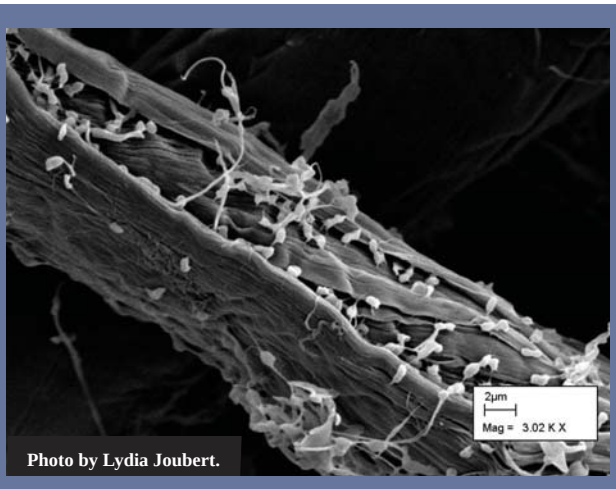
In many ways, when you feed your cow you're really feeding the microbes in her rumen. The cow cannot directly utilize most feed components, even simple sugars. She relies on rumen microbes to convert feeds to volatile fatty acids (VFAs) that the cow absorbs and uses to make energy and milk. About two-thirds of feed digestion takes place in the rumen, and 90 percent of fiber digestion – all with the aid of microbes.

While rumen microbes help cows ***digest*** feed, they're also an important ***source*** of feed for cows. They're typically about 55 percent protein; on some rations the microbes provide 90 percent of the total dietary protein requirement of the cow. Even more amazing is the fact that rumen microbial protein has an ideal mixture of amino acids – one that is not

easily duplicated in any ration. And rumen microbes are a particularly rich source of lysine and methionine, two amino acids that are difficult to supplemnt in dairy cattle rations.

Rumen microbes, cows and other ruminants can make use of feed, like alfalfa and grasses, that people can't eat. Many of these plants are grown on land that isn't suitable for other crops. Thus, cattle produce food from land that might otherwise be under utilized. Cows are also useful consumers of by-products from human food production (like citrus or beet pulp) and from grain ethanol production (distillers grains). ❖

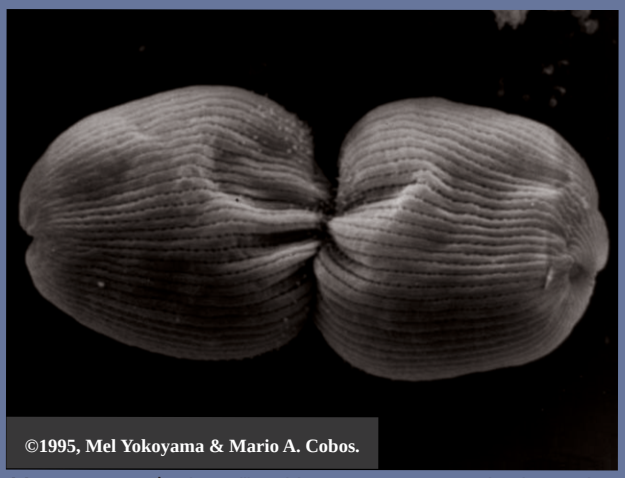
Photographs of rumen microbes



Bacteria attacking a strand of fiber that was taken from a cow's rumen.

This is one of the biggest rumen protozoa. Protozoal cells are much larger than bacterial cells; some can almost be seen with the naked eye.

The large microbe is a type of protozoan. The creature that looks like a tadpole attached to the side of the protozoan is a fungal spore. The smaller, rod-shaped beasts lining the underside of the protozoan are bacteria.



Most rumen microbes, like this protozoan, grow by increasing their cell size, then splitting in half to make two cells. Under ideal conditions, most species of rumen bacteria can double their populations in a few hours.

The hairy stuff on the outside of this protozoal cell is “cilia” – Latin for eyelash. These cilia wave back and forth and either propel the microbe through the water or propel food toward it. This protozoan has several chains of bacteria that are stuck to it.

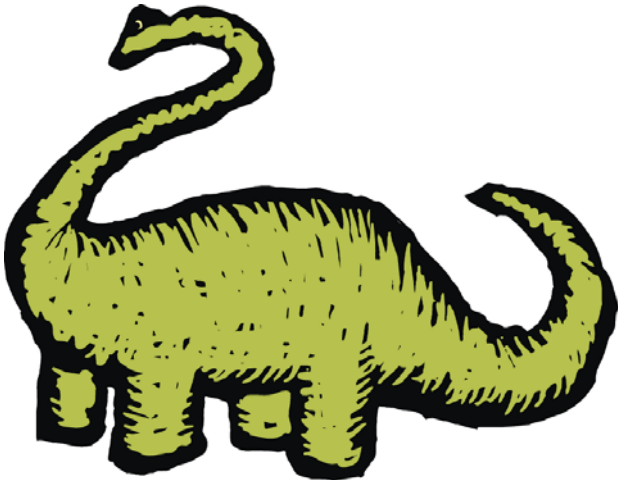
This rumen protozoan is covered with chains of bacteria. Some protozoal and bacterial cells actually have a symbiotic relationship, each giving the other something that it needs to survive.

Q: Did dinosaurs have rumens?

A: With films like Jurassic Park and Lost World, there has been an increased interest in dinosaurs. Some of the dinosaurs were meat-eating carnivores, but the largest ones were plant-eating herbivores. Because ruminant animals (cattle, sheep, goats, deer, etc.) utilize rumen microorganisms to digest plant material, it is reasonable to ask the question "Did dinosaurs have a rumen?"

The script to Jurassic Park indicated that dinosaurs were "giant cows," but there are scientific reasons for disputing this idea.

All animals have microorganisms in their gastrointestinal tracts, but the rumen is a highly specialized organ that facilitates the digestion of fibrous plant materials. Adult ruminants have a very large rumen compartment, and the rumen usually accounts for 1/10 to 1/7 of the animal's weight. By having a large rumen, ruminants are able to retain feed material for a long time, and this gives the microorganisms a greater chance to digest food.



Rumen retention time increases with the size of the ruminant. The duiker is a very small ruminant that lives in Africa, and mature adults have a weight that is less than 10 pounds. Because duikers have very small rumens, they are not able to eat as much fiber as large ruminants like cattle and buffalo. Duikers are browsers, and they consume tender leaves that are rich in sugars and other readily digested carbohydrates.

The strategy of having an ever larger rumen so the microorganisms will have more time to digest fiber has another constraint. Acetic acid (the acid found in vinegar) is the primary end-product of rumen fermentation, and the ruminants use this acid for energy. If the rumen retention time gets too long, a bacterium called *Methanosarcina* is able to convert acetic acid to methane and carbon dioxide. Since these gases are belched away, the animal would loose most of its energy. Nature has prevented the problem of acetate conversion to gas by making sure that retention time is never too long and is short enough to wash *Methanosarcina* out of the rumen.

> **"The problem with dinosaurs having a traditional rumen is their very large body size."**

The problem with dinosaurs having a traditional rumen is their very large body size. The largest plant-eating dinosaurs weighed more than 25 tons. Based on the observation that feed retention time in the rumen and body size appears to be a linear function, the retention time of a dinosaur rumen could be greater than 1250 hours, a time clearly not compatible with efficient acetate utilization.

Some large herbivores have a different strategy for digesting plants. The cecum is an enlarged pouch that extends from the intestines, and it too can serve as a habitat for microorganisms. Humans have a very small cecum (the appendix), but horses and elephants have enlarged ceca. Cecal fermenters do not retain feed as long as or digest plant materials as completely as ruminants, but this may not have been a problem for dinosaurs. Large plant-eating mammals had not yet evolved, and plants may have been more digestible. ❖

Q: Do belching cows contribute to global warming?

A: The rapid conversion of fossil fuels to carbon dioxide has lead many scientists to believe that the earth is becoming a global greenhouse. Because even small changes in average temperature would have catastrophic effects on climate, ocean depth and other aspects of our environment, the U.S. government has undertaken a detailed examination of all facets of global warming. Carbon dioxide is clearly the most abundant "greenhouse gas," but it is not the most potent one. Methane is 15 times more potent than carbon dioxide in trapping the sun's radiation.

Methane gas is derived from a variety of sources that include natural gas leaks from the petroleum industry as well as biological sources. Biological methane, or "biogas," is emitted from wetlands, rice paddies, termite guts, and ruminants. Estimates of ruminant methane production have changed considerably. Early estimates indicated that ruminants might be producing as much as a third of the total, but more recent estimates are 15 to 20%.

Methane is a natural end-product of rumen fermentation and feed digestion. Cattle produce as much as 50 liters of gas per hour and as much as 1/3 of this gas can be methane. Because methane represents a significant loss of feed energy, nutritionists sought ways of reducing methane production in the early 1970s. Rumen bacteria that produce methane are very sensitive to chlorinated hydrocarbons like chloroform, and there is enough chloroform in three medicated cough drops to completely inhibit the methane production of a sheep. The ability of chloroform to inhibit rumen methane production was, however, confounded by the ability of rumen bacteria to adapt. Ruminal bacteria eventually reduced and inactivated the chloroform.

It the mid-1970s a veterinary pharmaceutical company developed a product called monensin to treat an intestinal disease, *coccidiosis*, in pigs and chickens. However, researchers noted that this antibiotic could also improve feed efficiency of sheep. Monensin and other ionophroes don't completely inhibit methane production, but reductions as great as 33% have been reported. Monensin has a different mode of action from chloroform. Methane-producing bacteria are not sensitive to monensin, but it prevents carbohydrate-fermenting bacteria from producing hydrogen, a precursor of rumen methane.

> **"The impact of American cattle on global biogas production and global warming is still being analyzed, but the effect is probably insignificant."**

The rumen is normally well buffered by saliva, but rumen pH can decline (acidosis) if the rate of starch fermentation is rapid. Low rumen pH causes a variety of problems (discussed in forthcoming segments), but it has at least one positive effect. Low rumen pH inhibits methane-producing bacteria. Animals with low rumen pH produce less methane and retain more of their feed energy.

The impact of American cattle on global biogas production and global warming is still being analyzed, but the effect is probably insignificant because American cattle consume very little feed per unit of production (pounds of milk or meat), often have a rumen pH that inhibits methane production, and are sometimes fed additives that inhibit methane production. ❖

Q: If a cow can digest cellulose, why can't I feed her sawdust?

A: Cellulose is the most abundant polymer in nature, but mammals do not produce enzymes that can digest this material. Ruminant animals, by developing a symbiotic relationship with cellulose-digesting bacteria, have developed considerable capacity to digest cellulose, and ruminant animals can be fed diets that are rich in cellulose and other fibrous materials.

Dr. Paul Weimer (U.S. Dairy Forage Research Center) estimated that the global rumen volume of domesticated ruminants is approximately 100 billion liters (2 billion animals with 50 liters per animal). Based on this estimate, the rumen is the largest commercial fermentation vat in the world.

> **"Rumen bacteria have the capacity to digest virtually any type of cellulose, but the cellulose digestion rate of mature and highly lignified plant cells can be very slow."**

Cellulose and starch are both repeating glucose structures, but the two molecules have different bonds and physical structures. Starch chains can bind to other starch chains, but the hydrogen bonding is relatively weak and can be easily disrupted with mild treatments (e.g. heat). Any person who has made gravy has watched the starch dissolve when the water was heated. By contrast, cellulose chains are so strongly bonded to other cellulose chains that only strong acid can cause solubilization.

The propensity of cellulose molecules to form insoluble bundles reduces the surface area exposed to enzymes, and this feature is the rate limiting step in rumen cellulose digestion. The animal rechews the cud (ruminates) on an almost continual basis and increases the surface area of the cellulose, but this mechanical process of particle size reduction has clear cut and obvious limitations. The plant cell walls of forage crops can be easily disrupted and rumen bacteria typically digest plant cells from the inside out. Woody plant cells are nearly completely filled with cellulose, and have much less surface area for microbial attack.

Another feature that limits the digestion of cellulose and other cellulosic materials is lignin. Lignin is a highly branched compound that binds to hemicellulose. Lignin cannot be degraded by ruminal bacteria and it acts to block the enzymes of rumen microorganisms from ever using some of the carbohydrate. Woody plants (sawdust) typically have a much higher lignin content than forage crops and are inherently less digestible.

Rumen bacteria have the capacity to digest virtually any type of cellulose, but the cellulose digestion rate of mature and highly lignified plant cells can be very slow. Sawdust would provide some energy to a cow, but not enough to maintain her. Corn stalks and poor quality hay provide enough energy for maintenance but not enough for rapid weight gain or lactation. Rapidly growing and heavily lactating animals can utilize high quality forages, but they usually need at least some very rapidly fermented carbohydrate (e.g. starch). ❖

Q: Why do cattle waste so much pasture protein?

A: Domestic ruminants have been allowed to graze pasture since ancient times, but pasture utilization declined when farmers had mechanized methods of harvesting, storing, and feeding forages during the summer months. In the 1980's, rotational grazing gained popularity, and pasture management was purported to decrease the costs of production.

Grazing ruminants can do well on pasture, but it should be recognized that forages are inherently mismatched with respect to carbohydrate and protein degradation. Rumen bacteria grow optimally when approximately 14% of the potentially digestible organic matter is protein and 84% is carbohydrate. Since lush grass and other forage crops have a composition similar to the amounts preferred by rumen bacteria, pasture would, in theory, be an ideal substrate for rumen fermentation. These simple compositions, however, ignore the dynamics of rumen fermentation.

> "This imbalance of carbohydrate and protein digestion provides rumen bacteria with twice as much protein as they can efficiently use. "

Lush forage contains some sugar and other readily fermented carbohydrate, but the bulk of the forage carbohydrate is fiber (cellulose and hemicellulose). The rumen degradation rate of fiber is so slow that only half of it is digested in the rumen. Forage protein is, by contrast, almost completely digested in the rumen. This imbalance of carbohydrate and protein digestion provides rumen bacteria with twice as much protein as they can efficiently use. The excess protein is wastefully converted to rumen ammonia.

Ammonia is a highly volatile substance that is readily absorbed from the rumen into blood. The animal transports the ammonia to the liver and kidney and converts it into urea, a less toxic substance. The animal then excretes the urea as urine. Because grazing ruminants stand still when they urinate, the amount of nitrogen applied to the soil can be extremely high on a g N/square foot basis. If the nitrogen percolates through the soil before the plant captures it, the excess rumen protein ends up in the ground water as urea, nitrate or other types of nitrogen. If the grazing density is high, ruminants can be a very significant source of environmental pollution.

Animals that are not allowed to graze pasture are typically fed total-mixed rations, and under these conditions it is fairly easy to match the rates of rumen protein and carbohydrate degradation. If the ration has an abundance of forage protein, one can simply increase the rate of carbohydrate fermentation by adding high moisture corn or some other rapidly fermented carbohydrate. Animals grazing on pasture could be supplemented in a similar fashion, but this practice has not yet been widely adopted. ❖

Q: Can too much starch in the diet kill my cow?

A: Wild ruminants do not consume large amounts of starch, but cereal grains are often added to domestic rations. Starch increases the overall rate of fermentation, provides a mechanism of balancing the rates of carbohydrate and protein degradation, and usually causes an increase in animal performance. Starch also causes dramatic decreases in rumen pH, acute indigestion, depressions in food intake, founder, and even death of the animal.

Forage-fed cattle have low numbers of *Streptococcus bovis*, a bacterium that grows very rapidly on starch; but its numbers can increase as much as 1,000-fold if a large amount of starch is added to the diet. Because *S. bovis* produces large amounts of lactic acid, the buffering capacity of the rumen is overpowered and pH declines. When the pH is 5.6 or lower, the normal microorganisms are inhibited, and *S. bovis* has an even greater advantage.

Acute acidosis is a calamity. When the rumen pH declines, the blood pH also decreases, and this effect causes organ damage. Because tissues above the horny hoof are particularly sensitive to changes in blood pH, sore feet are also a symptom. If the rumen pH is less than 5.0, water rushes from the blood into the rumen. This flux of water causes hemoconcentration and death. The best prevention for acute acidosis is gradual adaptation to starch and the timely feeding of carefully formulated rations. Under these conditions the normal rumen flora utilizes the starch more effectively, *S. bovis* numbers do not increase so dramatically and lactate does not accumulate.

"The best prevention for acute acidosis is gradual adaptation to starch and the timely feeding of carefully formulated rations."

Ruminants can also develop a subclinical form of acidosis that does not involve *S. bovis* or lactate accumulation. Subclinical acidosis is caused by a very high volatile fatty acid concentration. Researchers have debated the cause of subclinical acidosis, but a decline in rumen motility and absorption is a likely cause. When starch increases, forage decreases, and forage provides tactile stimulation needed to trigger rumen movements. If the rumen does not mix, acids are absorbed more slowly. The role of tactile stimulation in acid absorption is illustrated by the work of Dr. Loerch at Ohio State University. He put nylon pot scrubbers in the rumen and demonstrated an increase in pH.

Hay is the typical remedy for acidosis, but the syndrome can be very difficult to reverse. Because normal rumen microorganisms are eliminated by low rumen pH, the fermentation tends to produce lactate even after the starch is removed. Once the animals have sore feet, they tend to lie down most of the time. When the animals get up, they eat their meals very quickly and this feeding pattern tends to bring pH down. Chemical methods for preventing acidosis but have been incomplete. Rumensin, an additive that inhibits S. bovis only moderates rumen pH somewhat. Sodium bicarbonate is often marketed as a rumen buffer, but it really stimulates water intake. When water intake increases, acid and unfermented starch can be washed out of the rumen. ❖

Q: What role do rumen bacteria play in grass tetany?

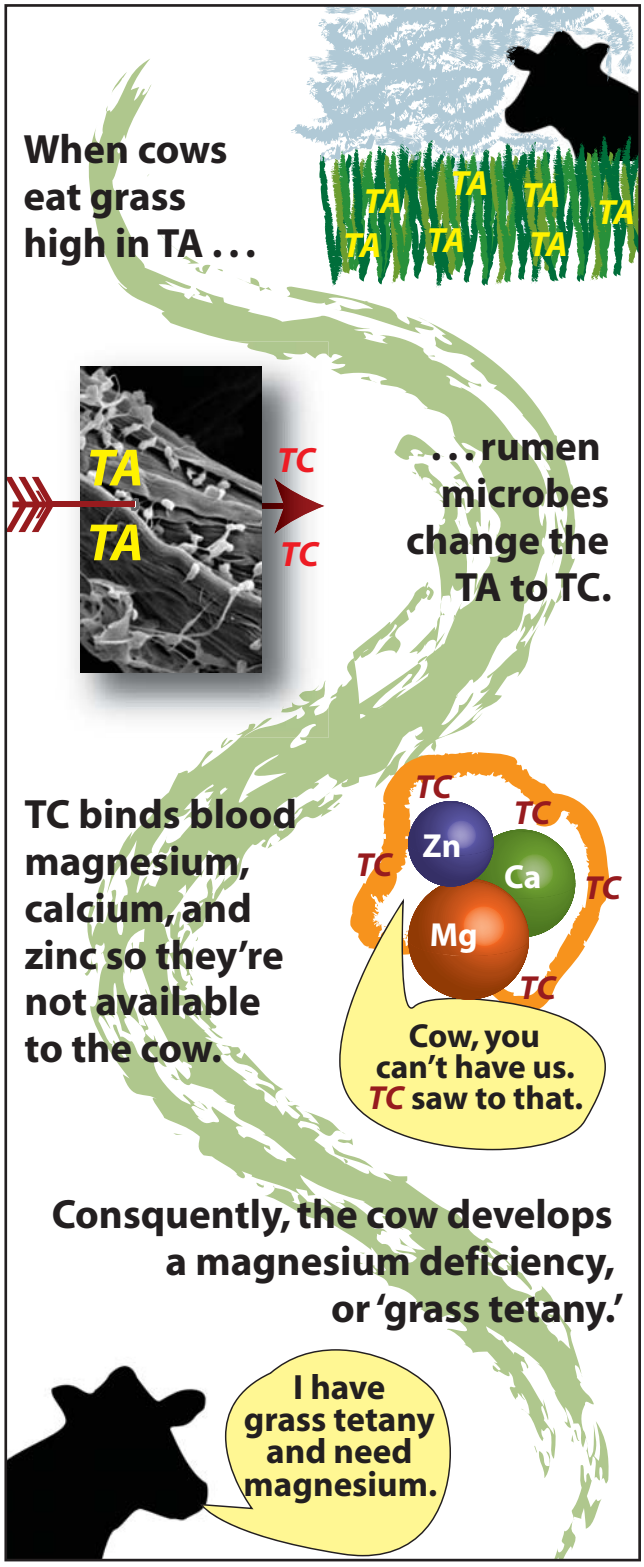
A: Lactating cattle that graze lush grass sometimes develop a severe form of magnesium deficiency commonly called "grass tetany." Grasses causing tetany are low in magnesium, but this deficiency alone could not explain the problem. USDA researchers Dave Grunes and Floyd Horn correlated the incidence of tetany with the presence of an unusual acid in plants, trans-aconitic acid (TA). These grasses accumulated as much as 6% TA, and this acid can bind magnesium.

> "Grasses causing tetany are low in magnesium, but this deficiency alone could not explain the problem."

In the 1980s, we examined the ability of rumen bacteria to metabolize TA and noted that TA was rapidly converted to another acid, tricarballylic acid (TC). Animals absorbed TC, but they were unable to degrade it. TC bound blood magnesium, calcium and zinc and increased urinary excretion. *Selenomonas ruminantium*, a bacterium common in grass-fed cattle, converted TA to TC.

The reason for TA accumulation in plants is not entirely clear, but it may be linked to overfertilization and high soil potassium. When grasses and small grain forages are cultivated with high soil potassium, they take up more potassium and this positively charged ion must be balanced by a negatively charged ion. Some plants of the grass family use trans-aconitate as a negatively charged ion.

Some years ago we isolated a bacterium from the rumen (*Acidaminococcus fermentans*) that was able to convert TA into acetic acid, a normal end-product of rumen fermentation. *A. fermentans* is found at very low numbers in the rumen; it is a rapidly growing organism when TA is available. *A. Fermentans* has potential as a rumen inoculant. If *A. fermentans* numbers in the rumen could be increased, cattle would have a mechanism to protect them from grass tetany. ❖



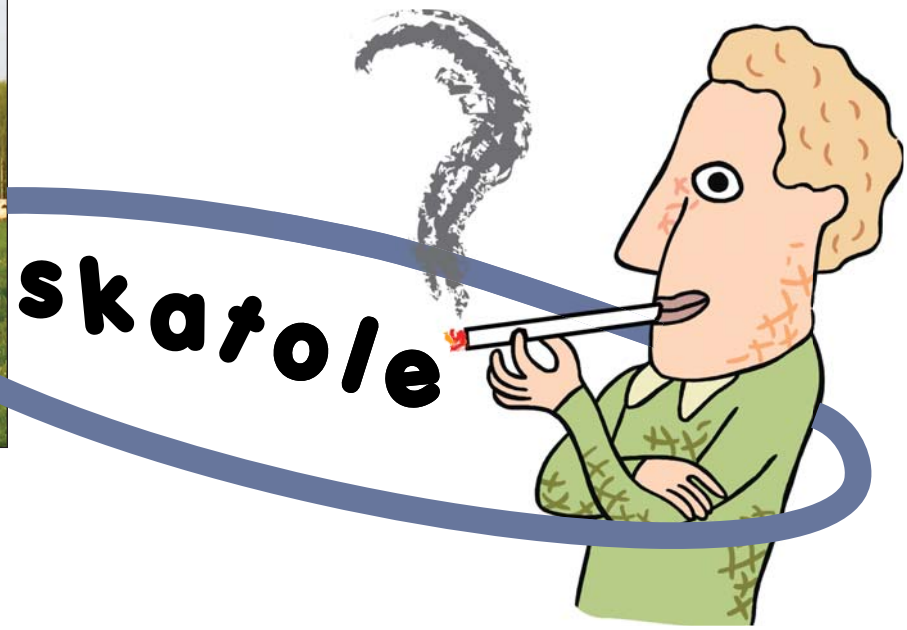
Q: What do cattle and cigarette smokers have in common?

A: In the western United States, cattle are often taken from the range to irrigated pastures in the fall, and this abrupt change in diet can cause a condition known as acute bovine emphysema. Researchers initially suspected some type of mold or fungus, but none could be correlated with the incidence of this disease. Subsequent work by J.R. Carlson and his colleagues at Washington State University demonstrated that rumen bacteria were converting the amino acid tryptophan into skatole, a highly selective lung toxin that is also found in cigarette smoke.

Skatole production in the rumen appears to be a two step process. When normal rumen bacteria are abruptly given large amounts of soluble protein (lush irrigated pasture), they ferment tryptophan and convert it to indoleacetic acid. A highly specialized lactobacillus then takes up the indoleacetic acid and converts it to skatole.

A variety of antibiotics (chlortetracycline, penicillin, neomycin) were able to inhibit skatole production in the laboratory, but rumen bacteria quickly become resistant to traditional antibiotics. Lactobacilli, however, can also be inhibited by the feed additives Rumensin and Bovitec, and resistance does not seem to be a problem. Rumensin and Bovitec have decreased the incidence of bovine emphysema, but these additives can be difficult to administer to grazing cattle. Wise pasture management and gradual adaptation is still the most effective method of controlling this potentially fatal syndrome. ❖

> **" . . . rumen bacteria were converting the amino acid tryptophan into skatole, a highly selective lung toxin that is also found in cigarette smoke."**



Q: How much urea can cattle safely use?

A: Mammals never developed the capacity to synthesize all 20 of the amino acids commonly found in protein and require at least 8 amino acids. Because most rumen bacteria are able to synthesize all 20 amino acids, ruminant animals can be maintained on diets that are protein-free. If the rumen bacteria have sufficient non-protein nitrogen (ammonia, urea, etc.), microbial protein flow from the rumen provides a continuous supply of all 20 amino acids.

Virtually all of the rumen bacteria utilize carbohydrates as an energy source to synthesize amino acids, and urea utilization is directly proportional to the supply of ruminally degraded carbohydrate. Poor quality diets that are rich in fiber supply approximately half as much rumen available carbohydrate as diets based primarily on cereal grains.

Rumen bacteria are never able to utilize more than 7 g of urea per 100 g of rumen degraded carbohydrate. If less carbohydrate is being degraded in the rumen or if the rumen bacteria have a source of ruminally degraded true protein, the animal will need less urea. Dr. Larry Satter of the U.S. Dairy Forage Research Center indicated that cattle receiving 14% true protein receive no benefit from dietary urea supplements.

Adapted animals can protect themselves from excess dietary urea, but excess urea may decrease the energetic efficiency. The animal must expend energy to synthesize and excrete urea as urine. The Cornell Net Carbohydrate and Protein System and the 1996 Beef NRC have a rumen sub-model that allows users to monitor ammonia accumulation in the rumen as well as the cost of urea synthesis by the animal. ❖

When cattle receive more urea than the rumen bacteria use, ammonia accumulates in the rumen and eventually enters the blood. Ammonia is a highly toxic compound, and the animal detoxifies the ammonia by converting it to urea in the liver and kidney. The enzymes needed to synthesize urea are inducible. If the flux of ammonia into blood has been low, the animal will have a low activity of urea cycle enzymes. The animal can synthesize additional urea cycle enzymes, but a period of adaptation is required.

"When cattle receive more urea than the rumen bacteria use, ammonia accumulates in the rumen and eventually enters the blood."

If animals consuming poor quality diets are given a large dose of urea, the rumen bacteria will not be able to utilize all of the rumen ammonia. If the animal is unadapted, ammonia flux into blood can be so great that it kills the animal. The cause of death is still being debated, but the depletion of α-ketoglutarate (a metabolite in brain tissue) appears to be involved.

Q: If cows utilize non-protein nitrogen, why do we feed true protein?

A: Rumen bacteria can utilize non-protein nitrogen sources like urea and supply the animal with amino acids, but animals fed only non-protein nitrogen never attain high levels of production. Highly productive animals have very high amino acid requirements, and the flow of microbial protein from the rumen is inadequate. By supplying protein that is protected from rumen degradation, producers have in many cases increased amino acid supply and improved animal productivity.

When animals are fed rations containing high quality forages or soybean meal, rumen microorganisms often have more true protein than they can utilize, and the remainder is converted to excess rumen ammonia and eventually urinary urea. Because rumen bacteria can utilize non-protein nitrogen, many ruminant nutritionists thought that virtually all of the true protein should be protected from rumen degradation.

Rumen microorganisms can utilize non-protein nitrogen, but they prefer to utilize true protein. Because rumen microorganisms grow faster and more efficiently when they have true protein, the net return of amino acids to the animal can be greater if ruminally degraded protein is added to the diet. The optimal ratio of non-protein nitrogen, ruminally degraded true protein, and rumen undegraded true protein is not easy to calculate. Cornell Net Carbohydrate and Protein System and the 1996 Beef NRC have calculations for determining potential benefit of rumen degraded protein to the microorganisms and this relationship is based on efficiency of rumen degradable carbohydrate as well as pH. Users are able to monitor the protein or energy allowable production, the overall and rumen amino acid balance, as well as total nitrogen excretion.

> **"Rumen microorganisms can utilize non-protein nitrogen, but they prefer to utilize true protein."**

Silages often contain large amounts of non-protein nitrogen, but this fraction can have a large amount of peptides and amino acids. Dr. Rich Muck of the U.S. Dairy Forage Research Center indicated that the non-protein nitrogen of high quality silages can be as much as 2/3 amino nitrogen. Because rumen microorganisms use amino acids as efficiently as other forms of rumen degraded protein, the potential benefit of silage crude protein can be underestimated. ❖

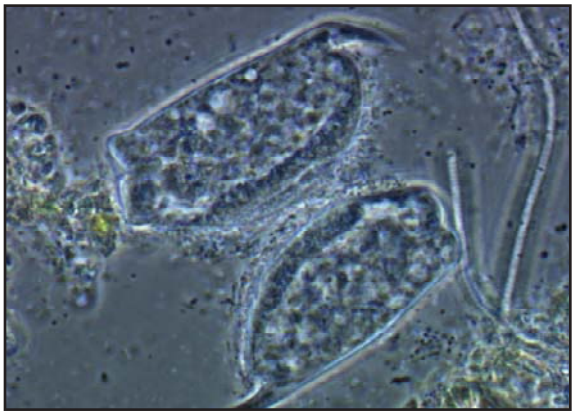
Q: Are rumen protozoa bad for my cow?

A: The rumen is one of the most constant and carefully controlled habitats in all of nature. The cow consumes food almost continuously, the temperature is maintained at 39˚ C, the pH is controlled with the bicarbonate of saliva, acids are absorbed directly across the rumen wall, gases escape by eructation, and mixing motions mix the feed with microorganisms. This constancy has selected for a highly diverse microbial population in the same way that the tropical rain forest has selected for a broad range of plant life.

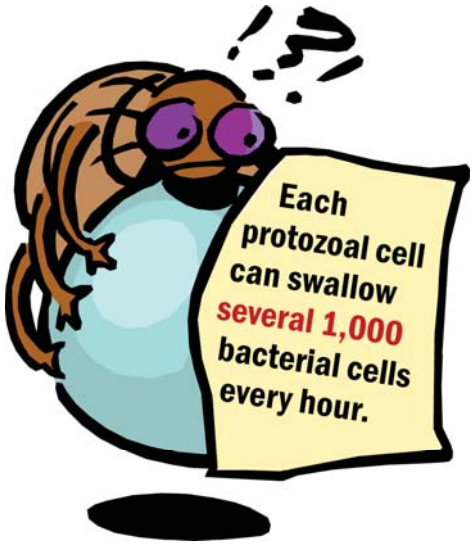
The rumen has at least 50 species of bacteria, 11 genera of protozoa and at least 5 types of fungi. Bacteria are clearly the most important microorganisms in the rumen, and they are responsible for most aspects of the fermentation. Protozoa are much larger than bacteria, and they grow much more slowly. Nonetheless, the protozoa can be a very significant part of the population. In many cases, protozoa can account for as much as half of the microbial protein in the rumen.

The rumen protozoa are predators that graze on bacteria as well as plant materials. When protozoa ingest bacteria, they digest the microbial protein. This turnover of bacteria increases the ammonia concentration and decreases the overall flow of microbial protein from the rumen. The protozoa are a relatively unstable population, and the spontaneous breakdown of protozoa also causes an increase in ammonia.

Protozoa can be removed from the rumen in at least two ways. If calves are taken from their mothers immediately after birth or by Caesarean section and raised in isolation, the rumen is not inoculated. The protozoa can also be removed by chemicals (dimetridazole), but these treatments can kill the animal. ❖



"The rumen protozoa are predators that graze on bacteria as well as plant materials. When protozoa ingest bacteria, they digest the microbial protein."



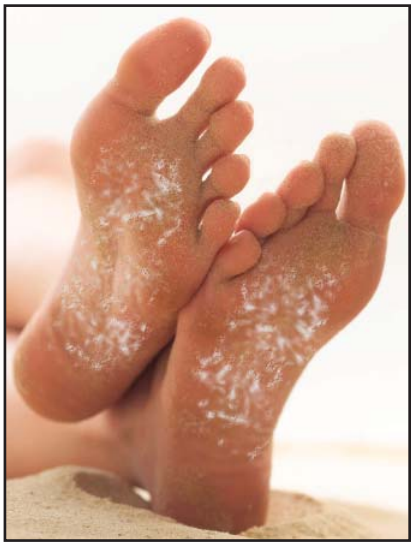
Q: What do rumens have in common with athlete's foot?

A: The mental association of fungi and athlete's foot is a strong one, but it should be realized that fungi are capable of occupying many habitats. Anyone who has walked along the floor of an old growth forest has observed the activity of cellulose-digesting fungi. White and brown rot fungi attack dead and fallen trees and are able to digest even the most crystalline and highly lignified forms of cellulose.

Rumen microorganisms also digest cellulose, but the role of fungi in rumen fermentation was only recently recognized. Cellulose digestion could be explained by the activity of cellulose-digesting bacteria and protozoa, and the involvement of fungi seemed to be precluded by the observation that most fungi need oxygen, a nutrient that is not present in the rumen.

Scientists had observed large swimming microorganisms with tails (flagella) in rumen fluid , but these cells were classified as protozoa rather than fungal zoospores. In the 1970s, Dr. Colin Orpin became convinced that fungi were living and growing in the rumen. Feed particles were sometimes covered with a dense mat of fungal rhizoids, the flagellated cells arose from fungal sacks called a sporangia; and chitin, a carbohydrate unique to fungi, was detected. Since Dr. Orpin's pioneering work, many species of anaerobic fungi have been isolated from the rumen and gastrointestinal tract.

The importance of fungi in rumen fermentation has been at times hotly debated. Proponents cited the fact that fungal enzymes had more activity than the ones possessed by bacteria or protozoa, and they observed that fungi could burrow directly into feed particles. On the other hand, fungi seem to grow slowly in the rumen and are usually present at low numbers.



Animals consuming cool season grasses (e.g. Northern United States) generally have very low numbers of fungi, but fungi numbers increase as the climate becomes more tropical. Warm season grasses have thick, highly lignified cell walls and fungi are better suited to digest this material. ❖

"Rumen microorganisms also digest cellulose, but the role of fungi in rumen fermentation was only recently recognized. "

Q: Do rumen bacteria cause polio in cattle?

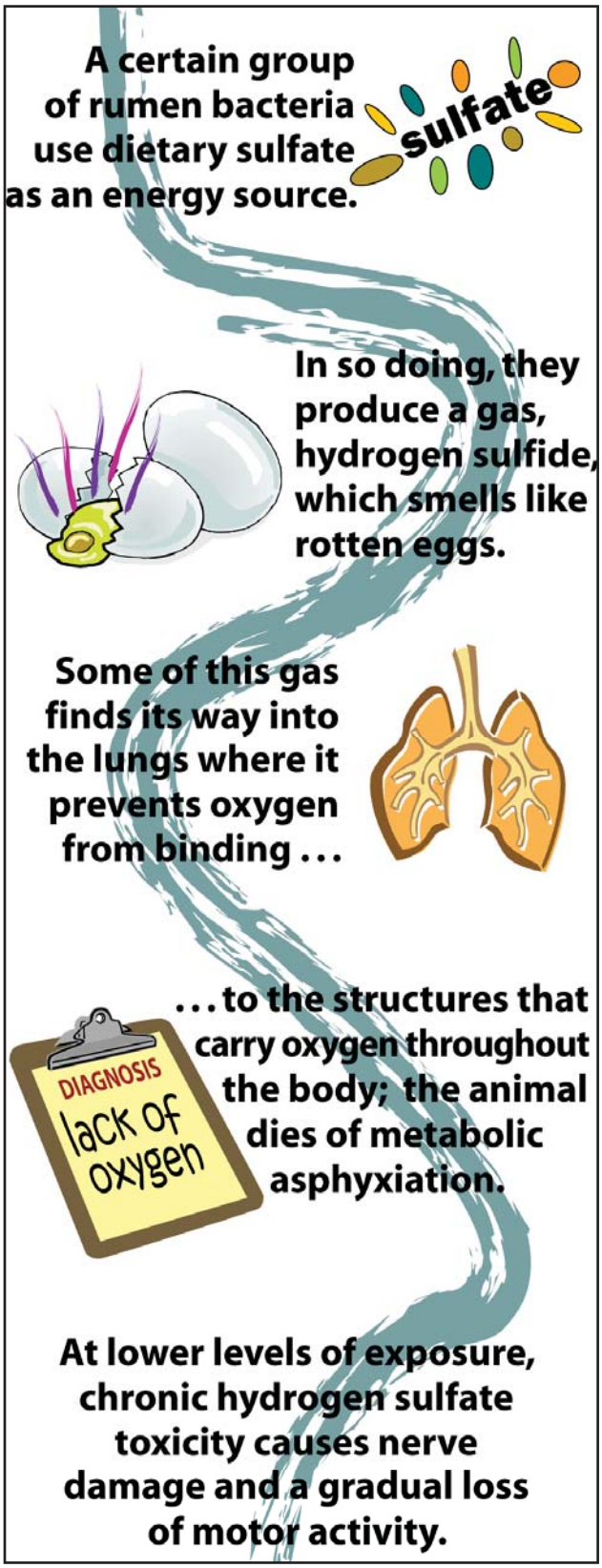
A: Rumen fermentation produces gases (chiefly carbon dioxide and methane) that are normally belched away, but some gas always passes into the lungs. If the rumen gas contains hydrogen sulfide, there can be an immediate and acute effect on the animal that is very similar to polio in humans. In ruminants, excess rumen hydrogen sulfide causes a type of polioencephalomalacia (PEM) and brain lesions virtually identical to the viral PEM that was common in humans before a vaccine was developed.

> "If the rumen gas contains hydrogen sulfide, there can be an immediate and acute effect on the animal."

Hydrogen sulfide is the gas responsible for the stench of rotten eggs, and in the rumen this gas is produced by a specialized group of bacteria that use dietary sulfate as an energy source. How does this gas affect the animal? Hydrogen sulfide prevents oxygen from binding to mitochondrial cytochromes. When these structures cannot bind oxygen, the animal dies of metabolic asphyxiation. At lower levels of exposure, chronic hydrogen sulfate toxicity causes nerve damage and a gradual loss of motor activity.

Because rumen bacteria and ruminant animals need dietary sulfate to grow, PEM is not always easy to prevent. Nutritionists usually recommend sulfur to nitrogen ratios less than 1 to 10, but this arbitrary guideline does not differentiate whether the sulfur is coming from sulfate or other sources. If the nitrogen is derived from urea, higher ratios can be required.

Bacteria-producing hydrogen sulfide can be inhibited by a class of chemicals called anthraquinones, but these compounds have not yet been adequately screened for use in cattle diets. Because anthraquinones are fat soluble compounds, they could very well accumulate in meat or milk. ❖



Q: What do rumen bacteria do with nitrate?

A: The overuse of chemical fertilizers or manure has in many cases increased the nitrate concentration of soil and ground water, and nitrates can be a problem for cattle. Forage crops tend to accumulate nitrate, and it has long been recognized that oat hay can be poisonous. Green oat hay is a particular problem, but virtually any forage crop can accumulate nitrate if the soil concentration is high and the rate of plant growth is rapid.

Nitrate itself is not a toxic substance, but it can be reduced to nitrite by rumen bacteria. Nitrite is a potent poison. Nitrite combines with blood hemoglobin and prevents it from transporting oxygen to the tissues. Symptoms of nitrate poisoning include trembling, staggering, rapid breathing and death. Nitrate concentrations in plants greater than 0.02% can be toxic, but this value is highly dependent on other ingredients in the diet as well as adaptation and feeding behavior.

Nitrate-reducing bacteria are always found at high numbers in the rumen, and many of these bacteria can use nitrate as a electron acceptor to increase energy production. Nitrate can be used as a nitrogen source to synthesize amino acids, but this process is a three step conversion. Nitrate must first be converted to nitrite, the nitrite is converted to ammonia, and the ammonia is then used to synthesize amino acids:

When cattle consume nitrates rapidly (lush forage or hay), step 1 is much faster than step 2, and nitrite accumulates in the rumen. Step 2 is somewhat adaptable. If animals are gradually adapted to increasing amounts of nitrate, step 2 increases, and the animal can tolerate more nitrate in the diet. The potential impact of nitrates on cattle can also be decreased by making sure that the diet has an abundance of ruminally degradable carbohydrates (starch and sugars). When rumen bacteria have an ample amount of carbohydrates, they can increase their rate of growth and amino acid synthesis. ❖

> "Nitrate itself is not a toxic substance, but it can be reduced to nitrite by rumen bacteria. "

3-step conversion, nitrate to amino acids

nitrate → step 1 → nitrite → step 2 → ammonia → step 3 → amino acids

Q: Can ruminants be inoculated with better bacteria?

A: It is generally agreed that bacteria were the first forms of life to inhabit the earth, and this highly adaptable group of organisms can grow in even harsh habitats (for example, hot springs with temperatures near boiling and the gastric stomach of man). Because bacteria are ubiquitous in nature (present everywhere at all times), microbiologists had generally assumed that inoculation of natural environments would be a futile exercise. If the environment would permit growth, the bacteria would already be there.

Leucaena is a leguminous plant that it commonly found in the tropics. Because the leaves are rich in protein and highly digestible, *Leucaena* has potential as a forage crop for ruminant animals. *Leucaena*, however, had one serious drawback. Its leaves and seeds had a toxic amino acid called mimosine. Mimosine caused low weight gain, hair loss, goiter and ulcers in the esophagus. Dr. Raymond Jones from Australia noted that Hawaiian goats were able to consume Leucaena without the toxic side effects, and he hypothesized that these goats might have mimosine degrading bacteria in their rumens. In vitro studies indicated that this hypothesis was correct, and in vivo inoculation of Australian cattle with rumen bacteria from Hawaiian goats corrected the toxic symptoms of *Leucaena* consumption.

The success of rumen inoculation as a means for correcting mimosine toxicity in Australia indicated that bacteria were not always "ubiquitous in nature," but this example is probably rare. In the 1960s, Robert Hungate, the father of rumen microbiology traveled extensively in Africa and sampled rumen contents from a variety of wild ruminants. Dr. Hungate found the same types of bacteria in wild ruminants as had been obtained from domestic ruminants in the United States, and no new species were obtained. Later surveys of other geographic locations supported the conclusion that wild and domestic ruminants had the same types of bacteria.

Since the early 1980s, there has been considerable interest in improving rumen bacteria via genetic engineering, but this has been a difficult task. Rumen bacteria are very good at degrading the various substrates in feed, and in most cases, surface area on the feed particles is the rate limiting step in the fermentation. There is, however, at least one case when rumen bacteria are not up to the task.

When cattle are fed large amounts of cereal grain, rumen pH can decrease to the point where cellulose is no longer digested. The enzymes needed for cellulose digestion are still active, but the bacteria producing these enzymes (cellulases) are unable to maintain a favorable intracellular pH and grow. Some years ago we identified a rumen bacterium that was able to grow at low pH, and this bacterium produces a very weak cellulase.

We have been attempting to improve the acid-resistant rumen bacterium to increase its rate of cellulose digestion. We were able to genetically modify the cellulase so it would digest cellulose at a faster rate, but transfer of the reconstructed gene back into the rumen bacterium has been a tedious exercise. Rumen bacteria do not readily take up genetic material, and many of them have different mechanisms for expressing genes than *E. coli*, the work-horse of genetic engineering. ❖

"Since the early 1980's, there has been considerable interest in improving rumen bacteria via genetic engineering, but this has been a difficult task. "

Q: Can bacteria prevent kidney stones and oxalate poisoning?

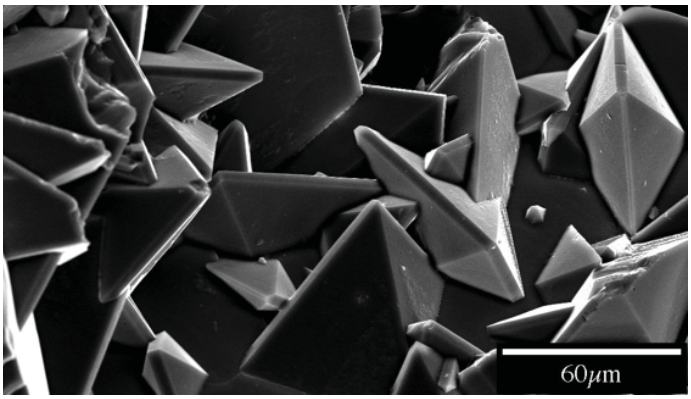
A: Some forages (halogeton, turnip tops, mangolds) and human foods (tea, spinach, rhubarb) have relatively high concentrations of oxalate, an organic acid that can be toxic to animals and man. High oxalate intake causes diarrhea, distress and even death. In humans, oxalate forms a insoluble complex with calcium, and this material can accumulate in the kidney as "stones." Kidney stones are more than 70% calcium oxalate.

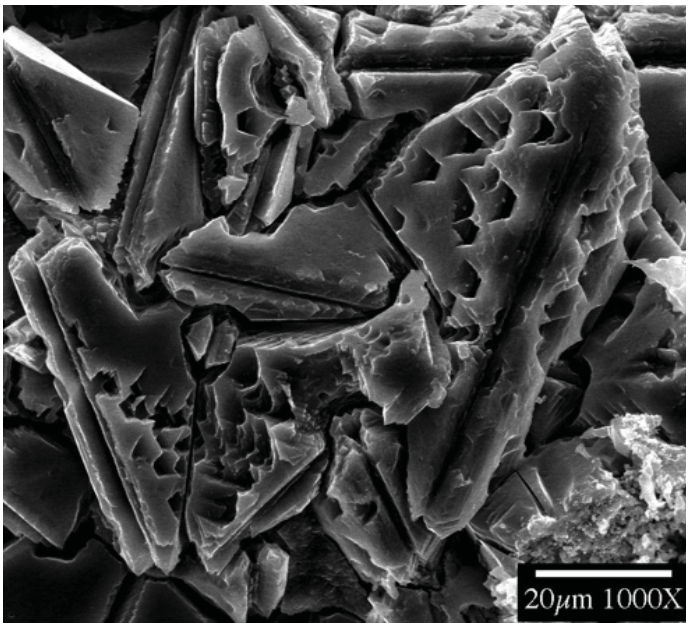
In the early 1980s, Dr. Milton Allison (USDA, Ames, IA) adapted cattle and sheep gradually to increasing amounts of oxalate and demonstrated an enhanced degradation by rumen bacteria. Later work with rabbits, guinea pigs, and horses indicated that oxalate-degrading bacteria were also found in the intestines, but oxalate-degrading bacteria are not always present. Wild rats degrade oxalate, but at least some strains of laboratory rats cannot.

Oxalate is a highly oxidized acid that cannot be degraded by most rumen or intestinal bacteria. The only gut bacterium that appears to degrade oxalate is *Oxalobacter formigenes*, and this bacterium grows slowly and cannot utilize other substrates. The role of *O. formigenes* in oxalate degradation is supported by the observation that inoculation increased the degradation rate of oxalate in unadapted animals.

Enteric diseases such as Crohn's disease or ileal bypass surgery increases a person's risk of kidney stones and other aspects of oxalate poisoning. Physicians explained the increased uptake of oxalate by a depression of fat absorption. Undigested fats bind calcium and increase the amount of free oxalate, but there may also be a complete loss of oxalate-degrading bacteria. When the colon is removed, bile salts are not absorbed as well, and these salts seem to kill *O. formigenes*. Recent work has also indicated that antibiotic therapy may inhibit *O. formigenes* and increase the risk of kidney stones. ❖

"In humans, oxalate forms a insoluble complex with calcium, and this material can accumulate in the kidney as 'stones.' . . . The role of *O. formigenes* in oxalate degradation is supported by the observation that inoculation increased the degradation rate of oxalate in unadapted animals."





Kidney stones. Source: www.sdm.buffalo.edu/scic/gallery (South Campus Instruemntation Center, University at Buffalo, State Universtiy of New York.

Q: Do rumen bacteria cause liver abscesses?

A: Cattle that are fed large amounts of cereal grain (starch) have a higher incidence of liver abscesses than cattle that are fed forages, and in the United States over 3 million livers are condemned each year. The economic impact of the abscesses is much greater than the loss of the liver itself. Cattle with liver abscesses have lower weight gain, feed intake, and feed efficiency than normal animals; and the dressing pecentage and carcass grade are often reduced.

Diets causing liver abscesses alter end-products of rumen fermentation. Rumen fermentation usually produces volatile fatty acids (acetic, propionic and butyric), but starch-digesting ruminal bacteria can also produce large amounts of lactic acid. Lactic acid is a very strong acid, and it can cause a pronounced decrease in rumen pH. When pH is very low, the rumen wall is irritated. In severe cases, the rumen wall deteriorates to the point of chronic bleeding.

Lactate accumulation in the rumen also promotes the growth of *Fusobacterium necrophorum*, a lactic acid-utilizing species. *F. necrophorum* is able to attack the rumen wall and pass into the blood. Blood supply from the rumen carries *F. necrophorum* to the liver where it produces abscesses. The genetic technique, restriction fragment length polymorphism (RFLP), was a key part of the O. J. Simpson trial, and rumen microbiologists have used RFLP to identify bacteria. Dr. T. G. Nagaraja of Kansas State University has shown that *F. necrophorum* isolates from the rumen wall are similar to those from the liver but unrelated to those in rumen fluid. These findings indicate that only certain *F. necrophorum* strains cause liver abscesses, but the exact nature of this difference has yet to be defined.

The study of liver abscess disease is complicated by the fact that other bacteria are often involved in the infection, but the incidence can be substantially reduced by a variety of antibiotics (bacitracin, chlortetracycline, Tylosin, etc.). Good feeding management can also be an effective preventative measure. Liver abscesses are most common when cattle are abruptly shifted to "hot rations" after a period of starvation (e.g. trucking). ❖