Chapter 1 General Introduction

In ruminants, all feed is subjected to fermentation and enzymatic digestion. The primary (or pre-gastric) fermentation occurs in the reticulo-rumen before gastric digestion takes place in the abomasum. Additional (or post-gastric) fermentation of undigested material from the reticulo-rumen takes place in the caecum and the colon after passing through the small intestine. The process of fermentation includes a combination of physical and microbiological activities which convert carbohydrates (starch, non-starch polysaccharides and other fibrous material) into volatile fatty acids (VFAs), methane (CH_4) and carbon dioxide (CO_2). Volatile fatty acids are efficiently absorbed from the digestive tract and provide an important source of substrates for animal growth and metabolism. With forage diets, VFAs provide 50-80% of the metabolisable energy used by ruminant animals with 66-80% of the total energy being derived from fermentation in the rumen (Church 1988).

The process of fermentation depends on a dense population of micro-organisms (mainly bacteria) in the digestive tract. While neutral pH conditions are optimum for the fermentative microbes and for normal healthy function of the gut wall, the maintenance of neutral pH conditions depends on the balance between the rates of acid production and absorption. It is also influenced by the buffering capacity of the gut contents. The buffering capacity depends upon balance between rates of (1) acid production and absorption, (2) input of buffers from saliva, and (3) presence or release of buffers or bases from the feed.

The process of fermentative digestion evolved to cope with fibrous material in the diet which could not be broken down by the animal's digestive enzymes. It is therefore a system and a process ideally suited to handle the structural carbohydrates of plant cell walls and, under normal conditions, the rates of VFA absorption and outflow are well balanced with the rate of VFA production from the fermentation of structural carbohydrates. The pH in the fermentation compartments of the gut also normally remains between pH 6.5 and 7.0. However, this fermentative

digestion system is not ideally suited or necessary for the digestion of soluble carbohydrates such as sugars and non-structural carbohydrates such as starches. For animals well adapted to a diet rich in readily fermentable carbohydrates, the fermentative environment undergoes significant changes to cope with the rapid fermentation of readily fermentable carbohydrates. Such changes include microbial species and population densities, fermentative processes, rates of VFA production, rates of absorption, and passage rates.

If the ruminant abruptly ingests large amounts of readily fermentable carbohydrates or when the time period of adaptation to readily fermentable carbohydrates is insufficient, the fermentation of readily fermentable carbohydrates increases suddenly, and VFAs are produced at a rate higher than their removal (absorption and passage through) leading to VFA accumulation and reduced pH. These acidic conditions can lead to proliferation of lactic acid-producing bacteria such as *Streptococcus bovis* and Lactobacillus which leads to the accumulation of lactic acid and the development of the condition known as lactic acidosis. The severe effects of lactic acidosis are well known in terms of stock deaths during the drought-feeding of sheep and cattle. A range of more minor effects of acidity in the fermentation compartments includes:

- marked reduction or cessation of feed consumption (anorexia) (Huntington 1988);
- loose faeces or diarrhoea (Huntington 1988);
- risk of lameness (laminitis) (Rowe *et al.* 1994);
- a listless, depressed or distressed appearance (Huntington 1988); and
- possible effects on health via inflammation of the gut wall and/or accumulation of endotoxins within the gut (Rowe 1997).

The prevention and treatment of acidosis are based on:

(1) controlling the rate of fermentation achieved by feeding animals diets low in readily fermentable carbohydrates, by controlling the amount of grain, or by controlling the pattern of eating (Huntington 1988);

(2) controlling build-up of bacteria-producing lactic acid by addition of antibiotics (Beede and Farlin 1977a);

(3) converting lactic acid to VFAs to be absorbed using microbiological methods, such as probiotics (Newbold 1990; Whitehead and Cotta 1993);
(4) maintaining stable pH by addition of buffers (Reid *et al.* 1957).

Although many treatments are available for preventing and controlling fermentative acidosis, they are not all totally effective. Virginiamycin has been identified as an effective compound for controlling lactic acid accumulation and low pH in sheep (Rowe *et al.* 1989, Godfrey *et al.* 1992) and cattle (Thorniley *et al.* 1996). A dietary supplement, infusion or injection of sodium bicarbonate has been used to treat fermentative acidosis in ruminants and humans for a long time.

It is well established that lactic acid accumulates in the rumen during lactic acidosis. However, little is known about the acidosis of the caecum and the colon which are also important compartments for fermentation in ruminants (Elsden *et al.* 1946). It is likely that both the caecum and the rumen are important in fermentative acidosis. However, while saliva, containing buffers, is secreted into the rumen, no saliva is secreted into the caecum. While feed is fermented in the rumen, the caecum which is associated with the colon, is just responsible for fermentation of undigested residues which pass from the small intestine into the caecum and the colon. There is therefore normally far less starch and sugar fermentation in the hindgut than in the rumen, and the hindgut is less well adapted than the rumen for the effects of rapid fermentation.

Fermentative acidosis depends on a number of factors, including the amount of readily fermentable carbohydrates consumed, rates of production and absorption of acids (lactic acid, VFAs and other acids), and buffering capacity. All of these factors should be understood in order to determine the critical factors influencing the development of acidosis. This understanding is also fundamental in developing effective methods of prevention and treatment of acidosis. Two important aspects of the biology and biochemistry of caecal and rumen fermentation are the absorption of VFAs and lactic acid from the caecum and the rumen, and the buffering capacity in these digestive compartments.

This thesis covers three main areas related to the problem of fermentative acidosis in the caecum and the rumen of sheep. These are:

(1) measurement of the absorption rates of lactic acid and VFAs in both the caecum and the rumen;
(2) determination of buffering capacities in the caecum and the rumen; and
(3) the integration of the main information derived from (1) and (2) above with the information already available in the literature to develop a computer model accounting for the critical factors leading to acidosis.

A general hypothesis explored in this thesis is that the rate of production and accumulation of lactic acid is more important than the buffering capacity within the gut in the development of fermentative acidosis.

This thesis has been divided into six chapters. Chapter 1 provides a general introduction. In Chapter 2, the literature on the factors associated with lactic acidosis is reviewed. Chapter 3 describes a study on the absorption of lactic acid from the rumen and the caecum of sheep. Chapter 4 reports an investigation of the buffering capacity of caecal and rumen digesta from sheep grazing pasture or fed roughage-based diets. Chapter 5 presents a computer model of rumen fermentation which focuses on the development and control of acidosis. A general discussion of the experimental work and the model are brought together with the conclusion in Chapter 6.

Chapter 2 Review of the Literature

Ruminants include sheep, cattle, goats, deer and antelope. They are a highly developed and specialised group of herbivores able to use fibre and other carbohydrates unavailable to non-ruminant digestion (Van Soest 1994). The digestive system of the ruminant is characterised by pre-gastric retention and fermentation with symbiotic micro-organisms and absorption of microbial metabolites from the reticulo-rumen. Undigested structural carbohydrate and starch which pass from the rumen can be fermented and absorbed in the caecum and colon (Figure 2-1).

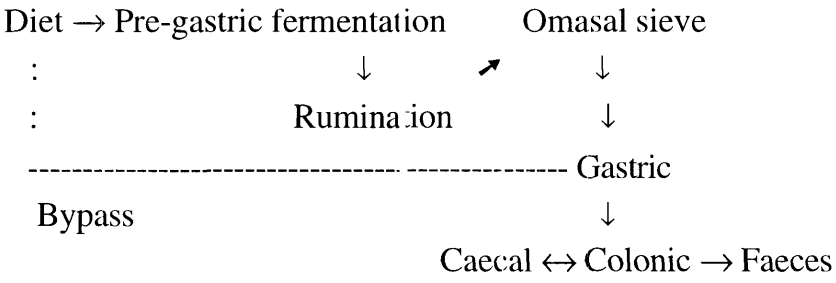


Figure 2-1. Sequence of digestion in ruminants (from Van Soest 1994).

Therefore, this review of fermentation in the gastrointestinal tract will focus on the reticulo-rumen and the caecum of the ruminant and will cover their structure, fermentation, absorption, buffering capacity, lactic acidosis, and the prevention and treatment of lactic acidosis.

2.1 The reticulo-rumen and the caecum in ruminants

The digestive tract of ruminants is mainly composed of stomach, small intestine, caecum, colon and rectum (Figure 2-2). The four compartments of stomach, which consist of the rumen, reticulum, omasum and abomasum, distinguish ruminants from non-ruminant herbivores. The rumen and reticulum are usually considered as reticulo-rumen or simply rumen since they are only partly separated by the reticulo-rumen fold,

allowing a free exchange of contents between the two compartments. The reticulo-rumen volume varies between animal species and individuals. Generally speaking, the rumen fluid volume of an adult sheep is from 3 to 5 litres (SE = 1.0) (Hydén 1961; Church 1988) which is 9-13% of the body weight (Table 2-1). The large reticulo-rumen supports a dense and diverse population of microbes which ferment the feed and allows retention of material to facilitate the breakdown of fibres through fermentation (Parra 1978; Stevens 1988).

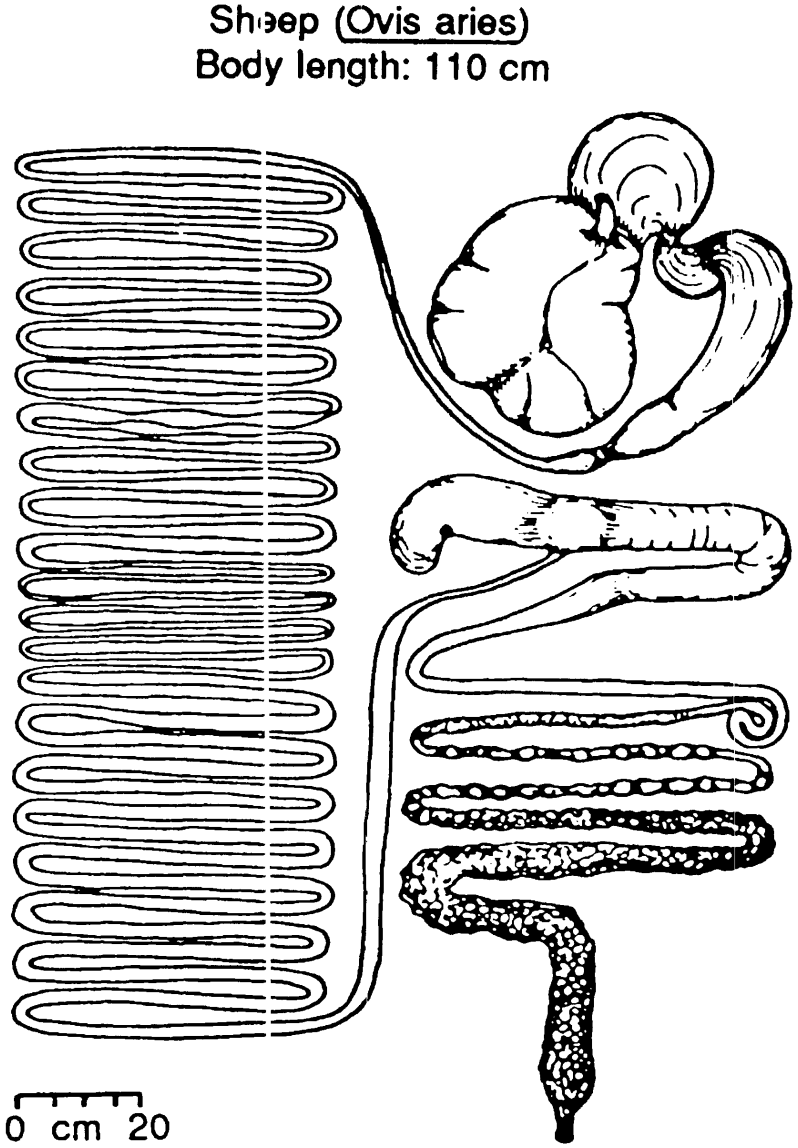


Figure 2-2. The digestive tract of sheep, illustrating the fermentation compartments of the reticulo-rumen, the caecum and the colon connected by the small intestine (from Stevens 1988).

Table 2-1. Volume of various parts of the digestive tract of sheep and cattle expressed as a percentage (%) of total body weight (Parra 1978).

Species	Sheep	Cattle
Total digestive tract	12-19	13-18
Reticulo-rumen	9-13	9-13
Omasum	0.1-0.3	1.1-2.8
Abomasum	0.7-1.6	0.5
Small intestine	1.0-1.6	0.9-2.3
Caecum	0.9-1.6	0.8
Colon	0.5-0.7	0.8-1.5

The rumen includes bacteria, protozoa and fungi which are responsible for fermentation of ingestive feed. The conditions in the reticulo-rumen are very favourable for microbial growth. The absence of oxygen and production of reduced end-products (e.g. sulphide and hydrogen) in the rumen creates a highly reduced environment (Eh = -250 to -450 mv) that is suitable for the growth of anaerobic bacteria (Van Soest 1983). The moist conditions and relatively neutral pH (6 to 7) are favourable for many micro-organisms. The temperature (39-40°C) is near optimum for many microbial enzyme systems (Van Soest 1983). The availability of substrate is more or less continuous and contractions of the reticulo-rumen help to bring the micro-organisms in contact with freshly ingested or ruminated feed. The end-products of fermentation are removed by absorption and pass out of the rumen, thus reducing possible inhibition of microbial growth through accumulation of toxic waste products. As a result of this favourable environment, a dense population of micro-organisms thrives in the reticulo-rumen (Church 1988) resulting in an extensive microbial fermentation. In addition, the saliva from the salivary glands possesses lipase and pre-gastric esterase which are capable of preferentially degrading short-chain fatty acids such as butyrate or caproate from triglycerides. The importance of the rumen fermentation as a means of digestion is demonstrated by the high proportion (64 - 71%) of

digesta residing in the rumen relative to the whole digestive tract in cattle and sheep (Table 2-2).

Table 2-2. Fermentative capacity expressed as percentage (%) of the total digesta in the gastrointestinal tract with active fermentative digestion for a range of different animals (Parra 1978).

Species	Reticulo-rumen	Caecum	Colon & rectum	Total fermentation
Sheep	71	8	4	83
Cattle	64	5	5 - 8	75
Horse	-	15	54	69
Pig	-	15	33	48
Capybara	-	71	9	80
Guinea pig	-	46	20	66
Rabbit	-	43	8	51
Rat	-	32	29	61
Man	-	-	17	17
Cat	-	-	16	16
Dog	-	1	13	14

The structure of the reticulo-rumen wall is adapted for the favourable absorption of nutrients and consists of a serous membrane, a muscular layer and the epithelium which is the site of absorption (Figure 2-3). The cells of the ruminal surface tend to be keratinised and the keratinisation is normally offset by specialised cells that are important in the absorption of VFAs. The luminal surface includes papillae in the rumen and reticular ridges in the reticulum (Figure 2-4). The papillae increase the absorptive surface area of the rumen. The internal structure of a papilla is shown in Figure 2-5. These features make the reticulo-rumen well suited for the absorption of nutrients as well as for fermentative activity. It has been estimated that about 88% of VFAs produced in the rumen of sheep are directly absorbed from the rumen and only about 12% flow to the omasum (Sutherland 1963; Sutherland *et al.* 1964).

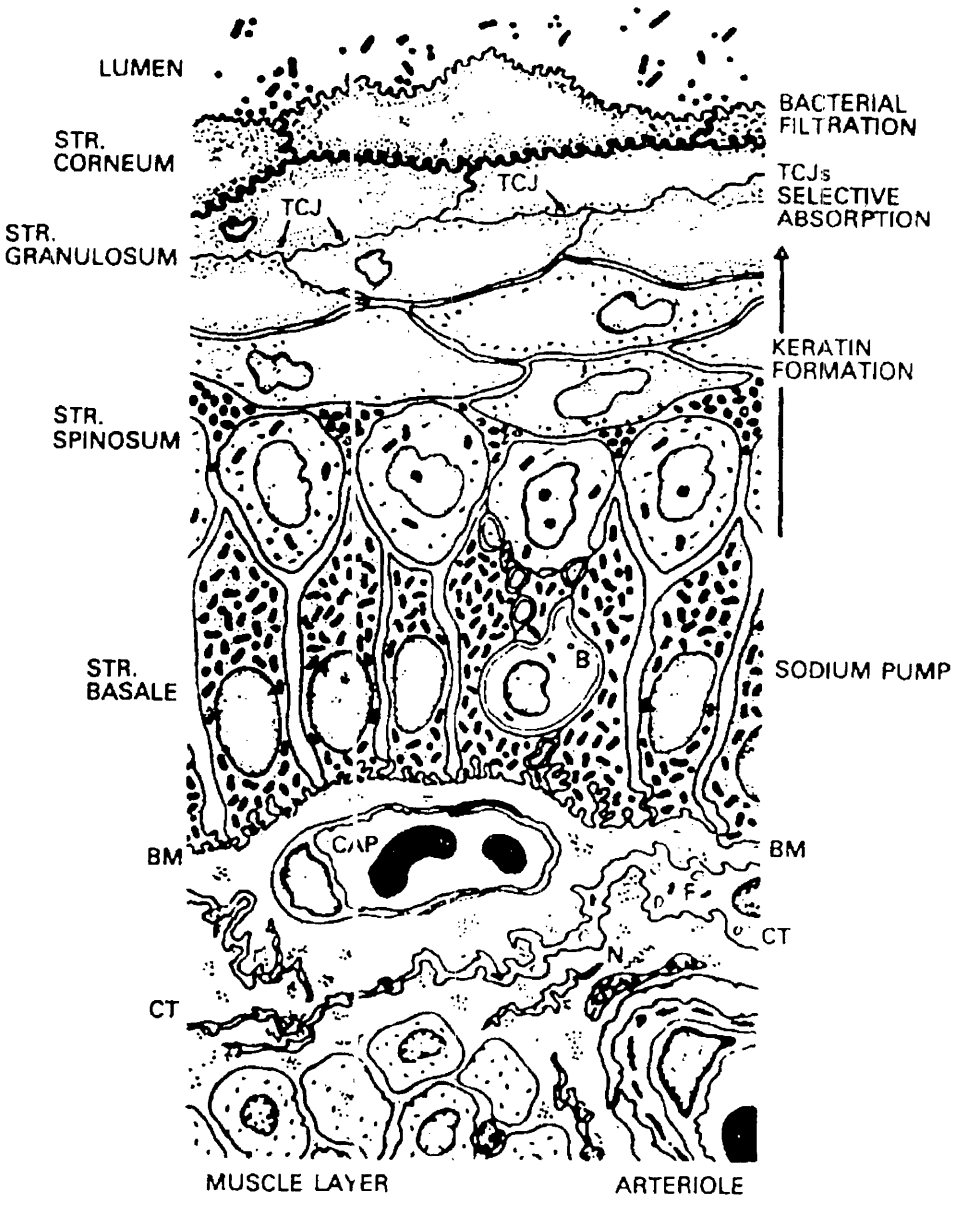


Figure 2-3. Cross-section of the fully developed rumen wall depicting the types of cells and layers present (Van Soest 1994 from Steven and Marshall 1973). Details of the cell junctions are omitted. B = branching cell; BM = basement membrane; CAP = capillary; CT = connective tissue; F = fibro-blast; N = nerve trunk; TCJ = tight cell junct on.

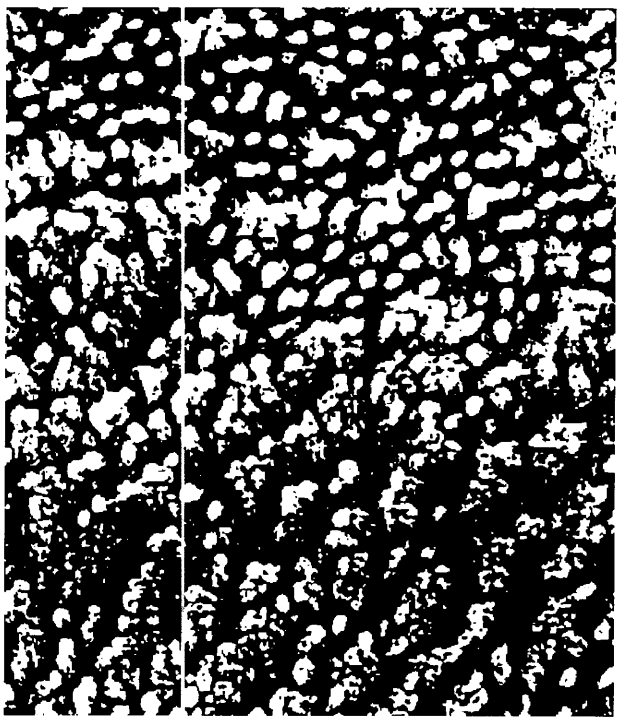


Figure 2-4. Filiform atrium papillae of a one-day-old goat kid (intermediate feeder) (from Hofmann 1988).

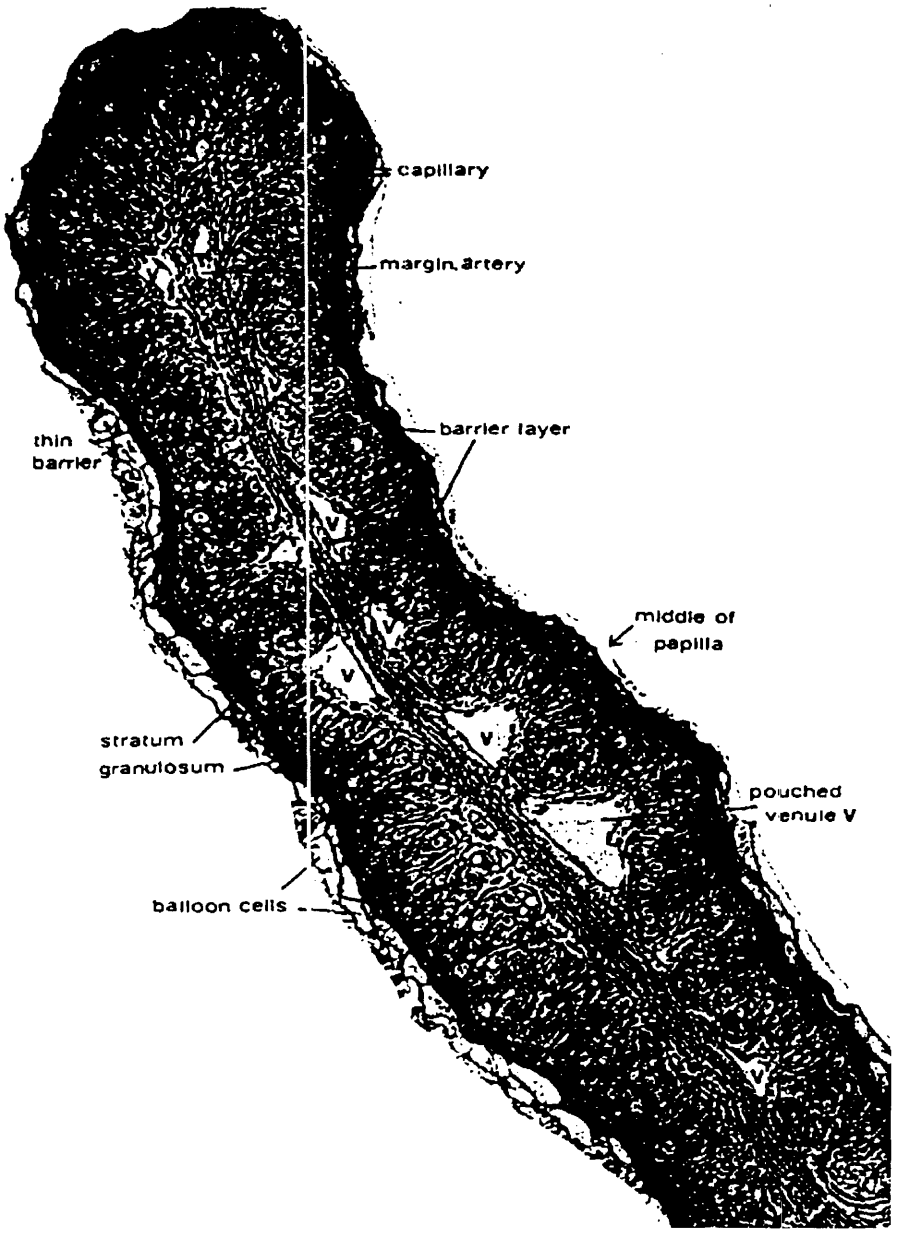


Figure 2-5. Microstructure of an absorptive ruminal papilla (semithin transverse section, x 145.5) (from Hofmann 1988). Note the few epithelial cell layers with denser barrier, and superficial balloon cells containing ruminal bacteria, and the extensive vascular system (mainly venules with fenestrated endothelium; i.e. absorptive type).

Similarly, the structure and conditions of the caecum are adapted to allow secondary fermentative activity and the absorption of nutrients. The caecum of all ruminants is a blunt-tipped tube which is continued without external demarcation into the colon from the ileocolic junction (Figure 2-2). The relative volume of the caecum depends on the diet of the animal. The largest caecal volume is found in highly selective ruminants like giraffe, dikdik or roe deer and the smallest caecal volume exists in sheep on grass and roughage (Church 1988). The fluid volume of the caecum is usually from 300 to 600 mL in an adult sheep grazing on grass. Like the rumen, the caecum is 39-40°C and normal pH is about 7.5. The caecum also supports a dense and diverse population of microbes which ferment undigested feed and allows for retention of material and fermentative digesta.

The caecum, like all other portions of the large intestine, is distinguished by the absence of villi, by semicircular mucosal folds, by an increasing proportion of goblet cells and by long tubular crypts of Lieberkühn (Church 1988). There is, however, a relative reduction of goblet cells and an increase in the density of sub-mucosal and mucosal blood vessels in the caeco-colon (the distal fermentation chamber, DFC) of several concentrate selectors (e.g. roe deer, dikdik) or intermediate mixed feeders (IM) (e.g. caribou, goat) These characteristics are favourable for absorption of materials and fermentative end-products, especially for rapid absorption of VFAs (Church 1988).

Most carbohydrate is fermented in the rumen as a result of microbiological activity, the carbohydrate that leaves the rumen is mainly digested by a range of enzymes and simple sugars are absorbed from the small intestine. Some undigested carbohydrate enters the caecum and colon and is broken down by microbiological fermentation (Church 1988, Van Soest 1994).

2.2 Microbial fermentation of carbohydrates

Fibrous carbohydrates must be digested by gut micro-organisms in all higher animals that have not evolved cellulases, hemicellulases, or pectinases (Prins and Clarke 1980; Sutton 1980). Most carbohydrates

consumed by ruminants are polymers of glucose present in the form of cellulose or starch. However, large amounts of hemicellulose and pectin may be present in some diets. Most carbohydrates must therefore be hydrolysed by microbial fermentation in the rumen to produce VFAs which are mostly absorbed from the rumen during this process. Synthesised microbial protein can be digested and absorbed from the small intestine to provide a valuable source of amino acid. Carbohydrates which pass from the rumen and are undigested in the intestines can be fermented to VFAs and absorbed from the caecum and colon. However, the microbial protein produced in the hindgut is excreted in the faeces (Van Soest 1994).

2.2.1 Fermentative microbes

The relative numbers of the different classes of rumen microbes vary depending on the diets and rumen environment. Up to 10^{11} viable bacterial cells/mL of rumen fluid have been reported by Hungate (1966).

Fibrobacter (formerly *Bacteroides*) *succinogenes, Ruminococcus albus* and *Ruminococcus flavefaciens* are the most common cellullolytic bacteria in the rumen (Church 1988; Dehority 1993). The cellulase(s) produced by *Ruminococcus* species are believed to be extracellular because of the clear zones produced around distinct colonies when incubated in cellulose agar media. The cellulase(s) produced by *Ruminococccus albus* degrade only amorphous cellulose while those produced by *Ruminococcus flavefaciens* can hydrolyse crystalline cellulose (Pettipher and Latham 1979). These bacteria also produce a non-hydrolytic protein which enhances the action of cellulase. *Fibrobacter succinogenes* is the most common cellulolytic bacterium in the rumen when diets are high in crystalline cellulose. This is due to the greater activity of cellulase(s) from *Fibrobacter succinogenes* compared to that from *Ruminococci.*

The most common amylolytic and dextrinolytic bacteria in the rumen are four bacterial species, *Ruminobacter* (formerly *Bacteroides*) *amylophilus, Streptococcus bovis, Succinimonas amylolytica* and *Succinivibrio dextrinosolvens* (Baldwin and Allison 1983; Dehority 1993). These bacteria ferment starch to maltose and some glucose, and the rate of fermentation is greatly affected by the source of starch and the

type of feed processing. Once starch is degraded to maltose, it is fermented rapidly by saccharolytic microbes. The most common saccharolytic bacteria in the rumen are *Prevotella* (formerly *Bacteroides*) *ruminicola, Butyrivibrio fibrisolvens* and *Selenomonas ruminantium* (Church 1988; Dehority 1993).

Common hemicellulose degraders are the same bacterial species which hydrolyse cellulose. However, some cellulolytic bacteria like *Fibrobacter succinogenes* do not themselves ferment the released pentoses. Hemicellulose appears to be degraded by cellulase through a non-specific hydrolysis of ß -1, 4 xylosidic linkages (Church 1988; Dehority 1993).

Pectins are degraded by *Butyrivibrio fibrisolvens, Prevotella ruminicola* and *Lachnospira multiparus* as well as by several genera of protozoa (Dehority 1993).

2.2.2 Fermentative process of carbohydrates and end-products

Rumen bacteria do not have a complete citric acid cycle since they cannot oxidise acetate to CO_2 and H_2O. In fact, portions of the cycle, namely malate to succinate, tend to operate in reverse as compared with aerobic organisms. Formation of oxaloacetate is an important step and may be accomplished by phosphoenolpyruvate (PEP) fixation of carbon dioxide and to a lesser degree by transcarboxylation. The pathways of microbial fermentation of carbohydrates in the caecum and large intestine are very similar to those occurring in the rumen (Church 1988). Fermentation of glucose and other monosaccharides occurs mainly by the Embden-Meyerhof (EMP) pathway (Figure 2-6).

The conversion of glucose (Baldwin 1970) associated with energy production (Bergen and Yokoyama 1977) in the gut is described as follows:

$$\text{Glucose} + 4\,\text{Pi} + 4\,\text{ADP} \rightarrow 2\,\text{acetate} + 4\,\text{ATP} + 2\,CO_2 + 8\,\text{H}$$
$$\text{Glucose} + 6\,\text{Pi} + 6\,\text{ADP} + 4\,\text{H} \rightarrow 2\,\text{propionate} + 6\,\text{ATP}$$
$$\text{Glucose} + 3\,\text{Pi} + 3\,\text{ADP} \rightarrow \text{butyrate} + 3\,\text{ATP} + 2\,CO_2 + 4\,\text{H}$$
$$CO_2 + 8\,\text{H} \rightarrow CH_4 + 2\,H_2O$$
$$\text{Net: } 3\,\text{glucose} + 13\,\text{Pi} + 13\,\text{ADP} \rightarrow 2\,\text{acetate} + 2\,\text{propionate} + \text{butyrate} + 13\,\text{ATP} + 3\,CO_2 + CH_4 + 2\,H_2O$$

This is in the case where the acetate-to-propionate ratio is 1 : 1 and the methane-to-glucose is 1 : 3. If acetate production increases 3 times and propionate and butyrate are unchanged, that is, acetate-to-propionate is 3 : 1, and methane-to-glucose is 3 : 5, then the conversion of glucose associated with energy production in the gut will be the following:

3 Glucose + 12 Pi + 12 ADP → 6 acetate + 12 ATP + 6 CO_2 + 24 H
Glucose + 6 Pi + 6 ADP + 4 H → 2 propionate + 6 ATP
Glucose + 3 Pi + 3 ADP → butyrate + 3 ATP + 2 CO_2 + 4 H
3 CO_2 + 24 H → 3 CH_4 + 6 H_2O
Net: 5 glucose + 21 Pi + 21 ADP → 6 acetate + 2 propionate + butyrate + 21 ATP + 5 CO_2 + 3 CH_4 + 6 H_2O

In microbial fermentation, carbohydrates, proteins and all other fermentable substrates are converted simultaneously into VFAs (the principal fatty acids in descending order of abundance are acetic, propionic, butyric, isobutyric, valeric, isovaleric and traces of various higher acids), microbial protein, CH_4 and CO_2. Church (1988) pointed out that in sheep fed dried grass cubes, 5.3% of total VFA production occurred in the caecum. In addition, the proportion of acetate in caecal VFAs tends to be higher than in the rumen, reflecting the greater proportion of structural polysaccharides arriving at the caecum.

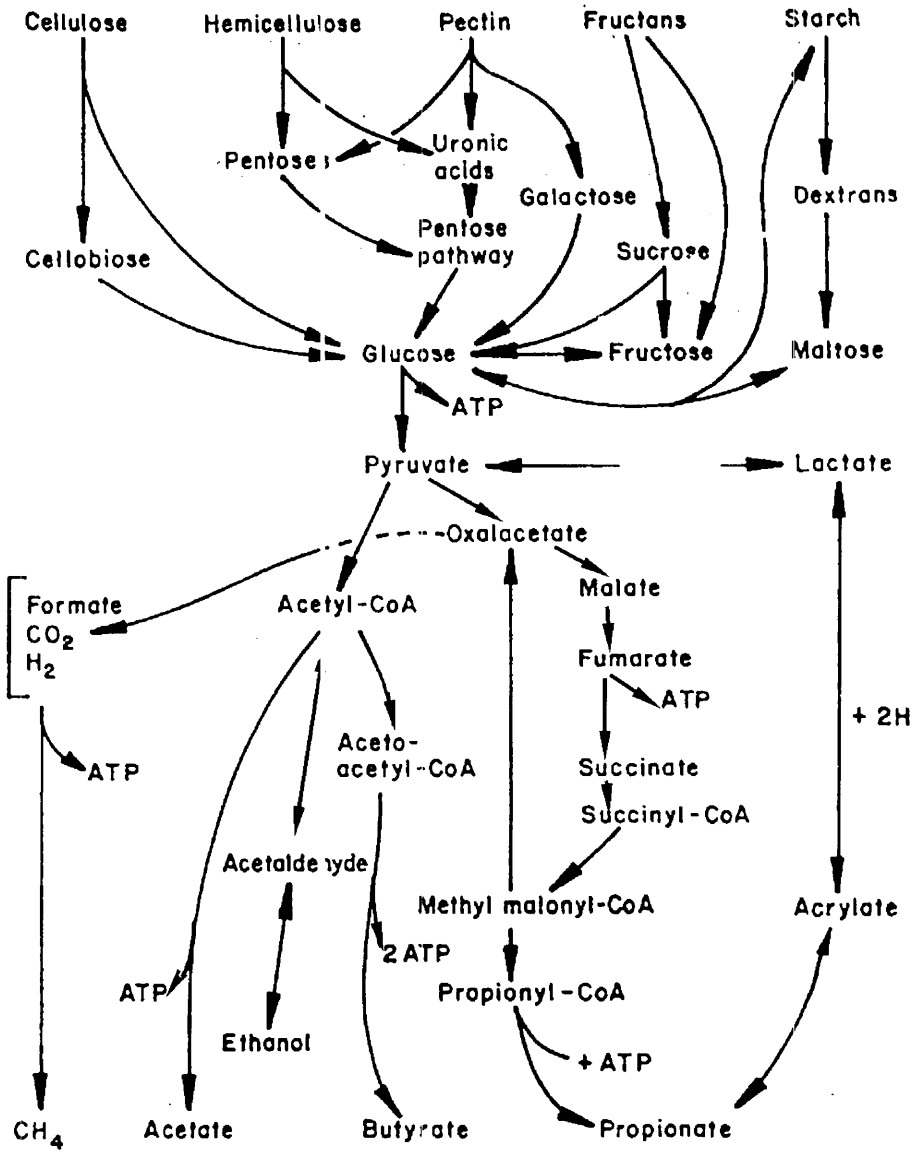


Figure 2-6. Fermentation of carbohydrate in the rumen (from Van Soest 1994).

Compared to the formation of VFAs mentioned above, fermentation of carbohydrate to lactic acid releases relatively little energy in a form available for microbial growth and much of the energy is lost as heat (Lehninger 1977).

Glucose + 2 Pi + 2 ADP $\rightarrow$ 2 lactate + 2 ATP + 32.4 kcal (135.5 kJ)

Thus, under conditions where lactic acid is the major end-product of ruminal fermentation, a relatively large amount of carbohydrate must be fermented to lactate for sustained microbial growth (Leng *et al.* 1980).

Therefore, fermentation of lactic acid is not an "economical" way and lactic acid does not normally accumulate in the rumen (Gill *et al.* 1986; Rowe 1997).

Although the fermentation of glucose by rumen micro-organisms results in lactic acid production, this metabolite is subsequently converted to VFAs to be absorbed (Elsden 1945). The isotope results of Gill *et al.* (1986) indicated that about 90% of total lactate is converted to VFAs.

2.2.3 Rate of fermentation

The rate and the extent of carbohydrate fermentation in the rumen determine the amount of energy which is available for both the microbes and the host animal. The main factors influencing rate and extent of carbohydrate fermentation are:

(i) type of substrates;
(ii) source of carbohydrate (e.g. maize starch is fermented more slowly than barley starch);
(iii) method and extent of processing (e.g. cracking, rolling, hammer-milling or other processing method increase surface area or gelatinise starch and make carbohydrate more rapidly fermentable);
(iv) passage rate to the omasum;
(v) rumen environment (e.g. pH, temperature), the population and proportion of different classes of microbes.

Van Soest (1994) defined the rate of fermentation to set the amount of feed energy per unit time for rumen bacteria. The rate of fermentation of the respective carbohydrates is significantly correlated to microbial efficiencies which represent production of microbial protein per unit of feed digested in the rumen. The digestibility of various carbohydrates versus the associated rumen microbial yield is expressed in Figure 2-7.

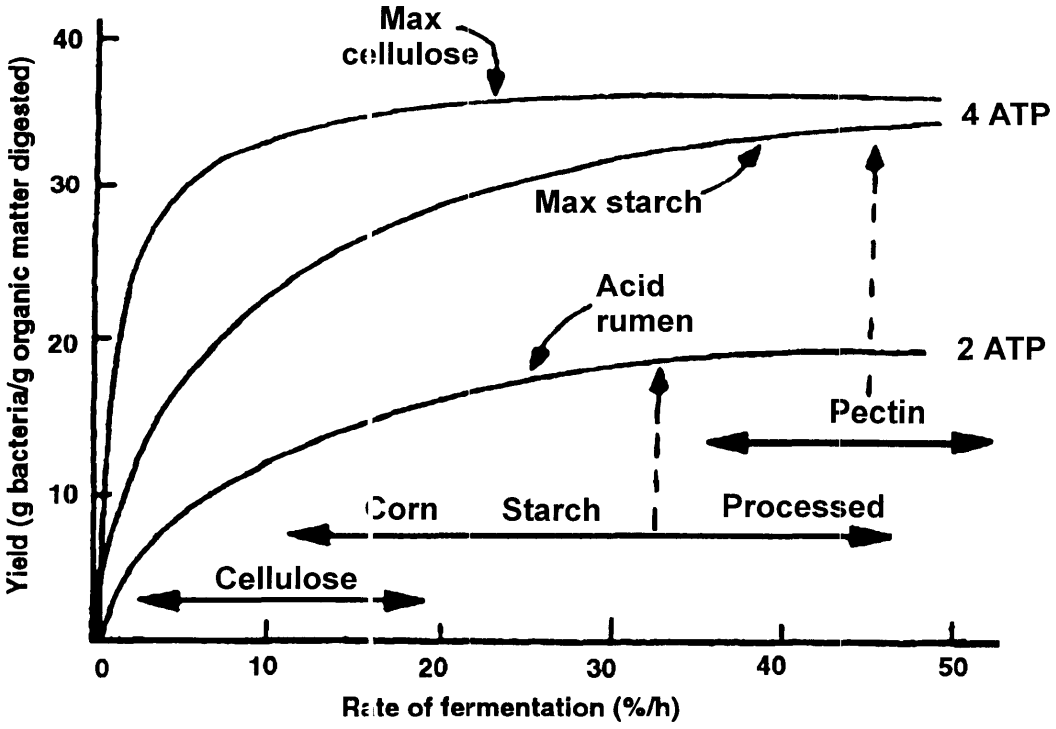


Figure 2- 7. The relationship between the fermentative rate and the amount of microbial protein produced per unit of feed fermented in the rumen (from Van Soest *et al.* 1991).

2.3 Absorption

In the non-ruminant animal, the digestion and absorption of food takes place mainly in the small intestine, particularly in the duodenum. However, in the ruminant, fermentation in the reticulo-rumen makes a large proportion of the substrate of structural components of plants available in the forms which are directly used by the tissues of the animal. At the same time, the reticulo-rumen is a major absorption site of some nutrients, like VFAs, glucose, ammonia etc., although the absorption also occurs in the other parts of the gastrointestinal tract.

2.3.1 Absorption of VFAs

Volatile fatty acids originate from anaerobic fermentation of complex carbohydrates in the rumen and caecum and colon. Volatile fatty acid

concentrations are similar in the reticulo-rumen and large intestine (Bugaut 1987). While VFAs are major substrates for energy metabolism in ruminants, they are also important in mono-gastric herbivores (Bergman 1990). Therefore, VFA absorption from the reticulo-rumen and hindgut is essential for these animals. Although the histology and structural organisation of the ruminal and the large intestinal epithelium are quite different, VFAs are rapidly and effectively absorbed from the hindgut (Argenzio *et al.* 1975; Rechkemmer *et al.* 1988; Engelhardt *et al.* 1989) as well as from the reticulo-rumen (Barcroft *et al.* 1944; Stevens 1970). The functional processes involved in trans-epithelial VFAs passage appear to be similar.

Volatile fatty acids are transported across the rumen and hindgut epithelium in both the dissociated (anions) form by electro-neutral anion exchange systems and in the undissociated (free) form by non-ionic diffusion (Rechkemmer *et al.* 1995). However, the relative contribution of these different pathways in different species is still not known. Volatile fatty acids are weak mono-carboxylic acids with a pKa around 4.8, and most of the VFAs are present as anions at physiological pH where they constitute the major anions in the fluid phase of the digesta in the rumen and hindgut (Engelhardt *et al.* 1989). However, only in their undissociated form, VFAs may easily permeate the lipid bi-layer of epithelial cell membranes by non-ionic diffusion. Non-ionic diffusion is a passive process with the direction of transport dependent on the concentration gradient across the epithelium. The transport of anions across epithelial membranes is a more complex process (Figure 2-8) since the lipid solubility of ions is generally fairly small and thus specific systems, incorporated in the membrane, are required for efficient trans-membrane transport. For transport of ions not only the prevailing concentration gradients are of importance, but also the trans-membrane (trans-epithelial) potential difference (PD) may affect the driving force. Moreover, transport of VFA anions appears to be linked to the operation of a non-selective, electro-neutral anion exchanger, and this system seems to be somehow linked with chloride and bicarbonate transport, although the nature of this anion exchanger is not known.

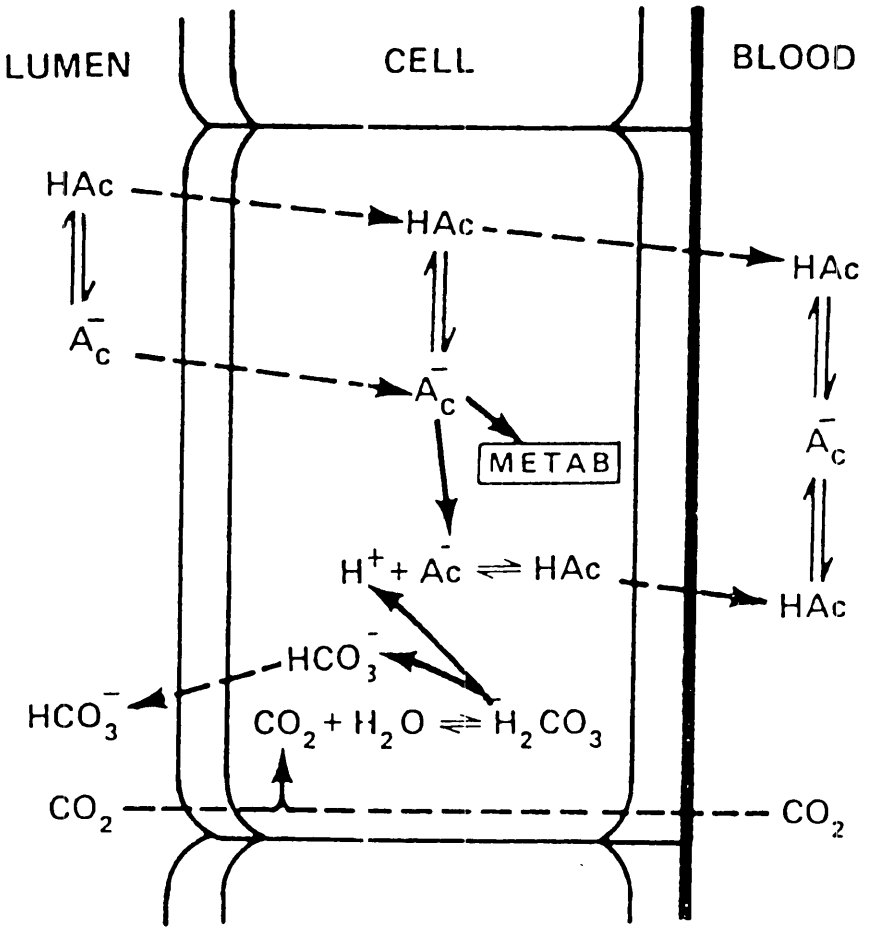


Figure 2-8. Hypothetical model of VFAs transport across the rumen epithelia. The membrane on the luminal side is permeable to both free and anion forms of the acid while the membrane facing the blood is permeable only to the free acid. Metabolism of fatty acids by epithelial cells influences the concentration gradient on both sides of the membrane. Carbonic acid formed from CO_2 produced by cellular metabolism or absorbed from rumen or blood acts as a H donor for VFAs transport (Church 1988 from Stevens 1970).

2.3.2 Absorption of lactic acid

There is no information on caecal absorption of lactic acid in ruminants and there is a range of different ideas about the absorption of lactic acid from the rumen. Dobson and Phillipson (1956) found no evidence for absorption of lactate from solutions buffered at pH 4 in the isolated rumen of sheep. In further studies in sheep, although ruminal lactic acid concentration rose and rumen pH fell following infusion of lactic acid or

administration of grain meal or glucose, Juhász and Szegedi (1968) did not observe any change in blood pH and blood lactic acid level.

On the other hand, Hueter *et al.* (1956) observed a rapid increase in the concentration of lactic acid in blood after intra-ruminal administration of sodium lactate in cattle, and suggested that this was due to direct absorption of lactic acid. Williams and Mackenzie (1965) reported that the mean absorption rate of lactic acid was about 8% that of VFAs from the ligated washed-out fore-stomach of sheep at about pH 5. Similar observations were recorded by Argenzio and Whipp (1980) who found the rate of lactic acid absorption to be 10% that of VFAs in pigs.

It is possible that the trace concentration of D-lactate in blood resulting from carbohydrate overload may be due to the absorption of lactic acid from the small intestine, abomasum and colon. Lactic acid has been found to be absorbed from the abomasum of sheep (Herden 1980) and cattle (Huntington and Reynolds 1986). Absorption of both isomers of lactic acid from the small intestine is more rapid than from the fore-stomach (Dunlop 1970). The flow of lactic acid from the rumen and its absorption from the abomasum and intestines could therefore explain the results reported by Hueter *et al.* (1956), Williams and Mackenzie (1965), and Argenzio and Whipp (1980).

Acidity (pH 4.0 - 4.5) of the rumen liquid during lactic acidosis may inhibit rumen motility (Shinozaki 1958). This would reduce the flow of rumen fluid to the small intestine and may therefore limit the post-ruminal absorption of lactic acid. However, under conditions of very severe lactic acidosis, gross structural changes occur in the rumen wall in sheep (Lee *et al.* 1982) and similar changes have been reported in the caecal wall in horses (Krueger *et al.* 1986). It is likely that this damage may make it possible for lactic acid to be absorbed directly from the rumen and caecum. This suggestion is supported by the results of Godfrey *et al.* (1992, 1995) who found that high levels of blood D-lactate only occurred under conditions of very low pH in the rumen. On the other hand, the retention time of digesta in the colon is longer than that in the caecum (Warner 1981) and it is possible that some lactic acid may be absorbed from the colon.

Lactic acid is transported as free (undissociated) acid (Giesecke and Stangassinger 1980; Newbold *et al.* 1984; Gill *et al.* 1986). Free lactic acid is more readily absorbed than the anion because free acid is less polar (Williams and Mackenzie 1965; Dunlop 1972; Giesecke and Stangassinger 1980). The pH of the gut fluid influences the absorption of lactic acid by affecting the proportion of the free and anionic forms of lactic acid in the gut. Williams and Mackenzie (1965) reported that D-lactic acid may be absorbed slightly more rapidly than L-lactic acid. They suggested that this effect may be due to the lower initial concentration of D-lactate in blood relative to L-lactate. Absorption of lactic acid by diffusion is considered to be influenced by blood flow rates for that organ (Dobson and Phillipson 1956). Gut osmolarity also influences the absorption of lactic acid from the gut (Williams and Mackenzie 1965). In many animal experiments, the gut contents were emptied which may cause different degrees of trauma and/or a change in osmolarity, and these factors may influence the absorption of lactic acid.

The absorption mechanism of lactic acid has not been studied thoroughly. It is usually assumed that lactic acid is absorbed by diffusion (Dobson and Phillipson 1956; Giesecke and Stangassinger 1980). However, recent studies by Ullrich *et al.* (1982), and Siebens and Boron (1987), indicated that most epithelia possess both H-lactate and Na-lactate co-transporters, and that lactate is transported from the lumen to the blood using the Na-lactate co-transporter to take lactate out of the lumen, and the H-lactate co-transporter to move lactate from the cells to the blood.

2.3.3 Absorption of ammonia

Significant quantities of ammonia (NH_3) are produced in the rumen as a result of microbial degradation of dietary protein, hydrolysis of dietary and endogenous non-protein nitrogen (NPN), and through the turnover and degradation of microbial cells. Although the NH_3 produced enters a pool which may be utilised as a source of nitrogen for amino acid biosynthesis by rumen microbes, a large proportion of NH_3 the is not taken up by the microbes and is absorbed directly through the rumen wall. McDonald (1948) first demonstrated the absorption of ammonia from the rumen into ruminal venous blood. Ammonia is also produced in the caecum and colon as an end-product of fermentation and is absorbed

from the hindgut in the same way as from the rumen (Dixon and Nolan 1982).

Free ammonia (NH_3) is absorbed by diffusion across cell membranes much more rapidly than in the case of the ammonium (NH_4^+) ion. Ammonia is absorbed through the rumen wall strictly by passive diffusion and the quantity absorbed is positively related to ruminal NH_3 concentrations and to rumen pH (Visek 1968). Ammonia exists in a state of equilibrium with NH_4^+ in solution.

$$NH_3 + H^+ \leftrightarrow NH_4^+$$

The extent of ionisation is highly pH dependent. Ammonia is a weak base with a pKa in the vicinity of 8.80 to 9.15 (Bromberg *et al.* 1960; Bloomfield *et al.* 1963) and increased pH causes the NH_4^+ to be converted to NH_3 (Hogan 1961; Bloomfield *et al.* 1963; Swales *et al.* 1970; Nolan 1981). Consequently, increased rates of absorption occur at higher pH due to the increase in concentration of free NH_3. The rate of transport of NH_3 across the rumen wall at pH 6.5 is approximately three times greater than at pH 4.5 (Visek 1968). In addition to pH, osmolarity also affects the absorption rate of NH_3. Swales *et al.* (1970) found that hypertonic solutions inhibited NH_3 absorption from the rat jejunum, ileum and colon perhaps due to change in gut permeability.

2.3.4 Absorption of glucose

Compared to non-ruminants, absorption of glucose in ruminants is generally less important quantitatively. In adult ruminants virtually all of the soluble sugars (lactose, sucrose etc.) in the diet, as well as a large proportion of the dietary starch, are fermented by rumen microbes. Tsuda (1956) found that glucose appeared to be absorbed slowly from the rumen. In animals fed high concentrate diets at high levels of intake, as much as 50% of the dietary starch may escape ruminal fermentation and be presented to the lower gut for digestion and absorption (Johnson and Bergen 1982). Digestion of this "bypass" starch by pancreatic and intestinal amylases mainly occurs in the small intestine where absorption of glucose is also efficient (Church 1988). There is no report of glucose absorption from the hindgut where fermentation of soluble sugars and starch is likely to be rapid.

Glucose as well as other monosaccharides derived from intestinal digestion are absorbed by an active process which appears to be coupled to Na^+ transport. Although the actual mechanism of glucose absorption is still largely undefined, substantial evidence supports the model of glucose transport shown in Figure 2-9.

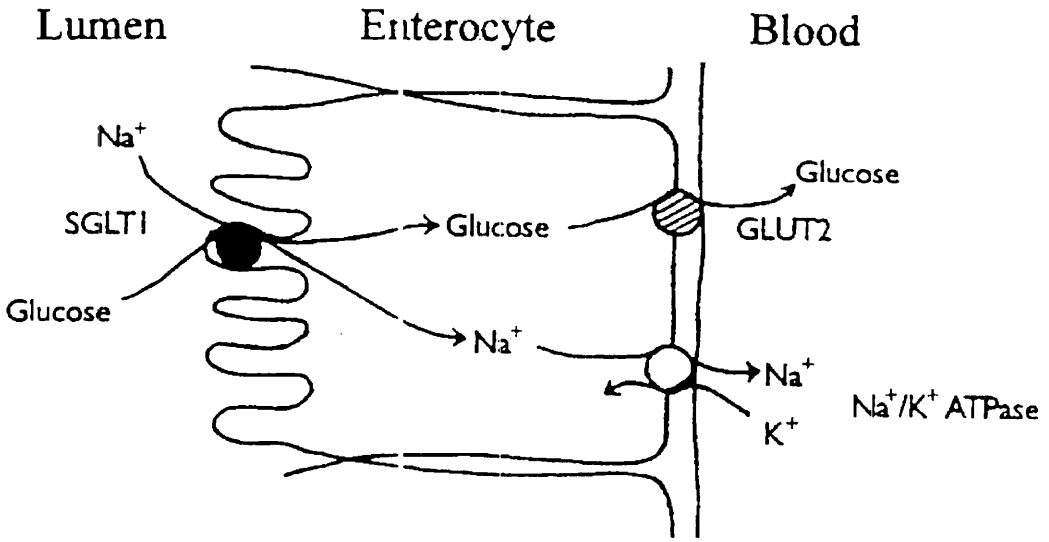


Figure 2-9. Trans-cellular transport of D-glucose across the enterocyte. D-glucose is transported across the brush border membrane by the Na^+-dependent D-glucose co-transporter (SGLT1) and across the basolateral membrane, down their concentration gradient via a facilitated sugar transporter (GLUT2). The Na^+/K^+-ATPase maintains the low intracellular Na^+ concentration to drive SGLT1 to transport glucose against its concentration gradient (from Shirazi-Beechey *et al.* 1995).

2.4 Buffering capacity

A buffer is a solution whose pH remains essentially constant despite the addition of a small amount of acid or base. Its buffering principle is described as follows:

$$HA + HCO_3^- \leftrightarrow A^- + H_2CO_3$$
$$B + H_2CO_3 \leftrightarrow BH^+ + HCO_3^-$$

where HA is an acid and A^- is an anion of acid, B is a base and BH^+ is a salt.

Normally, gut pH is relatively constant and this is very important to maintain normal functioning of the gut. The maintenance of the gut at a relatively constant pH depends on the buffering capacity of the animal. Buffering capacity provided by buffers in the gut plays a key role, although respiratory and excretory systems also function in pH regulation via blood, for example, carbon dioxide (CO_2) is exhaled from the lung and excess H^+, NH_3 and K^+ are excreted in the urine (Dunlop 1972; Rawn 1989).

Buffering capacity in the different parts of the gut varies depending on the amounts and components of buffer. Saliva is rich in mineral ions, particularly Na^+, HCO_3^-, HPO_4^{2-}, K^+ and Cl^-, which help to buffer the rumen contents. Secretion from some salivary glands is strongly buffered, and mixed saliva is a good buffer between pH 5.5 and 7.5; thus saliva is a poor buffer against alkali but a good buffer against acid. However, it is not very effective against a stronger acid such as lactic acid which is normally present when rumen pH drops below 5.5 (Church 1988). While saliva is directly secreted into the rumen, no saliva is secreted into the caecum and this is a major difference in the buffering mechanisms between the caecum and rumen. Bicarbonate in the tissues and the blood can move into the gut to provide an additional buffering function. Urea can also play a buffering role through its conversion to ammonium bicarbonate by microbial ureases and this also allows for nitrogen recycling, which is important in the economy of protein and nitrogen balance in the ruminant (Church 1988; Van Soest 1994). Feed proteins and non-protein nitrogen (NPN) fractions of forage rich in glutamate, aspartate, glutamine and asparagine may contribute to buffering capacity. The plant cell wall also has a cation exchange capacity which may contribute to gut buffering (Van Soest 1983, 1994). Reabsorption of Na^+, HPO_4^{2-} and other recycled ions is necessary for the maintenance of acid-base balance in the host and occurs in the omasum and other sites farther down the digestive tract.

Generally, the main buffer systems in the gut are based on bicarbonate ($NaHCO_3/H_2CO_3$) and phosphate systems (Na_2HPO_4/NaH_2PO_4). In cattle, bicarbonate and phosphate buffers account for about 90% of anion content and Na^+ is at least 18 times the concentration of K^+ (Church 1988). HCO_3^-/H_2CO_3 play a central role in maintaining a relatively stable pH in the gut.

2.5 Lactic acidosis

Normally, there is no measurable lactic acid in the rumen of animals fed hay or in those well adapted to grain-based diets. This is because both L and D isomers are rapidly converted to VFAs through acetyl-CoA (Gill *et al.* 1986). Doetsch *et al.* (1953) did not find lactic acid in the rumen liquor of normal cattle fed hay and grain except in trace amounts. Similarly, no lactic acid was detected in the rumen of Holstein steers fed grass or lucerne hay (Waldo and Schultz 1956; Jayasuriya and Hungate 1959). Only traces of lactic acid, less than 1 mg/100mL rumen liquor (i.e. 0.11 mmol/L), were found in the rumen of dairy cows fed high-roughage diets (Balch and Rowland 1957). Ryan (1964a, b) found that lactic acid constituted less than 2% of the ruminal organic acids in sheep on the hay diet. Huntington (1988) reported that in forage-fed ruminants or in ruminants adapted to high-grain diets, lactate was not found or was present in trace amounts of ruminal fluid.

However, if the ruminant abruptly ingests large amounts of grain or starch or other feed that is rich in readily fermentable carbohydrates, much lactic acid will be formed and this is associated with a fall in pH in the gut leading to fermentative acidosis. Some average concentrations of lactic acid in different parts of the gut and blood of the ruminant fed concentrates and grains are shown in Table 2-3. It should be noted that the table does not include the examples of very serious lactic acidosis.

Table 2-3. Some average concentrations of lactic acid in different parts of the gut and blood of ruminants

Animal	Diet	Organ	Lactic acid (mmol/L) (D)	(L)	Reference
Heifer	50% corn 50% silage	Rumen	0	0	Ward *et al.* 1961.
		Abomasum		0.2(DL)	
		Small intestine		0.5(DL)	
		Caecum		3.1(DL)	
		Colon		2.8(DL)	
Calves	milk replacer	Rumen	2	5-10	Juhász *et al.* 1976.
		Abomasum	2	20-30	
Calves	milk concentrates & hay	Rumen	13	4-8	Juhász *et al.* 1976.
		Abomasum	24	5-20	
Cattle	50% barley feedlot	Rumen	6	8	Oginmoto 1977.
Cattle	barley	Blood	0.5-2.3	1.1-5.9	Oginmoto 1977.
Sheep	75% maize, 25% hay	Rumen	2	6	Giesecke & Stangassinger 1976.
Sheep	1.4 kg barley	Rumen	15.4	19.3	Godfrey *et al.* 1992.
		Small intestine	17.6	41.6	
		Caecum	8.2	12.7	
		Colon	6.8	9.9	
		Plasma		2.37	

Accumulation of lactic acid in the gut associated with a fall in gut pH is considered to be due to faster and complete fermentation of readily fermentable carbohydrates in the gut. Lactic acid only accumulates if its rate of production exceeds the rate of utilisation or conversion to VFAs. This can result either from a proliferation of lactate producers (Gram-positive bacteria) or a failure of lactate utilisers (Gram-negative bacteria) to proliferate rapidly enough to utilise the increased quantity of the acid. This occurs when there is a build-up of VFAs and a reduced pH (Rowe *et*

al. 1989; Rowe 1997). *Streptococcus bovis* and *Lactobacillus* spp. (Gram-positive bacteria) are two major lactate producers in the fermentation of carbohydrate in the rumen of ruminants (Schwartz and Gilchrist 1974). Actually, several species of bacteria have been found to be able to ferment carbohydrates to lactic acid as end-product and have been isolated from ruminal contents. *Streptococcus bovis* is one of the most commonly isolated organisms with this capacity. Other lactic acid producing ruminal bacteria include *Butyrivibrio fibrisolvens, Lachnospira multiparous*, *Lactobacillus* spp., *Selenomonas* strains and the holotrich protozoa (Mackenzie 1967). All of these organisms have been shown to produce lactic acid during *in vitro* experiments. Although protozoa, mainly *Isotricha* and *Dasytricha* spp., may ferment much of the soluble sugar to lactic acid (Cuthbertson 1969), both low ruminal pH and high ruminal osmotic pressure have been cited as factors limiting protozoal survival in the rumen (Hungate 1966; Slyter 1976).

An extensive range of carbohydrates has been reported to produce lactic acid either *in vivo* or *in vitro,* and is listed in Table 2-4. Any food-stuff rich in soluble carbohydrates is capable of acting as a lactic acid precursor in the acidosis syndrome. However, sugars are more toxic than starches (Dunlop 1972) and this is likely to be due to their greater solubility and their more rapid rate of fermentation. In addition, hexose sugars, both monosaccharides and disaccharides, result in greater accumulation of lactic acid than does fermentation of pentose (Cullen *et al.* 1986). Cereal grains are the most common source of readily fermentable carbohydrates (RFC) that cause fermentative acidosis. Results of fermentation of grains and by-product, in order of greatest to least potential to produce lactic acid, were steam-flaked barley = barley = wheat > citrus pulp > beet pulp = corn > high moisture corn = sorghum grain (Cullen *et al.* 1986). Furthermore, steam flaking, rolling, popping or other processing methods increase surface area or gelatinise starch and make carbohydrates more rapidly fermentable, thereby increasing the potential for fermentative acidosis (Huntington 1988).

Table 2-4. Dietary components reported to produce lactic acid

Food	Reference
Oat	Dougherty *et al.* 1975; Rowe *et al.* 1994.
Barley	Szegedi and Juhász 1968; Godfrey *et al.* 1995.
Wheat	Reid *et al.* 1957; Patra *et al.* 1996.
Corn	Phillipson 1952.
Sucrose	Phillipson and McAnally 1942; Suda *et al.* 1996.
Pear	Ahrens 1967.
Cabbage	Phillipson 1942.
Mangold	Scarisbrick 1954.
Starch	Whanger and Matrone 1966.
Glucose	Szegedi and Juhász 1968.
Fructose	Phillipson and McAnally 1942.
Maltose	Phillipson and McAnally 1942.
Mixed concentrate feeds	Dunlop 1972.
Apple	Dunlop 1972.
Oral re-hydration solution	Gentile 1995.

Lactate is produced during tissue metabolism and from glucose. The metabolism of skeletal muscle is a major source of endogenous lactate although lactate can also be produced in the metabolism of erythrocytes, kidney medulla, retina and gut epithelium. Pennington and Sutherland (1956) have shown that rumen epithelial tissue converts propionate to lactate. The conversion of propionate to L-lactate by rumen epithelium can contribute considerable amounts of endogenous lactate after functional rumen development (Giesecke and Stangassinger 1980). Incubation experiments with rumen epithelium *in vitro* suggest that as much as 50% (Bergman and Wolff 1971; Leng *et al.* 1967) or as little as 3-5% (Weekes 1974; Weigand *et al.* 1972) of rumen propionate production may be metabolised to lactate during absorption. McCarthy *et al.* (1958) found that blood lactate contained 9.7% of the radioactivity of infused labeled butyrate. Endogenous L-lactate rapidly equilibrates with the extracellular fluid (Reilly and Chandrasena 1978) and it is possible that the ruminant body tissue may contribute to the rumen lactate pool through the flux of lactate into the rumen. Even if this does occur, the

quantity of lactate is relatively small, less than 2 mg lactate per 100 mL rumen fluid (Williams and Mackenzie 1965).

Lactic acid can be consumed in diets such as silage. However, the concentration of lactic acid in the rumen of sheep fed silage was found to be very low (0.208 mmol/L) at all times (Counotte 1981; Gill *et al.* 1986). This highlights the rapid conversion of lactic acid to VFAs under normal conditions.

2.6 Adverse effects of lactic acidosis on the animal

Lactic acidosis is a condition with severe consequences for the animal. Acidosis can cause a series of biochemical, physiological and histopathological effects, and the clinical signs of acidosis vary depending on the severity of the disease (Braun *et al.* 1992). The conditions of acidosis can be acute, posing a life-threatening situation, or chronic (sub-acute), resulting in reduced feed consumption and weight gain that can be summarised in Figure 2-10.

Intake of large amounts of readily fermentable carbohydrates
↓
Increase *Streptococcus bovis* → **Decreased Gram-negative organisms**
↓ ↓
Increased *Lactobacillus* spp. **Increased endotoxin**
↓
Increased rumen organic acids → **Fermentative acidosis**
(Lactic acid, VFAs etc.) → **Increased rumen osmotic pressure**
↓ ↓
Decreased rumen pH **Decreased extracellular fluid volume**
Rumen stasis ↓
↓ **Dehydration**
Ruminitis **Decreased cardiac output**
↓ **Decreased peripheral perfusion**
Liver abscesses **Decreased renal blood flow**
↓ ↓
Pulmonary thromboembolism **Shock**
↓
Death

Figure 2-10. An outline of adverse effects of lactic acidosis.

2.6.1 Biochemical effects

Increased concentration of lactic acid and VFAs and lowered pH have been reported in the rumen liquor of acidotic sheep (Phillipson and McAnally 1942; Phillipson 1952; Bond 1959; Dunlop and Hammond 1965) and cattle (Balch and Rowland 1957; Dirksen 1965; Oltjen *et al.* 1967) and goats (Sen *et al.* 1982; Lal *et al.* 1991). This was attributed to rapid fermentation of readily fermentable carbohydrates in the rumen.

Increased concentrations of urea and total protein in the blood have been observed in experimentally acidotic sheep (Juhász and Szegedi 1968). A significant increase in serum urea is an index of a decreased glomerular filtration rate in acidotic sheep, possibly due to renal damage or reduction in effective renal blood flow, and a fall in the arterial blood pressure which results in subnormal renal function (Dunlop 1972). The significant increase in serum protein may be attributable to the state of dehydration due to passage of water from the intra-vascular compartment into the rumen (Parthasaarathy and Phillipson 1952).

Elevated activities of amylase, creatinine phosphokinase (CPK) and gamma glutamine trans-peptidase (GGTP) were revealed in serum enzyme profiles of acidotic sheep (Patra *et al.* 1996). Change in serum amylase may be attributed to change in carbohydrate metabolism (Kaneko 1989) and lowering of rumen pH (Slyter 1976). Creatinine phosphokinase activity is one of the most specific indicators of muscle damage (Kaneko 1989) and hence increased activity of this enzyme may be a pointer to the muscle damage in acidosis Increased activity of GGTP, a membrane-bound, liver-specific enzyme, reflects hepatobiliary damage (Patra *et al.* 1996).

Moreover, pyruvic acid inorganic phosphate, and haematocrit value increased, whereas blood Na^+ and Cl^- concentration declined, in sheep overloaded with fermentable carbohydrates (Juhász and Szegedi 1968). Elevated histamine was found in acidotic animals (Dirksen 1965). Sugar levels in blood increased which may be an indication of internal metabolism initiated by acidosis in the rumen and mediated via toxic substances such as lactate and histamine (Dirksen 1986; Cao 1987; Abdel-Rasek 1988; Nikolov 1996).

2.6.2 Physiological effects on the animal

Voluntary feed intake decreases and even results in death in severely acidotic ruminants (Silanikove and Tadmor 1989; Blood and Radostits 1989). Lactic acidosis can kill ruminants through dehydration due to excessive water loss in the faeces (scouring) in the short term, with symptoms showing within 12 hours of engorgement. If animals survive this shock, rumenitis (loss of rumen lining) permits microbial contamination of the blood and animals may die after some weeks. Rumenitis also leads to a high incidence of liver abscesses (Figure 2-11) in feedlot cattle. Laminitis (shedding of hooves) is also associated with acidosis. Anorexia, rumen-stasis, nasal discharge, dullness, distended rumen, lameness, and increased pulse and respiration rate have also been observed in acidotic cattle (Hejlasez *et al.* 1984) and sheep (Shiskov 1984; Crichlow 1989). The severity of clinical signs appears to reach a maximum between 12 and 36 hours in sheep (Patra *et al.* 1995).

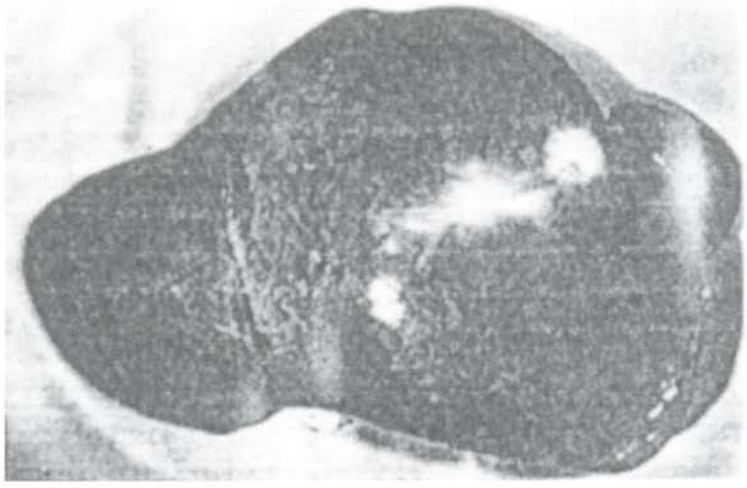
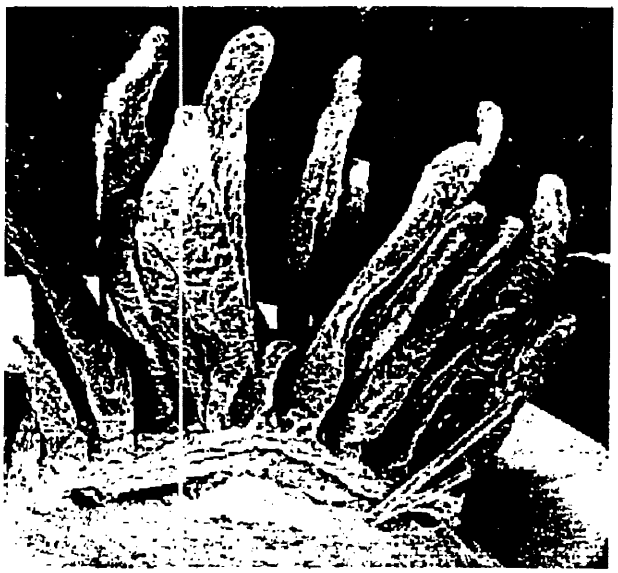


Figure 2-11. A beef liver with two large abscesses on the surface (Courtesy of Elanco Products Company. From Church 1988).

2.6.3 Histopathological effects

There are still a series of histopathological changes in different organs of acidotic ruminants. Vesweber and Leipold (1974) and Patra *et al.* (1995) reported that the rumen showed denudation and broken continuity. There was micro-vesiculation in the stratum lucidum with infiltration of polymorphs in and around vesicles. A few vesicles coalesced to form larger ones. Mucosal papillae revealed infiltration of polymorphs and mononuclear cells. Ruminal papillae showed hydropic degeneration of epithelial cells (Figure 2-12) that started in the deeper layer. With time, micro-vesicles increased in size and moved towards the superficial layers with loss of integrity of epithelium in many areas. The liver was congested with the evidence of haemorrhage in the parenchyma; sinusoids were distended with red blood cells and there was peri-portal and peri-vascular mononuclear infiltration and degenerative and focal necrotic changes in hepatocytes.

The severity and the signs of lactic acidosis are different from animal to animal. Although rumen acidosis (rumen pH < 5.4) was recorded in some goats exposed to *ad libitum* concentrates in the post-partum period, no clinical signs of acidosis or laminitis were observed (Mgasa and Arnbjerg 1993). This appears to be because adult goats have a stable rumen structure and are resistant to laminitis even when exposed to high amounts of concentrates.



A

B

Figure 2-12. Comparison of normal (A) and abnormal (B) rumen papillae (14.2 - 16.5 x) of calves 4 - 6 weeks of age (Courtesy of M.D. McGavir and J.L. Morrill. Kansas State University. From Church 1988).

2.6.4 Primary cause of adverse effects of lactic acidosis

Many of the symptoms, diarrhoea, haemoconcentration and pronounced dehydration associated with the grain engorgement syndrome have been shown to be attributable to an increase in osmotic

pressure of the rumen and not due to the absorption of lactic acid or any other toxic factor (Morrow *et al.* 1973; Dougherty *et al.* 1975; Suber *et al.* 1979; Patra *et al.* 1995). Ahrens (1967), Elam (1976), and Silanikove and Tadmor (1989) reported osmotic pressures in excess of 500 mOsmol/L in ruminal fluid of a heifer experimentally engorged with wheat. Over 60% of increased rumen osmolarity may be due to increased ruminal osmolar concentrations of lactic acid in sheep (Huber 1976).

However, the effect of lactic acid in blood and tissue from the rumen is generally considered a primary factor causing adverse effects associated with grain feeding and acidosis (Blood and Henderson 1963). This adverse effect of lactic acid is also thought to be exacerbated by the slower clearance by the body of D-lactic acid originating from gut microbes and referred to as "D-lactic acidosis" (Dunlop and Hammond 1965; Whanger and Matrone 1966). However, the concentrations of D-lactate in blood during episodes of rumen lactic acidosis are normally very much lower than that of L-lactate (Godfrey *et al.* 1992). It is possible that L-lactic acid predominates in the fermentative production (Ryan 1964b; Huntington and Britton 1973, 1979; Byers and Goodall 1979) or both D- and L-lactic acid are produced in approximately equal amounts by microbes in the gut. On the other hand, only L-lactate is produced by metabolism of animal tissues and the conversion of propionate by gut epithelium (Giesecke and Stangassinger 1980). Therefore, the concentration of L-lactic acid is normally higher than that of D-lactic acid (Ganter *et al.* 1993). Cori and Cori (1929) and Dunlop and Hammond (1965) found that there was no detectable difference in absorption between the two isomers and suggested that the peak entry rate of L- and D-lactic acid into the blood should occur at the same time. However, liver tissue was found to be able to synthesise carbohydrate from D-lactic acid but hardly from L-lactic acid (Meyerhof and Lohmann 1926). L-lactic acid is utilised 4 times more slowly in the rat than D-lactic acid because D-lactic acid can be deposited as liver glycogen (Cori and Cori 1929). Hence, it is not just D-lactate which disturbs the acid-base status during acidosis. It appears that the result of Cori and Cori (1929) was incorrectly interpreted by Emery *et al.* (1966) and Dunlop (1972) who concluded that there was evidence for D-lactate to be more slowly metabolised than L-lactate.

Juhász and Szegedi (1968) attributed the adverse effects of acidosis to the local effects of pH and excess lactic acid in the rumen. Excess CO_2 formed in the rumen during fermentation in the pathological process also plays an important role. Excess CO_2 formation causes distension of the rumen which hinders the movements of the diaphragm as well as respiration and circulation. Suppression of respiration is brought about also by a marked rise of the blood CO_2 level since excess CO_2 is absorbed into the blood. Pathological respiration may cause cardiac or circulatory insufficiency, fall in blood pressure, impairment of tissue oxygen supply and tissue metabolism. Accordingly, reduction of blood pH and blood standard bicarbonate causes, through paralysing the respiratory centre, a relative oxygen deficiency (hypoxia) and absolute accumulation of CO_2 in the tissue so that the affected animals actually die of asphyxia.

The severity of acidosis is related to total acid production and not only lactic acid production. It is important to emphasise the contribution of all acids to disruption of the acid-base status during acidosis (Harmon 1983). Rowe (1997) suggested that a new condition, "acidic gut syndrome" (AGS), should be recognised. This is characterised by the accumulation of acids in the gut at concentrations that have not previously been considered harmful to animals or humans. The detrimental effects initiated by lactic acid and low pH may be mediated through direct action on the gut wall, through the production of bacterial endotoxin, through the combination of acids and endotoxins, or through other factors.

2.7 Prevention and treatment of lactic acidosis

Acidosis is an important management disease of ruminants. Immediate therapy for acidosis is to remove the source of readily fermentable carbohydrates and provide afflicted animals with good quality forage or a diet low in readily fermentable carbohydrates. In acute cases involving low numbers of animals, intravenous infusion of electrolytes may be practical. Antibiotics in the diets or by injection may aid in treatment of secondary involvements resulting from acidosis (Huntington 1988). Extensive research on the aetiology and prevention of acidosis has resulted in the development of several approaches to its treatment and these are summarised below.

2.7.1 Gradual adaptation

Current practices to prevent acidosis in livestock depend largely on gradual adaptation to diets high in readily fermentable carbohydrates and careful management while feeding such diets (Huntington 1988). Adaptation is based on changes in microbial species and relative population densities in response to changes in substrate. Gradual adaptation to a carbohydrate-rich diet over a period of 2 - 3 weeks is accompanied by a microbial succession that eventually sees the rise of lactic acid-metabolising bacterial populations and achieves an ecological balance between production and utilisation. In steers adapted to a grain-based diet, *Selenominas ruminantium* subsp. *lactilytica* was reported to be the major lactate-utilising bacterium, followed by *Megasphaera elsdenii* and *Peptococcus asccharolyticum* (Huber *et al.* 1976). While in sheep adapted to a high grain diet, *Anaerovibrio* spp. were observed to be the major lactate-utilising bacteria (Mackie *et al.* 1978).

The detrimental effect of large intakes of carbohydrates has been treated by transferring rumen contents from animals adapted to grain into those about to receive grain for the first time (Allison *et al.* 1964; Huber 1974). Intra-ruminal inoculation with crude rumen fluid from animals already adapted to a high grain diet not only prevented lactic acidosis in lambs (Huber 1974), but also increased daily feed intake by 35% in heifers (Cook *et al.* 1975). Godfrey *et al.* (1992) also reported successful cross-inoculation, using rumen fluid from adapted animals, to provide a practical and effective method of reducing the adverse effects associated with the high intake of carbohydrate

However, direct application of rumen fluid transfer is problematic because of the amount of rumen fluid needed for inoculating large numbers of animals and the extreme variability of the procedure. Godfrey *et al.* (1992) pointed out some logistical problems in collecting a suitable inoculum and in the transfer of a sufficient volume. Under feedlot conditions, gradual adaptation to a carbohydrate-rich diet is complicated by animal to animal variation in adaptation rate and is accompanied by reduced weight gain in adapting animals (Wiryawan and Brooker 1995).

2.7.2 Application of antibiotics to control lactic acidosis

Antibiotic compounds can be used to prevent and treat lactic acidosis. It appears that antibiotics act through controlling the populations of the major lactate-producing organisms, *Streptococcus bovis* and Lactobacillus, to inhibit the production of lactic acid (Figure 2-13).

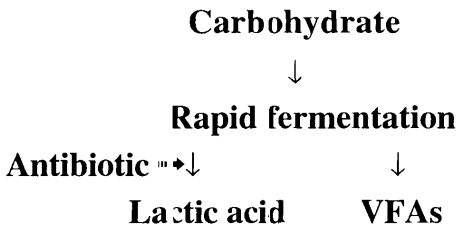


Figure 2-13. Schematic representation of how antibiotic inhibits formation of lactic acid.

Antibiotics such as virginiamycin, lasalocid, monensin, thiopeptin, etc. have been used to reduce lactic acid production during fermentation of carbohydrate in the rumen. The efficiency of antibiotics varies depending on antibiotics, dosage, animal and whether the experiment is *in vivo* or *in vitro*.

2.7.2.1 Effects of antibiotics vary depending on antibiotics

The effects of 16 antibiotics were evaluated by Beede and Farlin (1977a) in terms of their effect on lactate and VFA production in an *in vitro* rumen fermentation system. Of the antibiotics evaluated, bacitracin methylene di-salicylate, capreomycin disulfate, novobiocin and oxamycin effectively decreased lactate production, while erythromycin, hygromycin B and tylosin tartrate appeared to lower lactate production moderately. In contrast, apramycin, bacitracin, chlortetracycline, iodinine, monensin, the penicillins, streptomycin sulfate and thiram did not decrease lactate production.

A procedure for a large capacity disc assay of antibiotics was conducted by Muir and Albert Barreto (1979). Of the antibiotics evaluated, the penicillins, such as penicillin G and ampicillin, and the sulfur-containing peptide antibiotics, such as thiopeptin, sulfomycin,

thiostrepton, siomycin, sporangiomycin and taitomycin, were the most active. The sulfur-containing peptide antibiotics, especially thiopeptin, sulfomycin and thiostrepton, are highly active against *Streptococcus bovis* and appear to be excellent candidates for the control of ruminal *Streptococcus bovis*.

Muir *et al.* (1980) investigated the efficacy of antibiotics in preventing lactic acidosis in lambs challenged by intra-ruminal administration of ground wheat at 40 g/kg body weight. They found that thiopeptin given as a single dose completely prevented lactic acidosis by reducing rumen lactate 80-90%. In addition, thiopeptin permitted "normal" rumen fermentation to continue as indicated by a significant increase in VFAs. The minimum effective dose of thiopeptin to control acute lactic acidosis was 0.18 mg/kg body weight. Other members of the thiopeptin class, including sulfomycin, sporangiomycin, siomycin and taitomycin, prevented lactic acidosis in a manner similar to thiopeptin. Penicillin, however, inhibited ruminal VFA production as well as lactate synthesis.

Lasalocid and monensin have an advantage over other antibiotics because they inhibit not only *Streptococcus bovis* but also other major lactate-producing rumen bacteria such as *Lactobacillus, Butyrivibrio* and *Lachnospira* (Dennis *et al.* 1981). Moreover, both lasalocid and monensin exert favourable effects on rumen fermentation by enhancing propionate formation (Richardson *et al.* 1976; Bartley *et al.* 1979) and inhibiting methanogenesis (Richardson *et al.* 1976; Chalupa 1977).

Virginiamycin has been widely studied *in vivo* and *in vitro* and reported to be effective against lactic acid-producing bacteria (*Streptococcus bovis* and *Lactobacillus* ssp.) in the rumen (Nagaraja *et al.* 1987). Virginiamycin (Eskalin Ptizer) is an antibiotic type compound derived from a strain of *Streptomyces virginiae*. It has been shown to increase fermentative efficiency in that it acts on Gram-positive bacteria (Boucque *et al.* 1990) and decreases protozoal numbers (Murray *et al.* 1992). Rowe *et al.* (1989) designed an experiment using rumen fluid from sheep and examined the effects of virginiamycin using an *in vitro* fermentation model and found that virginiamycin controlled lactic acid production even at a concentration of 0.5 mg/ml. Rowe and Zorrilla-Rios (1993) included virginiamycin at a concentration of 20 mg/kg in a complete diet containing 80% barley to cattle even without a gradual

increase in grain content of the diet and observed no signs of acidosis. The feed additive virginiamycin (as the granular formulation Founder-guard™) is effective in controlling the pattern of acid build up and accumulation of lactic acid in horses given high levels of grain (Rowe *et al.* 1995). However, in the studies of Nagaraja *et al.* (1995), virginiamycin actually decreased ($P < 0.01$) ruminal pH and increased ($P < 0.05$) lactate and propionate concentrations following grain challenge. Nagaraja *et al.* (1995) hypothesised that the effect of virginiamycin on the pattern of rumen fermentation in this experiment indicated redirection of electrons away from methane production and into lactate and propionate. Godfrey *et al.* (1994a) noted that virginiamycin reduced fermentation of soluble carbohydrate in some sheep. The addition of virginiamycin to the diet in the experiment was associated with a decrease in feed intake (Godfrey *et al.* 1995) during the first 10 days with a return to normal after approximately 2 weeks.

2.7.2.2 Effects of antibiotics vary depending on dosage and animal species

When oxamycin dosages of 0 (control), 77 (low) and 770 (high) mg/kg wheat were designed to treat sheep with acidosis induced by engorging ground wheat at a rate of 34.5 g/kg body weight, ruminal pH increased linearly with increased dosage levels of oxamycin ($P < 0.05$). The low dosage also effectively reduced lactate concentrations compared to controls at all sampling times. Volatile fatty acid concentrations tended to be higher after longer times with higher dose rates, although total VFAs concentrations were not altered by oxamycin treatment ($P > 0.05$) (Beede and Farlin 1977b).

Intra-ruminal administration of lasalocid or monensin at the level of 1.3 mg/kg body weight effectively prevented glucose or corn-induced lactic acidosis in cattle. However, there was substantial variation between animals (Nagaraja *et al.* 1981). Cattle required larger doses of virginiamycin than sheep to achieve similar levels of protection against lactic acidosis. When sheep were drenched with virginiamycin at 2.6 mg/kg live weight, rumen L-lactate levels were suppressed for 4 - 5 days. However, in cattle drenched with virginiamycin at 5.2 mg/kg live weight (double that of sheep), rumen L-lactate was suppressed only for 3 days (Thorniley *et al.* 1996).

2.7.2.3 Effects of antibiotics vary between *in vivo* and *in vitro* experiments

Penicillins were found to be the most active *in vitro*, whereas they would be destroyed and rendered relatively ineffective *in vivo* by an active penicillinase contained in the rumen (Muir and Albert Barreto 1979).

Supplemental monensin in a diet (50% concentrate mix and 50% corn silage) of steers did not significantly alter rumen fluid dilution rate or ruminal pH but did decrease the molar proportion of acetate and increase that of propionate (Rogers and Davis 1982a). However, in an *in vitro* fermentation using a purified diet and a mixed culture of ruminal micro-organisms, the addition of monensin prevented the accumulation of lactate and maintained higher pH than in untreated controls. After 22 hours of fermentation, total VFA concentration was greater in culture with monensin than in untreated controls (Tung and Kung 1993).

2.7.3 Use of probiotics

Probiotics have principally been investigated in terms of their effect on rumen fermentation to prevent grain poisoning. The use of probiotics is based on the theory that bacterial pre-treatment can prevent acidosis by increasing lactate utilisation in animals.

The probiotic Yea Sacc 1026 (Alltech) was shown to reduce the accumulation of lactic acid in the rumen fermentation of starch by Newbold (1990), Williams and Newbold (1990), Girard *et al.* (1993), and Newbbold *et al.* (1996). However, in the studies by Godfrey *et al.* (1992) the Yea Sacc did not cause any changes in the pattern of fermentation when compared to untreated control animals.

Carro *et al.* (1992) reported that the inclusion of yeast culture (10 g/day) did not affect rumen pH, ammonia or VFA concentration as well as molar proportions of acetate, propionate, butyrate and valerate in dairy cows fed grass silage and concentrates. However, Andrighetto *et al.* (1993) stated that yeast supplements (20 or 40 g/day) decreased rumen

pH ($P < 0.05$) and increased total VFA concentration ($P < 0.05$) in sheep fed a high concentrate diet.

In the studies of Wiryawan and Brooker (1995), when acute grain feeding was preceded by inoculation of the rumen with 10^8 colony forming units of *Selenomonas ruminantium* subsp. *lactilytica* strain JDB201, ruminal lactate was undetectable and ruminal pH was stabilised at 6.3 - 6.5 for up to 24 hours. Inoculation of the rumen with a mixture of 10^8 colony forming units of *Selenomonas ruminantium* subsp. *lactilytica* strain JDB201 and *Megasphaera elsdenii* strain JDB301 was shown to be more effective than *Selenomonas ruminantium* subsp. *lactilytica* strain JDB201 alone and maintained ruminal stability following acute grain feeding for up to 4 days. Since *Megasphaera elsdenii* strain JDB301 ferments lactate but not glucose, whereas *Selenomonas ruminantium* subsp. *lactilytica* strain JDB201 ferments both, it is possible that an interaction between these micro-organisms explains their effectiveness. The ability of the mixture of these bacteria to prevent lactic acidosis may also be due to stimulation of growth of other rumen bacteria, thereby reducing the domination by *Streptococcus bovis*. A continuous culture model of acidosis was also developed to test the effect of probiotic inoculation in combination with 0.75 mg/ml of virginiamycin in preventing lactate accumulation and establishing a stable fermentation *in vitro*. The data suggest that although probiotic treatment is effective, a combination of probiotic and antibiotic may be the best approach to achieve rapid ruminal adaptation during acute grain-feeding of sheep. However, these results need to be confirmed *in vivo*.

In addition, *Aspergillus oryzae* fermentation extract (AO; 2 g/day) caused a small reduction in L-lactate concentration in a high barley diet fed to sheep, but rumen pH was not changed significantly (Newbold *et al.* 1992).

2.7.4 Genetic engineering of rumen microbes

The genetic engineering of rumen bacteria is a technology essentially to change rumen bacteria by transferring DNA. The topic has been discussed extensively (Hespell 1985; Teather 1985; Russell and Wilson 1988; Gregg 1989), and a number of laboratories have begun research on this topic. The results of early work in this field suggest that methods for

genetic manipulation of rumen bacteria will probably be developed to a highly practical level within the next few years.

The physiology of the rumen, governed by its immensely diverse population of micro-organisms, provides a number of obvious possibilities for improvement by gene manipulation. Foremost among these is the opportunity to alter the way in which grain and plant materials are processed in this initial digestive organ. The natural role of the rumen bacteria is to ferment food before it passes through the rest of the digestive tract. This provides an opportunity to make genetic alterations to the bacteria, which could produce important changes to the host animal.

Russell and Wilson (1988) and Hazlewood and Gilbert (1989) have had some successes in transferring DNA to rumen bacteria. However, the capacity of those particular bacterial strains to survive within the rumen is uncertain. Whitehead and Cotta (1993) have tested a cloned amylase gene from the ruminal strain *Streptococcus bovis* JB1 as a potential DNA probe for rapid and accurate identification of *Streptococcus bovis* strains from all sources. The probe also hybridised with variants of *Streptococcus bovis* that did not grow on starch. Gregg *et al.* (1997) inoculated test sheep with a mixture of four genetically modified strains of *Butyrivibrio fibrisolvens*, which attained a combined population level of 10^6-10^7 cells per mL in each animal. The bacteria had been modified to express the enzyme fluoro-acetate dehalogenase and the results showed clearly that rumen bacteria were capable of detoxifying the poison fluoro-acetate, significantly reducing the effect of this poison upon the host animals. This is an example of the possibility in transferring DNA to rumen bacteria. The application of biotechnology has the long-term potential to make dramatic improvements in the efficiency and the ecological compatibility of animal production.

2.7.5 Use of buffers and other chemicals

Some buffers and other chemicals have been studied and applied to prevent acidosis. So far the buffers and other chemicals summarised in Table 2-5 are the most common and effective in the treatment of acidosis.

Table 2-5. Some buffers and other chemicals used in treating acidosis

Chemical	Reference
Carbonate	Reid *et al.* 1957; Nicholson *et al.* 1996.
Bicarbonate	Rogers and Davis 1982a; Bigner *et al.* 1996.
Rumen 8®	Xu *et al.* 1994.
Alkaten®	Xu *et al.* 1994.
Carbicarb	Kamel 1996.
Dehydrate alfalfa	Stroud *et al.* 1985.
Slaframine	Hibbard *et al.* 1995.
Sodium chloride	Hemsley *et al.* 1975.
Calcium hydroxide	Offer and Offer 1992; Boukila *et al.* 1995.
Magnesium hydroxide	Boukila *et al.* 1995.
Sodium bentonite	Horn *et al.* 1979.

2.7.5.1 Carbonate

The administration of suitable doses of sodium carbonate (Na_2CO_3) into the rumen can eliminate the accumulation of lactate in the ingesta and prevent the pH fall after feeding sheep with rations containing high proportions of wheaten starch (Reid *et al.* 1957). Crab meal could be a useful rumen buffer in sheep and cattle since the crab meal consists mainly of calcium carbonate (Nicholson *et al.* 1996).

2.7.5.2 Bicarbonate

Bicarbonate, mainly sodium bicarbonate ($NaHCO_3$), has been used to manipulate rumen fluid pH by dietary supplement and intra-ruminal infusion. Sodium bicarbonate infusion increased ruminal pH compared to water control in steers on both high-grain and high-roughage diets, but the increase was more dramatic when the high-grain diet was fed (Rogers and Davis 1982b). In infusion therapy for acute ruminal acidosis in cattle by Rossow *et al.* (1994), 1.3 % $NaHCO_3$ solution was applied at a rate of 0.5 mmol/kg live weight/h. After 50% of the estimated daily amount of required buffer was applied within 6 hours, the remaining buffer quantity was infused at a lower rate or was administered orally, depending on the clinical conditions of the animal. The correction of acidosis by infusing

$NaHCO_3$ also partially improved insulin resistance in rats with chronic renal failure (Mak 1996).

Dietary supplementation with 5% $NaHCO_3$ or 33 ppm monensin or 5% $NaHCO_3$ plus 33 ppm monensin (dry matter) increased rumen pH, feed intake, water intake, fluid dilution rate, and decreased both molar proportion and production rate of propionate in the rumen of steers fed basal diet (50% concentrate mix and 50% corn silage) (Rogers and Davis 1982a). That $NaHCO_3$ supplement (0.75-1%) increased ruminal pH was also reported by Boerner *et al.* (1987) and Zinn (1991). Furthermore, total VFA and pH of rumen fluid increased with increasing levels of dietary $NaHCO_3$ in lambs (Kovacik *et al.* 1986) and in goats (Cetinkaya and Unal 1992). The correction of acidosis by oral administration of $NaHCO_3$ prior to glucose tolerance testing also increased blood pH (Bigner 1996).

In cattle, supplemental $NaHCO_3$ increased ruminal pH and the latter, in turn, increased ruminal digestion of fibre (Harrison *et al.* 1976; Rogers *et al.* 1979) and protein (Okeke *et al.* 1983; Loerch *et al.* 1983). The benefits of higher ruminal pH to fibre digestion may be due, in part, to the effects of $NaHCO_3$ that are detrimental to fibre digestion such as increased rate of passage (Mertens 1978).

The addition of $NaHCO_3$ to a grain diet was reported to increase average daily gain (ADG). Lofgreen (1976) observed a 7% increase in ADG and an 8% decrease in dry matter (DM)/gain by the addition of 0.75% $NaHCO_3$ to a steam-rolled barley-based finishing diet (90% concentrate). Brethour *et al.* (1986) reported an 11.6% increase in ADG and a 5.3% decrease in DM/gain by the addition of 1.05% $NaHCO_3$ to a finely rolled wheat and sorghum-based finishing diet. Zinn (1991) observed a 5.9% increase in ADG and a 4.6% increase in DM intake by the addition of 0.75% $NaHCO_3$ to a steam-flaked corn or sorghum-based finishing diet (DM/gain was not affected). Sodium bicarbonate was found to have a short-term effect on weight gain (Russell *et al.* 1980) and has been attributed to an adjustment of the animal to a bicarbonate load.

However, there are different ideas on the effect of dietary $NaHCO_3$. Russell *et al.* (1980) and Haaland and Tyrell (1982) reported that up to 2% $NaHCO_3$ supplement had no effect on ruminal pH. Zinn and Borques (1993) found $NaHCO_3$ (0.75% of dry matter) not to influence ($P > 0.10$)

ruminal or total tract digestion of organic matter (OM), starch, nitrogen (N), ruminal pH or ruminal VFA molar proportions in feedlot steers. Xu *et al.* (1994) reported no significant differences in rumen fluid pH or molar percentage of VFAs between cows fed buffered diets (2.2% $NaHCO_3$ of dietary DM) and those fed regular diets. In the studies by Orozco *et al.* (1994), $NaHCO_3$ at 2% of DM reduced the digestibility of most nutrients without affecting pH of rumen fluid or degradation of DM. An experiment of Clayton *et al.* (1997) showed no significant effect of 2% $NaHCO_3$ supplement on rumen pH, faecal pH and L-lactic acid accumulation in rumen fluid. These differences may be accounted for by the differences in experimental animals, conditions, techniques, *in vivo* and *in vitro*, and the quantities of buffer used.

2.7.5.3 Rumen 8® and Alkaten®

The buffering function of bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) was also investigated using mixed rumen buffers, Rumen 8® (36.3% Na_2CO_3 and 26.5% $NaHCO_3$. Agchem Commission Co.) and Alkaten® (43.4% Na_2CO_3 and 34.4% $NaHCO_3$. Church and Dwight, Co., Princeton, NJ). In determining the effects of Rumen 8® and Alkaten® on buffering capacity, Xu *et al.* (1994) found titratable acidity and acid buffering capacity of Alkaten® significantly higher ($P < 0.01$) than those of Rumen 8®. This difference may be explained by the higher proportions of CO_3^{2-} and HCO_3^- in Alkaten®. Subsequently, Xu *et al.* (1994) found that the addition of Rumen 8®or Alkaten® not only significantly increased the pH of the diets, but also helped consume more feed in cows fed high grain total mixed rations.

2.7.5.4 Carbicarb and dehydrate alfalfa

The administration of Carbicarb (0.75 mmol) led to higher pH, in which Carbicarb is a new alkalising agent that has been proposed as a replacement for $NaHCO_3$ (Kamel 1996).

The influence of dehydrated alfalfa as a buffer to improve steer performance and ruminal characteristics was studied by Stroud *et al.* (1985). They considered that dehydrated alfalfa was an effective ruminal buffering agent and that changes in the rumen environment might be manifested in improved animal performance. Supplementing dehydrated

alfalfa with 1% $NaHCO_3$ could further increase ruminal pH, buffering capacity, and bicarbonate concentration. In addition, blood pH and bicarbonate concentration also increase.

2.7.5.5 Slaframine and sodium bentonite

Injections of 66-100 mg of slaframine/kg live weight increased salivary flow approximately 50% compared with controls and reduced the decrease in ruminal pH associated with subacute acidosis in growing beef steers, but slaframine did not reduce the risk (or severity) of acute ruminal acidosis (Hibbard *et al.* 1995).

Supplementing a corn silage ration with sodium bentonite increased pH values and decreased lactate concentrations in the rumen of Holstein steers (Horn *et al.* 1979).

2.7.5.6 Sodium chloride

In the studies by Rogers *et al.* (1979), a sodium chloride (NaCl) infusion at 200 g/day appeared to increase ruminal pH in steers fed a high-grain diet but had no effect in steers fed a high-roughage diet. High NaCl infusion (600 g/day) either had no effect (high-grain diet) or caused a decrease in rumen pH (high-roughage diet). The pH in the rumen was increased significantly and the concentrations of VFAs were decreased in steers fed the high concentrate diet during the infusion of NaCl. Rogers *et al.* (1979) attributed the decreased concentrations of VFAs to the increase in rumen fluid pH. Moreover, Garg and Nangia (1993) reported that the higher rumen pH values, lower concentration of ruminal VFAs, increased acetate and reduced propionate molar proportions were associated with 200 g/day NaCl supplementation.

2.7.5.7 Calcium hydroxide and magnesium hydroxide

Addition of 1% calcium hydroxide [$Ca(OH)_2$] or 0.79% magnesium hydroxide [$Mg(OH)_2$] to the diet alone or in combination 0.5% $Ca(OH)_2$ and 0.39% [$Mg(OH)_2$] improved the systemic acid-base status of sheep fed a high-barley diet and was associated with increases in DM intake (Boukila *et al.* 1995). However, Offer and Offer (1992) reported that $Ca(OH)_2$ treatment greatly improved the nutritional characteristics of malt

distillers' grains (MDG) giving substantial improvements in apparent digestibility, intake and performance, but rumen pH, VFA concentrations or hay digestibility in sac were not significantly affected by the supplement.

2.7.6 Other methods

Dunlop (1972) considered that the only satisfactory treatment for severe acidosis cases was to empty the rumen by surgery or lavage in order to permit a normal fermentation to be re-established in ruminants. When Sanz-Mareno and Botella (1995) treated 13 ill persons with paired filtration dialysis (PFD), the biochemical results were excellent with a stabilisation of all parameters and a better control of acidosis (statistically significative after 6 months). The clinical tolerance was also excellent and the technique was simplified.

Groundnut cake maintained significantly ($P < 0.001$) higher rumen pH (6.58) in cattle fed 4% urea treated wheat in the experiments of Dutta and Singh (1994).

Fron *et al.* (1996) reported that if cattle were fed condensed distillery by-products for several weeks, rumen microbiology and metabolism were affected. Cultivable counts of starch-degrading and lactic acid-utilising bacteria increased and this coincided with a two-fold increase in the *in vitro* rate of lactic acid fermentation. These data suggest that distillery by-products could be used to selectively manipulate the rumen microbial population and improve the capacity of the rumen micro-organisms to utilise lactic acid, the causative agent of acute ruminal acidosis and morbidity in cattle.

2.8 Summary

At present, prevention of acidosis centres on gradual adaptation to diets high in readily fermentable carbohydrates and careful management while feeding such diets. A though the problems of lactic acidosis have been recognised in ruminants, the characteristics and adverse side-effects of acidic gut syndrome have not been understood in animal production.

An understanding of how acidosis affects the animal is a fundamental requirement in developing effective methods of prevention. Two important aspects of the biology and biochemistry of caecal and rumen fermentation are the absorption of VFAs and lactic acid from the caecum and the rumen, and the buffering capacity in these digestive compartments. A computer model of rumen fermentation to predict lactic acid production and the efficacy of treatment of acidosis has a potential role. These were studied in this thesis and described in the following chapters.

Chapter 3 No Lactic Acid Is Absorbed from the Caecum and Rumen of Sheep

3.1 Introduction

It is generally thought that lactic acidosis, associated with high levels of grain intake in ruminants, is the result of lactic acid absorption from the rumen (Dunlop 1972), and that it is the effect of this strong acid in blood and tissue which is a primary factor causing adverse effects associated with grain feeding and acidosis (Blood and Henderson 1963). This adverse effect of lactic acid is also thought to be exacerbated by the slower clearance by the body of D-lactic acid originating from gut microbes (Dunlop and Hammond 1965; Whanger and Matrone 1966). The aetiology of lactic acidosis relies on the rapid absorption of considerable quantities of lactic acid from the rumen. However, there is evidence which suggest that this may not be the case. Firstly, the concentrations of D-lactate in blood during episodes of rumen lactic acidosis are reported to be much lower than that of L-lactate (Godfrey *et al.* 1992; Ganter *et al.* 1993). Both D- and L-lactic acid are produced in approximately equal amounts by microbes in the gut, whereas only L-lactate is produced by animal tissue (Giesecke and Stangassingor 1980). Cori and Cori (1929) and Dunlop and Hammond (1965) found that there was no detectable difference in absorption between the two isomers and suggested that the peak entry rate of L- and D-lactic acid into the blood should occur at the same time. However, liver tissue was found to be able to synthesise carbohydrate from D-lactic acid but hardly from L-lactic acid (Meyerhof and Lohmann 1926). L-lactic acid is utilised 4 times more slowly in the rat than D-lactic acid because D-lactic acid can be deposited as liver glycogen (Cori and Cori 1929). The second line of evidence suggesting that absorption of lactic acid from the gut may not be of primary importance in the aetiology of lactic acidosis is based on existing data on the absorption of lactic acid from the rumen. Under normal conditions of fermentation in the rumen, there is little or no measurable lactic acid. Gill et al. (1986) showed that, even when lactic acid constitutes over 13% of the

dry matter in silage diets, metabolism of lactic acid within the rumen is rapid and complete. Therefore, the absorption of lactic acid from the rumen, even when it is a major dietary component, is not likely to occur. On the other hand, the absorption of VFAs from the rumen is known to be efficient and influenced by a range of factors including chain length, pH, the effect of absorptive area, osmolarity and the concentration of individual VFAs (Leng 1970; Bugaut 1987; Argenzio 1988; Bergman 1990). Dobson and Phillips (1956) found no evidence for absorption of lactate from solution buffered at pH 4 in the isolated rumen of sheep.

However, Hueter *et al.* (1956) observed a rapid increase in the concentration of lactic acid in blood after intra-ruminal administration of sodium lactate, and these authors suggested that this was due to direct absorption of lactic acid.

On the other hand, the potential importance of the caecum in lactic acidosis has received little attention. It is clear that lactic acid can accumulate in the caecum of sheep fed grain, even when there is no lactic acid in the rumen (Ward *et al.* 1961; Godfrey *et al.* 1992). Studies on the absorption of VFAs from the caecum in sheep (Faichney 1968), rats (Berggren *et al.* 1993), horses and donkeys (Horspool *et al.* 1994) show this process is efficient and similar to absorption from the rumen. However, there has been no study of the absorption of lactic acid from the caecum.

The rate and extent of lactic acid and VFA absorption from the gastro-intestinal tract need to be quantified in order to understand and manage conditions of grain poisoning and acidosis in ruminants and other animals. The hypothesis explored in this chapter is that lactic acid is absorbed far more slowly than VFAs from both the rumen and the caecum, and that the primary problem in lactic acidosis is low pH within the gut rather than absorption of a large quantity of lactic acid.

3.2 Materials and Methods

3.2.1 Animals and diet

Nine Merino wethers, around 5 years old and 35 to 40 kg live weight, were penned individually and given oaten chaff (900 g) and urea (1%) once a day for two months before the experiment.

3.2.2 Experimental design and procedures

The sheep were weighed and anaesthetised with halothane/O_2 before the peritoneal cavity was opened using standard surgical procedures. Pouches were prepared as follows. The caecum was divided into two pouches: the blind end pouch referred to as Caecum 1, and the second pouch, Caecum 2 which was between Caecum 1 and the ileo-caecal junction. One pouch was made in the caudal end of the dorsal sac of the rumen. The pouches were prepared by first gently stripping digesta from the site for the pouch and then sealing that compartment from the remainder of the organ with outline suturing and ties which were located to maintain normal blood supply. The rumen pouch was sealed by inserting a soft rubber ball (30 mm diameter) through an incision in the rumen wall and tightening the sutures and ties against the rubber ball which formed the bottom plug for the pouch. The pouches were opened and washed thoroughly using 37°C physiological saline and then sealed with a silicon catheter in place for injecting and sampling the test solutions. Following washing with 37°C physiological saline and rinsing with the test solution, 50 mL of the test solution (37°C) was introduced into each pouch and left for a period of 50 minutes to measure absorption (Absorption Period 1). Samples (5 mL) were taken from each pouch at 10 minute intervals. The pH was measured immediately after sampling. Sub-samples (1 mL) were stored at 4°C for glucose analysis and the remaining 4 mL was acidified with 0.1 mL of 98% sulfuric acid (H_2SO_4) and stored at -20°C for analysis of lactate, VFAs, ammonia and Cr-EDTA.

At the end of Absorption Period 1, the remaining test solution was removed from the pouches and the volume recorded. The pouches were

then washed with 37°C physiological saline and rinsed with the next test solution before introducing 50 mL of the test solution. Absorption Periods 2 (50 minutes) and then 3 (50 minutes) were carried out in the same manner as described above for Absorption Period 1 using different test solutions for each period.

During the experimental period, the animals were monitored for core temperature and blood pressure via a carotid transducer (Cobe Disposable Transducer, Lakewood, Colorade Biopac Systems Inc., Goleta, California, USA). Physiological saline was administered intravenously and continuously at a rate of approximately 5 mL per minute.

At the end of Absorption Period 3, the animals were euthanased with valaborb (Euthanasia Solution, Pitman-Moore Australia Limited, 71 Epping Road, North Ryde, NSW) by overdose. The pouches were removed, opened and washed with tap water before drying surface moisture with paper towel. They were then weighed and their area was measured. These measurements were used to calculate absorption rates per unit of surface area and per tissue weight.

Test solutions were prepared containing constant concentrations of acetic acid (70 mmol/L), propionic (20 mmol/L), sodium n-butyrate (10 mmol/L), D-glucose (4 mmol/L), ammonium chloride (7 mmol/L), potassium hydroxide (5 mmol/L), potassium and sodium dihydrogen orthophosphate (30 and 22 mmol/L, respectively). Cr-EDTA (5 mg/L) was used as a marker in order to measure fluid movement in or out of the pouches and, if required, to correct the absorption rates of test solutions for fluid movement. The test solutions varied in the concentrations of lactic acid (12.5, 25 or 50 mmol/L), osmotic pressure (280, 420 or 460 mOsmol/kg) and pH (4.5, 5.0, 5.5 or 6.5). These factors were adjusted by the addition of DL-lactic acid, sodium chloride and concentrated sodium hydroxide solution, respectively. The DL-lactic acid contained equal concentrations of D- and L-lactic acid. Each solution was tested at least once in both the rumen and caecum, and the solutions of pH 5.0, 420 mOsmol/kg and varying lactic acid concentrations were tested 3 times in each of three sheep.

Under normal conditions of fermentation, lactic acid only accumulates at pH below 5.5. For this reason, most results presented are for the test solutions at pH 5.0.

3.2.3 Assay and measurement

Osmotic pressure of test solutions was determined with a 5100B Vapour Pressure Osmometer (Wescor, Inc, 459 So. Main Street, Logan, Utah 84321, USA) and the pH of samples was measured immediately after sampling, using a pH-meter with a glass electrode (20.9 B, Activon Digital pH/mv Meter, Activon Scientific Products Company Pty. Ltd., 27 Oakleigh Avenue, Granville, NSW).

Concentrations of VFAs were determined using a gas-liquid chromatography (GLC, Model 304, Pye Unicam Ltd, Cambridge, Cambs) based on the method of Erwin *et al.* (1961). Lactic acid and D-lactic acid concentrations were measured using a Cobas Mira Auto-analyser (Roche Diagnostics Inc., Frenchs Forest, NSW) and enzyme kits (D-Lactic acid/L-Lactic acid kit, Cat. No. 1112821, Boehringer-Mannheim, Mannheim, Germany). Glucose was determined using enzymatic UV test HK/G6P-DH method on a Cobas Mira Auto-analyser (Roche Diagnostics Inc., Frenchs Forest, NSW). Ammonia-nitrogen (NH_3-N) was analysed on an Auto-analyser (Kjeltec 1030 Auto-analyser, Perstop Analytical Ltd, Maidenhead, Berks) (Nolan and Leng 1972). Cr-EDTA was determined by atomic absorption spectroscopy (Udén *et al.* 1980).

3.2.4 Calculation of absorption rates and statistical methods

Absorption rates are expressed as an average percentage per minute (%/min) or as micro-mole per square centimetre per minute (µmol/cm^2.min). The calculations are as follows:

Absorption rate (%/min) = absorption percentage (%) / absorption time (50 min)

Absorption rate (µmol/cm^2.min) = absorption micromoles / pouch surface area (cm^2).absorption time (50 min)

The former (%/min) was used to compare averages of caecal and rumen values, while the latter (μmol/cm^2.min) was used to compare values between the caecum and rumen.

Data in Tables 1 - 2 and Figures 1 - 5 were analysed by analysis of variance with Fishers Protected LSD Posthoc Test (SuperANOVA V1.11 Folder) (Abacus Concepts, In., 1984 Bonita Avenue, Berkeley, California, 94704-1038, USA). Regression analysis was used to examine the relationships between pH, osmotic pressure, lactic acid concentration and absorption (StatView 4.02) (Abacus Concepts, In., 1984 Bonita Avenue, Berkeley, California, 94704 - 1038, USA).

3.3 Results

Neither L-lactic acid nor D-lactic acid was absorbed from the caecal or rumen pouches throughout 50 minute absorption period. There was, in fact, a slight increase in both isomers of lactic acid, and the increment of L-lactic acid was more than that of D-lactic acid in the caecum and rumen (Figure 3-1). There was no difference between the two caecal pouches for any measurements and the data from these two pouches have been combined in all tables and figures.

The increase in lactic acid and the absorption of VFAs, glucose and ammonia from the caecal and rumen pouches occurred continuously throughout the 50 minute absorption period (Figure 3-2).

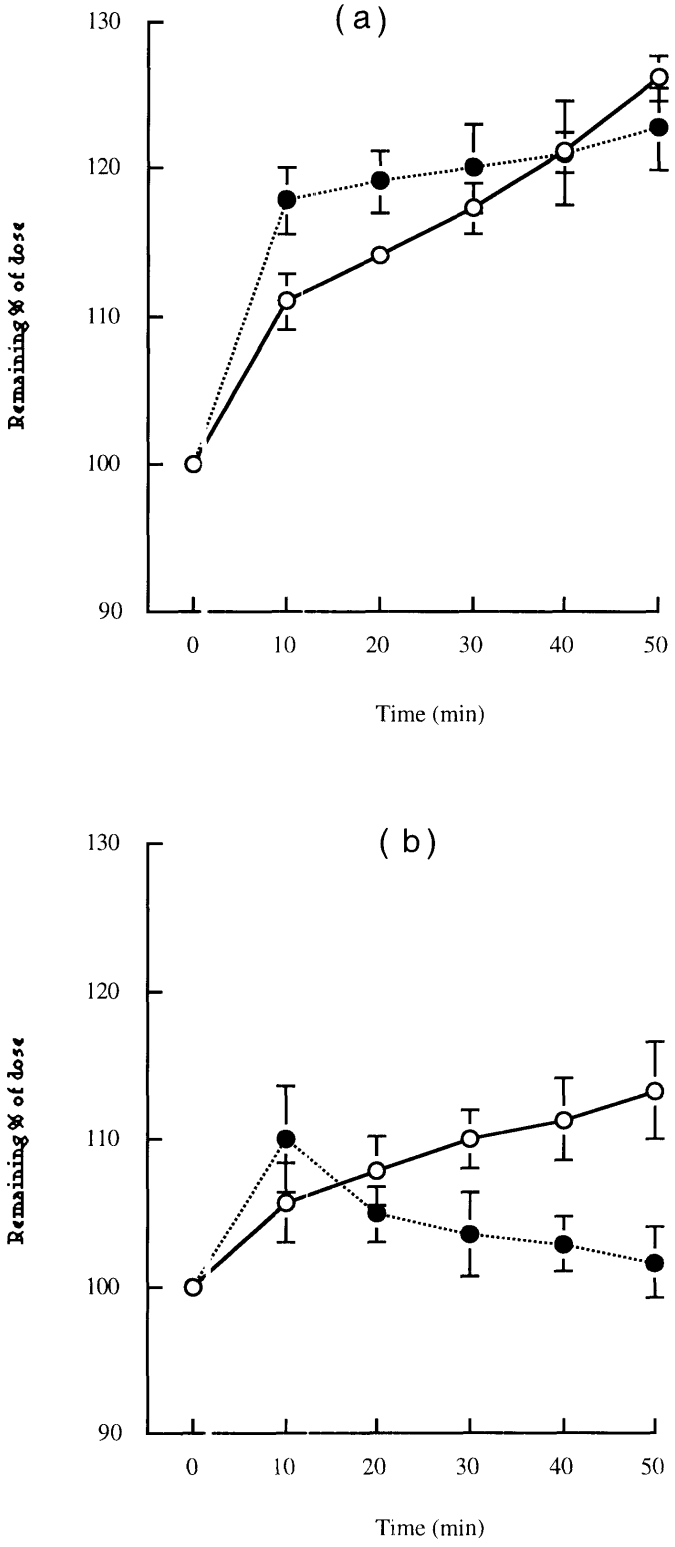


Figure 3-1. Remaining percentages of dose L- (○) and D-lactic acid (●) in the caecum (a) and rumen (b) over time within 50 minute absorption period in the test solutions with pH 5.0, 420 mOsmol/kg and 25 mmol/L lactic acid.

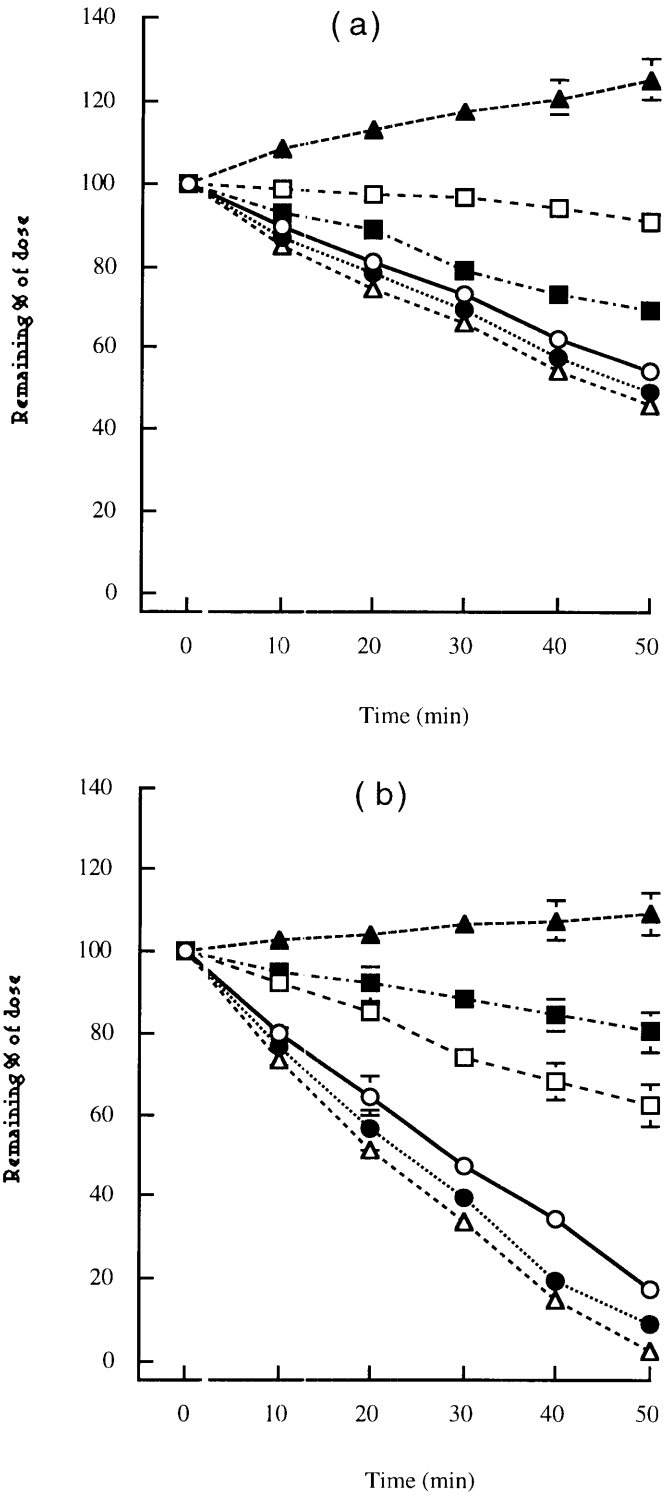


Figure 3-2. Percentages of dose remaining in the caecum (a) and rumen (b) of acetic (○), propionic (●), butyric (Δ) and lactic acids (▲), glucose (□) and ammonia (■) over time within 50 minute absorption period in the test solutions with pH 5.0, 420 mOsmol/kg and 25 mmol/L lactic acid. The data for L- and D-lactic acid have been averaged.

There was a significant effect of pH on the absorption rate of test substances both in the caecum and rumen (Figure 3-3). The average increase in lactic acid for the caecum and rumen was much higher at pH 4.5 (0.34%/min) than that at pH 6.5 (0.04%/min). The mean absorption rate of VFAs from the caecum and rumen at pH 4.5 (1.63%/min) was about 1.7 times higher than that at pH 6.5 (0.94%/min), while the mean absorption rate of ammonia for the caecum and rumen at pH 6.5 (0.99%/min) was about 2.6 times higher than that at pH 4.5 (0.38%/min). There was a small gain in the amount of glucose in the caecal and rumen pouches at pH 4.5 (0.10%/min), and glucose was apparently absorbed at pH 6.5 (0.35%/min). There was, however, no significant change in absorption rate between pH 5.5 and pH 6.5 ($P > 0.05$).

The influence of osmolarity on absorption is shown in Figure 3-4. There was a net gain in lactic acid at all levels of osmolarity, but the net gain of lactic acid with 460 mOsmol/kg solutions (0.56%/min) in the caecum and rumen was greater than that with 280 mOsmol/kg solutions (0.24%/min). On the other hand, absorption of VFAs and ammonia increased at lower osmotic pressures. For the rumen and caecum, the mean absorption rate of VFAs with a test solution of 280 mOsmol/kg (1.65%/min) was about 1.4 times greater than that with a 460 mOsmol/kg solution (1.17%/min). The mean absorption rate of ammonia with a test solution of 280 mOsmol/kg (0.41%/min) was higher than that with a 460 mOsmol/kg solution (0.12%/min).

The effects of lactic acid concentration in the test solutions on the absorption rate are shown in Figure 3-5. With increasing concentrations of lactic acid, there was less apparent entry of lactic acid into the test solution. Increasing concentration of lactic acid had little effect on the rate of VFA absorption but reduced the absorption rate of ammonia. In the case of glucose there was an apparent increase in absorption rate with increasing lactic acid concentration in the caecal pouch and a decrease in the rumen pouch.

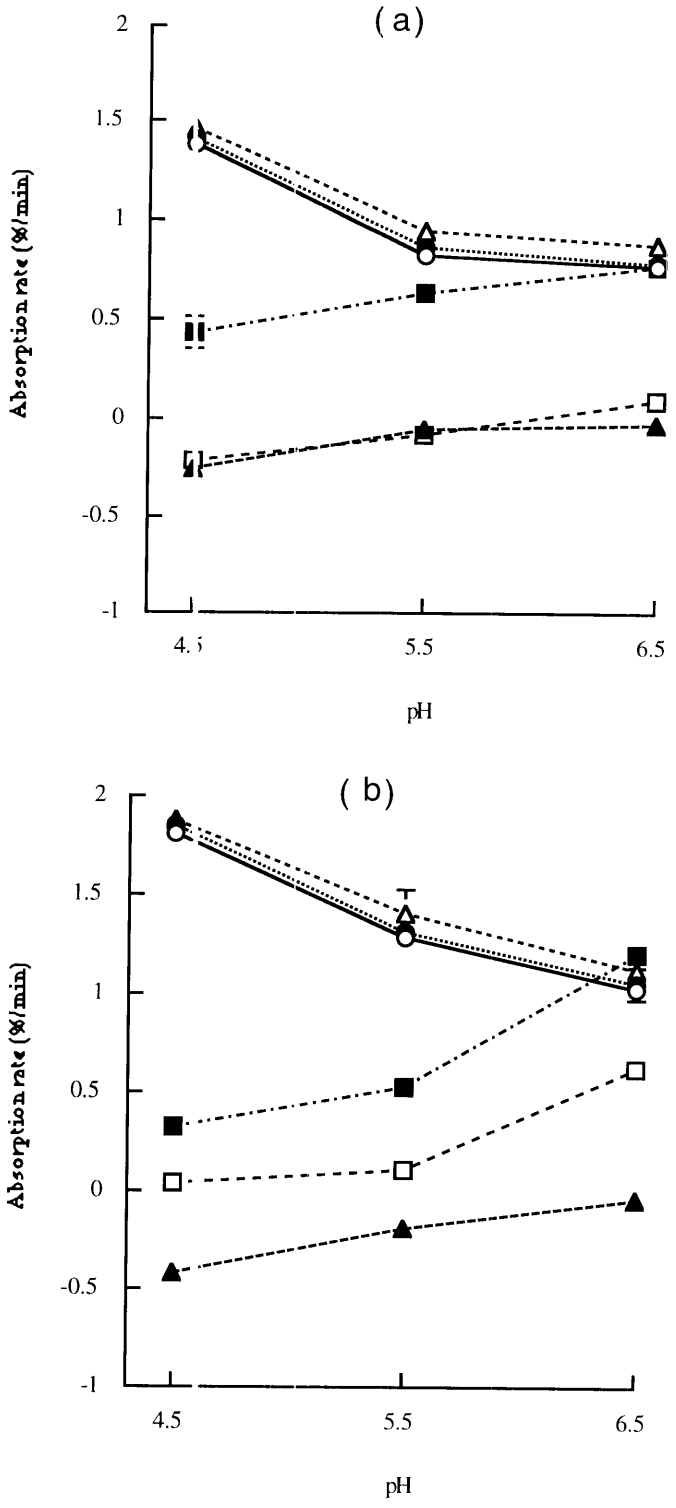


Figure 3-3. Effects of pH on the mean absorption rate of acetic (○), propionic (●), butyric (Δ) and lactic acids (▲), glucose (□) and ammonia (▮▮) in the caecum (a) and rumen (b) of sheep within 50 minute absorption period in the test solutions with 280 mOsmol/kg and 25 mmol/L lactic acid.

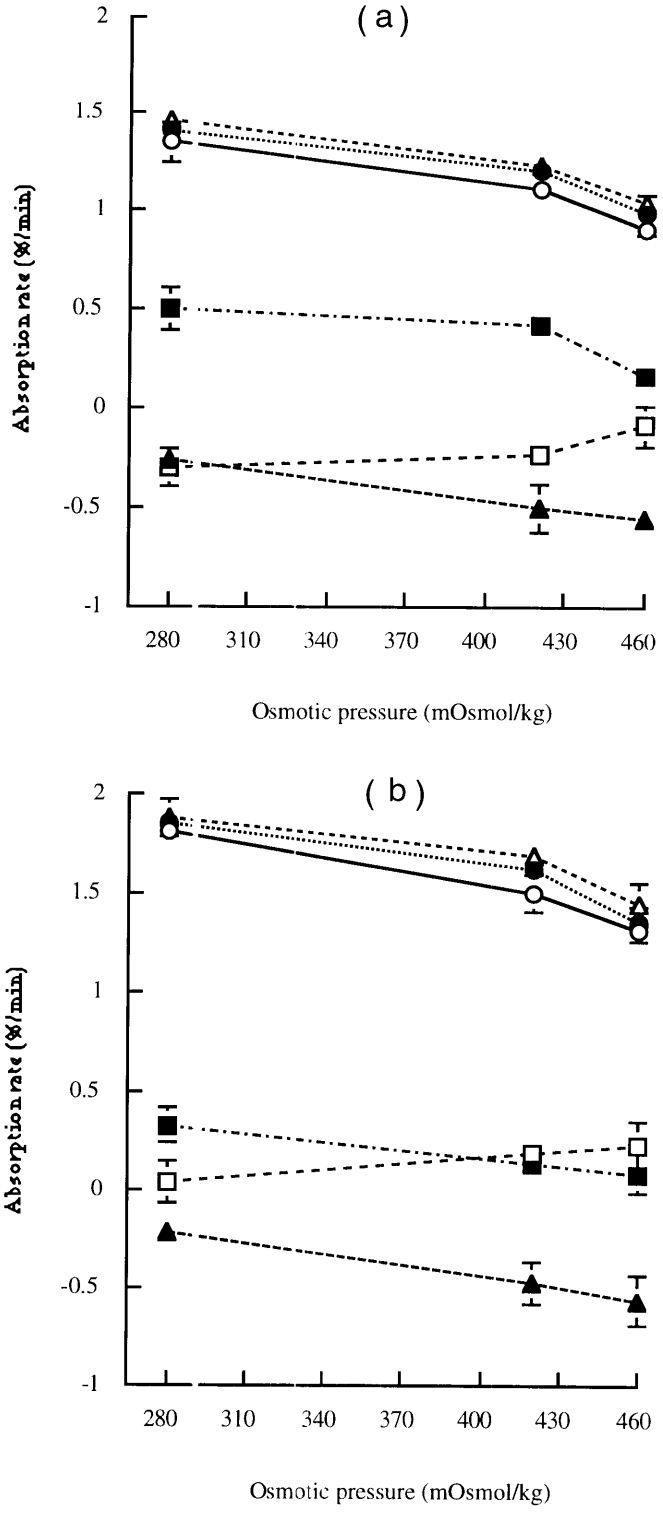


Figure 3-4. Effects of osmotic pressure on the mean absorption rate of acetic (○), propionic (●), butyric (Δ) and lactic acids (▲), glucose (□) and ammonia (■) in the caecum (a) and rumen (b) of sheep within 50 minute absorption period in the test solutions with pH 4.5 and 25 mmol/L lactic acid.

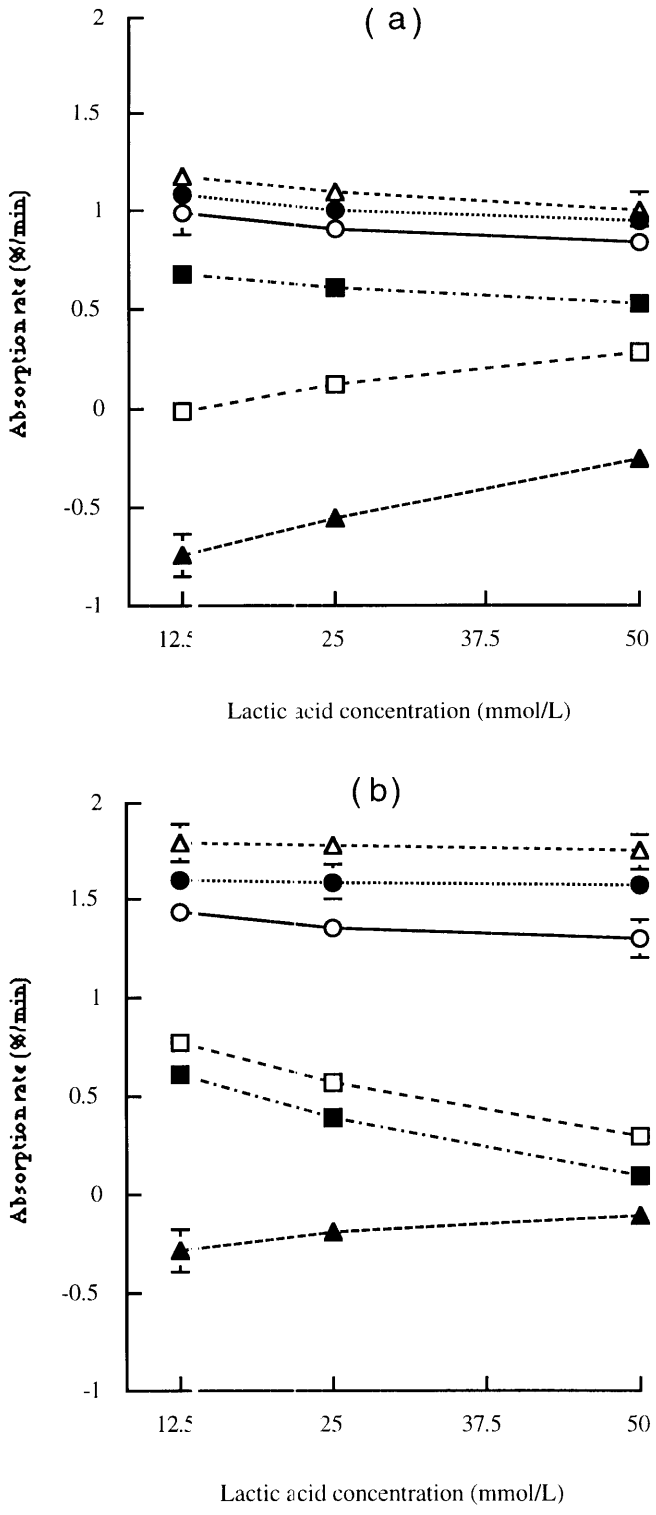


Figure 3-5. Effects of lactic acid concentration on the mean absorption rate of acetic (○), propionic (●), butyric (Δ) and lactic acids (▲), glucose (□) and ammonia (■) in the caecum (a) and rumen (b) of sheep within 50 minute absorption period in the test solutions with pH 5.0 and 420 mOsmol/kg.

Table 3-1 summarises the mean absorption rates (%/min) for all compounds in the test solutions starting with pH 5.0, 420 mOsmol/kg and 25 mmol/L lactic acid. There was a slight increase in both isomers of lactic acid. The rate of increase for L-lactate (0.39%/min) was greater ($P < 0.001$) than that for D-lactic acid (0.24%/min) averaged for the caecum and rumen values.

Table 3-1. Mean absorption rates (%/min) of initial concentration of VFAs, lactic acid, glucose and ammonia in caecal and rumen pouches of sheep in the 50 minute absorption period in the test solutions with pH 5.0, 420 mOsmol/kg, and lactic acid 25 mmol/L. Row comparisons of different test substances are highly significantly different ($P < 0.0001$) except the rows of acetic, propionic, butyric and D-lactic acid ($P > 0.05$). Column comparisons of different pouches are not significantly different ($P > 0.05$). Both within and between row comparisons, means followed by the same letter are not significantly different ($P > 0.05$). Negative values indicate a net increase as opposed to absorption.

Test substance	Absorption pouch		s.e.m.
	Caecum	Rumen	
Acetic acid	0.91a	1.40a	0.09
Propionic acid	1.02a	1.62a	0.10
Butyric acid	1.08a	1.77a	0.12
L-Lactic acid	–0.52b	–0.26b	0.08
D-Lactic acid	–0.45b	–0.03c	0.10
Glucose	–0.02c	0.55d	0.14
NH_3–N	0.61d	0.39d	0.07
s.e.m.	0.14	0.18	

Absorption varied depending on the sites of digestive tract and the effective surface area of absorptive tissue and this is summarised in Table 3-2. The data in Table 3-2 indicate that, for the caecum and rumen, the mean increase in the rate of L-lactic acid (0.05 μmol/cm^2.min) was twice as high as that of D-lactic acid (0.025 μmol/cm^2.min). The rate of increase for

the caecum (0.06 μmol/cm² min) was much higher than that for the rumen (0.015 μmol/cm².min) based on the average of L- and D-lactic acid ($P < 0.0001$). The absorption rate of ammonia from the caecum (0.60 μgN/cm².min) was 2.5 t mes higher than that for the rumen (0.24 μgN/cm².min) ($P < 0.0001$. However, the absorption rates of individual VFAs for the caecum (0.49, 0.17 and 0.08 μmol/cm².min for acetic, propionic and butyric acids, respectively) and rumen (0.48, 0.16 and 0.08 μmol/cm².min for acetic, propionic and butyric acids, respectively) were very similar ($P > 0.05$). G ucose was apparently absorbed from rumen pouches (0.18 μmol/cm² min) but not from caecal pouches (-0.01 μmol/cm².min), and this difference was statistically significant ($P < 0.0001$).

Table 3-2. Mean absorption rates (μmol/cm².min and μgN/cm².min for NH_3) of VFAs, lactic acid, glucose and ammonia per minute in caecal and rumen pouches of sheep in 50 minute absorption period in the test solutions with pH 5.0, 420 mOsmol/kg, and lactic acid 25 mmol/L. Row comparisons of different test substances are highly significantly different ($P < 0.0001$). Column comparisons of different pouches are not significantly different ($P > 0.05$). Both within and between row comparisons, means followed by the same letter are not significantly different ($P > 0.05$). Negative values indicate a net increase as opposed to absorption.

Test substance	Absorption pouch		s.e.m.
	Caecum	Rumen	
Acetic acid	0.49a	0.48a	0.02
Propionic acid	0.17b	0.16b	0.01
Butyric acid	0.08b	0.08b	0.01
L-Lactic acid	–0.08c	– 0.02c	0.01
D-Lactic acid	–0.04c	– 0.01c	0.01
Glucose	–0.01c	0.18b	0.04
NH_3–N	0.60a	0.24b	0.07
s.e.m.	0.06	0.04	

The weight of rumen wall per cm^2 ($0.66g/cm^2 \pm 0.06$) was 2 times greater than that of the caecal wall ($0.33g/cm^2 \pm 0.015$).

3.4 Discussion

The results confirm the hypothesis that lactic acid is absorbed from the rumen and caecum far more slowly than VFAs. There was effectively no lactic acid absorption from either rumen or caecal pouches and, in fact, there was a net increase in lactic acid at lower pH in both the rumen and the caecum. At the same time, there was rapid and apparently normal absorption of VFAs and ammonia.

Increased lactic acid in the pouches might be due to the conversion of VFAs or glucose to lactic acid and/or the movement of lactic acid into the pouches as a result of metabolism of VFAs and glucose in the rumen or caecal wall (Pennington and Sutherland 1956). It is unlikely that bacterial conversion of glucose, or VFAs, to lactic acid can explain the apparent lack of absorption and the net gain in lactic acid in the pouches. The pouches were very thoroughly washed before introducing the test solutions including glucose and, while a small number of bacteria may have remained attached to the gut wall, it is unlikely that they would have had a large initial effect on lactic acid concentration and then no subsequent effect. It is also relevant that there were no differences in apparent lactate absorption between Periods 1, 2 or 3. If the conversion of glucose to lactic acid was related to the bacterial conversion of glucose and/or VFAs, then the extent of lactic acid accumulation would be expected to be different during the different periods. Either there would have been a build up of bacteria with time resulting in more lactic acid being produced in Periods 2 and 3 compared with period 1. Alternatively the additional washing of the pouches in the 3 periods would have removed bacteria and therefore reduced the capacity to produce lactic acid in Periods 2 and 3 compared to Period 1. In addition, if the increase in lactic acid was solely due to bacterial fermentation, it would be logical to expect the bacterial population to increase exponentially with time and for a corresponding increase in the concentration of lactic acid over each 50 minutes test period. This pattern was not observed in Figure 1 or 2. In addition, Table 3-2 indicates that there was no net loss of glucose from

the caecum while at the same time there was an increase in lactic acid concentration of approximately 0.12 μmol/cm².min. The data therefore indicate that it is most unlikely that fermentation of glucose could account for the apparent gain in lactic acid and suggest the movement of glucose into the test solution from the tissue. The fact that L-lactate concentration increases more rapidly than D-lactate confirms that tissue metabolism may be more important than bacterial fermentation.

Accumulation of lactic acid in the rumen and caecum occurs during rapid fermentation when the pH drops to the point where bacteria utilising lactic acid are no longer able to function normally, while those bacteria producing lactic acid thrive and continue to produce lactic acid. The fact that the lactic acid is not absorbed from the rumen or caecum means it will have a major adverse effect on pH within these compartments. It therefore appears likely that lactic acid may have its primary adverse effect on the animal through direct local effects within the gut rather than through systemic acidosis through its absorption from the gut or both effects. Julιász and Szegedi (1968) also considered the adverse effect of lactic acidosis to be a local effect of pH and excess lactic acid production. Low concentrations of D-lactate in blood resulting from carbohydrate overload may be due to the absorption of lactic acid from the small intestine, abomasum, and colon (Dobson 1961; Annison 1965; Whanger and Matrone 1966; Warner 1981). The flow of lactic acid from the rumen and its absorption from the intestine, abomasum, and colon could explain the results reported by Hueter *et al.* (1956), and Williams and Mackenzie (1965). The absorption of lactic acid from the abomasum of sheep has been reported by Herden (1980), and Huntington and Reynolds (1986). In the hindgut, the retention time of digesta in the caecum is shorter than that in the colon (Warner 1981) and lactic acid may be absorbed from the colon rather than the caecum. The final conclusion should be drawn from further studies.

Acidity (pH 4.0 - 4.5) of the rumen fluid during lactic acidosis may inhibit rumen motility (Shinozaki 1958). This will reduce the flow of rumen fluid to the small intestine and therefore limit the post-ruminal absorption of lactic acid. However, under conditions of very severe lactic acidosis, gross structural changes occur in the rumen wall in sheep (Lee *et al.* 1982), and similar changes have been reported in the caecal wall in horses (Krueger *et al.* 1986). It is likely that this damage may make it

possible for lactic acid to be absorbed directly from the rumen and caecum. This suggestion is supported by the results of Godfrey *et al.* (1992, 1995) who found that high levels of blood D-lactate only occurred under conditions of very low pH in the rumen.

On the other hand, Tsuda (1956) reported that approximately 30 - 50 percent of 0.154 M lithium lactate infused into a washed pouch of a goat rumen was absorbed after 4 hours. Firstly, the lactate may be conversed to VFAs if the bacteria were not removed clearly. Secondly, this may indicate differences between animals, effect of lactate concentration, and the role of time.

The present study shows that, in the caecum and rumen, the rate of absorption of individual VFAs was inversely related to chain length when considered in terms of the percentage of initial concentration, i.e. butyric > propionic > acetic (Table 3-1). This is consistent with the results of others (Pfander and Phillipson 1953; Williams and Mackenzie 1965; Stevens and Stettler 1966). This pattern of absorption is related to the relative lipid solubilities of the different VFAs (Bergman 1990). The mean absorption rates of VFAs from the caecum and rumen were similar to the rumen when calculated on the basis of absorptive surface area (Table 3-2). Decreasing pH and osmotic pressure in the test solution resulted in increasing absorption rate of VFAs (Figures 3-3, 3-4, and 3-5) and is a finding consistent with the results of Williams and Mackenzie (1965) and Lopez *et al.* (1994). With decreasing pH, the degree of dissociation of the acids decreases and lipid solubility increases which, in turn, increases the permeability of the ruminal and caecal mucosa for VFAs (Engelhardt *et al.* 1968).

McDonald (1948) first demonstrated the absorption of ammonia from the rumen into the ruminal venous blood. In the present study, ammonia was absorbed from the caecum about 2.5 times faster than that from the rumen, based on absorptive surface area (Table 3-2). Because ammonia is a weak base with a pKa in the vicinity of 8.80 to 9.15 (Bromberg *et al.* 1960; Bloomfield *et al.* 1963; Visek 1968), increased pH causes the ammonium to be converted to ammonia in which form it is absorbed (Hogan 1961; Bloomfield *et al* 1963; Swales *et al.* 1970; Nolan 1981). The pattern of increased rate of absorption with increasing pH was confirmed in the present study. The mean absorption rate of ammonia measured at

pH 6.5 was about 2.6 times faster in both the rumen and caecum than that at pH 4.5. The decreased absorption rate of ammonia with higher osmotic pressure is consistent with the results of Swales *et al.* (1970) who found that hypertonic solutions inhibited ammonia absorption from the rat jejunum, ileum and colon, perhaps due to change in gut permeability.

In the present study, glucose was apparently absorbed from rumen pouches, but not from caecal pouches (Tables 3-1 and 3-2). This result agrees with that of Tsuda (1956) who found that glucose appeared to be slowly absorbed from the rumen.

The concentrations of lactic acid and VFAs used in the pouches were physiological and the results show clearly and consistently that lactic acid was not absorbed under these conditions. This finding emphains the importance of lactic acid in the accumulation of acids within the gut during rapid fermentation of starch and sugars.