4 REVIEW OF PHYSIOLOGY, ANATOMY AND FUNCTION OF THE RUMINANT DIGESTIVE TRACT

4.1 The animal

The grazing pattern of ruminants defined by evolution demanded that food be eaten quickly, in order to evade predators, but due to the type of food eaten it had to be digested slowly in relatively safe surroundings. Complex mechanisms have developed in the ruminant to satisfy these conflicting demands. A large 'container'(rumen) has evolved where fermenting fibrous foods can reside for up to 24 h to achieve the breakdown of cellulose, developed in front of the true stomach (abomasum). The ruminant also has the capacity for the regurgitation of part of the solid digesta and so that it can chew this during periods when it is not grazing but is safe from predators.

The characteristic of ruminant digestion is that the ingested pasture is subject to microbial degradation in the rumen. The result of this microbial attack on the feed ingested is the production of energy substrates, in the form of VFAs, that are released my microbes into the rumen fluid. These VFAs are then absorbed across the rumen and lower gut walls. Protein, in the form of synthesised microbial protein, eventually passes from the rumen to the abomasum, then to the intestines, for digestion and absorption. The bacterial protein is synthesised in the rumen from the true protein and non-protein N in the feed sources. When the bacteria move out of the rumen into the abomasum, the bacterial protein is subjected to enzymatic digestion in the abomasum and small intestine and the peptides formed are either absorbed in the small intestine or are further degraded to amino acids and absorbed.

4.2 The stomach

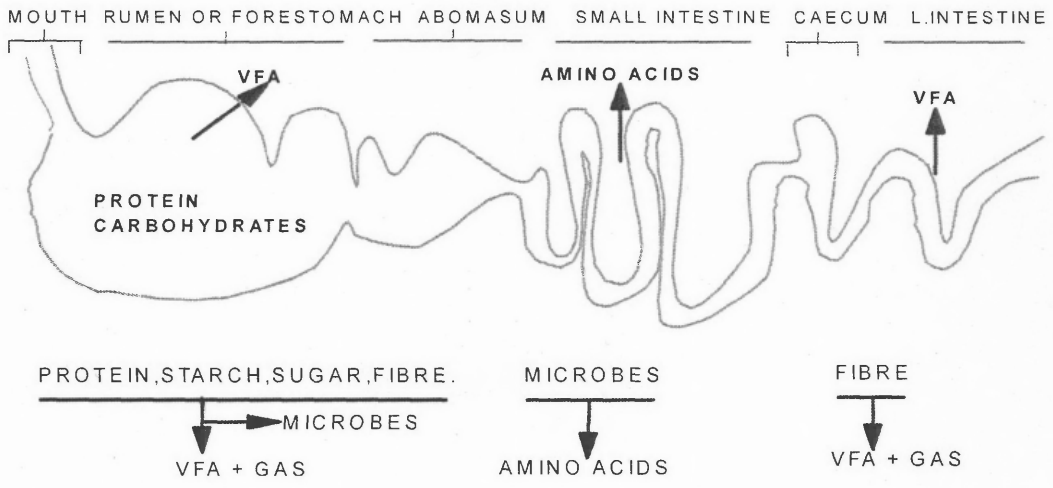
In the cow the stomach is very large, occupying about 75% of the abdominal volume. There are four parts, *viz.* the rumen, reticulum, omasum and abomasum. The capacity of the stomach varies from birth, where the rumen and reticulum together are half as large as the abomasum.

By the time the calf has reached 12 weeks of age this ratio is reversed. By 18 months of age the rumen attains its ultimate capacity of 80%, the reticulum 5%, the omasum 8% and the abomasum 8% of total gut volume (Sisson and Grossman 1968).

The 'reticulo-rumen' will be referred to as the 'rumen' in the following discussion.

The rumen and omasum are lined with mucous membrane, covered with stratified squamous epithelial cells and are non-glandular. Thus the rumen is set up mainly with an absorptive function. The abomasum, on the other hand has a glandular mucous membrane, secretion is the main function and absorption of the digested food mostly occurs further down the tract. The abomasum is actually the true stomach (see Fig. 7).

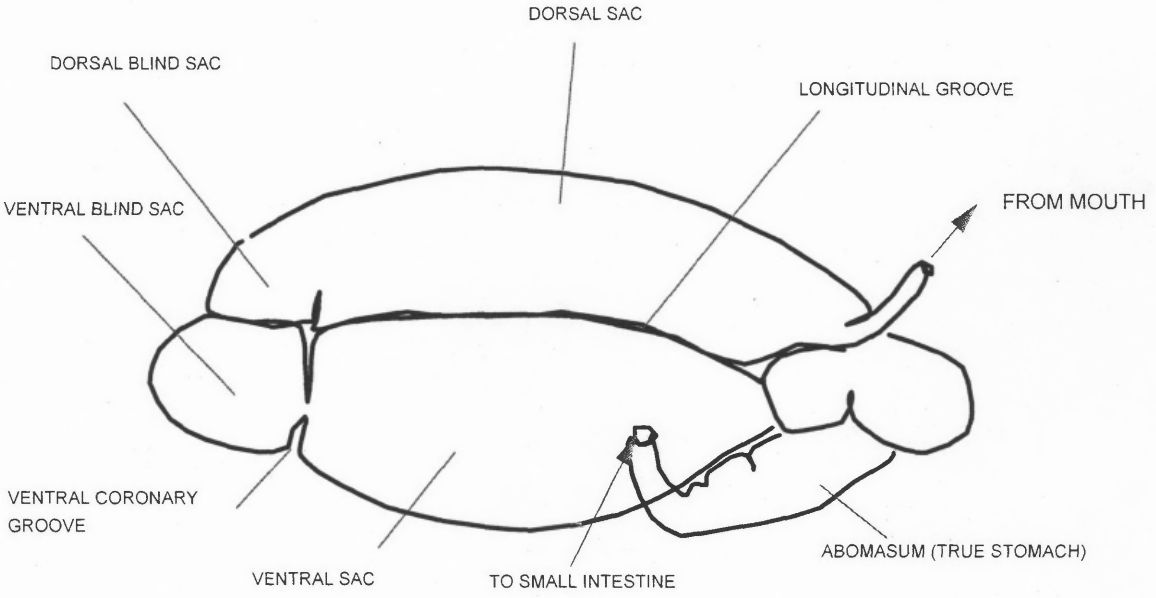
Figure 7 Intestinal tract of the ruminant



Source: Leng (1983)

Thus, the fibrous food of ruminant animals is subjected to pre-gastric and post-gastric microbial action, with the reticulo-ruminal fermentation taking place before reaching the true stomach (abomasum). The stomach complex of the ruminant animal is shown diagrammatically, viewed from the right side.

Figure 8 Anatomy of the rumen



DIAGRAMATIC VIEW OF THE OUTSIDE OF THE RUMEN (RIGHT SIDE) , ALSO SHOWING THE RETICULUM , THE OMASUM AND THE ABOMASUM . tHE GROOVES OUTSIDE CORRESPOND TO THE SEPARATION INTO SACA INSIDE THE ORGAN (ADAPTED FROM CZERKAWSKI 1986)

When ruminants are young (and still suckling and under certain conditions when they are older) these 'lips ' along the length of the groove from the oesophagus to the reticulum can close enabling liquids to flow directly from the oesophagus to the omasum, thus by -passing the rumen. When ruminants begin to eat grass this groove opens up allowing digesta into the rumen thus stimulating the function of fermentation to begin. The two orifices, the esophageal opening and the opening to the omasum are quite close to each other.

4.3 Rumen contents

The contents of the rumen are very heterogeneous and include a large proportion of semi-solid digesta, particularly in the region of the longitudinal pillar, where the semi solid digesta forms a 'raft 'of solid material. The contents above and below the raft and in the reticulum are more fluid, but contain varying quantities of particles. There is usually some gas in the upper regions of the rumen (the gas cap). Normally the raft is tightly packed and since it occupies a region with a complicated pillar structure it is difficult to see how it could be stirred by simple

mechanical means and certainly not by a tumbling action. However, the solid contents are not stagnant and the mechanism for their controlled flow involves two important processes, the rumen movement and rumination.

4.4 Ruminal movements

The rumen movement is bought about by a series of complex muscular contractions. They start with a brisk double contraction of the reticulum, at approximately 1 min intervals. At the height of this contraction the reticulum is reduced in size and its contents propelled into the anterior blind sac that dilates and then into the dorsal sac of the rumen. As the reticulum relaxes, the anterior blind sac relaxes and the cranial pillar contracts, so that some of the liquid contents flow back to the reticulum. Next, during the primary rumen contraction, the dorsal sac, the dorsal blind sac and the dorsal coronary pillar contract, while the ventral rumen is relaxed. The contraction of the longitudinal pillar region will effectively hold the raft in position and the liquid digesta will be forced through the raft to the ventral region of the rumen. Finally, the dorsal sacs are relaxed and the ventral sacs contract, forcing the liquid digesta through the raft to the dorsal rumen and then to the reticulum. The whole rumen relaxes before the next cycle. The contractions of the rumen are more frequent than the double contractions of the reticulum (approximately 2 cycles/min).

The raft of digesta that is formed above the fluid contents is so solid that it is doubtful that it can be stirred by rotation in the vertical plane, although contraction and relaxation of the pillars could rotate it slowly in the vertical plane (Czerkawaski 1986). Rumen liquid is forced through the raft. The liquid contains a dense microbial suspension and feed particles of varying sizes. Some of the particles pass through the raft but many are trapped and a proportion will also be released during the reversal of flows of the liquid. Studies in sheep have shown that although the total volume of the liquid in the rumen is 5 to 8 liters at any one time, the amount of liquid flowing through the raft could be as much as 10,000 L/d. This internal flow is important in the efficient functioning of the rumen (Sisson and Grossman, 1968).

During rumination, a proportion of the solid ingesta is regurgitated, mixed with saliva and chewed by the animal. This process can occur for as much as 8 h per day in the animal. The

partly digested mass is mixed with saliva and squeezed and the liquid that has been squeezed from the mass is swallowed first, with part being swept from the reticulum to the dorsal rumen and part passing into the omasum and down the gut. The squeezed bolus is swallowed and carried to the caudal part of the dorsal rumen during rumen movement.

There are considerable amounts of fermentation gas produced in the rumen, (approximately 30 L of methane and 90 L of carbon dioxide per day in sheep). Some of the gas accumulates in the dorsal part of the rumen and a variable amount is trapped in the raft of the digesta. This gas has to be removed as failure to do so causes an accumulation, a dilation and distention of the organ and the potentially fatal condition of 'bloat' if not corrected.

The gas is removed normally by a process called eructation. After contraction of the dorsal rumen, the gas is forced to the caudal area and the cardia relax admitting the gas to the thoracic oesophagus and then to the nasopharynx. A great deal of the eructed gas enters the respiratory passages and then is breathed noiselessly out through the nose.

A flow of liquid through the raft of digesta will help in the removal of gas both in the form of bubbles or as dissolved gas and interference with this process, (for example by formation of stable froth) may result in bloat.

4.5 Absorption from the rumen

The internal surface of the reticulum is covered by papillae and is raised into a characteristic honeycomb pattern. The internal surface of the rumen is also covered by papillae, varying from short tongue - like forms to 'flattened' leaf - shape forms. These structures greatly increase the surface area for absorption from the rumen and so give a greater area of contact to the digesta (Sisson and Grossman, 1968).

The structure of the rumen wall is such that it can resist the action of the microorganisms present but at the same time provide absorptive and metabolic activities. The sequence of contractions is not just a mechanism for stirring the contents but also a sophisticated mechanism for controlled transfer of suspensions of particles within the rumen in such a way that there is provision for longer retention of particles needing further digestion. The

contractions are also a systematic process that separates the large and small particles and ensures that large particles do not leave the rumen.

Rumination increases the digestibility of fibrous feeds and helps in the microbial colonisation of these feeds. The system also provides excellent conditions for the microbial population by maintaining ideal temperature and pH (saliva) and by efficient removal of end products of microbial metabolism.

5 MICRO-ORGANISMS

Most large animals, are in fact not the single
Individuals they seem to be. They are walking
Menageries, whole communities of different
Species which, in their various ways, are
Committed by evolution, for better or for
Worse, in sickness and in health, to live
Together

(David Attenborough, 1990)

5.1 Overview

The rumen contains one of the most varied and dense microbial populations known in nature. The microbial ecosystem can be divided into three major groups, bacteria, protozoa and fungi. Viruses and bacteriophages also occur but will not be considered here.

The complex or structural carbohydrate reserves in plants are digested by the activities of the microbes possessing enzymes capable of breaking the B-1-4 glucosidic bonds between the

molecules of cellulose and hemi-cellulose. In this relatively stable environment and with a continual supply of plant material, the microbial populations supply the ruminant with the readily metabolisable end products including both substrate energy as VFAs and a source of protein and energy (lipids and polysaccharides) from the microbial biomass. This is a true symbiotic relationship. The benefit to the animal is that it gains the ability to create true protein from non-protein nitrogen and the ability to degrade and 'release' energy from cellulose.

At least 200 species of bacteria (flora) and 20 species of protozoa (fauna) have been identified, although only 20-30 species are predominant. The strained contents of the rumen may consist of a microbial suspension with 10^{12} bacteria and 10^{6} protozoa per ml (Theodorou and France, 1993), but this is not uniform as considerable numbers of protozoa and bacteria are associated with the solid digesta in the rumen. The concentrations of organisms within the solid matrix are greater (g/g) than in free suspension and the fungi in the vegetative form are associated with solid digesta and only their zoospores are free in solution.

Normally the bacterial population density in the rumen forms the majority of the microbial biomass (70%), although protozoa can sometimes make up 40% of the biomass. The fungal biomass, under ideal conditions of no deficiencies for fungal growth, in the rumen, appears to contribute less than 8% of the total (Theodorou and France, 1993).

5.2 Bacteria

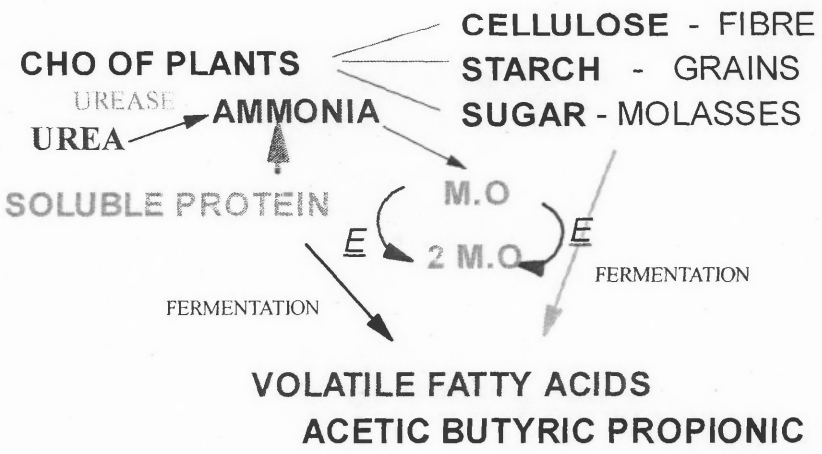
The rumen bacteria vary in size and shape from rods, small rods and small ovals to round cocci. The bacteria may form chains, rosettes and other associated groups. The preferred substrates, species and fermentation end-products have been reviewed (e.g. Hungate, 1966; Russell and Hespell, 1981: Baldwin and Allison, 1983) and the diet eaten by the ruminant affects the composition of the microbial population. In diets high in cell wall polymers - cellulose, hemicellulose and pectin, the bacterial species involved in cellulose, (cellulolytic) degradation being *Bacteroides succinogenes, Ruminococcus albus, R flavefaciens*, *Eubaterium cellosolvens* (Theodorou and France, 1993) and *Fibrobacter succinogenes* (Leng, 1990).

The micro-organisms degrading fibrous feeds appear to have minimal requirements for amino acids and maximal requirements for ammonia and 98% of species studied could grow only in the presence of adequate ammonia (Bryant and Robinson,1962; Allison et al. 1987).

Hemicellulose is degraded by *Butyrivibrio fibrisolvens* and *Bacteroides ruminicola* in addition to the use of hemicellulose by some of the cellulolytic microorganisms (Hungate, 1966). The pectin degrading bacteria include *Butyrivibrio fibrisolvens, Lachnospira multiparus* and protozoa (Williams and Withers, 1991; Wojeciech et al. 1982; in Theodorou and France, 1993).

There are many complex interrelationships between microbial species in the rumen. Some reactions are sequential, whereby the end product of metabolism of a given substrate by one microbial type becomes the substrate for another microorganism. The rumen micro-organisms degrade complex, very often low-quality, highly lignified feeds and convert them to end products of use to the animal and obtain energy for their own growth (fermentation, see Fig. 9). The host animal uses the end products of microbial metabolism as its major source of energy and heavily relies on the microbial mass as its main source of protein as well as energy.

Figure 9 **Energy and protein transactions in the rumen**



The above diagram shows the dual activity of the micro-organisms fermenting the carbohydrate to VFAs, while also growing, using some of the energy and intermediate products of the fermentation to incorporate ammonia and minerals to produce further microbial protein.

5.3 Methanogens

Between 6 and 10% of the energy of the ruminant diet is converted to methane energy in the rumen and lost by eructation: balancing the nutrient mix in the rumen results in decreased methane and heat production (Leng,1991; see Fig. 11). Methane production in the rumen is one of three main disposal routes for hydrogen produced from the fermentation of hexose to pyruvate and eventually to VFAs (Czerkawski, 1986). Beever (1993) states the amount of excess hydrogen produced and thus available to be converted to methane from the overall fermentation reaction is dependent on :

1. The fermentation reaction and the partition of the reaction apportioned to microbial cell production relative to volatile acid production.

2. The amount of propionate relative to acetate formed.

The effect of diet then, is immediately obvious. More propionate is produced from concentrate-based diets where the molar proportion of propionate : acetate is typically 75:100 compared to 16:100 on forage-based diets of low digestiblity (Preston and Leng,1987).

3. The involvement of hydrogen in the saturation of long-chain fatty acids within the rumen.

Beever (1993) demonstrated the effect of increasing microbial growth relative to VFA production in an experiment in a dairy cow where he examined the effect of a high-concentrate and a high-fibre diet on methane production. The effect of changing the ratio of microbial cells to VFA produced (P:E) on methane production was also measured.

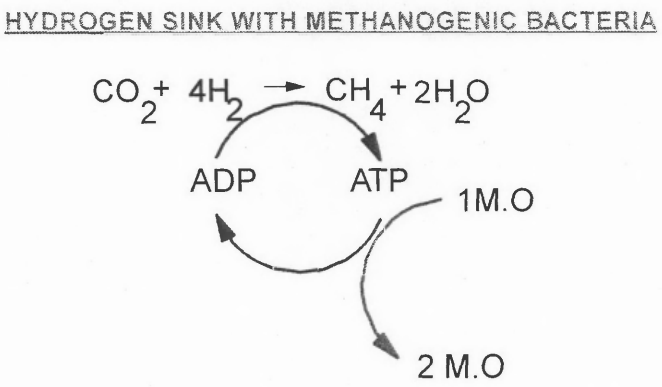
Table 5: **The effect of diet and microbial cell : VFA production on methane production (mol/d). (after Beever, 1993)**

Ratio Microbial cells: VFA produced	0.4:1	0.6:1	0.8:1	1:1	1.2:1
Mol. hexose fermented	35	30.6	27.2	24.5	22.3
High- forage diet (methane production)	21.4	18.7	16.6	14.9	13.6
High-concentrate diet (methane production)	13.3	11.6	10.3	9.3	8.5

In the above table, the improved microbial cell production (higher P: E) is associated with decreased production of methane. This occurrs because less free hydrogen is produced when microbial growth is maximised. The effect of a high forage (fibrous) diet compared to a concentrate diet also showed the above trend, but less methane was produced from the high-concentrate diet at every microbial: VFA ratio compared to the fibrous diet. This was due to the higher propionic: acetate ratio produced in the rumen from the concentrate diet.

The consortium of microorganisms associated with a fibrous diet, eventually converts complex carbohydrates to relatively more acetic acid, which is less reduced than the substrate and carbon dioxide. The methanogens use hydrogen and reduce the carbon dioxide to produce methane and ATP and use the ATP for their growth.

Figure 10. **Methane production by hydrogen utilisation by bacteria**

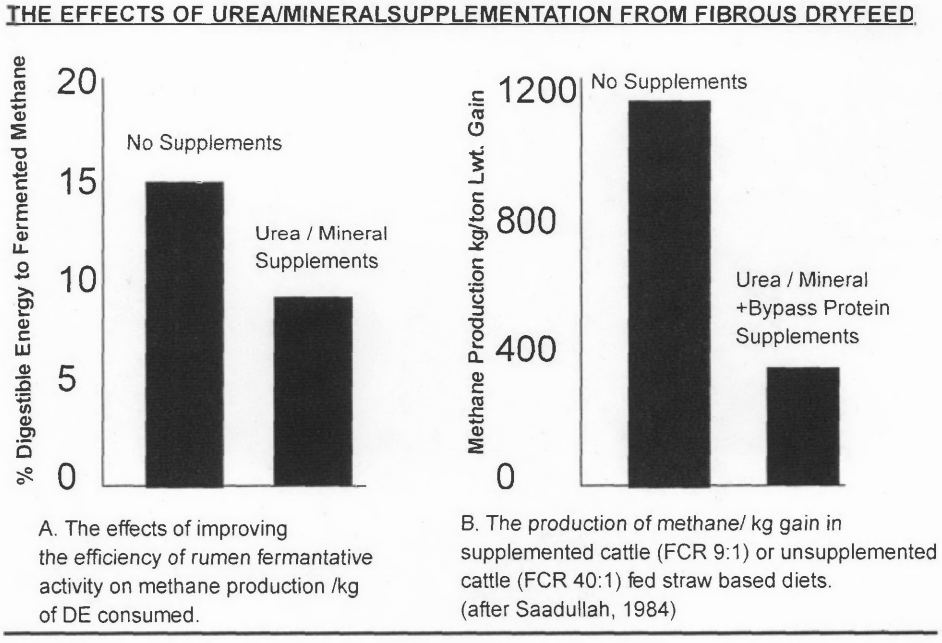


The generation of methane provides a means of disposing of the hydrogen produced by fermentation. Hydrogen can be released in the rumen but generally the hydrogen that

participates in the production of methane arises as reduced co-enzymes in organisms producing VFAs. Increased methane production from ruminants feeding on unsupplemented fibrous pastures is a net loss of energy to the animal, decreasing the efficiency of nutrient availability to the animal.

The major source of methane production is from methanogenic bacteria using hydrogen. Thus improving the propionate:acetate ratio and increasing the microbial growth relative to VFA production are the main options available to improve production by reducing available hydrogen ion and thus methane production in animals grazing dry pastures. This would imply that by increasing the amounts of concentrates fed in the diet in the former option this would occur. This is not an option on the fibrous feeds occurring in Northern Australia due to economic restraints. However, supplementation of fibrous diets, with nitrogen and minerals, to promote efficient fermentation of these diets, increasing the microbial cell : VFA produced, would decrease methane generation per unit of feed of digested feed (Leng,1991; see Fig. 11).

Figure 11. **Effects of supplementing low quality dry feed on methane production**

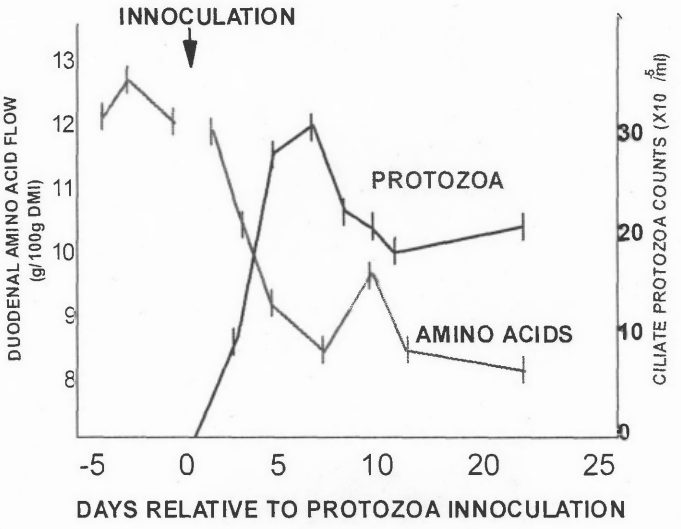


After Leng (1991)

5.4 Protozoa

Protozoa are generally on average 100 times larger than bacteria and can be divided into three main groups, *viz.* the rumen flagellates, the holotrichs (with hair like cilia over their entire body) and the entodiniomorphs. The holotrichs are mobile, use simple carbohydrates and can store excess carbohydrate as starch. The entodiniomorphs are more exacting in their nutritional requirements and appear to be morphologically more complex (Czerkawski,1986). The rumen flagellates have been the least studied and some are now considered to be zoospores of rumen fungi (Theodorou and France,1993). Protozoa can ingest small feed particles and bacteria, thus contributing to the microbial turnover in the rumen.

Figure 12. Changes in duodenal amino acid flow and protozoal numbers from 6 protozoal free sheep (Caesarian section), (day -5 to 0,) and the effect of inoculation with protozoa (day 0 to 25)



After Veira et al. (1984)

The role of protozoa depends on the diet that the animals are being fed, but in diets low in true protein and by-pass protein, they have a negative effect (Bird and Leng, 1984). Protozoa ingest and digest bacteria and reduce the bacterial biomass in the rumen (Hungate, 1966; Coleman, 1975) and therefore the outflow of bacterial protein from the rumen to the

abomasum. Protozoa are also preferentially retained in the rumen (Weller and Pilgram, 1974). They decrease the protein to energy ratio from the ingested pasture and decrease the amount of true protein absorbed from the diet and thus decrease the protein availability from the diet (Bird and Leng, 1984). Ruminants grazing fibrous pastures, low in protein, would therefore have a decreased efficiency of utilisation nutrients from the feed due to protozoal numbers and this in turn would affect the nutrients available to the animal for growth and milk production (Leng,1982, 1991).

In optimal pasture conditions, the number of protozoa in the ewe is less than the number of bacteria by a factor of about 10,000 but because of their larger size their biomass can be up to 50% of that of the bacterial biomass.

The effect of protozoa in high starch diets will not be discussed, but their biomass usually increases with increasing levels of soluble sugars or starches in the diet. The effect of protozoa on animals grazing increasing amounts of fiber in the diet may be substantial due to their predation of the bacteria (Theodorou and France, 1993) and thus the effect on protein availability. Bacterial and fungal numbers are often already depressed in animals on fibrous diets due to deficiencies of nitrogen, phosphorus and sulphur in these diets. Although protozoa are able to utilise plant nutrients, much of their energy and nitrogen requirements are derived from the ingestion of other microbes.

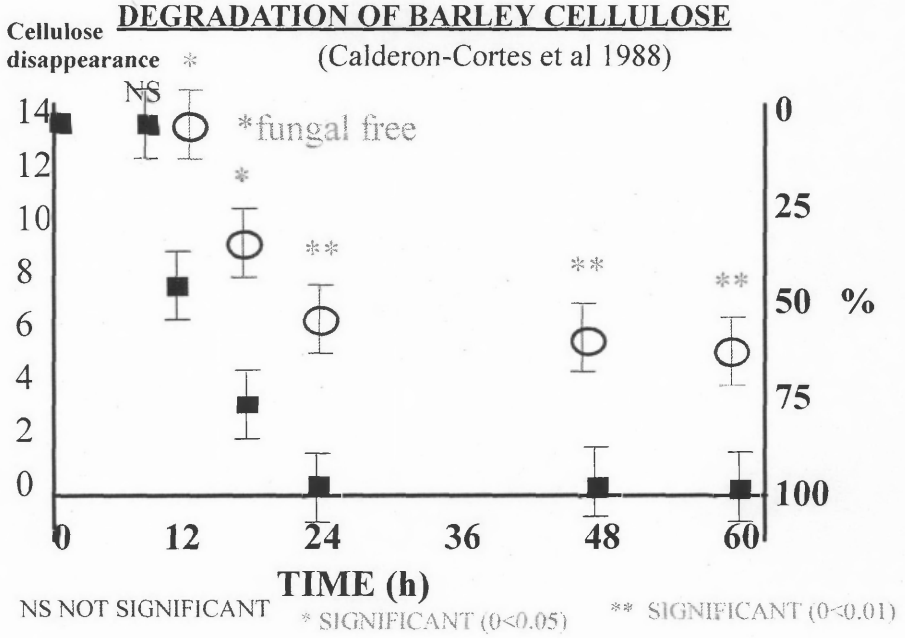
Juul-Nielson (1981) suggested that by increasing the pool of cellulolytic bacteria by feeding small amounts of highly digestible fibre, the increased pool of cellulolytic bacteria would in turn increase the rate and extent of fermentation in the rumen, counterbalancing the effect of the protozoa. However, this is totally impracticable in most situations due to the high cost of cartage to remote areas, especially in northern Australia.

Cattle grazing low protein, fibrous pastures are undoubtedly affected by the predation and engulfment of the cellulolytic bacteria by the protozoa. In addition the sequestration of protozoa in the rumen reduces their contribution to the supply of protein into the duodenum. Supplementation with a protein source to potentiate microbial growth in these cattle would have a positive effect, combatting the low protein in the pasture and the protein loss caused by the protozoa (Leng,1991).

5.5 Anaerobic fungi

Anaerobic fungi are considered to be immensely important to the utilisation of poor quality, high fibre pasture crops and residues by ruminants (Gordon and Phillips 1995). Their importance is based on their demonstrated ability to colonise lignified cell walls and to weaken fibrous plant tissues in the rumen (Akin and Booreman, 1990; Akin et al. 1983, 1990). They also degrade the structural components of plant cell walls to fructose, glucose and xylose (Phillips and Gordon, 1988; Teunissen and Op den Camp, 1993; Wubah et al. 1993) and ferment these monosaccarides (Phillips and Gordon, 1988).

Figure 13 **The effect of fungi in the rumen on barley cellulose degradation**



The amount of cellulose digestion was measured from barley straw in nylon bags suspended through rumen fistulas into the rumens of animals with normal bacterial flora but with and without fungal elements in the rumen. By 60 h, 100% of the straw was degraded in animals with full fungal complement, whilst with out fungi approximately 60% has been degraded.

The fungi were recognised to exist in the rumen as both zoospore sporangia (Bauchop, 1979; Orpin, 1975). Motile zoospores germinate on the ingested food material, then grow to a larger

vegetative fungal thallus comprising of a sporangium and rhizoid. The time for a fungal life cycle is 24-26 h (Lowe et al. 1987).

Ruminants grazing young unlignified pasture may have few anaerobic fungi compared to the same animals when the pasture matures and the plants become lignified and lower in moisture (Bauchop, 1979). The fungi become increasingly important in digestion as the fibrous component increases in a diet.

In ruminants fed silage diets fungal numbers are lower (Grenet et al.1989) as with diets based on carbohydrates (grain and molasses). These diets also support large numbers of protozoa in the rumen (Gordon, 1985). The increase in fungal numbers in the rumen of animals on fibrous feed is thought to occur because there is a decreased predation of fungal protein by the reduced protozoal numbers (Newbold and Hillman, 1990. However, it is likely that without the increase in fungi, the numbers of bacteria would decrease since the surface area of the feed exposed to bacterial fermentation is decreased. This in turn makes less substrate available to sustain bacterial numbers.

The sulphur content of hay appears to affect the fungal population density in the rumen (Akin et al. 1983; Gordon, 1985). When the sulphur content in the diet was < 1g S/kg of organic matter (OM), anaerobic fungi were not present in the rumen of sheep being fed on *Digitaria* pasture hay (Akin et al. 1983). When sulphur-containing fertiliser was applied to this pasture prior to hay making and the hay fed to the sheep, the fungal population in their rumen increased dramatically (Akin et al. 1983).

Similar increases in fungal populations also occurred when sulphur was supplemented to hay initially low in sulphur (Gordon, 1985). Under these conditions the *ad libitum* intakes of forage increased by up to 83% with sulphur supplementation (Akin et al. 1983; Gordon, 1985). This occurred due the increased digestibility of the hay caused in part because of increased fungal activity affecting cellulose breakdown, exposing more surface area to microbial attack (see Fig. 13).

Morrison et al. (1990) found that cattle grazing spear grass (*Heteropogon contortus*), deficient in sulphur, had undetectable fungal ruminal populations, while abnormally low fungal populations were also found in cattle eating wheat bran, also low in sulphur (Gordon, 1985; Gulati et al. 1985; Weston et al. 1988). However, after supplementation with sulphur, greatly

increased populations of fungi occurred. This resulted in increased voluntary intake of digestible dry matter. There was little or no change in the density of the bacterial or ciliate protozoal populations, (Akin et al. 1983; Gulati et al. 1985; Morrison et al. 1990).

Sometimes elemental sulphur does not improve feed intake but methionine can, (1g/day in sheep grazing *Digitaria* sp., (Gordon, 1985). Gulati et al. (1985) showed that increased anaerobic fungi numbers occurred with methionine supplementation while Weston et al. (1988) successfully increased fungal numbers using sulphate. Sulphate supplemented spear grass hay also supported higher ruminal fungal populations than unsupplemented hay (Morrison et al. 1990).

When anaerobic fungi are grown *in vitro* they require reduced forms of sulphur (Orpin, 1977) indicating that reduced sulphur is needed in the rumen before it is available to the fungi; however, in the rumen, sulphate would be quickly reduced to sulphide.

Owing to the demonstrated effect of increased densities of anaerobic fungi in the rumen, resulting in the corresponding increase in the utilisation of poor quality mature herbage diets, considerable potential exists to influence the increase of fungal numbers and activity in the rumen. Thus resulting in increased production responses from this feed source (Gordon and Phillips, 1995).

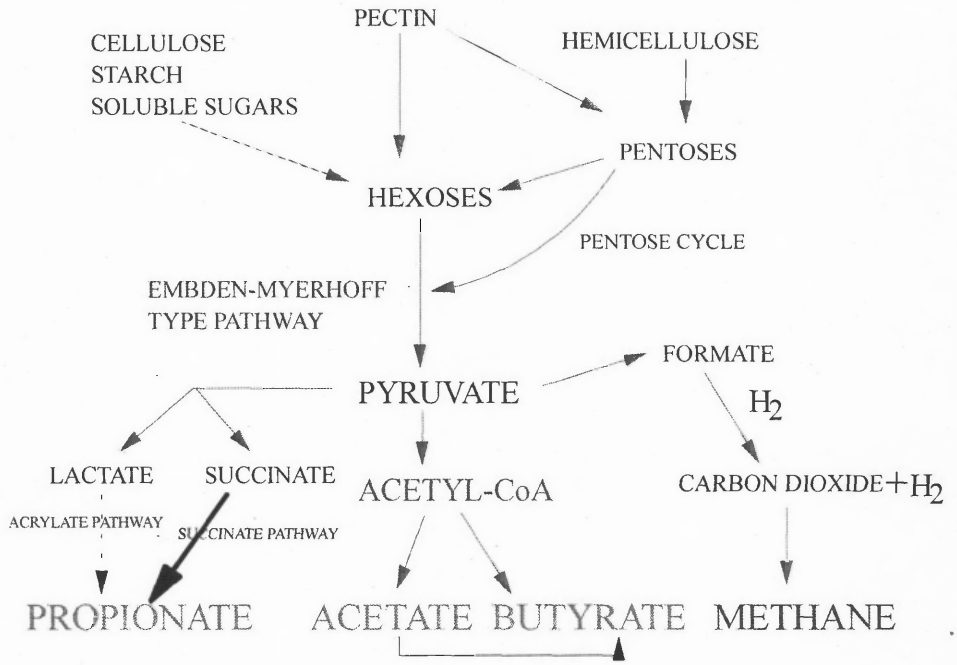
5.6 Carbohydrate fermentation

Cellulose, hemicellulose, pectin, starch and soluble sugars occurring in plants, represent the major carbohydrate sources in the ruminant diet. These are degraded to their constituent hexoses and pentoses before being fermented to VFAs from pyruvate. Regardless of the carbohydrate source, the universal end products of fermentation are VFAs (VFAs), methane (CH_4) and carbon dioxide (CO_2). During this fermentative process multiplication of microbial cells occurs. Soluble proteins are also largely fermented to VFAs with the production of ammonia.

The biochemical pathways for the production of VFAs and methane from simple sugars have been well-documented (Baldwin 1965; Leng 1970; Prins 1977; Baldwin and Allison, 1993).

The sugars are mainly converted through the Embden- Meyerhof pathway of glycolysis to pyruvate, which is a common intermediate in the production acetate, butyrate, lactate and propionate, although pyruvate rarely appears free in the ruminal fluid.

Figure 14 **Pathway for cellulose degradation**



(After Czerkawski, 1986)

The rumen micro-organisms are capable of degrading all carbohydrates in plants, including those that are structural and converting them to end-products that are of use to the animal.

5.7 Volatile fatty acid production

Potential energy is generated as ATP (adenosine triphosphate) in the breakdown of carbohydrate to VFA.

The ATP is the energy source that enables bacteria and protozoa to survive and multiply. The products of this process are VFAs (VFAs), principally acetic acid, propionic acid and butyric acids, but also lesser amounts of valerate, caproate, isobutyrate, isovalerate, 2 -

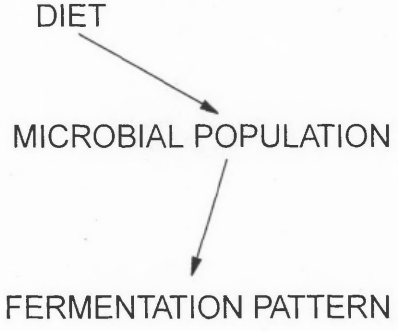
methylbutyrate are also produced (France and Siddons, 1993), the latter group being produced mainly from degradation of amino acids.

Although, from the point of view of the microbes, the VFAs are waste products, these substrates represent the major source of energy in the animal's tissues where they are assimilated and used for maintenance and polymer syntehsis. To the host animal, the VFAs are essential, accounting for between 60% and 75% of the dietary fermentable energy disappearing in the rumen. The remaining 25–40 % is lost as heat and methane or is conserved in microbial cell mateerials. This energy supply accounts for 50–70% of the digestible energy intake of the animal (Sutton, 1972, 1979; Thomas and Clapperton, 1972).

Of the carbohydrate and proteins fermented in the rumen a proportion are converted to the building blocks for microbial growth and the rest converted to VFAs and a variable amount of methane and heat is produced, depending on the efficiency of the microbial growth. The relative conversion of carbohydrates to microbial growth depends on the substrates and the availability of critical growth factors. When ammonia is deficient in the rumen, about 10% of the carbohydrate can go to cell synthesis and 90% to VFA production (Preston and Leng, 1987). Russell and Strobel (1993) reported that, when bacterial growth was limited by factors other than energy, maintenance energy requirements of the cells were much higher and thus yields of bacterial cells were lower. 'Energy spilling' or 'uncoupling' are terms used to describe the observed phenomenon where energy sufficient bacterial cultures have higher yields. It appeared that energy-sufficient cultures required more energy for maintenance, but used the remaining energy more efficiently than the energy limited ones.

5.8 Fermentation pattern

Figure 15 Effect of diet on fermentation pattern



The fermentation pattern is determined by the composition of the microbial population that is in turn influenced by the diet, particularly the source of dietary carbohydrate.

High fibre diets encourage the growth of bacterial species producing acetate, increasing the lipogenic (fat) precursors. Propionic acid producing diets (starch and concentrates) increase the glucogenic (glucose) precursors. An exception occurs when protozoa proliferate under certain conditions on concentrate diets. In this instance an increase in butyrate occurs rather than propionate (France and Siddons, 1993).

Because of the fermentative digestion, ruminants on fibrous feeds normally absorb little or no dietary carbohydrate as hexose sugar (Bergman et al. 1970). Van der Walt et al. (1983) and Bergman (1973) state that, even when diets contain high amounts of grain, the absorption of glucose from the gut accounts for less than one-third of the whole body turn over of glucose. However, in animals given corn (maize) and sorghum, some grain escapes fermentation in the rumen and so the unfermented starch is absorbed directly from the small intestine. This is in contrast to barley that is totally fermented in the rumen (Preston and Leng, 1987). The aspects of glucose availability will be discussed later in this thesis where the effect of glucose on ovarian activity will be highlighted.

The fermentation pattern differences in molar proportions of VFAs that determine the VFA proportions from fibrous diets compared with grain diets has been shown by Siciliano-Jones and Murphy (1989) and Leng and Brett (1966) (see Table 6).

Table 6 **Dietary effects on VFA proportions**

Diet	Acetic acid %	Propionic acid %	Butyric acid %
Grain : forage 4:1	73	18	9
Grain : forage 1:4	67	22	12

The effects of fibrous diets with their high acetate:propionate ratios, affecting lipid levels, progesterone, the ovary and reproduction will also be discussed later.

5.9 Propionate

Propionate synthesis proceeds by two pathways of fermentation (Fig. 10) – the main one being via succinate and the alternative involving acrylate (France and Siddons, 1993). The succinate pathway is used by most propionate-producing microorganisms and is the prominent pathway in the rumen, especially with forage–based diets. Production by this pathway involves the carboxylation of pyruvate (C3) to oxaloacetate (C4). The acrylate pathway involves the step-wise reduction of pyruvate to propionate, lactate being formed as an intermediate and this conversion is reversible.

5.10 Acetate

Acetyl CoA is an intermediate in the formation of acetate (Fig. 10). During the cleaving of pyruvate to form Acetyl-CoA, formate is produced and this is rapidly converted to CO_2 and H_2. A second method, involves the pyruvate-ferredoxin oxidoreductase enzyme system, where pyruvate is oxidised to acetyl CoA, CO_2 and reduced ferredoxin. Acetyl-CoA is subsequently converted to acetate with the release of 1 mol of ATP / mol acetate produced (Prins, 1977; Baldwin and Allison, 1983).

5.11 Butyrate

Butyrate has the same fermentation pathway to the production of acetyl CoA as acetate (Fig. 10). The fermentation then proceeds via a reversal of the B-oxidation pathway (Leng, 1970). In this fermentation, 2 mols of NADH are oxidised converting acetyl-CoA to butyrate and 2 mols of pyruvate are converted to 1 mol of butyrate.

5.12 METABOLISM OF SHORT CHAIN FATTY ACIDS

5.12.1 Propionate

A sheep on a maintenance diet of lucerne pellets (800 g/d) produces 30-45 mmol of propionate per hour (Judson and Leng, 1973a; Steel and Leng, 1973) of which it absorbs

18-24 mmol of propionate per hour (Bergman et al. 1966; Bergman and Wolff, 1971). This amounts to 40–60% of ruminal production (Judson and Leng, 1973a; Steel and Leng, 1973). A substantial amount of the propionate produced in the rumen is converted to glucose in the liver. Half of the plasma glucose and plasma lactate in sheep were found to be derived from ruminal propionate (Leng et al. 1970). However, only about 15% of the lactate is derived from propionate (Weekes and Webster,1975). Up to 90% of the absorbed propionate is converted to glucose (Judson and Leng, 1973b; Steel and Leng, 1973; Veenhuizen et al. 1988). In pregnant animals the fraction of propionate used for glucose synthesis is higher than in non–pregnant animals, *i.e.* 40–50% v. 50–60% (Judson and Leng, 1973b).

The nutritional implications of increased need for propionic acid production for the animal are important. When stress is high on the animal, either climate (extreme heat or cold), or physiological (pregnancy, lactation), blood glucose levels have to be maintained for the animal to supply glucose for essential functions.

The need for glucogenic precursors is likely to be critical in ruminants on dry pasture during late pregnancy when the uterine and foetal demands for glucose are increasing and the fibrous nature of the pasture is also increasing. This tends to more favour acetate production rather than propionic from the bacterial fermentation of the more fibrous pastures, resulting in a decrease in glucogenic precursors and the consequent increase in metabolism of fat with ketone bodies increasing in the blood. This is occurring when the ruminant, physiologically, is in a period where glucose needs are maximal. The lack of glucogenic precursors causing a fall in blood glucose concentration at a time of increased glucose utilization can lead to increased metabolism of fat stores and an increase in blood ketone concentration. These increased concentrations of ketone bodies can lead to the medical conditions of ketosis in cattle and lambing sickness in sheep as well as exacerbate the clinical conditions of hypocalcaemia and hypomagnesaemia.

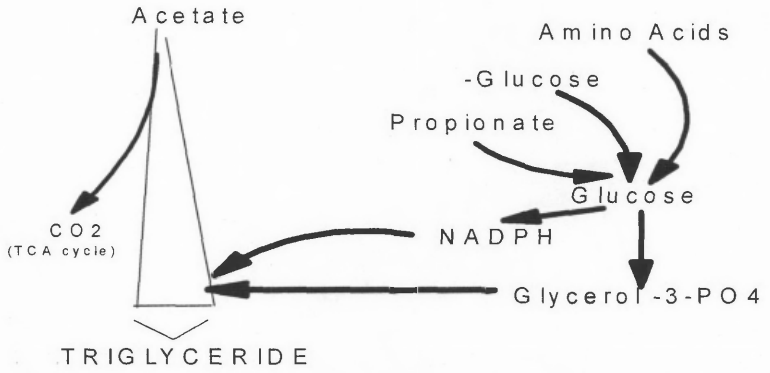
5.12.2 Acetate and butyrate

Within the host animal's tissues, absorbed acetate and butyrate are used mainly as energy sources through oxidation in the citric acid cycle. Acetate is also a principal substrate for fat synthesis (lipogenesis). The balance between the supply of the glucogenic propionate relative

to that of the non–glucogenic acetate and butyrate influences the efficiency with which the VFA are used for productive purposes (Orskov, 1975; MacRae and Lobley, 1982).

The implications of this observation are that not only is an adequate supply of VFAs important but also the molar proportions are important for their tissue partition, metabolism and utilisation.

Figure 16 **Acetate and gluconeogenesis**



Relationship between gluconogenic precursors and Acetate utilisation from Preston & Leng (1987) p 52

With the production of milk, acetate, butyrate and long chain fatty acids are the precursors for milk fat, NADPH is also need for fat synthesis but the majority is formed from the oxidation of glucose to glycerol-3-phosphate in the Embden–Meyerhof pathway. Growth of animal tissue includes both fat and protein synthesis with acetate, butyrate and dietary long chain fatty acids being the precursors of fat deposition.

There are no reliable means by which the composition of rumen VFA can be accurately predicted on the basis of diet composition,(Beever, 1993). Isotopic methods to measure the individual VFA have been satisfactorily employed with small ruminants (Weller et al. 1967; Leng and Brett, 1966), but the reliability of the limited studies conducted on cattle, especially dairy cattle, is still open to question.

5.13 STOICHIOMETRY OF FERMENTATION

The fermentation of fibrous feeds depends on a stepwise degradation polysaccharide to cellobiose. Then1 mol of hexose is degraded to given 2 mol pyruvate with a net production of 2 mol ATP and 2 mol of reduced NADH.

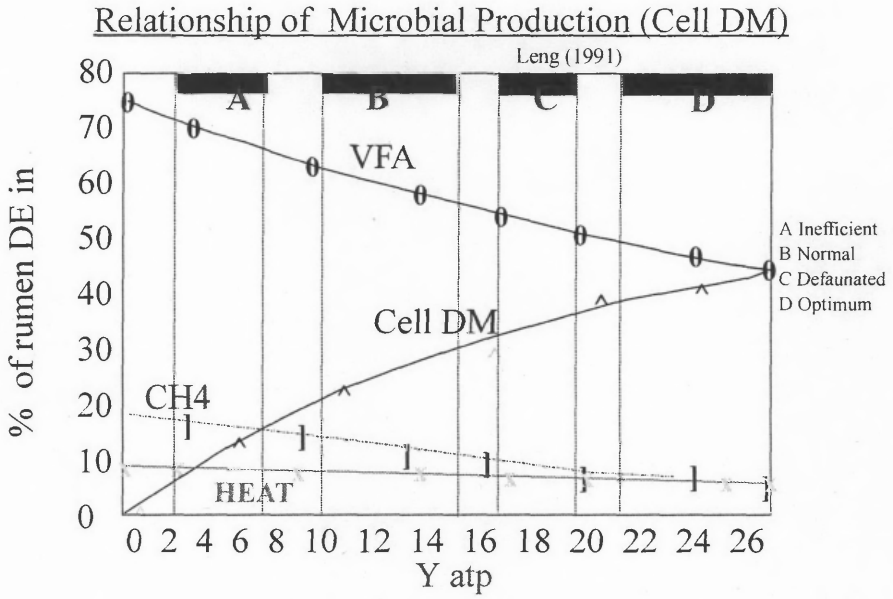
From each mol of pyruvate either 1 mol of propionate, 1 mol of acetate or 0.5 mol of butyrate is formed, although the molar amounts of ATP production are not fully understood. There is some disagreement about the exact availability to the microbes of ATP in the various pathways by which carbohydrates are progressively degraded to VFA in the rumen (Preston and Leng 1987). However, in general the following is proposed, *i.e.*

$$\text{HEXOSE} \rightarrow 2\ \text{PYRUVATE} + 4[H] + 2\ \text{ATP}$$

1. $2\ \text{pyruvate} + 2H_2O \rightarrow 2\ \text{Acetic Acid} + 2\ CO_2 + 2H_2 + 2\text{ATP}$
2. $2\ \text{pyruvate} + 8[H] \rightarrow 2\ \text{propionic Acid} + 2H_2O + 2\text{ATP}$
3. $2\ \text{pyruvate} + 4[H] \rightarrow 1\ \text{Butyric Acid} + 2H_2 + 2CO_2 + 2\text{ATP}$
4. $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O + \text{ATP}$

The efficiency of production of microbial biomass in the rumen increases as the nutrients required for their growth approach optimum levels in the rumen. When conditions are optimal, there are no micro- or macro-mineral deficiencies and ammonia levels in the rumen are such that the rate of assimilation of ammonia is not concentration-limited. Provided cellulose is not limiting, with increasing ammonia and adequate minerals, the microbial cells use ATP more efficiently for growth so more of the fermentation intermediates are diverted for microbial growth so less are converted to VFAs (Fig. 17).

Figure17 **Microbial cell synthesis**



Y_{atp} = grams of dry microbial cells produced for each mol of ATP produced .This can vary from 10 (Y_{atp}10) to 26 (Y_{atp} 26) which is theoretically the optimum (Leng, 1982), although $Y_{atp} > 20$ may be unlikely to achieve (Harrison and M^{c}allan,1980).

The area A in the diagram represents the amount of biomass produced when there is a deficiency of nitrogen or an element critical to microbial growth. The partitioning of ATP is such that where fermentation proceeds in a situation of substrate deficiency for microbial growth the production of VFAs takes preceedence **(A)** so that the protein / energy ratio is low. However, with increasing availability of substrates the ability of the microbes to grow increases (*i.e.*get more growth of microbes for each mol of ATP available)**(B) and** so with increasing microbial biomass, VFA production decreases relative to the microbial biomass as ATP is diverted to microbial synthesis.

The unfaunated state after removal of protozoa) **(C)** has been shown to increase the availability of protein in the small intestine (Bird and Leng, 1984). The increased protein availability comes because no microbial protein is being ingested by the protozoa and this causes an increase in microbial cell concentration in the rumen and consequently more microbial protein becomes available to the animal. Leng (1982) further calculated the maximum theoretical microbial output relative to VFA production **(D)**. The heat production from an inefficient rumen can be considerable (**A**).

Brouwer (1958) attempted to calculate the heat of frementation occurring in the rumen by assuming that the carbon dioxide formed during fermentation and the heat were constantly proportional to the amount of methane formed. Czerkawski (1986) also calculated the heat of fermentation without taking this relationship into consideration, although he acknowledged that such a relationship undoubtedly existed. However the total heat production in the animal would be a function of the heat produced in the body (t(b)) and the heat produced during the fermentation in the rumen (t(f)) so that total heat production would be:

$$T\,(tot) = t(b) + t(f)$$

Thus in hot environments such as the Northern Territory, when ruminants are ingesting dry, fibrous pastures, the heat of fermentation could contribute significantly to total body temperature. Cattle in hot environments, grazing unsupplemented dry pastures would experience a depression in intake due to the heat production from a rumen deficient in nitrogen or minerals (Fig. 18). This would also occur with a supplement inadequate in supplying the rumen requirements.When environmental temperatures are high there is only one way an animal can minimise heat load and that is to decrease feed intake and preserve body water.

Leng (1991) postulated the difference in heat production according to the amount of microbial cell production, ammonia concentration and methane produced. He found that the heat produced was inversely proportional to the concentration of microbial cells, Y_{ATP} and proportional to the methane level (see Table 7).

Table 7 **Efficiency of microbial multiplication and heat production**

$Y_{(atp)}$	Microbial protein	VFA energy (MJ)	Methane energy (MJ)	Heat energy (MJ)	Protein/energy (g protein /MJ)
8	498	55.5	9.4	6.4	9
14	798	46.8	8.5	5.1	17
19	1008	40.8	8.0	4.3	24
25	1212	34.5	3.1	3.1	34

From Leng (1991)

From the above calculations (also Fig. 17), it can be seen that with a decreasing number of bacteria, as would occur with a low protein content in dry grass and /or mineral deficiencies,

the amount of bacterial cells is less. However, the production of VFAs, methane and heat increases (Y_{atp} =8). The incremental heat reduction that occurs with increasing efficiency of microbial production has a positive effect on feed intake intake. Not only is heat production lower but the increased P/E signals a more balanced rumen, resulting in increased digestibility of the fibrous material (see Fig. 20).The protein to energy ratio is higher with higher Y_{atp} values that occur in a balanced rumen. Conversely in the unbalanced rumen due to low rumen ammonia concentration, Y_{atp} values are low as is the P/E ratio (Preston and Leng, 1987).

The implications for production of cattle in the hotter and or humid areas are profound because, without supplements, the total dry matter intake may also be decreased as a thermo-regulatory mechanism to lessen the effect of environmental heat. This may also have implications for high producing cattle such as dairy cows, in hot/humid areas under reproductive or lactation stress, grazing fibrous pastures.

The amount of energy produced by hind gut fermentation can be considerable and should not be under-rated but is not considered here.

6 PROTEIN – NITROGEN METABOLISM

6.1 Overview

Protein ingestion and availability to the animal is intimately associated with carbohydrate fermentation in the rumen but, for the purpose of discussion, it will be examined separately.

True protein when ingested by the ruminant is initially subjected in the rumen to microbial extra-cellular protease and peptidase action. This produces peptides, some of which are degraded further to amino acids in the bacteria. These are either used as such for microbial protein synthesis or are fermented to ammonia and VFAs. The VFAs are mainly discharged from the bacteria as with the ammonia, but some ammonia is used intra-cellularly to synthesise new amino acids that are then used by the bacteria. The ammonia released from some microbes into the rumen fluid may be taken up by others and converted into bacterial protein. When the bacteria or the undegraded dietary protein pass down into the abomasum