

RUMEN MICROBIOLOGY AND FERMENTATION

- *References: Allison (1993) & Leek (1993) in "Dukes' Physiology of Domestic Animals" by Swenson & Reece, ed. (1993), "http://arbl.cvmbs.colostate.edu/," and others.*

INTRODUCTION

(Herbivorous strategies or utilization of forages in General;
<http://arbl.cvmbs.colostate.edu/>)

- **Professional Fermentors?** - Two distinct strategies evolved for "professional fermentors"
 - A. Cranial fermentors (or ruminants) - e.g., Cattle, sheep, and deer.
 - 1) Have a large, multi-compartmented section of the digestive tract between the esophagus & true stomach.
 - 2) The forestomach can house a very complex ecosystem that supports fermentation.
 - B. Caudal fermentors, aka cecal digestors - e.g., Horses & rabbits
 - 1) Similar to pigs & humans through the stomach and small intestine.
 - 2) But, their large intestine, where fermentation takes place, is complex and exceptionally large.
 - C. Similarities & differences?
 - 1) The process and outcome of fermentation are essentially identical in the rumen of a cow or the cecum of a horse.
 - 2) However, the position of the "fermentation vat" in relation to the small intestine has very important implications for the animal's physiology and nutrition.
 - 3) Summary?

| Function | | |
|---|-----|-----|
| Ability to efficiently digest and extract energy from cellulose | Yes | Yes |
| Ability to utilize dietary hexose sources directly | No | Yes |
| Ability to utilize the protein from fermentative microbes | Yes | No |

- ☛ Remember? The small intestine is the only site where simple sugars and amino acids can be absorbed in all animals!?

- 4) Utilization of dietary starch?

- a) Horses? - Starch to glucose by amylase & maltase in the SI, and glucose is absorbed into circulation.
 - b) Ruminants? - Very little is absorbed as glucose, and starch & others are fermented to VFA in the forestomach.
- 5) Protein?
- a) The bodies of microbes can be a source of high quality protein!
 - b) Because the fermentation vat of a horse is behind the small intestine, all their microbial protein is lost - ?
 - c) Ruminants - Microbes can flow into the stomach and small intestine, where they are digested and absorbed as amino acids and small peptides.

MICROBIOLOGY OF THE RUMEN

1. Introduction

- A. Gastrointestinal tracts of ruminant species (& also others)? - Colonized by a diversity of microorganisms, and the use of fibrous feedstuffs by microbes depends on the metabolic activities anaerobic microbes in the rumen and the large intestine.
- B. Rumen & large intestine? - Occupied by highly concentrated populations of bacteria, and also by protozoa and anaerobic fungi.
- C. Gastrointestinal tract? - Perhaps, the most intimate environment that animals are exposed to, and has a profound impact on the physiology and health of the host animal.

2. Forestomach Fermentation

- A. In the simple stomach species? - Before reaching the acidic stomach, fermentation is limited to the ethanolic or lactic acid type, which may have minor impacts on the nutrition of the animal (. . . obviously, some exception though!).
- B. Forestomach fermentation? - Occur at nearly neutral pH, and may be separated from the acidic region.
- C. Ruminants:
 - 1) Are the most diverse (about 155 species) and best known of the herbivores with extensive forestomach fermentation systems.
 - 2) But, there are also others such as Camelidae (camel, llama, alpaca, guanaco, and vicuna), hippopotamuses, tree sloths (*Choloepus* and *Bradypus*), and leaf-eating monkeys.
- D. Reticulorumen:
 - 1) A fermentation chamber, in which bacteria and protozoa are located.
 - 2) Can convert plant materials to volatile fatty acids (VFAs), methane, carbon dioxide, ammonia, and microbial cells.

E. Some advantages of fermentation in the reticulorumen?

- 1) Allows digestion and then absorption of fermentation products that are of value to the host (e.g., microbial cells, VFAs, and B vitamins) before the acidic abomasum.
- 2) Change poor quality protein/N compounds to a "good-quality" microbial protein.
- 3) Selective retention of coarse particles extends fermentation time and allows for further mechanical breakdown during rumination (cud chewing).
- 4) Release of fermentation gas (mostly CO₂ & CH₄) from the system by eructation.
- 5) Toxic substances in the diet may be attacked by the microbes before being presented to the small intestine.

3. Ruminal Microbes

- A. Available information? - Obtained mostly from studies of cattle and sheep.
- B. Knowledge on wild ruminants is largely limited to that obtained by microscopic observations, but predominant bacteria species in rumen contents of deer, reindeer, elk, and moose are ones also found in cattle and sheep (based on cultural studies).
- C. Important bacterial species in cattle and sheep and their fermentative properties:

1) Fermentative properties of ruminal bacteria: (Hespell, 1981)

| Species | Function* | Products¶ |
|---|---------------|----------------|
| <i>Fibrobacter (Bacteroides) succinogenes</i> | C,A | F,A,S |
| <i>Ruminococcus albus</i> | C,X | F,A,E,H,C |
| <i>Ruminococcus flavefaciens</i> | C,X | F,A,S,H |
| <i>Butyrivibrio fibrisolvens</i> | C,X,PR | F,A,L,B,E,H,C |
| <i>Clostridium lochheadii</i> | C,PR | F,A,B,E,H,C |
| <i>Streptococcus bovis</i> | A,S,SS,PR | L,A,F |
| <i>Ruminobacter (Bacteroides) amylophilus</i> | A,P,PR | F,A,S |
| <i>Prevotella (Bacteroides) ruminicola</i> | A,X,P,PR | F,A,P,S |
| <i>Succinimonas amylolytica</i> | A,D | A,S |
| <i>Selenomonas ruminantium</i> | A,SS,GU,LU,PR | A,L,P,H,C |
| <i>Lachnospira multiparus</i> | P,PR,A | F,A,E,L,H,C |
| <i>Succinivibrio dextrinosolvens</i> | P,D | F,A,L,S |
| <i>Methanobrevibacter ruminantium</i> | M,HU | M |
| <i>Methanosarcina barkeri</i> | M,HU | MC |
| <i>Treponema bryantii</i> | P,SS | F,A,L,S,E |
| <i>Megasphaera elsdenii</i> | SS,LU | A,P,B,V,CP,H,C |
| <i>Lactobacillus sp.</i> | SS | L |
| <i>Anaerovibrio lipolytica</i> | L,GU | A,P,S |
| <i>Eubacterium ruminantium</i> | SS | F,A,B,C |
| <i>Oxalobacter formigenes</i> | O | F,C |
| <i>Wolinella succinogenes</i> | HU | S,C |

* C = cellulolytic; X = xylanolytic; A = amylolytic; D = dextrinolytic; P = pectinoiytic; PR = proteolytic; L = lipolytic; M = methanogenic; GU = glycerol-utilizing; LU = lactate-utilizing; SS = major soluble sugar fermenter, HU = hydrogen utilizer; O = oxalate-degrading.

¶ F = formate; A = acetate; E = ethanol; P = propionate; L = lactate; B = butyrate; S = succinate; V = valerate; CP = caproate; H = hydrogen; C = carbon dioxide; M = methane.

- 2) All of these bacteria are anaerobes & most are carbohydrate fermenters - Including gram-negative and gram-positive cells, sporeformers and non-sporeformers, and motile and nonmotile cells.
- 3) Obligatory anaerobic mycoplasmas (. . . cells enclosed by membranes rather than by rigid walls):
 - a) Some interest because detected only in rumen & can ferment starch and other carbohydrates.
 - b) But, minor in terms of proportions relative to total population components, and their contributions would be small.

D. Numbers and relative volumes of bacteria and protozoa:

- 1) Approximate average volumes and numbers of microbial groups in the rumen of sheep: (Warner, 1962)

| Organism | Avg. cell volume | Number/mL | % of total* |
|--|------------------|----------------------|-------------|
| Ciliate protozoa | | | |
| <i>Isotricha, Epidinium, Diplodinium</i> sp. | 1,000,000 | 1.1×10^4 | 33.55 |
| <i>Dasytricha, Diplodinium</i> sp. | 100,000 | 2.9×10^4 | 8.78 |
| <i>Entodinium</i> sp. | 10,000 | 2.9×10^5 | 8.79 |
| <i>Polyflagellated fungal zoospores</i> | 500 | 9.4×10^3 | 0.01 |
| Oscillospiras and fungal zoospores | 250 | 3.8×10^5 | 0.26 |
| Selenomonads | 30 | 1.0×10^8 | 0.09 |
| Small bacteria | 1 | 1.6×10^{10} | 48.52 |

*Total microbial volume was about 0.036 ml per milliliter of rumen fluid.

- 2) Protozoa are far less numerous than bacteria, but they are so much larger than the bacteria that they may occupy a volume nearly equal to that occupied by the bacteria.:
 - a) Most important ones are anaerobic ciliates that are differentiated on the basis of morphology. Most of them belong to two, "holotrichous & entodiniomorphid" protozoa.
 - b) Numbers and kinds of protozoa are markedly affected by diet, and the variability among protozoa populations tends to be greater than the bacterial population.

4. Rumen Ecology

- A. Rumen - An open ecosystem, and it is a dynamic system because conditions are continually changing (<http://arbl.cvmbs.colostate.edu/>).

- 1) Each milliliter of rumen content contains roughly:

- a) 10 to 50 billion bacteria,
 - b) 1 million protozoa, and
 - c) Variable numbers of yeasts and fungi.
- 2) The environment of the rumen:
- a) Anaerobic, and as expected, almost all these microbes are anaerobes or facultative anaerobes.
 - b) Fermentative microbes interact & support one another in a complex food web, with waste products of some species serving as nutrients for other species.
- 3) Bacteria? - Although many bacteria utilize multiple substrates, some of the major groups, each of which contain multiple genera and species, include:
- Cellulolytic - Digest cellulose
 - Hemicellulolytic - Digest hemicellulose
 - Amylolytic - Digest starch
 - Proteolytic - Digest proteins
 - Sugar utilizing - Utilize monosaccharides and disaccharides
 - Acid utilizing - Utilize lactic, succinic, malic acids, etc.
 - Ammonia producers
 - Vitamin synthesizers
 - Methane producers
- 4) Protozoa?
- a) Predominantly ciliates & seems to contribute substantially to the fermentation process.
 - b) Several studies have shown that lambs and calves deprived of their ruminal protozoa had depressed growth rates.
 - c) In general:
 - (1) Protozoa utilize the same set of substrates as bacteria.
 - (2) Different populations of protozoa show distinctive substrate preferences.
 - (3) Many utilize simple sugars and some store ingested carbohydrates as glycogen.
 - ☛ Some protozoa - Cannot regulate glycogen synthesis, and when soluble carbohydrates are in abundance, they continue to store glycogen until they burst.
 - (4) An additional feature of protozoa is that many species consume bacteria. Perhaps play a role in limiting bacterial overgrowth?
- 5) Microbial populations?

- a) Can vary with diet!
 - (1) Perhaps, reflecting substrate availability?
 - (2) e.g., Populations of cellulolytic bugs are depressed in animals fed diets rich in grain.
- b) Environmental conditions in the "fermentation vat" can have profound effects:
 - (1) Rumen fluid normally has pH between 6 and 7.
 - (2) But, may fall if large amounts of soluble carbohydrate are consumed.
 - (3) If pH drops to about 5.5, protozoal populations become markedly depressed because of acid intolerance.
 - (4) More drastic lowering of rumen pH, as can occur with grain overload, can destroy many species and have serious consequences to the animal!

B. Newborn animals:

- 1) Gloominess behavior among cud chewers may facilitate microbial transfer.
- 2) Strictly anaerobic bacteria (including cellulose digester) have been found in animals < 1 wk old.
- 3) Transmission of protozoa depends on close or direct contact, whereas normal rumen bacteria may be isolated from aerosols.

C. Established gastrointestinal populations create conditions that tend to exclude all but the most competent of "invaders."

D. Anaerobiosis (life in the absence of oxygen):

- 1) A fundamental property that limits both the kinds of microbes to colonize the fermentative system and reactions to occur.
- 2) Oxygen is metabolically removed by both bacteria and protozoa.
- 3) Short-chain VFA are the major end products of the fermentation simply because C skeletons cannot be completely oxidized to CO₂ in the absence of oxygen. (Also, the e-transport systems do not function, thus low ATP generation.)

5. Basic Fermentation Chemistry

A. Microbes that digest cellulose and other substrates also provide at least three other major "services?"

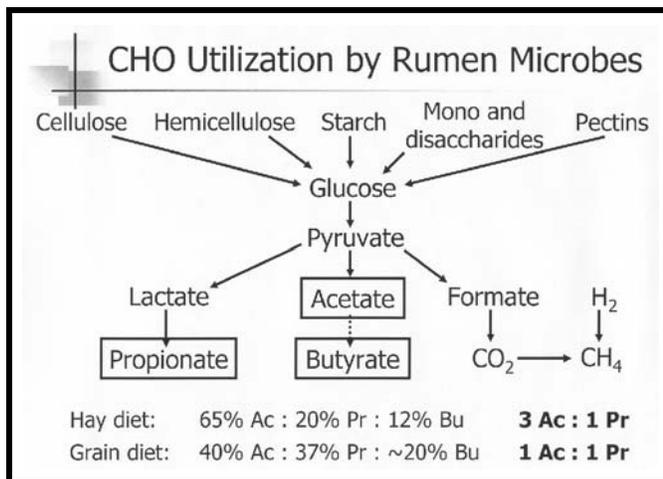
- 1) Synthesis of high quality protein in the form of microbial bodies:
 - a) i.e., Bacteria & protozoa, which can be digested and absorbed by the host animal.

- b) Animals need certain amino acids, which their cells cannot synthesize, "indispensable amino acids" - Fermentative microbes can synthesize & provide them to their host!
- 2) Synthesis of protein from non-protein nitrogen sources:
 - a) Fermentative microbes can, for example, utilize urea to synthesize protein.
 - b) In some situations, ruminants are fed urea as a inexpensive dietary supplement.
 - c) They also secrete urea formed during protein metabolism into saliva, which flows into the rumen and serves as another nitrogen source for the microbes.
 - 3) Synthesis of B vitamins:
 - a) Mammals can synthesize only a few B vitamins and require dietary sources of the others.
 - b) Fermentative microbes can synthesize all the B vitamins, and deficiency states are rarely encountered in some animals.

B. Substrates for fermentation (compiled by S. P. Schmidt, AU) - Also, may want to see subsequent sections on "Functions of Ruminal Bacteria & Protozoa and Manipulations of Ruminal Microbes!"

1) Carbohydrates:

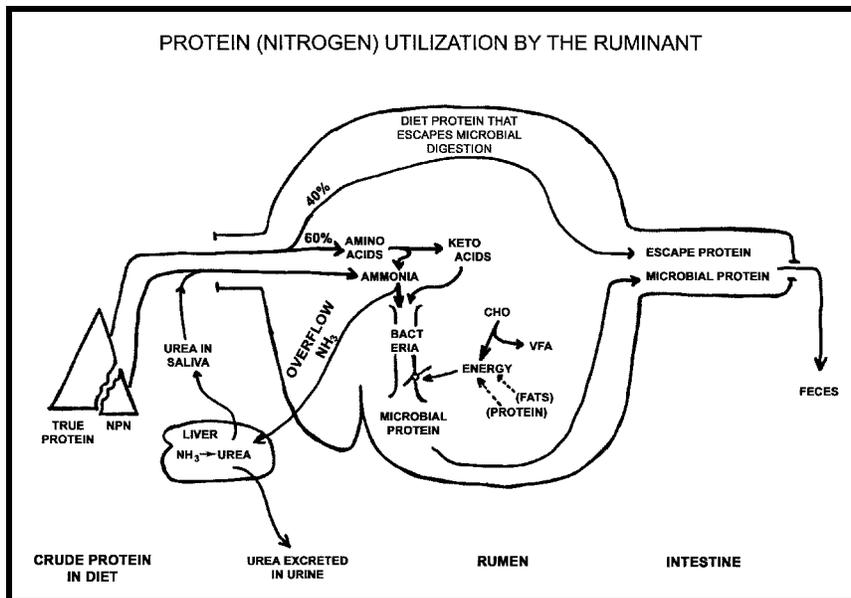
- a) Carbohydrate utilization by the ruminant (See the Figure).
- b) Most carbohydrates are utilized by rumen microorganisms, thus, very little glucose can be absorbed by ruminants.
- c) VFA account for ≈ 70% or more of animal's energy needs by:



- (1) Oxidation of VFA via TCA cycle.
- (2) Conversion of propionate to glucose, then oxidize glucose.

2) Nitrogenous substances

- a) Protein/N utilization by the ruminant:



b) Sources of rumen nitrogen:

- (1) Feed - Protein N (SBM, CSM, grain, forage, silage, etc.) and nonprotein N (NPN; usually, means urea, but from 5% of N in grains to 50% of N in silage and immature forages can be NPN).
- (2) Endogenous (recycled) N - Saliva and rumen wall.

c) Ruminal protein degradation/fermentative digestion - Enzymes of microbial origin:

- (1) Proteases and peptidases of microorganisms (MO) cleave peptide bonds and release AA.
- (2) AA deaminated by microbes, and release NH_3 and C-skeleton.
- (3) MO use NH_3 , C-skeleton, and energy to synthesize their own AA.
- (4) Formation of NH_3 is very rapid, and very little AA left in the rumen.

d) Limitations of microbial protein synthesis:

- (1) Two most likely limitations - Available energy and NH_3 . These need to be synchronized.
- (2) For diets containing urea, may also need: sulfur (for S-containing AA) and branched-chain C-skeletons (MO cannot make branched-chain C-chains). These are normally not a problem.

e) Protein leaving rumen:

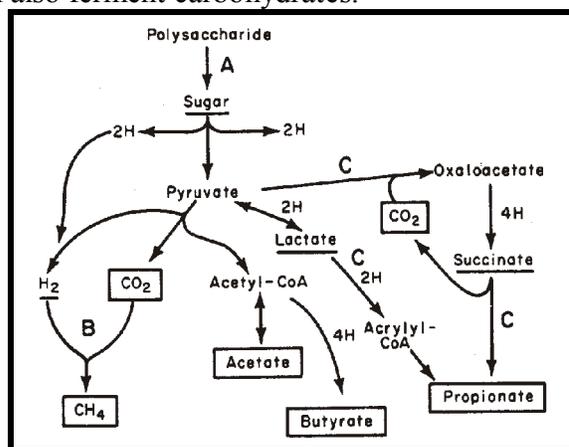
- (1) Microbial protein.
- (2) Escape protein (also called "bypass" protein).

- (3) Proteins enter abomasum & small intestine, and digested by proteolytic enzymes similar to nonruminants.
- (4) Escape vs. bypass protein? - Technically not "bypass."
- (5) Reticular groove? - Important for young ruminants.

6. Functions of Ruminal Bacteria

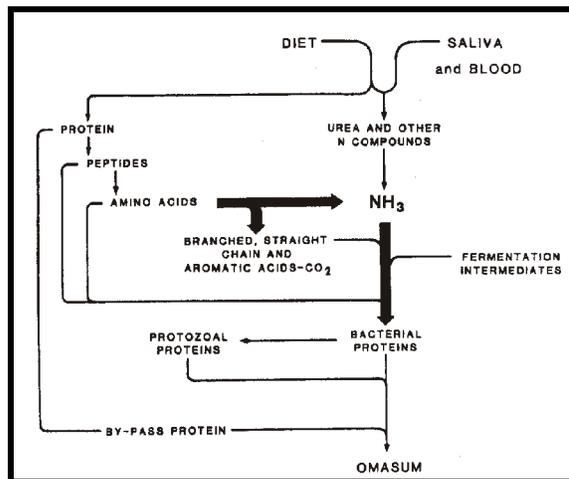
A. Fermentation of carbohydrate by diverse bacterial species (Allison, 1993):

- 1) "□" = Final product, and "—" = extracellular intermediate.
- 2) H = an electron plus a proton or electrons from reduced-pyridine nucleotides, A = carbohydrate fermenting species, B = methanogenic species, and C = lactate-fermenting species which often also ferment carbohydrates.
- 3) Catabolism by rumen microbes?
Hexose - The Embden Meyerhof glycolytic pathway; Pentose - the pentose phosphate cycle coupled with glycolysis with some by phosphoketolase pathway; Pyruvate - a variety of mechanisms to form acetate, butyrate, H₂, CO₂, and propionate.



B. Transformation of nitrogenous substances in the rumen (Allison, 1993):

- 1) Proteins are hydrolyzed by bacteria, protozoa, and anaerobic fungi. Bacteria are most important!
- 2) Protozoa? - A main function being metabolism of bacterial protein rather than exogenous protein.
- 3) Ammonia is produced during microbial metabolism, and is a major source of N used for biosynthesis of microbial cells.
- 4) Many ruminal bacteria can grow with ammonia as the main source of N, but some require amino acids.
- 5) Considerable interest in inhibition of microbial proteases so that more dietary protein would "bypass" the rumen.



7. Functions of Ruminal Protozoa

- A. Ruminant ciliate protozoa are metabolically versatile & capable of using all major plant constituents:
 - 1) Entodiniomorphid protozoa - Engulf particulate matter and have enzymes that attack cellulose, hemicellulose, etc.
 - 2) Holotrichs - Depend on nonstructural polysaccharide, especially, starch and soluble sugars.
 - 3) End products? - Various organic acids, CO₂, and hydrogen.
- B. Although bacterial predation is not important for protozoa, amino acids from ingested bacteria are used for synthesis of protozoal protein.
- C. Protozoa may not be essential for ruminant digestion, but:
 - 1) They do have a major influence on the overall microbial process!
 - 2) Protozoa may account for as much as one-third of ruminal cellulolysis, and their presence may enhance the cellulolytic activity of bacteria.

8. Manipulations of Ruminant Microbes

- A. Ruminant microbial protein:
 - 1) May be adequate for maintenance and during periods of slow growth or early pregnancy.
 - 2) When protein demand is high, animal productivity can be enhanced by increasing the amount of "rumen-escaped" protein.
- B. Some attempts have been made to find ways to manipulate the microbial population to minimize the degradation of feed protein, e.g.:
 - 1) Searches for chemicals that would inhibit the activity of microbial proteases or deaminases.
 - 2) Treatment of feedstuffs that would inhibit ruminal proteolysis such as the use of various drying procedures, heat, or treatment with chemicals. An example of the effort? - The increased efficiency of growth with formaldehyde-treated feeds!
- C. The use of some proteins to coat and protect fats from microbial attack to enhance yields of milk and to increase amounts of unsaturated fatty acids in milk or animal fat.
- D. The use of various chemicals to inhibit methanogenesis. About 10 percent of dietary energy may be lost as methane.
- E. The use of some compounds to increase the ratio of ruminal propionate to acetate.
 - ☛ The best example of successful manipulation via dietary inclusion? - Ionophore, monensin, which inhibits microbial methane production, proteolysis, and amino acid degradation and causes an increase in the ruminal propionate/acetate ratio.

9. Modification and Production of Toxic Substances in the Rumen

- A. Some poisonous plants are less toxic to ruminants because microbes can attack toxic compounds before being exposed to gastric digestion and absorption.
- B. Substances detoxified in the rumen:

| Substance | Source | Reactions | Organisms |
|---|---|--------------------------------|--|
| Nitrite | Nitrate | Reduced to ammonia | Various bacteria and protozoa |
| Oxalate | Oxalis and halogeton | Decarboxylated to formate | <i>Oxalobacter formigenes</i> |
| Ochratoxin A | Moldy feeds | Hydrolysis | Unidentified microbes |
| 3-Nitropropanol & 3-nitropropionic acid | Miserotoxin in many <i>Astragalus</i> sp. | Nitro-group reduction to amine | <i>Coprococcus</i> , <i>Megasphaera</i> , <i>Selenomonas</i> |
| Phytoestrogens | Subterranean clover and red clover | Degraded to p-ethylphenol | Unknown |
| Gossypol | Cottonseed meal | Bound to soluble protein | Unknown |
| Pyrrolizidine alkaloids heliotrine | <i>Heliotropium</i> | Reductive fission | <i>Peptococcus heliotrinreducans</i> |
| 3-Hydroxy-4(1 H)-pyridone | Mimosine from leucaena | Unknown | <i>Synergistes jonesii</i> , <i>Clostridium</i> sp. |

- C. Toxic substances produced in the rumen:

| Substance | Source | Organisms involved |
|---------------------------------------|---|--|
| 3-Methylindole (skatole) | Tryptophan in feeds | <i>Lactobacillus</i> sp. |
| Nitrite | Reduction of nitrates in feed | <i>Selenomonas ruminantium</i> , <i>Veillonella alcalescens</i> |
| Lactic acid | Rapidly degraded carbohydrates (in high-concentrate diets) | <i>Streptococcus</i> spp., <i>Lactobacillus</i> spp. |
| 3-Hydroxy-4(1 H)-pyridone | Degradation product of mimosine | Unidentified gram negative rod |
| Cyanide | Hydrolysis of cyanogenic glycosides | Gram-negative rods and gram-positive diplococci |
| Dimethyl disulfide | Degradation product of S-methyl-cysteine sulfoxide (Brassica anemia factor) | <i>Lactobacillus</i> spp., <i>Veillonella alcalescens</i> , <i>Anaerovibrio lipolytica</i> , <i>Megasphaera elsdenii</i> |
| Equol | Demethylation and reduction of formononetin (a phytoestrogen) | Unknown |
| Thiaminase | Microbial enzymes | <i>Clostridium sporogenes</i> ; <i>Bacillus</i> spp. and various anaerobes |
| 3-Nitropropanoic acid 3-nitropropanol | Hydrolysis of miserotoxins | Unknown |
| Goitrin | Hydrolysis of glucosinolates found in rapeseed meal and other crucifers | Unknown |

10. Small- & Large-Intestine Microbes

- A. Small intestine - Concentrations of viable bacteria in the small intestine content (10^4 to 10^6 /g) are much lower vs. the rumen & large intestine, and most are transients and the impact on digestion might be minimal.

B. Large intestine:

- 1) Concentrations of anaerobic bacteria (10^{10} to 10^{11} /g) are comparable with the rumen.
- 2) The diversity in microbes among animal species and diets.
- 3) Microbial fermentation - A major component of digestion in the LI, and main products include CO_2 , acetate, propionate, and butyrate (also, methane & H).
- 4) Genera of bacteria? - *Bacteroides*, *Fusobacterium*, *Streptococcus*, *Eubacterium*, *Ruminococcus*, and *Lactobacillus*. Also, *Treponema* & *Eschericia coli*.
- 5) Protozoa? - Similar (not identical though!) to rumen ciliates inhabit the LI of horses, rhinoceroses, tapirs, and elephants . . . but their roles - ???

11. Common, Well-Known Disorders in Ruminants**A. Bloat**

- 1) Eructation-inhibition reflex is initiated when proprioceptors in mucosa around the cardia are in contact with fluid or foam, thus gases cannot escape!
- 2) The buildup of pressure can be sufficient to interfere with movement of the diaphragm and also cause circulatory impairment, which can lead to death?
- 3) Causes? Rapid release of soluble proteins from the degradation of the forage (especially, legumes?) and rapid production of gas by the microbes:
- 4) Susceptibility differs, thus some research to control bloat include selection of plants that do not cause bloat, bloat-resistant animals, and the use of antifoaming agents.

B. Ruminal acidosis

- 1) Lactic acid accumulation in the rumen (& in the blood) if animals are overfed with, or are abruptly switched to grain or other readily fermented carbohydrate - Can be lethal!
- 2) A drastic shift in microbial populations from gram-negative predominance to gram-positive lactic acid producers (*Streptococcus bovis* and *Lactobacillus* sp.) in the rumen, cecum, and colon of overfed animals.
- 3) Ruminal pH may drop from more than 6 to 5 or less - Normal rumen microbes may not compete well with the pH less than about 5.5, and the resulting population is dominated by the more acid-tolerant lactobacilli.
- 4) Ruminal lactic acid concentrations may exceed 100 mM, which can increase the osmolarity of the rumen, thus water is drawn into the gastrointestinal tract from the systemic circulation, thus severe dehydration and circulatory collapse in 1 to 2 days?
- 5) Other toxic factors such as histamine or endotoxin, which is produced by lysis of the gram-negative anaerobes at low ruminal pH, may also be involved.
- 6) "Gradual adaptation" to high concentrate rations may occur without the above population shift, and several studies indicate that certain antibiotics, particularly those selective against *S. bovis*, may provide protection against lactic acidosis.

C. Acute pulmonary edema

- 1) Also called "foggage" or "fog fever" occurs naturally 2 to 10 days after mature cattle are switched suddenly from poor to lush pastures.
- 2) Animals suffer obvious respiratory distress. No effective treatment!
- 3) Avidly consumed lush pasture lead to abnormal ruminal fermentation - The significant feature being the conversion of Trp to 3-methylindole:
 - a) Absorbed & reaches the lung where it is oxidized to an active compound.
 - b) The compound binds to and kills alveolar & certain bronchial cells.
 - c) The cells slough off, allowing the airways to fill with frothy edematous fluid. The lungs would be enlarged, heavy, and rubbery.

D. Ketosis

- 1) Ketosis - A generic term for any condition in which ketone bodies (acetone & acetoacetate) are readily detectable in the body fluids and the expired breath.
- 2) Formed in liver mitochondria when acetyl-coenzyme A is being formed at a greater rate than it can be metabolized in the citric acid cycle.
- 3) Cause? - The inadequate provision of oxaloacetate to prime the cycle, and occurs when either oxaloacetate precursors, predominantly propionate, are deficient or available oxaloacetate is preferentially channeled for gluconeogenesis.
- 4) "Pregnancy toxemia" or twin-lamb disease, which occurs just before parturition in ewes that are carrying more than one fetus. Often fetal?:
 - a) The high metabolic demands of the twin-lamb pregnancy, which can be exacerbated by the cold and/or by a reduced availability of glucogenic fermentation products.
 - b) If there is an added nutritional or metabolic stress (. . . feed restriction, colder weather, etc.), likely to occur.
- 5) Acetonemia, which occurs at peak lactation in high-yielding dairy cows:
 - a) The demands for glucose as the precursor of lactose at peak lactation cause the available oxaloacetate to be used for gluconeogenesis at the expense of the needs of the citric acid cycle.
 - b) Exacerbated when the cow loses its appetite for concentrates, which would provide a relatively greater proportion of propionate.
 - c) As the disease progresses, the milk yield falls rapidly, so that the ketosis and its cause therefore decline.
 - d) The disease is usually not fatal, but the slowness of the self-cure and the consequential loss of peak milk production make therapy worthwhile.
- 6) Therapies? Includes dosing with propionate, intravenous administration of glucose, and the injection of the gluconeogenic corticosteroid hormones.

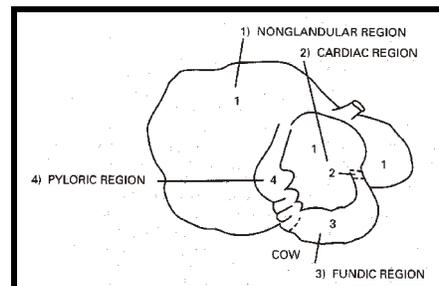
E. Ammonia toxicity

- 1) Arises most commonly when excessive amounts of urea are fed, especially at the time of low amylolytic fermentation. Ruminal urease rapidly convert urea to ammonia..
- 2) With cellulolytic fermentations, the VFA production rate is much lower, thus, less substrate for protein synthesis, and also microbial growth/division is much slower, thus less microbial protein synthesis.
- 3) The toxicity with ammonia arises after its absorption - Due in small part to a systemic metabolic alkalosis and in large part to central nervous system intoxication.
- 4) Toxicity is countered by oral administration of VFA and by feeding of grain.
- 5) The reduced ruminal acidity slows ammonia absorption, and the VFA provide carbon skeletons for microbial protein synthesis.

RUMINAL FERMENTATION IN GENERAL

1. Functional Anatomy of the Ruminant Stomach

- A. Ruminants [so named because they ruminate (chew the cud)] have a stomach consists of a non-secretory forestomach and a secretory stomach (abomasum).
- B. Forestomach - Consists of three compartments, the reticulum, the rumen, and the omasum, and serves as a microbial fermentation vat of the ingesta mainly by hydrolysis and anaerobic oxidation.
- C. Abomasum - Like the stomach of nonruminant species, largely concerned with the hydrolysis of protein by pepsin in an acid medium.
- D. A schematic diagram (Kellems and Church, 1998):



- 1) Reticulum - Spherical, and the esophagus enters dorsomedially at the cardia. [The reticular groove (not shown) runs ventrally from the cardia to the reticulomasal orifice.]
 - 2) Rumen - Divided into dorsal and ventral sacs (not shown). (The ruminoreticulum occupies the entire left side of the abdomen, and depending on the degree of filling, also extends ventrally on the right side.)
 - 3) Omasum - A kidney-shaped structure and consists of many leaves (laminae; bear small papillae), which enhance the internal surface area/volume ratio of the omasum.
 - 4) Abomasum - Consists of fundic, body, and pyloric regions.
- E. The ruminant stomach is highly vascularized, and innervated by vagal and splanchnic nerves, both of which provide sensory (afferent) and motor (efferent) pathways.

2. Benefits and Costs of Ruminant Digestion

A. Benefits?

- 1) Because of a pre-gastric fermentation, can use feeds too fibrous for nonruminants.
 - 2) Can use cellulose, the most abundant carbohydrate present, as a major nutrient.
 - 3) Can synthesize high-quality microbial protein from low-quality protein, nonprotein N, and recycled nitrogenous end products.
 - 4) Can provide all components of vitamin B complex, provided the presence of adequate Co for vitamin B₁₂ synthesis.
- ☛ Thus, ruminants can compete successfully with nonruminant grass eaters, and also occupy niches where the grass quality would be too low to support nonruminants

B. Disadvantages?

- 1) Spend a large part of its day chewing, i.e., chewing food 4-7 hr/day or chewing the cud about 8 hours a day.
- 2) Need complicated mechanisms to keep the fermentation vat working efficiently, e.g.:
 - a) Regular addition of large quantities of alkaline saliva.
 - b) Powerful mixing movements in the forestomach.
 - c) Mechanisms for the elimination of the gases of fermentation (eructation) for:
 - (1) the regurgitation of the cud (rumination), (2) absorption of end products, and (3) onward passage of portions of the ferment to the omasum.
- 3) Pathways of intermediary metabolism must be geared toward the use of the peculiar end products of fermentation:
 - a) In the case of all carbohydrates & some proteins - Volatile fatty acids (mainly acetic, propionic, and butyric acids).
 - b) Propionic acid - Can be converted to glucose, which is needed during milk production and the later stages of fetal growth.

| | | C-1 linkage | Straw | Grasses | Grains | Legumes |
|------------------|-------|-------------|-----------|-----------|-----------|-----------|
| Carbohydrates | | | | | | |
| Nonstructural | | | | | | |
| Starch | α | - | | 1 | 64 | 7 |
| Other | α & β | 7 | | 13 | 2 | 8 |
| Subtotal | | 7 | | 14 | 66 | 15 |
| Structural | | | | | | |
| Cellulose | β | | 32 | 24 | 8 | 14 |
| Hemicellulose | β | | 31 | 20 | 4 | 7 |
| Pectin | β | | 3 | 2 | - | 6 |
| Subtotal | | | 66 | 46 | 12 | 27 |
| Other components | | | | | | |
| Crude protein | | | 4 | 14 | 12 | 24 |
| Lipids | | | 2 | 4 | 4 | 6 |
| Lignin | | | 10 | 7 | 2 | 5 |
| Other | | | 8 | 14 | 5 | 21 |
| Subtotal | | | 24 | 39 | 23 | 56 |

MICROBIAL FERMENTATION

1. Dietary Substrates

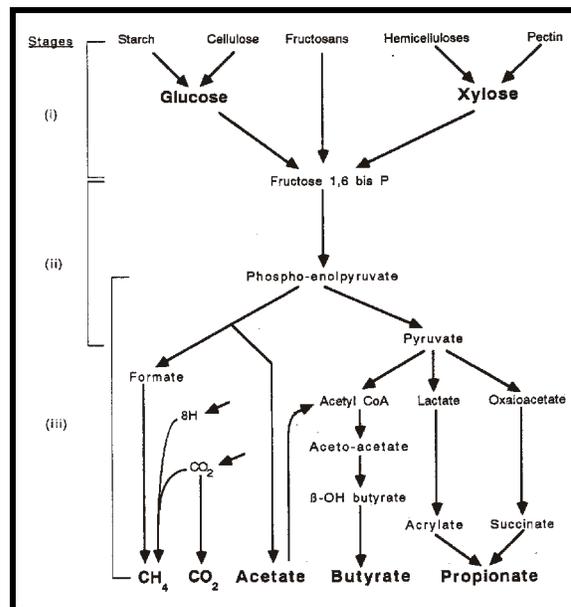
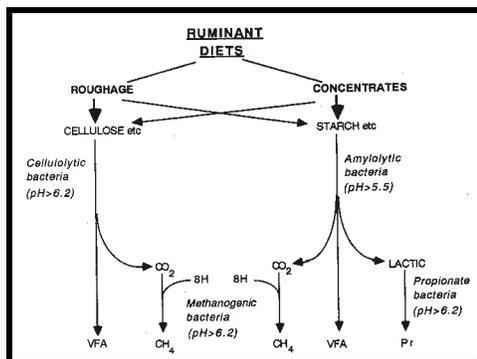
A.. The principal components of ruminant feedstuffs - See the table (Czerkawski, 1986).

- B. Nitrogen-fixing legumes are high in the protein content, but carbohydrates make up about 66% of the dry matter in non-leguminous plants & grains.
- C. Grains - Most of the carbohydrate is nonstructural, and intracellular stored energy (starches and fructosans) or intermediaries (simple sugars).
- D. Plant cell wall - Made up of cellulose fibers embedded in a hemicellulose matrix. The β -1 linkages are difficult to hydrolyze, and necessary enzymes (cellulase, hemicellulase, pectin lyase, and fructanase) are found only in microbes and plants.

2. Fermenting Microbes & Fermentation

A. Microbes:

- 1) Mainly of a mixed population of bacteria, but also yeast-like fungi and protozoa.
- 2) Primary bacteria - Degrade the diet constituents, and depending their preference for cellulose or starch, termed cellulolytic or amylolytic, respectively. (See the figure on the left: Leek, 1993))
- 3) Secondary bacteria - Use the end products of the primary bacteria, e.g., lactate-utilizing propionate bacteria.
- 4) Ruminal fungi - Little is known about the importance.
- 5). Protozoa - Feed on ruminal bacteria, plant starch granules & others, including perhaps PUFA, linoleic and linolenic acids.

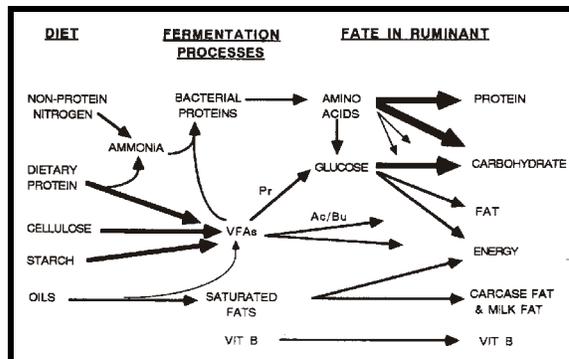


B. Fermentation

- 1) Three stages of a four-stage microbial process - See the figure on the right: Leek, 1993). The 4th stage of microbial activity being the synthesis of microbial compounds, especially amino acids, using intermediates from the stages 1 to 3, along with transamination.
- 2) Cellulose? - The degradation of the β -1 linked compounds (cellulose, hemicellulose, fructosans, pectin) by several species of cellulolytic bacteria.
- 3) Startch? - The degradation of the α -1 linked starches (amylose & amylopectin) and simple sugars by several species of primary amylolytic bacteria.

- 4) Protein? - Proteolytic bacteria represent only 12 to 38% of the total, and only about a half of the dietary protein is degraded in the rumen, thus the terms, "rumen-degradable protein & rumen-undegradable protein." Hydrolyzed to amino acids and(or) fermented to VFA, including branched-chain VFA from Leu, Ile, and Val.
- 5) Lipids? - Ruminal microbes rapidly hydrolyze dietary lipids, and, using unsaturated acids (oleic, linoleic, and linolenic) as H acceptors, quickly convert most of them to stearic acid.
- 6) Other important ruminal reactions?

- a) Vitamin B complex - Adequate Co needed for vitamin B₁₂ (required by both ruminants & some microbes) synthesis.
- b) S-containing amino acids - Adequate supply of S needed.
- c) Oxalates (toxic for nonruminants) - Converted to formate & CO₂.
- d) Dietary nitrate - Reduced to more toxic nitrite ion.



- 7) Overview/summary of the fermentation - See the figure (Leek, 1993).

CHANGES IN RUMINAL FUNCTION

1. Protected Nutrients, Antibiotics, and Probiotics

A. Protected nutrients:

- 1) Certain rumen undegradable protein (RUP) plants (e.g., corn) are poorly fermented in the forestomach, but readily digested in the abomasum and intestine.
- 2) Many denatured proteins also escape fermentation, which is being used commercially by subjecting, high-quality, rumen degradable protein (RDP) to denaturation (e.g., with formaldehyde) to increase RUP.
- 3) Lipids? Encapsulation by using a coating of protected protein, which prevents the saturation of polyunsaturated fatty acids & allows an increased lipid feeding.

B. Antibiotics

- 1) About 8% of total digestible dietary energy is lost as methane.
- 2) Thus, suppressing methane-producing bacteria would reduce the waste.
- 3) e.g., Monensin:
 - a) Affects electrolyte transport across the cell walls of methanogenic & other bacteria, while not affecting, e.g., propionate-producing bacteria.

b) Also, inhibits some bacteria responsible for proteolysis and deamination.

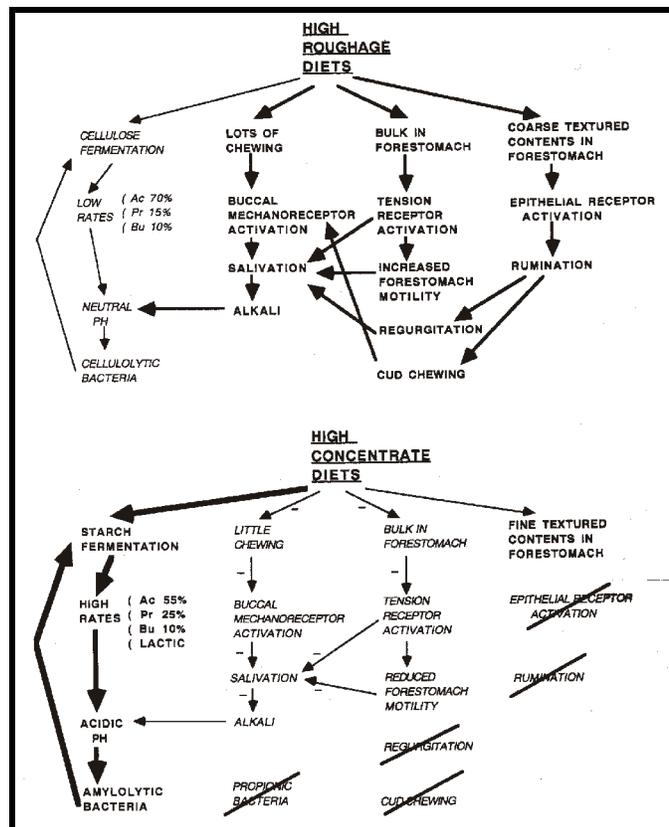
C. Probiotics:

- 1) Addition of selected, desirable active microbes. Undergo limited proliferation & cannot become established permanently, thus needed to add repeatedly.
- 2) Certain species of yeast:
 - a) Shown to be beneficial in concentrate-enriched roughage diets, perhaps, via less lactate production. Perhaps, pH effects & less H production, thus less methane?
 - b) Overall, the yeast supplementation increases the yield of VFA and microbial protein & decrease lactate & methane production.

2. Concentrate-Enriched Diets

- A. High-roughage vs. high-concentrate diets - See the figure (Ac = acetate, Pr = propionate, and Bu = butyrate; Leek, 1993).
- B. Increased energy and protein needs in ruminants (e.g., for lactation, pregnancy, & growth):

- 1) Part of the roughage component can be replaced by grain/grain-based concentrates.
 - 2) The potential energy is lower for concentrates vs. roughages (5 & 7.5 mol VFA/kg DM digested, respectively), but can compensate by the greater digestibility (75 to 90% vs. 45 to 70%) & higher fermentation rates via the amyolytic vs. the cellulolytic pathways.
 - 3) With adequate plant protein/NPN, expect a greater supply of VFA & a higher rate of microbial protein synthesis through microbial proliferation.
 - 4) The amyolytic pathways can lead to the increased lactic acid, but will be converted by bacteria to propionic acid if the intraruminal conditions remain above pH 6.2.
- C. On the other hand, grain and other concentrates undergo less chewing on ingesta than roughage diets, and their particle size is too small to evoke much rumination:



- 1) May lead to cessation of ruminoreticular motility (ruminal stasis), and may be sufficient to retard fermentation in some instance.
- 2) The high intraruminal osmolality may cause large movements of water into the forestomach, leading to tissue dehydration and diarrhea..
- 3) The high acidity gives rise to a systemic metabolic acidosis and also breaks down the integrity of the forestomach lining, e.g., multiple ulceration of the epithelium (rumenitis)?
- 4) The entry of largely anaerobic bacteria into the portal venous system:
 - a) At best - Cause multiple liver abscesses.
 - b) At worst - Swamp the animal's immune system & cause a fatal toxemia.

FATE OF THE END PRODUCTS OF FERMENTATION

1. Volatile Fatty Acids

A. End products:

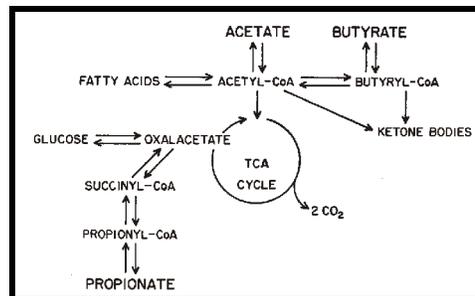
- 1) Carbohydrates - Converted to mainly acetic, propionic, and butyric acids, with a significant increase in the proportion of propionic acid when starch-rich concentrates are fed.
- 2) Proteins - Converted to:
 - a) Those acids mentioned for carbohydrates plus valeric acid (having a 5-carbon chain) and branched VFA (isoacids), isobutyric & isovaleric acid.
 - b) Additional VFA account for less than 5% of the total, and may be more valuable to the microbes for protein synthesis using NPN than to the ruminant/host directly.

B. Absorption:

- 1) Most of the VFA produced are absorbed across the forestomach wall.
- 2) Absorption rates are higher:
 - a) When ruminal pH is reduced, so that more compounds are present as "undissociated" acids.
 - b) As the chain length increases, so that the rate of absorption is butyric acid > propionic acid > acetic acid.
- 3) About one-half of the VFA absorbed by passive diffusion are in the undissociated state, and the remainder are effectively absorbed as anions by facilitated diffusion in exchange for bicarbonate (hydrogen carbonate) ions.

C. Metabolism?

- 1) Major metabolic pathways for VFA - See the figure [propionate is glucogenic, whereas acetate and butyrate are ketogenic; TCA = Tricarboxylic acid; Bergman (1993) in Swenson and Reece (1993)].



- 2) Butyric acid:

- Most? - Metabolized/oxidized to ketone body, β -hydroxybutyrate, in sheep (less so in cattle).
- Remainder? - Carried to the liver and metabolized similarly, thus absorbed butyrate appears in the general circulation almost entirely as β -hydroxybutyrate.
- Readily metabolized by most tissues & used to provide the first four carbon for the synthesis of about $\frac{1}{2}$ of the short- & medium-chain fatty acids (C4 - C14) in milk.

- 3) Propionic acid:

- About 30% of the propionate is metabolized by the forestomach wall to lactic acid, thus some lactic acid in portal venous originated as propionate in the rumen.
- The portal venous lactate and the remainder of propionate are almost completely removed by the liver.
- In the liver, converted to oxaloacetate & used in the Krebs' cycle or, together with lactic acid, converted to glucose, which is released into the circulation or stored in the liver as glycogen.
- Propionate is the only VFA that can be used for gluconeogenesis.

- 4) Acetate:

- The most abundant VFA in the general circulation and the prime metabolic substrate.
- A small amount of acetate is metabolized to CO₂ by the forestomach wall.
- Can be taken up by most body tissues to form acetyl Co-A for use in the citric acid cycle.
- In the mammary gland:

- Used for the synthesis of the short- and medium-length fatty acids.
- Account for about $\frac{1}{2}$ of the first four carbon units in each fatty acid chain (i.e., those not derived from β -OH butyrate) and all of the remaining carbon units in the chain.

2. Lactic Acid

- A. Along with VFA, lactic acid is produced by certain amylolytic bacteria during the degradation of starch.
- B. Normally, lactic acid is present transiently, and, therefore, only in low concentrations, as it is used by secondary bacteria to produce propionate.
- C. Lactic acid is a stronger acid than the VFA, thus ruminal pH tends to fall very quickly.
- D. Absorbed at only 10% of the rate for VFA, and the more common L(+) isomer is metabolized to pyruvate (en route to glucose and glycogen) by the liver faster than the D(-) isomer.
- E. Unmetabolized acid will cause "metabolic acidosis."

3. Gases

- A. Gases - Production reaches a peak of up to 40 L/h in cattle 2 to 4 hours after a meal when the fermentation rate is at its maximum. Eliminated almost entirely by eructation.
- B. Principal gases? - CO₂ (60%), CH₄ (30 to 40%), and variable amounts of N₂, with traces of H₂S, H₂, and O₂:
 - 1) Carbon dioxide - Arises from the decarboxylation of fermentation and neutralization of H⁺ by HCO₃⁻ ions entering the rumen in saliva & across the ruminal wall during VFA absorption.
 - 2) Methane - Arises from the reduction of CO₂ and formate by the methanogenic bacteria. Methane is a high-energy compound and its elimination as a waste product represents the loss of about 8 percent of the total digestible energy of the diet.
 - 3) Hydrogen sulfide - Arises from the reduction of sulfates and from sulfur-containing amino acids. A potentially toxic gas, even in small amounts.
 - 4) H₂ - Usually present in traces.
 - 5) Oxygen:
 - a) Via ingested food & water and also by diffusion from the blood.
 - b) Quickly used by the facultative anaerobic bacteria, so that ruminal concentrations are always low, which is essential because the majority of ruminal microbes are strict anaerobes.

4. Ammonia

- A. Arises from the deamination of dietary proteins, NPN, and urea derived from saliva and, across the forestomach wall, from blood.
- B. Feeding up to 30% total N as a urea supplement is usually well tolerated.
- C. With adequate and suitable VFA, NH₃ is incorporated into microbial protein. If not, can be absorbed, especially if the ruminal pH is alkaline.
- D. NH₃ (actually, NH₄⁺) must be removed from the portal blood and converted to urea. If not, ammonia toxicity can develop.

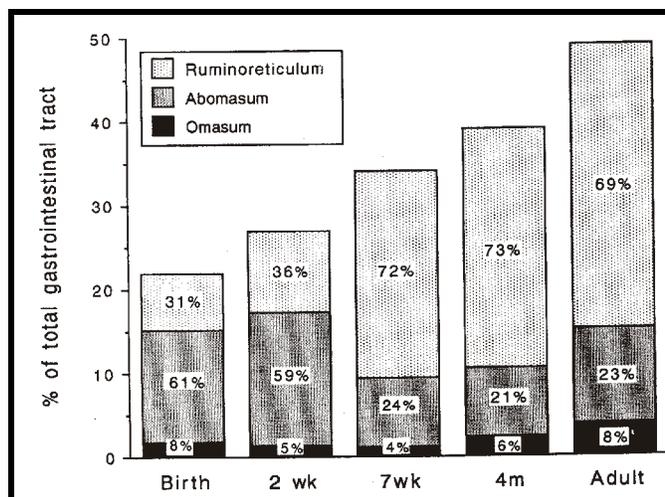
5. Other End Products

- A. Amino acids arising from fermentation are used by other microbes and are not immediately available to the ruminant.
- B. Microbes that pass out of the forestomach are digested in the gastrointestinal tract:
 - 1) Lysis of bacteria is started in the abomasum by the action of a lysozyme in the abomasal secretions.
 - 2) Microbes yield protein of high biological value, lipids (including some PUFA), polysaccharides (as starch), and vitamins.
 - 3) The protein content of the microbes is about 27 and 45% of the total DM in bacteria and protozoa, respectively.
- C. Fatty acids:
 - 1) Long-chain fatty acids - Absorbed and taken up by the adipose tissue and by the lactating mammary gland.
 - 2) Intraruminal hydrogenation of unsaturated fatty acids? - Causes ruminant carcass and milk fat to have a greater ratio of saturated to unsaturated fatty acids vs. nonruminants.

GASTRIC DIGESTION IN THE YOUNG RUMINANT

1, General

- A. In the young ruminant, the development of gastric digestion can be considered to have four phases: 1) the newborn phase (0 to 24 hr), 2) the preruminant phase (1 d to 3 wk), 3) the transitional phase (3 to 8 wk), and 4) the preweaning and postweaning phase (8 wk to adulthood).
- B. Changes in the proportion of the ruminant stomach - See the figure (Wardrop and Combe, 1960. J. Agric. Sci. 54:140-143)



2. The Newborn Phase (0 to 24 hr)

- A. Forestomach at birth:
 - 1) Nonfunctional, and represents a small proportion the total stomach.
 - 2) Contains no microbes, and rudimentary ruminoreticular papillae and omasal leaves.

- B. Diet? - Consists of colostrum, which is rich in immunoglobulins:
- 1) Abomasum - Secretes no acid or pepsinogen during the first day or so:
 - a) Thus, no gastric digestion, which prevents immunoglobulins (IgM antibodies, γ -globulins) from digestion/denaturation.
 - b) Also, the presence of trypsin inhibitors in the colostrum prevents the degradation of immunoglobulins in the intestine.
 - 2) Subsequently, colostral antibodies are absorbed intact through the intestinal mucosa by endocytosis/exocytosis.
 - 3) Ability to transport/absorb antibodies intact lasts only 24 to 48 hr after birth.
 - 4) Colostrum can be a rich source of vitamins A, D, and E, Ca, and Mg.
 - 5) Lactose is readily digested in the intestine to provide potential energy substrates, glucose & galactose.
 - 6) Colostrum contains mammary gland microbes (mainly lactobacilli species), and they would gain access to the intestine with each sucking period.
- C. Fecal contamination? - Provides a source of *Escherichia coli*, streptococci, and *Clostridium welchii*, which can be found in the intestine within 8 to 16 hr of birth.
- D. With no or insufficient colostral antibodies, the newborns would be susceptible to acute infections ("joint-ill" and "navel-ill") and then to diarrhea/scours.

2. The Preruminant Phase (1 d to 3 wk)

- A. The principal food is milk during this phase, but the young ruminant may start trying to taste solid foods during the latter half of this phase. Make only little contribution to its nutrient intake though!
- B. Compared with drinking from a bucket, sucking from a teat can lead to a greater secretion of saliva, and the saliva contains an esterase (pregastric esterase) that can start the hydrolysis of the milk lipids.
- C. Milk passing through the pharynx:
- 1) Stimulates chemoreceptors with afferent pathways in the glossopharyngeal nerve (ninth cranial nerve).
 - 2) The efferent vagal nerve output can cause the closure of the reticular groove and to relaxation of the reticuloomasal orifice and omasal canal.
 - 3) Contraction of the spiral lips of the reticular groove causes their shortening and apposition to produce a temporary tube connecting the cardiac and reticuloomasal orifices.
 - 4) Results? Milk can bypass the ruminoreticulum, and flow quickly through the relaxed rudimentary omasum, and end up in the abomasum.
 - 5) Factors affecting the closure of the reticular groove?
 - a) The hunger drive seems to be the main determinant.

- b) The closure is not consistently affected by other factors such as head position and whether feeding involves sucking from a teat or drinking from a bucket.
- D. The act of sucking and the presence of milk in the abomasum can lead to abomasal secretions:
- 1) Rate? - Proportional to the number of sucks, thus teat feeding may be more effective vs. bucket feeding.
 - 2) Consist of proteolytic enzyme, rennin/chymosin (no pepsinogen at this stage!), and HCl.
 - 3) Rennin can act on milk at pH 6.5 for 3 to 4 min to produce a hard clot/curd, which consists of butterfat and milk protein precipitated as Ca caseinate.
 - 4) Remaining fraction of the milk or whey? - Consists of whey proteins (albumins and globulins) and lactose, which enters the duodenum after each suck.
 - 5) Hard curd - Very slow degradation over the next 12 to 18 hr:
 - a) Butterfat can be hydrolyzed to fatty acids and glycerol by lipase in milk (mammary origin) and pregastric esterase from saliva.
 - b) Precipitated Ca caseinate - Subjected to further proteolysis by rennin at an optimum pH of 3.5.
 - 6) Curd & whey proteins are subjected to complete proteolysis in the intestine, and lactose is hydrolyzed by lactase to glucose and galactose.

3. The Transitional Phase (3 to 8 wk)

- A. Ingested milk is handled as described before during this phase.
- B. The animal starts to ingest progressively larger amounts of roughage, which can stimulate the development of salivary gland & ruminoreticulum:
 - 1) Salivary glands (especially, parotid glands) increase the size and secretion volume, and the composition of secretion becomes alkaline.
 - 2) Ruminoreticulum - Starts acquiring microbes:
 - a) "Early" microbes (at 1 wk after birth) are largely milk contaminants (lactobacilli), and give very low ruminoreticular pH values.
 - b) Transitional ruminants - Acquires normal microbes from the ingestion of food and water contaminated with some ruminal microbes.
- C. Microbial fermentation of roughage:
 - 1) Start producing VFA that is important for the development of the ruminoreticular papillae and omasal leaves.
 - 2) "Gas production?" - Necessary to develop the mechanisms of eructation.

- 3) Bulk factor of the roughage - Responsible for the size & muscular development of the ruminoreticulum, onset of cyclic motility, and effective rumination.

D. This period is critical for establishing the ruminoreticulum:

- 1) The biggest changes occur during this period.
- 2) By the end of the period, the animal will have the ruminoreticulum with all the basic features.
- 3) Intermediary metabolism - Moving away from the glucose-based toward the VFA-based, and blood glucose becomes less insulin-sensitive.

4. The Preweaning and Postweaning Phases (8 wk to Adulthood)

- A. The beginning of the preweaning phase? - Coincides with the natural decline in lactation. Progressively less milk would be available.
- B. Reticular groove? - Closure becomes erratic & absent in older animals . . . unless feeding milk regularly.
- C. The total empty stomach mass:empty gastrointestinal mass - Would become a progressively greater the animal approaches adulthood.
- D. "Forestomach motility cycles" - Start resembling the characteristics of adult animals.
- E. Pepsinogen/pepsin replaces rennin in the abomasal secretions.