Chapter 1. Introduction

There has been an increase in the number of cattle lot-fed in Australia in response to an increase in the demand for grain-fed beef and there are now around 700,000 head of cattle being fed in Australian feedlots (ALFA 2003).

Beef feedlots compete in the feed-grain market with demand from the pig, poultry and dairy industries. Profitable grain-feeding of beef cattle relies on efficient feed utilisation and the extent to which grain-feeding adds value to beef quality. In addition to the economic viability of feedlot production, there are community concerns about aspects of animal welfare in intensive management systems (Leng 1999) and the environmental impact of feedlots with respect to odour and waste management. The way that the starch component of grain is digested by cattle is a central factor influencing profitability, safety and feedlot waste.

1. Profitability of grain-feeding

   The aim of a feedlot operation will always be to produce cattle to meet particular market specifications at the lowest possible cost (Oddy 1995). Feed conversion efficiency, cost of the feed ingredients and the buying and selling price of the cattle all determine the profitability of a feeding system. As starch is the primary component of cereal grains, efficient animal production relies heavily on optimal starch digestion (Theurer 1986).

2. Safety of grain-feeding

   Rapid digestion and fermentation of starch leads to the risk of fermentative acidosis and this is a serious disease condition compromising animal welfare and production efficiency. There is a delicate balance in feeding grain to cattle between the objective of maximising animal performance and the risk of animals developing acidosis. It is therefore very important to understand how grain selection, processing and amount fed to cattle will effect starch digestion.

3. Feedlot waste

   Environmental pollution from intensive industries such as beef feedlots continues to be an important public concern. There are problems associated with methane (CH_4) production, the disposal of solid and liquid waste and odour produced from the fermentation of undigested starch in faeces. It is important to minimise such adverse effects.

A practical example can be used to highlight the quantitative importance of inefficient starch digestion. If a steer eats 8 kg of grain per day (approximately 6 kg of starch), a 1% increase in whole tract starch digestibility means 60 g/d less starch lost in the faeces. On the scale of a 20,000 head feedlot, 60 g/d per animal equates to 1.2 tonnes/d across the feedlot; or 437 tonnes/year. Considering that the fermentation of each kg of starch can produce up to 200 L of gas, it is clear that there are significant implications for both odour and methane production with inefficient starch digestion. There are also consequences in terms of the efficiency of grain utilisation by the animal and profitability of the enterprise. Increasing the efficiency of starch digestion by 1% is equivalent to saving 582 tonnes of grain (containing 75% starch) worth $145,500 (at $250/tonne) for a 20,000 head feedlot.

The focus of this thesis is on methods to 'improve the efficiency of starch digestion in beef cattle' based on the hypothesis that efficiency, safety and environmental impact of grain-feeding can be managed by grain selection and appropriate processing. In the longer term, selection of animals able to more efficiently digest starch may also be important in improving the efficiency of feed utilisation. The thesis consists of a literature review and a series of experiments designed to investigate aspects of starch digestion in cattle.

The literature review covers aspects of starch digestion in ruminants with a particular emphasis on the importance of the site of starch digestion. The review incorporates an assessment of the risks associated with grain-feeding and identifies the opportunities for improving the efficiency of starch digestion.

The experiments reported in this thesis include *in vitro* and *in sacco* investigations of the fermentative and digestive properties of different grains and the effects of processing. These studies were designed to identify sources of starch that carry a relatively low risk of causing rumen acidosis but maintain a high level of digestibility in the small intestine. Feeding experiments were conducted to provide a better understanding of starch digestion in cattle and the importance of variability in hindgut fermentation. A general discussion draws on information from the literature review and the experimental work to provide conclusions that will be useful to industry as well as providing a basis for future research.

Chapter 2. Review of the Literature

2.1 Features of Cereal Grain

The physiology of cereal grains and the chemical nature of starch have a significant effect on the processes of starch digestion and absorption and on changes arising during grain processing. Figure 2.1 shows the various components of a typical cereal grain. The seed coat (or pericarp) is the outermost surface of the grain. It protects the seed against moisture, insect attack and infection (Evers *et al.* 1999). Chewing or artificial processing is essential to crack this physical barrier in order to leave the starch exposed for digestion (Orskov 1986; Kaiser 1999).

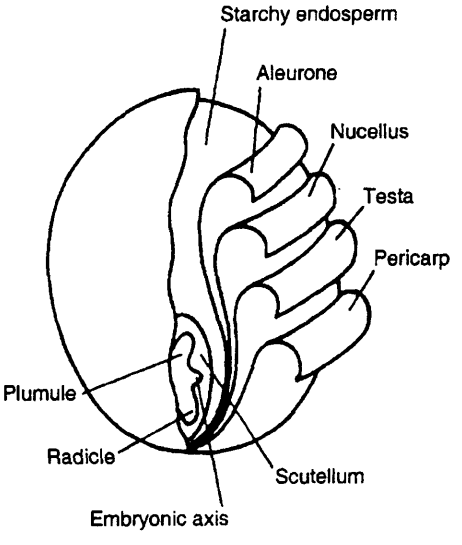
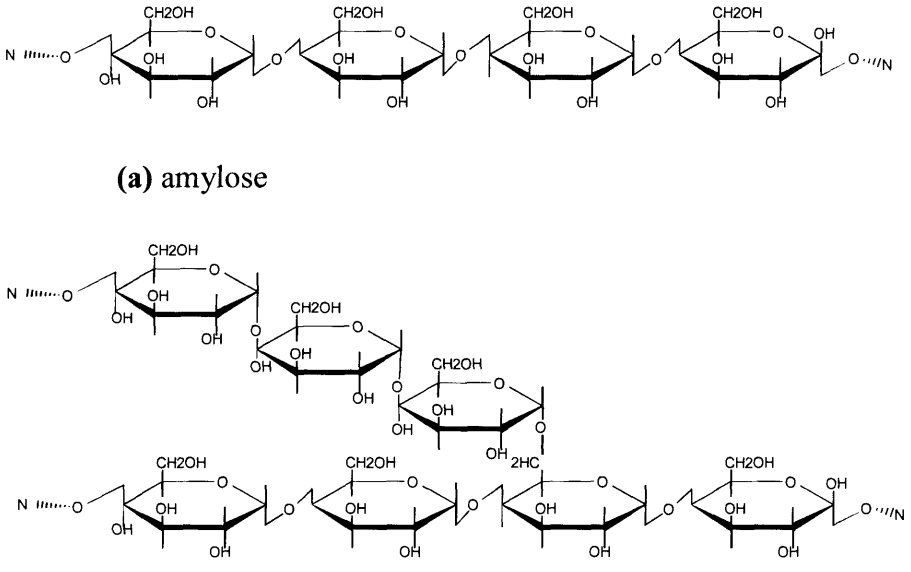


Figure 2.1 Generalised structure of a cereal grain. From Evers *et al.* (1999).

Starch is found within the area of the grain called the endosperm. The aleurone layer contains endogenous enzymes and enzyme inhibitors but no starch granules (Evers *et al.* 1999). Beneath this layer is the peripheral and corneous endosperm where starch is found embedded in a matrix consisting of protein and non-starch polysaccharides (NSP). The density and location of the protein contained within these parts of the endosperm influences the availability of starch for enzymatic digestion (Rooney and Pflugfelder 1986; Rowe *et al.* 1999). This is because the matrix can restrict the entry of water and hydrolytic enzymes. The floury endosperm, at the centre of the grain, contains a concentrated source of starch that is not embedded in a protein matrix and has little cellular structure (Huntington 1997).

Starch is a long chain polysaccharide, composed of glucose subunits, existing in two forms: amylose and amylopectin (Figure 2.2). The branched chain polymer, amylopectin, consists of α-1,4-linked D-glucose monomers with α-1,6 branch points every 20-25 glucose residues. Amylopectin is the major component of starch. The linear polymer, amylose, is made up of α-1,4-linked D-glucose monomers and is normally present in a lower concentration than amylopectin (French 1973; Rooney and Pflugfelder 1986; Rowe *et al.* 1999).



(a) amylose

(b) amylopectin

Figure 2.2 The chemical structure of (a) amylose and (b) amylopectin.

Amylopectin and amylose are held together by hydrogen bonds forming structured, starch granules (Rooney and Pflugfelder 1986). The digestive enzyme, amylase, and microbial endoglucanases are able to degrade amylopectin more rapidly than amylose because the structure of amylopectin is less crystalline and has a greater solubility than amylose (Rowe *et al.* 1999). In contrast, starch molecules in amylose chains are concentrated in tight bundles that inhibit water and enzymatic access. For this reason, starch digestibility is generally inversely proportional to the amylose to amylopectin ratio (Rooney and Pflugfelder 1986).

To manipulate starch digestion in cattle, there is a need to understand the principles of starch digestion as well as ruminant physiology relating to between-animal differences in the capacity to digest starch. There are three sites of starch digestion: the rumen; small intestine; and large intestine or hindgut (caecum and colon). Fermentative digestion by microorganisms occurs in

the rumen and hindgut whereas endogenous enzymes are involved in starch digestion in the small intestine.

2.2 Digestion and Fermentation of Starch in the Rumen and Hindgut

2.2.1 Introduction

When wheat, barley or oat-grain is fed to ruminants in the whole or dry rolled form, up to 90% of the starch can be digested in the rumen (Waldo 1973; Orskov 1986). The rumen is the initial site of digestion where the most accessible starch is usually fermented (Owens *et al.* 1986). The rate and extent of digestion in the rumen is important for two reasons. Firstly, the extent of rumen digestion governs the quantity and composition of starch entering the small intestine. Secondly, the risk of acidosis is closely related to the rate at which starch is fermented.

In most grain-feeding scenarios, a proportion of starch will escape digestion in the rumen. Some of this starch is not digested in the small intestine and passes into the hindgut. An assessment of the consequences of delivering more starch to the hindgut is important, as an increased starch supply to the hindgut can reduce overall efficiency of feed utilisation through energy loss during fermentation and increase the risk of fermentative acidosis (Rowe 1999). For these reasons, this section deals with fermentation of starch in both the rumen and the hindgut.

2.2.2 The rumen

The anaerobic rumen environment provides for extensive pre-gastric fermentation. The four stomachs of cattle make up approximately 71% of the total volume of the digestive tract (Bayley 1978). The volume of the rumen makes it the largest site of starch digestion in cattle. A 500 kg cow has at least 70 kg of rumen contents. Each day, approximately 60 L of saliva and a further 40 L of water pass through the rumen (Wolin 1981). Saliva contains large amounts of bicarbonate (HCO_3^-) and hydrogen phosphate ($H_2PO_4^-$) ions (Allen 1997). Consequently, the rumen is generally well buffered (Hungate 1966). The pH of the rumen is constantly fluctuating around an average of 6.4 and it is maintained at a constant temperature (39 – 40^{0}C) (Hungate 1966). The rumen wall is thick and muscular and the interior surface is a non-glandular keratinised, stratified squamous epithelium (Fell and Weekes 1975; Leek 1993; Rowe 1999). The structure of the rumen wall is important because this is the first part of the animal that has contact with acidic gut contents (Rowe 1999). The inner wall of the rumen is arranged in projections called papillae which greatly increase the surface area available for absorption of the end-products of fermentation (Hungate 1966). There is a continuous supply

of substrate/nutrients to the rumen that are generally well-mixed by muscular contractions and rumination.

Rumination is the physiological process of regurgitation, re-mastication and re-swallowing of reticular digesta (Hungate 1966). This serves to reduce digesta particle size and increase the surface area available for microbial attachment. Rumination also increases the buffering in the rumen because more saliva is added when digesta is re-masticated and re-swallowed (Hungate 1966; Allen 1997). In general, cattle ruminate for less than 10 h/d (Welch 1982) but this depends on what they are eating. Fibrous feeds of low digestibility require more rumination time for particle size reduction than concentrate-based feeds (Welch and Smith 1970).

There is a dense and diverse microbial population in the rumen including anaerobic bacteria, protozoa and fungi (Allison 1993). The relative biomass of different species depends on the substrate that is available and the rate of passage of digesta through the rumen. There are many interactions that occur within the microbial community. For example, some amylolytic bacteria can only ferment pyruvate to produce lactic acid. Other bacteria can then use lactic acid as a substrate and convert it to propionate (Leek 1993). Integration among bacterial species is required to maximise digestion of complex substrates because different species produce different enzymes and specialise in different parts of the digestive system (Allison 1993; Huntington 1997).

Bacteria perform the majority of digestion and fermentation in the rumen (Huntington 1997). *Streptococcus bovis*, *Lactobacillus* spp. and *Selenomonas ruminantium* are some of the key starch-degrading bacterium that are also implicated in the development of acidosis (Al Jassim and Rowe 1999). Bacteria need to attach to feed particles before digestion can commence (McAllister *et al.* 1994). The process of bacterial attachment is inhibited by the seed coat (Huntington 1997) but this is usually cracked during grain processing, mastication and rumination (McAllister *et al.* 1994).

Protozoa and fungi also exist in the rumen. Cattle fed all-concentrate diets *ad libitum* have been reported to have few, if any, protozoa in the rumen (Slyter 1976). However, more recent evidence indicates that protozoa can be present in significant numbers in the rumen of grain-fed cattle (Towne *et al.* 1990). Protozoa engulf and digest starch granules and any bacteria that may be attached (Hungate 1966; Huntington 1997). Mendoza *et al.* (1993) observed that the elimination of rumen protozoa (defaunation) improved starch digestibility in the rumen of sheep and suggested that the presence of protozoa may be advantageous if a reduction in the rate of starch digestion alleviates acidosis-related problems. Fungi produce hyphae that deeply

penetrate plant tissue (McAllister *et al.* 1994) and this activity exposes plant tissue that would otherwise be inaccessible to bacteria and provides a larger available surface area for bacterial attachment.

Rumen microorganisms require nitrogen and 80-90% of bacteria can grow on ammonia (NH_3) alone (Bryant and Robinson 1962). Even when ammonia nitrogen is not limiting, the supply of dietary peptides is important because nitrogen input from peptides and amino acids improves microbial growth (Argyle and Baldwin 1989) and efficiency (Cotta and Russell 1982; Fu *et al.* 2001; Kajikawa *et al.* 2002). Non-structural carbohydrate-fermenting bacteria require 67% of their nitrogen input from amino acids and peptides to optimise microbial growth and efficiency (Russell *et al.* 1983; Russell *et al.* 1992).

Sulphur is also essential to enable microorganisms to produce sulphur-containing amino acids such as cysteine and methionine. Microbial cells contain approximately 32% true protein, 10% small molecules (mainly nitrogen), 8% nucleic acids, 9% cell wall, 11% lipid, 17% polysaccharide and 13% ash (Czerkawski 1986) however storage polysaccharide content is variable (Nolan 1993).

Pathways of carbohydrate fermentation

The fermentable carbohydrate component of roughage diets comprises soluble and insoluble NSP. In pasture plants, most of the insoluble NSP is present as cellulose (Theander 1989). Polysaccharides are hydrolysed into monomers by the endoglucanases produced by rumen microbes and when animals are fed a grain-based diet, amylolytic bacteria and protozoa produce hydrolytic enzymes to degrade the starch.

Fermentation produces energy in the form of adenosine triphosphate (ATP) and carbon skeletons that are essential for microbial cellular growth. Heat is released during fermentation and volatile fatty acids (VFA), carbon dioxide (CO_2) and methane are end-products of the fermentation process. These end-products are of no use to the rumen microbes (Wolin 1981) and, with the exception of VFA, are also a waste to the animal.

There are a number of steps involved in the production of VFA. Carbohydrates must initially be hydrolysed into monosaccharides in an extracellular process by rumen microorganisms. These smaller sub-units are then converted to fructose-1,6-diphosphate before being degraded to pyruvate under anaerobic conditions (Leek 1993). Pyruvate plays a central role in the production of the major VFAs (acetate, propionate and butyrate) within microorganisms. The pathways are shown in Figure 2.3.

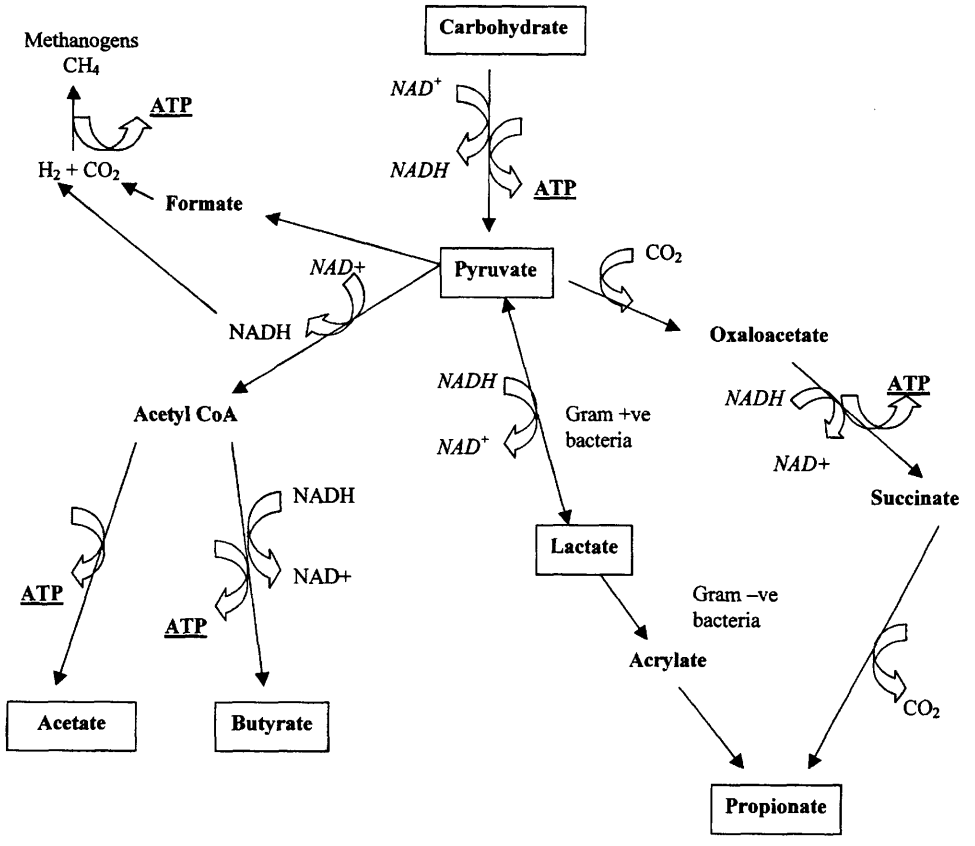


Figure 2.3 Microbial fermentation of carbohydrates in the rumen. From Hungate (1966) and Wolin (1981).

The animal absorbs and utilises VFA as the major source of energy and carbon for cell metabolism (Hungate 1966; Wolin 1981). Undissociated VFAs are absorbed by passive diffusion whereas anions are absorbed via facilitated diffusion in exchange for bicarbonate. At a rumen pH of 6.6, the anion to undissociated acid ratio is approximately 100:1 (Leek 1993). VFAs are absorbed at a faster rate when rumen pH is reduced because there is a greater amount of the undissociated acid at lower pH. The rumen epithelium uses VFA as an energy substrate and butyrate is converted into ketone bodies (β-hydroxybutyrate, aceto-acetate and acetone), acetate and carbon dioxide in the epithelium of the rumen (Fell and Weekes 1975; Leek 1993).

Acetate and butyrate are generally the major substrates used in oxidative metabolism within animal cells. Acetate is converted to acetyl CoA in body tissues and enters the citric acid cycle in this form of energy. On the other hand, propionate is an important substrate for glucose synthesis in the liver (Wolin 1981).

Within the rumen, fermentation pathways that utilise hydrogen are important as the reduced co-enzyme, NADH is produced during the catabolism of carbohydrates (Figure 2.3). The continual oxidation of this reduced pyridine nucleotide is essential for the fermentation process to continue. Therefore, to recycle the hydrogen carrier, oxaloacetate is reduced to malate then propionate. Hydrogen is also incorporated into cell polymers and removed by methanogens (Wolin 1981; Orskov 1986).

Fermentation of starch to propionate is a more efficient pathway for the animal than the production of acetate (Orskov 1975). This is because the pathway of propionate production utilises hydrogen whereas the production of acetate generates hydrogen that is lost from the body in the form of methane (Hungate 1966; Sutherland 1977). VFAs derived from the fermentation of cellulose are generally in the ratio of 70:15:10 for acetate, propionate and butyrate whilst the corresponding ratio for concentrate diets is typically 55:25:15 (Hungate 1966; Sutherland 1977; Orskov 1986; Leek 1993).

Methanogenic bacteria convert molecular hydrogen and carbon dioxide to methane with the production of ATP. Eructation eliminates methane and up to 10% of the digestible energy (DE) intake can be lost from the animal in this way (Wolin 1981). In addition to the negative aspect of lost energy, it is estimated (Howden and Reyenga 1999) that methane from ruminants constitutes 12% of Australia's greenhouse gas emissions. The environmental concern over methane and the greenhouse effect has stimulated interest in methods of decreasing methane production (Klieve and Hegarty 1999).

Protein and nitrogen in the rumen

Protein is hydrolysed into peptides and amino acids in the rumen (Sutherland 1976). These can then be directly utilised to build microbial protein or they may be subsequently deaminated to produce VFA and ammonia (Nolan and Leng 1972; Leng and Nolan 1984; Nolan 1993). Non-protein-nitrogen such as urea is also hydrolysed to ammonia in the rumen (Leek 1993). The extent of protein fermentation is influenced by the source of protein, its rate of solubilisation, processing treatment and factors such as particle retention time in the rumen (Hogan and Hemsley 1976; Sutherland 1976).

When ammonia is absorbed across the wall of the rumen it is converted by the liver to urea. Urea is excreted in urine but may also be returned to the rumen via the blood or saliva (Leng and Nolan 1984; Neutze *et al.* 1986; Nolan 1993).

Microbes draw on the pool of carbon intermediates created during digestion to produce monomers for the synthesis of cell biomass (Leek 1993). Microbial protein produced in the

rumen depends on the ATP produced in fermentation and availability of amino acids, peptides, ammonia and sulphur (Nolan 1993). Microorganisms that flow from the rumen can be digested in the small intestine and provide an important source of protein, B vitamins, polysaccharide and lipid for the animal (Wolin 1981).

2.2.3 The hindgut

Undigested dietary components, endogenous secretions and rumen microbes that escape digestion in the small intestine can all potentially be digested in the hindgut. The hindgut comprises the following regions: caecum, proximal colon, centripetal and centrifugal coils of the spiral colon, distal colon and rectum (Ulyatt *et al.* 1975). In contrast to the rumen, the hindgut mucosa has no villi however goblet cells secrete mucous to protect the epithelium and aid movement of digesta (Ulyatt *et al.* 1975). The organ is maintained at a relatively neutral pH (Ulyatt *et al.* 1975) although this does vary with diet. The caecum and to a lesser extent the proximal colon, are the most important sites of fermentation and absorption in the hindgut (Argenzio 1993b). The evidence is provided by the observations that VFA concentrations, organic matter (OM) and ammonia flow tend to decline as digesta progresses through the hindgut (Williams 1965; Dixon 1978).

Ulyatt *et al.* (1975) suggested that there are at least four types of activity that govern the motility of the caecum – localised contractions, regular coordinated contractions, peristaltic contractions which move digesta into the proximal colon and 'total' contractions which completely empty the caecum. MacRae *et al.* (1973) found that the contractions appear to originate at the ileo-caecal junction or from the blind pole of the caecum.

It is not known exactly how well digesta in the caecum and proximal colon are mixed but there is little mixing of digesta beyond the proximal colon (Ulyatt *et al.* 1975). Faichney (1969) and MacRae *et al.* (1973) found that radio-opaque materials introduced via ileal or caecal cannula in sheep became thoroughly mixed with the digesta in the caecum and proximal colon. Grovum and Hecker (1973) observed a DM gradient between the caecum and the distal portion of the proximal colon in sheep which suggests that the caecum and proximal colon are not a perfectly mixed pool. The findings of Grovum and Hecker (1973) also demonstrate the capacity for water absorption in the hindgut which is facilitated by the creation of an osmotic gradient during the active absorption of Na^+, K^+ and Cl^- ions (Hoover 1978). Most of the water that enters the hindgut is absorbed (Goodall and Kay 1965).

Digesta intermittently fills and empties from the caecum (Ulyatt *et al.* 1975). The flow of digesta to the caecum is influenced by the diet and the level of feed intake and is not believed

to be related to feeding activity or rumination (Goodall and Kay 1965). The volume of hindgut contents can be up to 20% of the volume of rumen contents (Hoover 1978).

The capacity for digestion in the hindgut is high but not limitless (Orskov *et al.* 1970b). Despite the fact that the substrate has already been subjected to rumen and intestinal digestion, 30 to 60% of the digesta DM reaching the hindgut may still be fermented (Owens *et al.* 1986). Karr *et al.* (1966) reported that approximately 11% (296 g) of dietary starch was digested in the hindgut of steers fed a diet containing 80% ground corn. Similarly, when a diet of 80% ground corn was fed to lambs, 7% (33 g) of dietary starch was digested in the hindgut (DeGregorio *et al.* 1982). In this study, 96% of the starch that reached the hindgut was digested there.

Patterns of fermentation in the hindgut

While the basic principles of digestion and fermentation in the hindgut are the same as in the rumen, there are some important differences between these sites in terms of the nature of the substrate and residence time (Orskov *et al.* 1970b; Allison *et al.* 1975; Wolin 1981). Compared to rumen microbes, the microbes in the hindgut are capable of producing more VFA from a pre-fermented substrate (Hoover 1978). Hindgut microbes generally have to scavenge the residues that have resisted digestion to reach this point (Bayley 1978) including cell wall components of bacteria and resistant plant material such as cellulose and lignin. Furthermore, the turnover time of digesta in the caecal pool is faster than in the rumen (Grovum and Williams 1973). Consequently, hindgut microorganisms have less time than rumen microorganisms in which to digest a substrate. There are no protozoa present in the caecum of ruminants (Kern *et al.* 1974; Ulyatt *et al.* 1975).

Compared to the rumen, the molar proportion of acetate is often higher and propionate lower in caecal contents (Williams 1965; Faichney 1968; Orskov *et al.* 1970b; Sharpe *et al.* 1975). Furthermore, DeGregorio *et al.* (1982) found that the pH of the hindgut was always about 1 pH-unit higher than rumen pH on both concentrate and forage diets. These two observations could be explained by the lower starch to fibre ratio in the digesta normally reaching the caecum compared to the rumen. Further evidence that the typically higher acetate concentration and pH in the hindgut is principally a substrate effect is provided by observations that infusions of starch directly into the caecum produce VFA proportions that are similar to those found in the rumen (Orskov *et al.* 1970b; DeGregorio *et al.* 1982) (Table 2.1).

It is generally considered that the host animal is not able to absorb microbial amino acids from the hindgut (Orskov *et al.* 1970b; Mason 1984). Undigested microbial cells (protein) and feed residues are excreted in the faeces and lost from the animal.

There is evidence that ammonia nitrogen can be absorbed from the hindgut (Nolan and Leng 1972). The capacity to recycle nitrogen is particularly important for ruminants eating low-nitrogen diets as maintenance and production of the microbial population can continue without any true dietary protein (Virtanen 1966; Neutze *et al.* 1986). A low pH in the hindgut would hinder the process of absorption because ammonia diffuses freely through tissues as the non-ionic ammonia, instead of the ammonium ion (NH_4^+) (Hoover 1978).

2.2.4 Starch digestion the hindgut

An increase in the proportion of grain in the diet usually results in more starch passing to the hindgut (DeGregorio *et al.* 1982; Siciliano-Jones and Murphy 1989b; Siciliano-Jones and Murphy 1989a). Level of feed intake (Grovum and Hecker 1973; Galyean *et al.* 1979; Huntington 1994) and the characteristics of particular grains and processing methods (Karr *et al.* 1966) also influence the amount of starch reaching the hindgut. Between 18 and 42% of the available starch in corn and sorghum-based diets has been reported to reach the small intestine (Owens *et al.* 1986). Similarly, heavily steam flaked grain is likely to be extensively fermented in the rumen but poorly flaked grain may deliver more starch to the small and large intestines. This has been demonstrated in cattle fed corn (Theurer *et al.* 1999b) and sorghum (Swingle *et al.* 1999). In these experiments, animals were fed relatively low-starch (generally <4 kg/d) diets and 2% of whole tract starch digestion occurred in the hindgut. Such intake levels may not truly reflect the starch intake of feedlot cattle.

There are a number of faecal measurements including faecal pH, DM and nitrogen content that may be useful for estimating the pattern of hindgut fermentation in a simple, non-invasive, and inexpensive way. Changes in caecal and faecal characteristics in response to starch escaping digestion in the rumen and small intestine are discussed in the following sections.

Acid concentrations

The information presented in Table 2.1 shows changes resulting from increasing levels of starch digestion in the hindgut. More starch digestion in the hindgut increases total VFA production (DeGregorio *et al.* 1982; Siciliano-Jones and Murphy 1989b) and the proportion of propionic acid while the proportion of acetic acid decreases (Ward *et al.* 1961; Orskov *et al.* 1970b; DeGregorio *et al.* 1984; Godfrey *et al.* 1992).

Table 2.1 Starch fermentation in the caecum of lambs fed diets containing three levels of corn. From DeGregorio *et al.* (1982).

Corn in diet (%)	Starch intake (g/d)	Starch digested in hindgut (g/d)	Total VFA (mM)	Acetate/ Propionate ratio	Lactic acid (mM)	Hindgut pH
0	67.5	0.7	46.3	4.7	0.4	7.2
40	263.7	17.1	91.2	3.2	0.6	6.7
80	486.9	33.0	134.8	2.6	0.7	6.0
Average	272.7	16.9	90.8	3.5	0.6	6.6

In a comparison of caecal VFA in sheep fed either dried grass or barley, Orskov *et al.* (1970b) showed that sheep eating grain had a decreased proportion of acetate and an increased proportion of propionate and butyrate. Similarly, sheep fed an 80% corn diet were shown to have a larger pool of caecal propionate and a faster rate of propionate and acetate production than sheep fed a 100% hay diet (DeGregorio *et al.* 1984). Caecal lactate concentrations have also been shown to rise in response to an increased supply of starch to the hindgut in cattle (Siciliano-Jones and Murphy 1989b) and sheep (DeGregorio *et al.* 1982).

Hindgut and faecal pH

Caecal and faecal pH in grain-fed sheep have been shown to be lower than that of sheep fed no grain (DeGregorio *et al.* 1982). Digesta pH has been reported to rise from the caecum along the colon and to the faeces in sheep (Lee 1977; Godfrey *et al.* 1992; Van Kessel *et al.* 2002) and in pigs (Pluske *et al.* 1998). In grain-fed sheep there is a clear association between faecal and caecal pH (Clayton and Rowe 1999) whilst Wheeler and Noller (1977) found a relationship (R^2=0.97) between faecal and colon pH in grain-fed cattle. Similarly, the data reported by Van Kessel *et al.* (2002) suggests a strong correlation between caecal and faecal pH (R^2=0.97) in cattle fed diets containing 90% hay.

The available information suggests that an inverse relationship exists between faecal starch concentration and faecal pH (Galyean *et al.* 1979; Zinn *et al.* 2002). However, the strength of the association appears to be variable. A number of researchers have reported significant correlations between faecal starch content and faecal pH: in steers fed diets based on whole shelled corn (r=-0.35) (Russell *et al.* 1980); steam flaked sorghum (r=-0.51) (Xiong *et al.* 1991); dry rolled barley, whole corn or cracked corn (r=-0.82) (Wheeler and Noller 1977); high

moisture corn (r=-0.94) (Wheeler and Noller 1977); dry rolled corn (r=-0.58) (Barajas and Zinn 1998) and steam flaked corn (r=-0.39) (Barajas and Zinn 1998).

Nitrogen

An increased supply of starch to the hindgut enhances the supply of energy, increases microbial activity and promotes the incorporation of ammonia into microbial protein (Mason *et al.* 1981; Mason 1984). Ammonia can be absorbed from the hindgut but microbial protein is not (Orskov *et al.* 1970b). Therefore, an increase in faecal microbial nitrogen (Orskov *et al.* 1970b; Orskov *et al.* 1972; Knowlton *et al.* 1998) and a reduction in caecal ammonia concentration (DeGregorio *et al.* 1982; Siciliano-Jones and Murphy 1989b) are likely to accompany an increase in the starch supply to the hindgut. This would generally result in reduced whole tract nitrogen digestibility.

Dry matter

An increase in caecal VFA concentrations will cause caecal osmolality to rise (Siciliano-Jones and Murphy 1989b) and during acidosis, the high osmotic potential of the gut contents causes diarrhoea (Huber 1976; Lee 1977; Godfrey *et al.* 1992). Sheep fed wheat have been shown to have a lower DM content in the colon than sheep fed lucerne (Lee 1977). Therefore, firm faeces (high faecal DM content) in cattle on grain-based diets could be a sign of reduced hindgut starch digestion and efficient pre-caecal digestion.

Hindgut fermentation is not the only factor that affects faecal DM content. The DM content of faeces in sheep has been observed to increase with infusion of large quantities of starch into the caecum (Orskov *et al.* 1970b). The authors believed the capacity for hindgut starch fermentation was overwhelmed when increments of starch in excess of 138 g/d were introduced into the hindgut and suggested that the excess starch was excreted in the faeces, thus increasing faecal DM content.

Faecal starch content

There appears to be a clear association between faecal starch concentration and whole tract starch digestibility (R^2=-0.95) in steers fed dry rolled and steam rolled wheat diets (Zinn 1994). In a summary of 64 trials involving steam flaked corn, Zinn *et al.* (2002) reported that the direct determination of faecal starch content explained 68% of the variation in rumen starch digestion, and 91% of the variation in whole tract starch digestion. The strength of this relationship suggests that measurement of faecal starch content could be used as a practical tool

for assessing the efficiency of grain processing and for determining differences between animals in the extent of rumen or whole tract starch digestion.

2.3 Fermentative Acidosis

2.3.1 Introduction

Acidosis (grain-poisoning) is a well-known risk associated with feeding grain-based diets to ruminant animals. Elam (1976) defined acidosis as *'a syndrome in ruminants which is brought about by excessive ingestion of feeds which are rich in readily available carbohydrates, such as starch and sugar'*. The condition can range from mild (sub-clinical or sub-acute acidosis) to extreme (clinical, acute, or lactic acidosis) depending on the amount of starch consumed and the rate at which it is digested then fermented (Nocek 1997; Rowe 1999). The fact that the severity of the problem is a continuum makes discussion of the consequences of acidosis difficult. Severe acidosis can often be fatal and will certainly have detrimental effects on animal production and health (Nocek 1997) whereas the effects of sub-clinical acidosis are more difficult to identify (Goad *et al.* 1998). Nonetheless, sub-clinical acidosis is believed to cause major reductions in the efficiency of feed utilisation in feedlot cattle (Beauchemin 2000) and it is possible that hindgut acidosis may also be important in the overall pathogenesis of grain-poisoning (Lee 1977; Godfrey *et al.* 1992).

The risk of acidosis varies dramatically depending on interrelated animal and dietary factors (Rowe 1997). These issues are discussed later in the review of factors that determine the extent/site of starch digestion. The aim of this section is to describe how and where acidosis arises in ruminants and the consequences for animal production. This major problem emphasises the importance of efficient small intestinal starch digestion and the impact this can have on overall efficiency of feed utilisation.

2.3.2 Cause of acidosis

Acidosis follows a common pattern of development when a large amount of fermentable starch enters the rumen. The rate of fermentation initially increases and there is an increase in VFA concentration. The problem begins when the ability of the animal to remove or neutralise acids produced from microbial fermentation is outweighed by acid production (Al Jassim and Rowe 1999; Beauchemin 2000). Acid accumulation causes rumen pH to decline towards 6.0 and this environment favours the growth of starch-degrading bacteria (Figure 2.4).

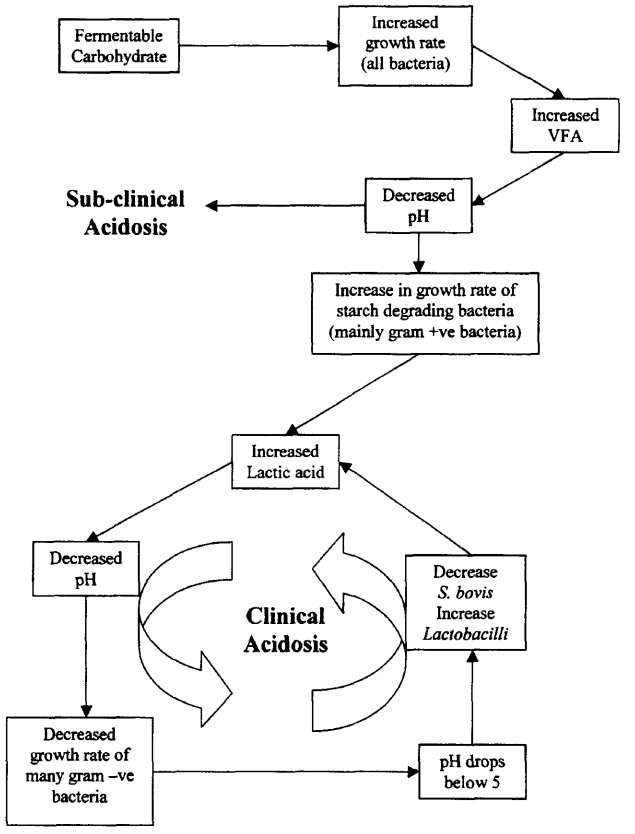


Figure 2.4 The chain of events leading to acidosis in the rumen or hindgut. From Nocek (1997).

Streptococcus bovis has been identified as playing a key role in starch fermentation and produces large amounts of acids, particularly L-lactate (Al Jassim and Rowe 1999). Intracellular concentrations of fructose-1,6-diphosphate in *Streptococcus bovis* are increased when the bacterium achieves high growth rates. This appears to activate the enzyme, lactate dehydrogenase and the organism then begins to produce lactic acid in preference to acetate (Russell and Hino 1985). *Lactobacillus vitulinus* and *Selenomonas ruminantium* are other important lactic acid-producing bacterium. The latter appears to be both a lactate producer and user, depending on the substrate.

Lactic acid is an intermediate in propionic acid production and the conversion is rapid and thermodynamically desirable under normal fermentation conditions (Sutherland 1977; Rowe 1997). Lactic acid is not usually absorbed across the rumen or hindgut (Ding 1997) and it is normally entirely dissociated at the normal pH of the rumen and blood (Huber 1976). D-lactic acid and L-lactic acid are the two isomers of lactic acid with the former being more slowly metabolised in the rumen (Giesecke and Stangassinger 1979; Nocek 1997). The main bacteria responsible for utilising lactic acid are gram-negative and are much more sensitive to low pH

than the gram-positive, lactic acid producers (Rowe 1997). The lactate utilisers are normally not present in sufficient numbers to cope with the levels of lactic acid being produced and/or they simply cannot function properly at the low pH. The production of lactic acid then quickly exceeds its rate of conversion into propionate. Lactic acid has a much lower dissociation constant than the VFAs (Russell and Hino 1985; Russell 1999) and has a profound effect on reducing pH when it accumulates (Leek 1993).

As pH falls below 6.0 the growth rate of *Streptococcus bovis* declines but the organism continues to produce lactic acid (Russell and Hino 1985). This is because when extracellular pH decreases, there is also a decline in intracellular pH which allows lactate dehydrogenase to remain functional preventing high concentrations of fructose-1,6-diphosphate (Russell and Hino 1985). Furthermore, pyruvate formate lyase is inhibited by low pH and the pathway for VFA production is effectively blocked (Russell and Hino 1985). *Lactobacilli* eventually take over from *Streptococcus bovis* and pH can decrease below 5.5 (Russell 1999). Wells (1997) hypothesised that this shift in the microbial population was due to the destruction of *Streptococcus bovis* by a bacteriocin produced by *Lactobacilli*, as opposed to differences in pH-resistance between the strains of bacteria.

2.3.3 Effects of acidosis

Acidosis reduces feed digestibility, animal production and compromises animal health and welfare. The severity of these effects varies according to acid load (Allison *et al.* 1964). Many of the following effects of acidosis are also closely interrelated.

Reduced pH

The normal buffering capacity of the rumen can be quickly exceeded when there is rapid production of acid (Slyter 1976). Urine and blood pH may also decrease if the acidic contents of the gut leak into the circulatory system. Blood pH falls to below 7.4 following a decrease in the base content of body fluids relative to acid content (Owens *et al.* 1998).

Acidity in the gut has a negative effect on fibre digestion (Slyter 1976) because the major cellulolytic bacteria (*Ruminococcus albus*, *Ruminococcus flavefaciens* and *Fibrobacter succinogens*) cannot function normally below pH 6.0 (Hungate 1966; Russell and Wilson 1996).

Rumen stasis

The amplitude and frequency of rumen contractions are reduced when rumen pH decreases below 5.0 (Elam 1976; Huber 1976). Inhibition of normal gut movement may be a protective

mechanism as the motility of the rumen influences the absorption of lactic acid (Huber 1976). Bruce and Huber (1973) suggested that when acid reaches the small intestine, hydrogen receptors in the duodenum release secretin which inhibits the motor activities of the forestomach. Intestinal motility may also be reduced during acidosis (Slyter 1976). In addition, more fermentable diets will increase rumen fluid osmolality due to a higher concentration of VFA. Osmolality values greater than 0.35 osmol have been reported to inhibit the normal functioning of the rumen (Welch 1982) but the mechanism behind this association is unclear.

Rumen stasis will decrease rumen dilution rate. A reduction in the motility of the rumen means a reduction in microbial synthesis will coincide with rumen stasis and less microbial protein will become available to the animal (Slyter 1976).

Reduced feed intake

Reduced feed intake is a direct result of low rumen pH (Cooper *et al.* 1999). Feed consumption quickly decreases as a consequence of rumen stasis and reduced fibre digestion because the gut remains distended (Elam 1976; Russell 1999). Reduced feed intake is typically one of the first physical signs that an animal is suffering from acidosis but this cannot be used as a diagnostic test for acidosis on its own.

Damage to the mucosa

The epithelium and lamina propria thicken in response to acidic conditions and this leads to the development of parakeratosis (Kay *et al.* 1969). This condition involves the excessive accumulation of keratinised epithelium (Hinders and Owen 1965). Rumen papillae clump and become necrotic and this is associated with ruminitis (Hinders and Owen 1965; Orskov 1986). There is a cost to the animal in maintaining the integrity of the gut and repairing damage associated with acid in the lumen. Repaired gut tissues will thicken and this effectively reduces the surface area available for absorption of VFA (Hinders and Owen 1965; Beauchemin 2000). In this way, an animal may never fully recover from a severe bout of acidosis.

Tissue damage has been shown to affect the permeability of the rumen wall of sheep (Lee *et al.* 1982) and the caecum of horses (Krueger *et al.* 1986). This leads to leakage of acids from the gut into the circulatory system and to compromised resistance to endotoxins, pathogens and parasites (Fell and Weekes 1975; Dougherty *et al.* 1975a; Owens *et al.* 1998). Lactic acid has a toxic effect when it enters the body of the animal (Godfrey *et al.* 1992). Liver abscesses in grain-fed cattle are an indication that bacteria have leaked into the animal's circulatory system. The major agent leading to liver abscesses is the lactate-utilising *Fusobacterium necrophorum*

(Russell 1999). Impaired liver function is likely to adversely affect the efficiency of production and carcass value (Nagaraja and Chengappa 1998).

A cytokine (inflammatory) and immune system response is likely to develop in response to tissue damage in the gut (Rowe 1999). This is a further cost to the animal as energy and protein are required to sustain such a response (Rowe 1997). Blood flow and hence, VFA absorption, may also be inhibited by an inflamed rumen wall (Huber 1971; Russell 1999).

Endotoxins

The growth rate of individual species of bacteria is pH-dependent (Russell *et al.* 1979). When pH decreases, gram-negative bacteria die and are replaced by a population of gram-positive bacteria. Death of bacteria is likely to result in elevated levels of microbial endotoxins (cell wall lipopolysaccharide) (Dougherty *et al.* 1975a).

Lameness/laminitis

Laminitis in horses is closely linked to acidosis caused by rapid hindgut fermentation (Garner *et al.* 1977). Lameness can also be a serious problem in grain-fed cattle suffering from clinical and sub-clinical acidosis (Nocek 1997). It appears that a systemic acid load in the body causes the release of vasoactive substances that trigger local mechanical changes and damage in the foot (Nocek 1997).

Disruption to acid-base and electrolyte balances

VFA and lactic acid contribute hydrogen ions to the host animal when absorbed into the body. Hydrogen ions have a direct effect on the systemic acid-base balance and on the balance between anion and cation concentrations (Rowe 1999). The kidneys play a critical role in maintaining the acid-base balance of the animal and in ruminants, the major mechanism for excretion of surplus acid is in the form of ammonium ions (Scott 1975). Amino acids need to be converted to ammonia before hydrogen can be excreted via this process (Baggott 1992) and this eventually constitutes a reduced supply of protein in the animal (Rowe 1999).

Increase in acid-resistant pathogens

Grain-fed cattle have been shown to have higher numbers of *Escherichia coli* in their faeces than cattle on molasses or roughage-based diets (McSweeney *et al.* 2002). *Escherichia coli* acid-resistance has also been shown to increase directly with acidity in the colon (Diez-Gonzalez *et al.* 1998). It is possible that animals on high-grain diets with fermentative acidosis in the hindgut can infect themselves with acid-resistant, coliform bacteria like *Escherichia coli*

that are capable of producing sub-clinical infection in the small intestine (Russell 1999). An infection of this nature would increase the requirements of the animal's immune system and, at the time of slaughter, the presence of *Escherichia coli* could pose a contamination/food safety risk for humans (Diez-Gonzalez *et al.* 1998).

2.3.4 Sub-clinical acidosis

Whilst clinical acidosis results in sick animals, ruminants can also experience sub-clinical acidosis (Slyter 1976; Rowe 1999). In this situation, animals generally appear to be healthy but feed intake and performance is reduced. Sub-clinical acidosis is believed to lead to chronic disease conditions such as diarrhoea and laminitis (Nocek 1997) which are readily observed in many feedlot cattle.

Sub-clinical acidosis has been defined to occur when rumen pH reaches 5.8 (Beauchemin 2000) or 5.5 (Goad *et al.* 1998) whereas clinical acidosis begins at a pH of 5.0. Reticulo-rumen pH values <5.8 have been reported for grain-fed cattle showing no clinical signs of acidosis (Wheeler and Noller 1977). Later examination of the rumen wall indicated that parakeratosis had occurred in some of these animals. After a feeding bout, the time for rumen pH to decrease to sub-clinical levels and remain low depends on many factors including the nature of the grain, the level of roughage and the buffering capacity of the animal. While it is common for rumen pH to drop below 5.8 within 2-3 h after feeding, it is the length of time that pH remains this low that dictates the severity of sub-clinical acidosis. The importance of diurnal fluctuations in rumen pH is uncertain although it does appear that sub-clinical acidosis persists for short periods during the day (Beauchemin 2000). In dairy cattle given total mixed diets, rumen pH drops after the morning feed and then begins to steadily rise. The second meal in the afternoon generally reduces rumen pH to a lower level than after the morning feed (Beauchemin 2000). Koenig *et al.* (2003) reported that the average time rumen pH was less than 5.8 in steers fed steam rolled barley-based diets was 10.5 h/d.

Lactic acid concentrations are commonly less than 5 mM during sub-clinical acidosis (Goad *et al.* 1998) and it appears that increased gut acidity during sub-clinical acidosis is more likely to be caused by high concentrations of VFA (Koenig *et al.* 2003). Goad *et al.* (1998) reported that VFA concentrations greater than 120 mM were causing sub-clinical acidosis 36 h after a grain challenge.

Sub-clinical acidosis is a particular problem in dairy cows and is estimated to cost the United States dairy industry between $500 million and $1 billion a year (Donovan 1999 cited by Beauchemin 2000). The condition may also affect early-entry feedlot cattle where fibre

constitutes a relatively large proportion of the diet. Modern feedlot diets typically contain at least 10 to 15% forage and even cereal grains contain more than 10% fibre (Russell and Wilson 1996). Hence, efficient fibre digestion is important in cattle given grain-based diets.

2.3.5 Hindgut acidosis

Feeding cattle grain-based diets may predispose them to acidic hindgut conditions. The colonic pH of cattle fed a diet containing more than 80% dry rolled corn (5.9 ± 0.6) was shown to be significantly lower than that of cattle eating hay (7.2 ± 0.1) or fresh grass (7.1 ± 0.1) (Diez-Gonzalez *et al.* 1998). Evidence for the increased incidence of hindgut acidosis rather than rumen acidosis is presented in Figure 2.5.

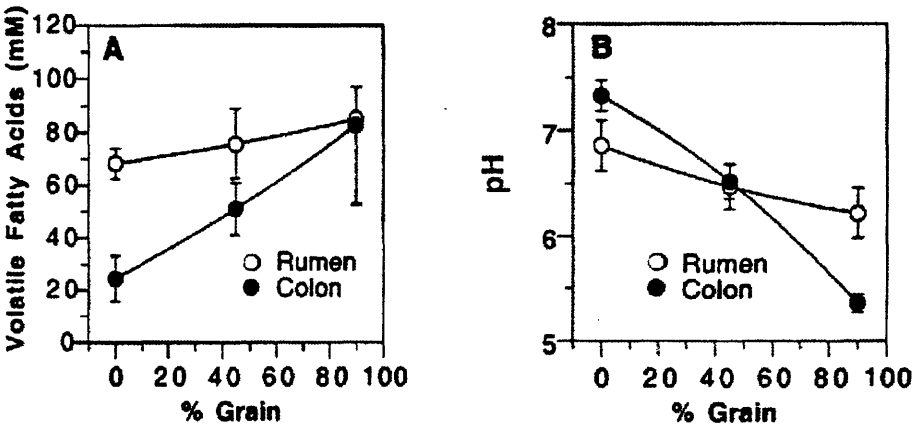


Figure 2.5 The effect of increasing amounts of dry rolled corn (0, 45 and 90% of dietary DM) on (A) VFA concentration and (B) pH, of rumen and colon contents. From Diez-Gonzalez *et al.* (1998).

Diez-Gonzalez *et al.* (1998) found that when cattle were fed increasing amounts of dry rolled corn (0, 45 and 90% of the diet), rumen VFA concentration did not increase significantly; however VFA concentrations in the colon increased approximately threefold (Figure 2.5). The pH of the colon decreased under these conditions whilst rumen pH remained relatively constant. This evidence suggests that the hindgut has a lower buffering capacity than the rumen.

Hindgut starch fermentation has also been shown to be a critical factor in the development of acidosis in grain-engorged sheep (Lee 1977) where caecal acidity was more severe than acidity in the rumen following high levels of grain consumption. Similar findings were more recently reported by Godfrey *et al.* (1992).

It is also understood that a number of important diseases and conditions in pigs (*Serpulina* infection – swine dysentery) (Pluske *et al.* 1996), poultry (wet litter) (Pluske *et al.* 1997) and horses (laminitis) (Garner *et al.* 1977; Garner *et al.* 1978) arise when carbohydrate is fermented in the large intestine.

The significance of hindgut acidosis in adapted feedlot cattle is not yet fully understood but the condition has been induced in experimental systems. Zust *et al.* (2000) infused large amounts of starch directly into the caecum of cattle which resulted in changes normally associated with acute acidosis such has high lactic acid levels and low pH in the caecum. There were also clinical signs of grain-poisoning normally attributed to rumen acidosis – inappetence, rumen stasis and general weakness.

Under normal feeding conditions, it is likely that any starch that reaches the hindgut would be relatively resistant to both microbial fermentation and digestion by endogenous enzymes. For example, in cannulated cattle adapted to steam flaked sorghum diets, the digestibility of starch entering the hindgut averaged 33% (Theurer *et al.* 1999b). Hindgut acidosis is more likely to be a problem in cattle consuming large amounts of highly digestible diets. Such dietary regimes may result in large amounts of digestible starch entering the hindgut.

2.4 Starch Digestion in the Small Intestine

2.4.1 Introduction

Starch digestion in the small intestine is important in any grain-feeding situation (Knowlton 2001). Normal starch intakes of feedlot cattle can be expected to vary between 1.5 and 6 kg/d (Huntington 1997). Theurer (1986) reported that between 4 to 60% of dietary starch reached the small intestine depending on the source and quantity of grain in the diet. Starch digestibility in the small intestine (expressed in relation to duodenal starch flow) was found to range from 63% in steers fed sorghum-based diets to 95% in steers fed wheat-based diets (Axe *et al.* 1987).

Before reviewing opportunities for improving digestion, it is important to understand the mechanisms of small intestinal digestion. The following section discusses features of the ruminant small intestine with particular emphasis on the digestion and absorption of starch. Limitations to starch digestion are also identified.

2.4.2 Structure and function

The small intestine comprises three segments - the duodenum, jejunum and the ileum. The length of the small intestine in cattle has been reported to be 46 m (Argenzio 1993c). The absorption of carbohydrates, lipids, protein, calcium and iron primarily occurs in the duodenum and jejunum whereas absorption of vitamin B_{12}, water, bile salts and electrolytes is concentrated in the ileum (Fox 1991). Within the small intestine, mucous is secreted from goblet cells (Figure 2.6) for lubrication while rhythmic segmentation and peristalsis function to mix and propel digesta towards the ileocaecal valve (Argenzio 1993b). On average, digesta spend less than 3 h in the small intestine of steers (Zinn and Owens, 1980 – cited by Owens *et al.* 1986). The mucosa of the small intestine is composed of many folds (visible to the naked eye) covered by microscopic villi (Argenzio 1993a). Each intestinal villus has a blood capillary, lymphatic capillary (lacteal) and nerve fibres (Figure 2.6). Microvilli cover the individual cells on each villi. These microscopic projections serve to improve digestion by increasing the surface area available for enzyme activity and absorption (Fox 1991). The cell membranes of the microvilli are embedded with digestive enzymes and the surrounding area is known as the brush-border. This is the cell extrusion zone shown in Figure 2.6.

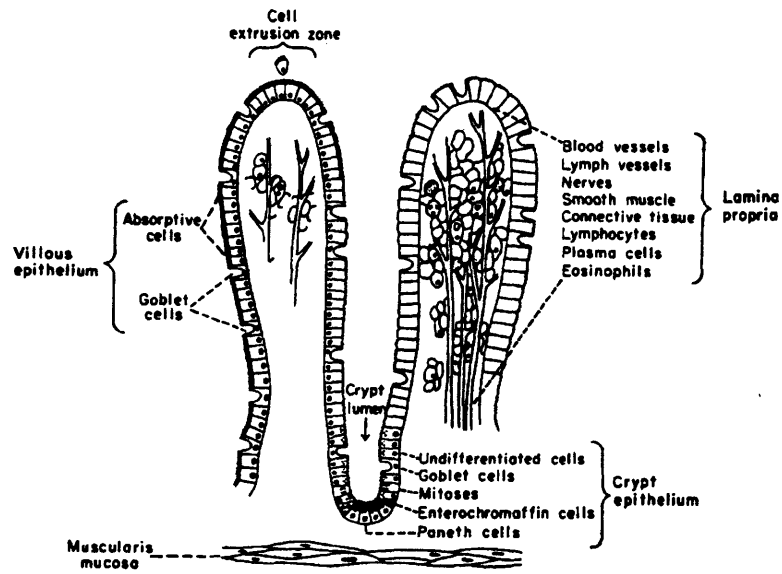


Figure 2.6 Cross-section of the small intestinal mucosa showing two sectioned villi. From Argenzio (1993a).

2.4.3 Enzymatic digestion of starch

The pancreas and the intestinal mucosa secrete α-amylase into the small intestine (Owens *et al.* 1986). The optimal pH for bovine pancreatic α-amylase is approximately 6.9 (Russell *et al.*

1981) which is maintained by the large amount of bicarbonate released in pancreatic juice (Fox 1991). Interestingly, distal ileal pH has been reported to decline with increasing post-ruminal flow of carbohydrate (Wheeler and Noller 1977; Russell *et al.* 1981). A decrease in ileal pH (measurement taken post-mortem at a site 5 m distal to the abomasum) may be a result of fermentation by bacteria within the small intestine (Nicoletti *et al.* 1984; Allison 1993; Walker and Harmon 1995). Wheeler and Noller (1977) suggested that an intestinal pH below 6.9 could reduce the activity of α-amylase and lead to an increase in the amount of starch reaching the hindgut.

The secretion of enzymes into the gastrointestinal tract is largely under the control of the autonomic nervous system (Argenzio 1993c). The hormone, secretin, is released when acid enters the duodenum from the abomasum and the pancreas is then stimulated to release bicarbonate, water and digestive enzymes.

Pancreatic α-amylase digests starch (amylose and amylopectin) to disaccharide (maltose), trisaccharide (maltotriose) and α-limit dextrin sub-units (Gray 1992; Harmon 1993; Rowe *et al.* 1999). The α-1,4 glucosidic bonds of starch are randomly hydrolysed by α-amylase (Rooney and Pflugfelder 1986). Isomaltase cleaves the α-1,6 branch points of amylopectin (Owens *et al.* 1986).

In the brush-border area of the small intestine, enzymes such as maltase, α-dextrinase and amyloglucosidase (collectively known as glycanases) degrade the di- and polysaccharides to monosaccharides – glucose, galactose and fructose (Owens *et al.* 1986; Huntington 1997; Rowe *et al.* 1999). Glycanases remain attached to the brush-border but expose the active sites inwards towards the digesta (Fox 1991; Gray 1992). Thus, when glucose is released from polymers, it will be adjacent to the brush-border for easy absorption (Argenzio 1993a).

2.4.4 Glucose absorption

The pathways of glucose transport in the small intestine of the ruminant are depicted in Figure 2.7. The major process whereby glucose crosses the brush-border membrane of enterocytes is via the Na^+-dependent glucose transporter, SGLT1 (Argenzio 1993a; Bird *et al.* 1996; Huntington 1997; Harmon and McLeod 2001). The system transports one molecule of glucose and two Na^+ in each of its cycles (Kimmich 1981; Huntington 1997; Harmon and McLeod 2001). Na^+ ions are transported down their electrochemical gradient by SGLT1 and this releases energy (Kimmich 1981). The Na^+ gradient is maintained by the activity of Na^+/K^+-ATPase (Harmon and McLeod 2001) which is located in the basolateral membrane (Figure

2.7). The energy released from the movement of Na^+ is then used to carry glucose against the concentration gradient from the lumen into the enterocyte.

Galactose absorption also relies on SGLT1 but fructose uses a different carrier (GLUT 5) (Figure 2.7) that is not Na^+-dependent (Argenzio 1993a).

Glucose and fructose within the enterocyte are then moved by Na^+-independent facilitated diffusion into adjoining blood capillaries. This is achieved using GLUT2 transporters that are embedded in the basolateral membrane (Figure 2.7) (Harmon and McLeod 2001).

Pentose sugars may also be absorbed by simple, paracellular diffusion (Figure 2.7) which is a process driven by differing solvent concentration gradients between the lumen and the blood. The process is often referred to as 'solvent drag'. Paracellular diffusion plays a very minor role in glucose transport and would be unlikely to exceed sodium-glucose transport unless glucose concentrations in the lumen exceeded 180 mM (Huntington 1997).

Glucose absorption is most efficient in the proximal area of the small intestine (Krehbiel *et al.* 1996; Huntington 1997) and amylase activity is also highest in this part of the tract. The density of glucose transporters is related to the position, orientation and number of intestinal villi with between 10^5 and 10^7 transporters per intestinal enterocyte (Huntington 1997).

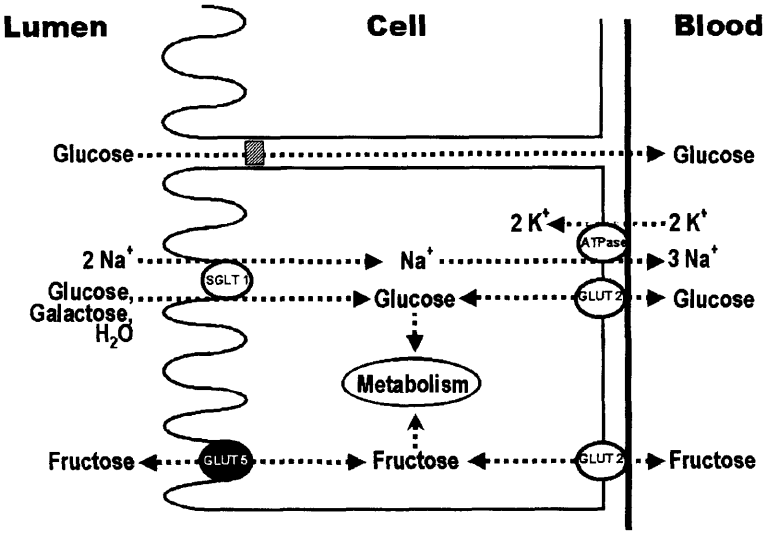


Figure 2.7 Glucose absorption in the small intestine. From Harmon and McLeod (2001).

2.4.5 Factors that limit starch digestion

Factors most likely to limit starch digestion in the small intestine are starch structure, α-amylase activity and glucose absorption capacity. It is not clear what the first limiting factor is

as this is likely to be diet-dependent. If α-amylase secretion and/or glucose absorption is limiting, understanding the regulatory mechanisms for these functions may provide a means to improve starch digestion.

There is little doubt that the structure of starch entering the small intestine affects its digestibility. Kreikemeier *et al.* (1991) infused corn-starch and corn-dextrin into the abomasum of steers at rates of 20, 40 and 60 g/h and measured starch disappearance in the small intestine. Corn-dextrin did not have the granular structure of corn-starch but the majority of the glucose remained polymerised (Kreikemeier *et al.* 1991). Table 2.2 shows that the percentage disappearance was reduced with increasing infusions of dextrin and starch. However, the decrease in the percentage disappearance was greater for starch than dextrin.

Table 2.2 Small intestinal disappearance (%) of corn-dextrin (partially hydrolysed corn-starch) and corn-starch infused into the abomasum of steers. From Kreikemeier *et al.* (1991).

	Infusion rate (g/h)		
	20	**40**	**60**
Corn-dextrin	93	85	79
Corn-starch	86	67	55

Differences in the extent of disappearance between the starch and dextrin were particularly obvious at the high infusion level and indicates that the more crystalline structure of the corn-starch was limiting small intestinal digestion (Kreikemeier *et al.* 1991).

The results of Kreikemeier *et al.* (1991) are consistent with the earlier conclusion of Owens (1986) that, for typical feedlot diets, the structure of starch particles is more likely to limit digestion than enzymatic activity. There are other studies that suggest that the physical structure of starch can limit post-ruminal digestion. Plascencia and Zinn (1996) reported that, in lactating dairy cows, 41% more starch was digested post-ruminally in cows fed steam flaked corn than in cows fed dry rolled corn, despite more starch reaching the small intestine in cows fed dry rolled corn. Likewise, Knowlton *et al.* (1998) showed that twice as much starch disappeared in the small intestine of dairy cows fed a high moisture corn diet than in cows fed a dry ground corn diet, even though the dry ground corn diet provided an extra 1500 g of starch to the duodenum. These results clearly show the effect that starch structure can have on small intestinal starch digestion.

Clary *et al.* (1969) showed that α-amylase concentration in pancreatic tissue was greater in cattle fed a concentrate diet than in cattle grazing pasture whilst studies by Russell *et al.* (1981) showed pancreatic α-amylase activity to be higher in steers fed corn than in steers fed lucerne. These results indicate that the small intestine of ruminants has the capacity to adapt to diets supplying increased amounts of rumen-bypass starch however the exact stimulus for increased pancreatic output of α-amylase has not been identified (Harmon 1993). The results of Clary *et al.* (1969) and Russell *et al.* (1981) suggest that α-amylase secretion increases in response to increased starch intake. However, when diets are formulated to provide the same amount of ME, forage-fed cattle have a higher concentration of α-amylase in the pancreas and an increased level of α-amylase secretion than grain-fed cattle (Russell *et al.* 1981; Harmon 1992). An increase in energy intake and not starch *per se*, may cause an increase in pancreatic α-amylase concentration (Russell *et al.* 1981; Kreikemeier *et al.* 1990b). The flow of starch and glucose to the small intestine of ruminants has actually been reported to reduce pancreatic α-amylase secretion (Kreikemeier *et al.* 1990b; Walker and Harmon 1995; Swanson *et al.* 2002).

Pancreatic adaptation may also be influenced by protein. A greater flow of protein from the duodenum in steers has been shown to result in an increase in starch disappearance from the small intestine (Richards *et al.* 2002) and an increase in the concentration and secretion rate of pancreatic α-amylase (Richards *et al.* 2003). These results help to explain why Taniguchi *et al.* (1995) observed an increase in the appearance of glucose in the portal drained viscera with increased protein flow to the small intestine.

Despite the ability of cattle to adapt to diets high in starch, the activity of pancreatic α-amylase may still limit small intestinal starch digestion (Orskov 1986; Huntington 1997). In the infusion studies by Kreikemeier *et al.* (1991) 15 times more starch than glucose flowed past the ileum when corn-starch and glucose were infused post-ruminally at a rate of 60 g/h.

Starch digestion may also be limited by the activity of glycanases such as maltase or isomaltase. There is evidence to suggest that brush-border glycanase activity does not increase in response to a high-starch diet (Russell *et al.* 1981) or increasing energy intake (Kreikemeier *et al.* 1990b). Russell *et al.* (1981) found that maltase activity did not increase in steers fed a corn-based diet for over 100 days.

There is evidence that α-amylase secretion does not limit starch digestion in the small intestine under feedlot conditions where cattle are adapted to high-starch diets. Zinn *et al.* (1995) showed that, in cattle fed a diet containing 75% dry rolled corn at either 1.6 or 2.4% of LW,

47% more starch entered the small intestine at the higher level of DM intake (DMI). Despite the increased starch supply, the proportion of starch digested post-ruminally was similar (66% vs 62%) for the two levels of DMI. The site of post-ruminal starch digestion in this experiment was unknown but it is reasonable to assume that the higher level of feed intake would have delivered more starch to the hindgut (Grovum and Hecker 1973) and that post-ruminal digestion was not confined to the small intestine. The inability to determine how much starch is digested in the small intestine is a major limitation in experiments that use cattle with rumen and duodenal cannulas only. Experiments using cattle fitted with rumen, duodenal and ileal cannulas (Knowlton *et al.* 1998; Swingle *et al.* 1999; Theurer *et al.* 1999b) provide more useful information on the site of post-ruminal starch digestion.

Studies examining starch disappearance should be viewed with a degree of caution because starch disappearance does not necessarily correspond to starch digestion, glucose absorption and utilisation by the animal. Microbial metabolism within the small intestine may account for some small intestinal starch disappearance (Kreikemeier *et al.* 1991; Harmon 1992). In theory, an increase in the digestibility of starch in the small intestine should result in a net positive appearance of glucose at the portal-drained viscera (Nocek and Tamminga 1991). However, the net absorption of glucose from the portal-drained viscera has been reported to be negative in studies of cattle fed high-starch diets (Huntington 1984; Reynolds and Huntington 1988; Lozano *et al.* 2000) which suggests that gut tissues are using more arterial glucose than is being absorbed into portal blood (Nocek and Tamminga 1991; Harmon 1992). McBride and Kelly (1990) noted that there were high energetic costs associated with maintaining the intestinal epithelium and warned that an increase in the protein demands of the gut could possibly negate the potential benefits of increasing nutrient delivery to the small intestine. Nocek and Tamminga (1991) suggested that, although an exogenous glucose supply may be used for visceral metabolism, this could potentially spare endogenously synthesised glucose for gut metabolism, allowing more glucose to be delivered to extrasplanchnic tissues.

The absorption of released glucose could possibly be a limitation to starch digestion in certain situations (Owens *et al.* 1986; Kreikemeier *et al.* 1991). Glucose transport activity is highest in the young ruminant and declines with age but older ruminants can quickly increase their capacity to absorb glucose (Shirazi-Beechey *et al.* 1991). The glucose transport systems of the ruminant small intestine are known to be stimulated by the presence of glucose in the lumen (Shirazi-Beechey *et al.* 1991; Harmon and McLeod 2001).

It appears that, in unadapted animals, transport activity limits the amount of glucose that can be absorbed from the small intestine (Huntington 1997). However, in adapted animals, it is

estimated (Huntington 1997) that the capacity of the intestines to digest starch limits the absorption of glucose.

2.5 Site of Starch Digestion

2.5.1 Introduction

There are two opposing views in the literature regarding the merits of promoting starch digestion in the rumen. One view is that the fermentation of readily digestible carbohydrates in the rumen should be minimised to reduce energy losses, the effect of low pH on fibre digestion and the risk of health problems from acidosis. An alternative viewpoint is that fermentation has positive benefits in terms of microbial protein synthesis and that there is potential to lose overall feeding value if grain is allowed to pass through the rumen as starch not completely digested in the small intestine may be fermented in the hindgut or lost in the faeces.

2.5.2 Differences in energetic efficiency – fermentation vs enzymatic digestion

Harmon and McLeod (2001) concluded that energy capture from starch or glucose fermented in the rumen was 70-75% as efficient as when starch or glucose is directly digested and absorbed from the small intestine. Black (1971) provided a detailed explanation of the ways in which energy can theoretically be lost when carbohydrate is either fermented in the rumen or digested in the small intestine of lambs. Microbial fermentation leads to DE losses associated with:

1. Microbial residue in the faeces (1%)
2. Methane production (10%)
3. Heat of fermentation (6%)

The amount of methane produced varies according to diet composition and DMI (Harmon and McLeod 2001). Generally, 0.35 moles of methane are produced per mole of fermented starch (Hungate 1966). In contrast, there is essentially no methane produced when starch is digested in the small intestine of ruminants (Hungate 1966).

When starch is fermented, energy is released in the form of heat and this is referred to as the heat of fermentation (Harmon and McLeod 2001). On the other hand, there is assumed to be no heat evolved from starch digestion in the small intestine although a small amount of energy is released when enzymes cleave glucosidic bonds (Harmon and McLeod 2001).

The amount of energy lost through methane and heat may not appear to be significant, when expressed as a percentage of total energy intake. However, when this loss of energy is expressed as a percentage of net energy for maintenance and production, the potential importance of methane and heat of fermentation assumes more significance. Black (1971) estimated that for concentrate diets, the amount of net energy available to a non-ruminant lamb (no fermentation) was 39% higher for maintenance and 22% higher for production than a ruminant lamb (fermentation). This estimate of the difference in efficiency between rumen and intestinal digestion is consistent with the conclusion of Owens *et al.* (1986) who reported that, in cattle fed processed corn and sorghum-based diets, the efficiency of starch utilisation was 42% greater when it was digested in the small intestine, as opposed to being fermented in the rumen.

Sources of energy loss that are common to both types of digestive system (but not necessarily in the same proportions) are endogenous faecal secretions, energy in urine, ATP requirement for urea formation, excretion and the utilisation of digestion end-products. Black (1971) concluded that energetic losses would be minimised if low fibre/high protein feeds could pass, undigested, to the abomasum and small intestine of ruminants. It is important to highlight that these estimates assume that the diets would be fully fermented in the rumen or completely digested by the host's endogenous enzymes. In reality, neither assumption will apply.

2.5.3 Starch digestion in the small intestine and marbling

There is evidence to suggest that glucose (rather than acetate) is the substrate for intramuscular fat deposition and therefore marbling in grain-fed beef (Smith and Crouse 1984). Extensive marbling is usually achieved by feeding a high-energy diet to cattle with a genetic propensity for marbling over 200-300 days. The process is expensive and any method of accelerating fat deposition at the marbling site certainly deserves close attention given the premiums paid for marbled beef, particularly in Japan. Although glucose can be synthesized from propionate and amino acids in the liver, it appears that glucose derived directly from the gut, rather than from hepatic gluconeogenesis alone, is more closely associated with the development of marbling fat depots (Pethick *et al.* 1997). Although the exact reason for the differences remains unclear, evidence is provided by the study of Pethick *et al.* (1995) who measured ATP citrate lyase activity in the subcutaneous adipose tissue of sheep fed various cereal grain-based diets. This enzyme is of central importance in the citrate cleavage pathway which stimulates fat synthesis from glucose. Enzyme activity was higher when a corn-based diet was fed than when lupins (negligible starch) or oats (extensively digested in the rumen) were fed (Pethick *et al.* 1995).

Similarly, Pethick *et al.* (1997) reported higher ATP citrate lyase activity and visual marbling score in cattle fed steam flaked corn and sorghum compared to the corresponding dry rolled grains. This was most likely due to an increased digestibility of starch in the small intestine and absorption of glucose along with increased DMI.

Evidence that starch digestion in the small intestine can potentially increase marbling was previously provided by Reddy *et al.* (1975). These researchers fed feedlot cattle various grain-based diets for 107 days. Cattle fed a corn-based diet had significantly higher levels of intramuscular and subcutaneous fat than cattle fed triticale or wheat-based rations.

2.5.4 Consequences of reducing starch fermentation in the rumen

Having considered the theoretical advantages of shifting the site of starch digestion to the small intestine, it is important to consider any potentially negative effects of reducing the fraction of starch digested in the rumen.

Microbial protein production

The amount of OM fermented in the rumen is an important factor determining the amount of microbial nitrogen flowing to the duodenum (Clark *et al.* 1992). Reducing starch digestion in the rumen will reduce microbial protein synthesis and this may have an adverse effect on production if protein is limiting. However, even if a greater proportion of starch escapes to the small intestine, protein supply may still be sufficient to meet requirements.

Rode and Satter (1988) reported that rumen microbial protein production in cows fed a barley diet was only higher than that in cows fed a corn diet when the rations contained a low proportion of concentrate. Processing techniques such as steam flaking will increase the exposure and digestibility of non-starch grain components, including protein (Zinn *et al.* 2002). The steam flaking of corn has been shown to increase post-ruminal protein and starch digestibility (Zinn 1990a; Barajas and Zinn 1998). Yang *et al.* (1997) showed that dairy cows eating a diet containing 60% steam rolled barley had a greater supply of microbial nitrogen to the duodenum than cows eating the same amount of a 60% steam rolled corn diet. The increased microbial protein production with barley was due to a higher level of fermentation in the rumen. However, more dietary protein flowed to the duodenum for the corn diet. Consequently, the passage of non-ammonia-nitrogen to the small intestine was not significantly different between these two diets. Other researchers have also observed similarities in post-ruminal flow of non-ammonia-nitrogen when barley replaced corn in diets fed to dairy cattle (Spicer *et al.* 1986; Rode and Satter 1988; McCarthy Jr *et al.* 1989). It is therefore considered

unlikely that there will be any significant adverse effect on protein supply to the grain-fed animal if there is a decrease in rumen digestion.

Hindgut fermentation

Lowering the rate of starch fermentation in the rumen would not be recommended if this resulted in increased starch flow to the hindgut. Increasing the quantity of starch escaping from the rumen increases the quantity of starch digested in the small intestine but starch digestibility (expressed in relation to duodenal starch flow) generally decreases (Karr *et al.* 1966; Nocek and Tamminga 1991; Kreikemeier *et al.* 1991). There are some reports indicating that an increase in starch flow to the small intestine results in a corresponding increase in small intestinal starch digestibility (Owens *et al.* 1986; Zinn *et al.* 2002). However these authors did not identify the site of post-ruminal starch digestion and it is possible that more starch was digested in the hindgut.

Whole tract starch digestion

It is argued by some that rumen fermentation needs to be maximised to achieve the highest level of whole tract starch digestion (Theurer 1986; Orskov 1986; Huntington 1997). This has been shown in studies comparing steam flaked and dry rolled grains where steam flaking consistently increased rumen and whole tract starch digestibility (Theurer 1986; Swingle *et al.* 1999).

Zinn (1990a) reported that decreasing flake densities of steam flaked corn improved post-ruminal and whole tract starch digestibility but did not change the digestibility of starch in the rumen. Although increased processing improved starch digestion, heavily steam flaked corn was associated with poorer animal performance. Zinn (1990a) suggested that a low rumen pH caused by heavily steam flaked corn was affecting feedlot performance via digestive disfunction in the rumen and reduced DMI. Similarly, DMI has also been shown to be affected by decreasing the flake density of steam flaked sorghum (Reinhardt *et al.* 1997; Theurer *et al.* 1999c; Swingle *et al.* 1999).

Whilst maximising whole tract starch digestion is a desirable goal, it should not be used as the only criterion for assessing the value of a grain. A reduction in whole tract starch digestibility may be acceptable if energy is conserved by having a greater proportion of starch digested in the small intestine and if acidic conditions in both the rumen and hindgut can be avoided.

2.6 Dietary Factors that Determine the Extent/Site of Starch Digestion

2.6.1 Introduction

From the previous sections it is clear that the site of starch digestion is an important criterion influencing the efficiency of animal production and health. The aim of the current section is to review the dietary factors that influence the site and extent of starch digestion in ruminants. An understanding of these factors will help to identify opportunities to manipulate starch digestion and fermentation.

There are numerous dietary and animal factors that influence starch digestion throughout the gastrointestinal tract. Although grain and animal factors are dealt with separately, it should be noted that many are interrelated.

2.6.2 Type of grain

Cereal grains differ in terms of starch structure, starch availability and NSP content (Rowe 1997). The nature of the seed coat and cell wall as well as the structure of the protein matrix can all affect starch digestion. Wheat and corn generally have the highest starch content (varying around 76% starch), followed by sorghum (75% starch), barley (61% starch) and oats (42% starch) (Rowe and Pethick 1994).

Notwithstanding the variation between grains, there is often a considerable range in starch characteristics within each grain type (Waldo 1973; Van Barneveld 1999). It is quite clear that no two batches of a grain are likely to have the same characteristics which may reflect environmental, varietal, age, storage or processing differences (Huntington 1997; Van Barneveld 1999). Sorghum cultivars have been shown to widely differ in digestive properties (Lichtenwalner *et al.* 1978; Bird *et al.* 1999). Corn, sorghum, barley, rice and millet have waxy (around 100% amylopectin) and non-waxy (around 75% amylopectin) genotypes (Rooney and Pflugfelder 1986) with waxy varieties generally having a higher starch digestibility in cattle (Huntington 1997). In addition to having a higher proportion of amylopectin, this improved starch digestibility may be due to consistently higher protein digestibility of waxy grain in the small intestine (Rooney and Pflugfelder 1986). The variation that exists in the characteristics of starch sources complicates the process of identifying the best grain to feed to cattle and the most appropriate processing method to apply.

2.6.3 Starch-protein interactions

Sorghum and to a lesser degree, corn, are far less extensively fermented in the rumen than barley or wheat (Orskov 1986; Rowe *et al.* 1999). The protein matrix that surrounds the starch in sorghum and corn is thought to contribute to this low digestibility (Rooney and Pflugfelder 1986; McAllister *et al.* 1993). When starch granules are embedded within a protein matrix, the susceptibility of the starch to enzymatic hydrolysis is reduced (Rooney and Pflugfelder 1986; Van Barneveld 1999). Corn, wheat and barley protein have been shown to be more digestible than sorghum protein (Hale 1973) whilst the protein matrix within sorghum and corn is more resistant to bacterial attachment and penetration than the equivalent structures in wheat and barley (McAllister *et al.* 1990; McAllister *et al.* 1994).

Pronase treatment of sorghum grain has been shown to improve the rate of starch hydrolysis (Lichtenwalner *et al.* 1978), most probably due to the starch-degrading enzymes being more effective once the protein matrix was disrupted. The digestion of isolated barley and corn-starch by microorganisms has been shown to be similar (McAllister *et al.* 1993) even though barley is usually more extensively digested than corn. This suggests that the protein matrix, rather than the starch granule structure, is the important determinant of the rate and extent of corn-starch digestion.

Interactions between starch and protein could also occur following grain processing as there is evidence that gelatinised starch can form complexes with protein (Thorne *et al.* 1983). Joy *et al.* (1997) observed that the degradation of feed nitrogen in the rumen was lower for steam flaked corn (0.39 kg/L) than for dry rolled corn. This was believed to be due to the formation of indigestible starch and protein complexes that occurred at this relatively mild level of steam flaking. Similarly, Zinn (1994) reported that the rumen degradation of dietary nitrogen was lower for steam rolled wheat than for dry rolled wheat.

2.6.4 Feeding regime and microbial adaptation

DMI in cattle is closely related to feeding practices. Feedlot cattle are often given a large meal, once or twice a day but starch digestibility is maximised when small meals are consumed at regular intervals throughout the day. A steady supply of substrate causes a slower rate of microbial fermentation than a large intake of a grain-based diet. The latter scenario also greatly increases the risk of acid accumulation and adapted animals can still die, or suffer from acidosis, if they overeat (Slyter 1976). Despite this information, the practical application of feeding small meals to individuals is limited in large feedlots.

Changes in diet composition need to be made gradually so that microbial populations are able to adjust to substrate changes. Slyter (1976) reported that when cattle are not inoculated with rumen ingesta from adapted animals, they can take at least a week to achieve feed intake levels of similar cattle inoculated with 2 L of rumen fluid from adapted animals. The transfer of rumen fluid from adapted sheep was shown to be as effective as a gradual introduction to grain in controlling L-lactate levels in the hindgut of sheep fed barley (Godfrey *et al.* 1992).

2.6.5 Roughage level

Roughages are included in feedlot diets to reduce starch concentrations and stimulate saliva flow. When dietary roughage levels are low, animals spend less time eating and ruminating whilst small grain does not stimulate rumen motility (Orskov 1986).

Starch is more likely to escape degradation in the rumen if fibrous roughages are included in the diet (Orskov 1986; Siciliano-Jones and Murphy 1989a). Thompson and Lamming (1972) showed that the amount of rumen by-pass starch could be increased by adding long or chopped straw to a basal diet of ground corn. The larger particles of straw stimulate the rumen epithelium and provide the tactile stimulation for rumen movement. Smaller grain particles are then more likely to escape through the reticulo-omasal orifice.

The physical form of forage will also affect the amount of starch digested in the different sections of the digestive tract (Siciliano-Jones and Murphy 1989a). Cole *et al.* (1976) used cottonseed hulls to vary the roughage levels in dry rolled and steam flaked corn-based diets fed to steers. Starch digestibility in the whole tract and small intestine was lower in steers given a diet with a roughage level of 21% than in steers given a diet without roughage. The high roughage diet increased DMI and Cole *et al.* (1976) suggested that the palatability of the rations had been improved. The steers may have also eaten more of the roughage diet to meet their energy requirements. Alternatively, enhanced gut stimulation due to the roughage and faster passage-rate of digesta may have facilitated this higher DMI. Increased passage-rate of digesta may offer some explanation for the reduction in starch digestibility observed for the high-roughage diets. McCullough and Matsushima (1973) found that the level of inclusion of corn silage (12% vs 0% of the whole ration) did not affect the site or extent of starch digestion in steers fed whole shelled or steam flaked corn-based diets. The results demonstrate how the optimal level of roughage inclusion varies with grain source, grain processing method and roughage source.

2.6.6 Feed additives

The ionophore antibiotic, monensin, is used widely as an additive in feedlot diets. This compound decreases lactic acid and methane production and simultaneously increases propionate production (Wolin 1981; Rowe and Pethick 1994). However, the use of in-feed antibiotics such as monensin raises concerns about the possible risks of antibiotic resistance developing and being transferred to human pathogens (Barton 2000).

Alternatives to antibiotics include vaccines, probiotics (living organisms that modify intestinal flora) and enzymes (to assist the digestion of particular dietary components) (Blackman 2000). Anninson (1997) suggested that there is scope for the use of exogenous enzymes in ruminant diets but noted that the complex nature of the rumen makes the application of this technology difficult. More research is required before such additives could effectively replace the role of the antibiotics currently used in ruminants.

2.6.7 Anti-nutritional factors

Silica, lignin derivatives, alkaloids, tannins, trypsin inhibitors and lectins may all have anti-nutritional effects in cattle. *In vitro* studies have shown that condensed tannins can form complexes with microbial enzymes and interfere with the process of microbial attachment (McAllister *et al.* 1994). Condensed tannins may also reduce protein digestibility in the rumen (Van Barneveld 1999) but this could benefit the animal if more dietary protein is supplied to the small intestine.

It appears that anti-nutritive components within grains are of less importance in ruminants than monogastrics. For example, the β-glucan content of barley has been shown to be unrelated to the extent of starch digestion and the efficiency of feed utilisation in ruminants (Engstrom *et al.* 1992) whilst it is thought to impair digestion in poultry (Rowe *et al.* 1999). It should be noted that the barley used by Engstrom *et al.* (1992) had a relatively low (<5%) β-glucan content. A grain such as hull-less barley can contain up to 13.5% β-glucan and may have a greater effect on the efficiency of feed utilisation in cattle. This may be the reason why steam rolled hull-less barley had a lower rumen starch digestibility than steam rolled covered barley in dairy cows (Yang *et al.* 1997). In contrast Zinn *et al.* (1996) had previously found no difference in the rumen digestibility of starch in steam flaked hull-less and covered barley.

2.7 Differences Between Grains and Effects of Processing

2.7.1 Introduction

As indicated earlier, the type and extent of grain processing influences starch digestion through changes in particle size, solubility, protein matrix structure, cellular distribution and the extent of starch gelatinisation. The key to successful grain processing is to make starch more accessible to microbial enzymes in the rumen and endogenous enzymes in the small intestine.

The following sections focus on the processes of dry rolling and steam flaking. Further information is readily available on the processes of high moisture treatment followed by rolling, pelleting, extrusion, ensiling, micronisation, (Rowe *et al.* 1999) and chemical treatments such as sodium hydroxide (Orskov 1986; Kaiser 1999).

2.7.2 Dry rolling and grinding

Dry rolling (cracking) serves to break the seed coat and reduce particle size. These changes provide bacteria and digestive enzymes with improved access to the starch within the endosperm. Reduced particle size also increases the rate of passage through the gastrointestinal tract (Galyean *et al.* 1981; Ewing *et al.* 1986) which can result in reduced starch digestibility in the rumen (McAllister *et al.* 1993). Even though small particles may have a reduced residence time in the rumen, low-pH conditions can quickly arise when microorganisms are able to ferment starch at a higher rate. For this reason, finely ground grains are rarely fed to ruminants.

The general pattern of starch digestion in cattle for dry rolled cereal grains is shown in Table 2.3. The digestibility of sorghum is relatively low compared with other grains. In contrast, the starch in oats, which is generally lower than the other grains, is almost totally digested (Rowe and Pethick 1994). Compared with sorghum, there is only a small benefit to be achieved by dry rolling wheat for cattle (Theurer 1986; Owens *et al.* 1997) whilst oats are generally fed whole (Rowe and Pethick 1994).

Table 2.3 Starch content and starch digestibility of dry rolled cereal grains fed to cattle. From Rowe *et al.* (1999).

Grain	Total starch in grain (% DM)	Rumen (% of starch intake)	Small intestine (% of starch entering)	Whole tract (% of starch intake)
Oats	42	92	76	98
Wheat	76	89	85	98
Barley	61	87	73	93
Corn	76	76	66	93
Sorghum	75	64	63	87
Average	66	82	73	94

2.7.3 Steam flaking

Steam flaking is commonly used to improve the digestibility of cereal grain in cattle. Grain is held within a steam chamber for 30-60 min while the moisture content is increased to 18-20% (Theurer *et al.* 1999a). Grains take up most of this moisture relatively early in the steaming process and there is a point at which any further steaming yields no additional benefit. For example, the nutritive value of corn is not improved by steaming beyond 34 min (Zinn 1990a). Following steaming, the grain is flaked between large, pre-heated rollers to a specific flake thickness. The degree of steam flaking is often referred to as flake bulk density (g/L) (Theurer *et al.* 1999b) with lower bulk densities corresponding to thinner steam flakes (Rooney and Pflugfelder 1986; Theurer *et al.* 1999a).

The benefits of steam flaking arise through changes in the granular structure of the starch and protein matrix, as well as exposing an increased surface area for digestion (Rooney and Pflugfelder 1986; Theurer 1986). McNeil *et al.* (1975) reported that the disruption of the protein matrix around the starch in sorghum may be just as important as changes in the starch itself.

Some of the available literature is difficult to interpret because of differences in the way in which the grain has been processed. Theurer *et al.* (1999a) explained that the steam rolling process involves steaming the grain for 15 min or less to increase the grain moisture content to approximately 15%. It is then run through rollers to produce a 'relatively thick flake' without specifying a flake density (Theurer *et al.* 1999a). Despite this definition, information in other

reports remains confusing. For example, specific flake densities for steam rolled wheat (Zinn 1994) and steam rolled barley (Zinn 1993) have been described in processing methods that are very similar to steam flaking descriptions. For the purposes of this discussion, steam flaking and steam rolling are considered together as the principle behind both processing methods and the final grain product is largely the same.

Gelatinisation

When starch absorbs water it swells and this process is usually reversible (French 1973; Evers *et al.* 1999). In the steam flaking process moisture, pressure and elevated temperatures interact to expand starch granules and irreversibly disrupt the surrounding protein matrix (Osman *et al.* 1970; Theurer 1986; Rowe *et al.* 1999). Excessive swelling breaks the intermolecular hydrogen bonds in the crystalline area of the starch (Rooney and Pflugfelder 1986) and new bonds are formed involving water (Joy *et al.* 1997). This process of gelatinisation ultimately results in a much less ordered starch structure (McNeil *et al.* 1975) and granules lose their crystallinity (French 1973). In addition, the flaking process flattens swollen starch granules that gel with other grain components to produce a flake (McDonough *et al.* 1997).

Retrogradation

Retrogradation occurs as starches cool and can be considered as the reverse of gelatinisation. Separated starch molecules may reassociate in an ordered structure, causing the water held within the starch gel to be released (French 1973; Rooney and Pflugfelder 1986). Some of the hydrogen bonds between amylose and amylopectin chains may reform. Starch structure and concentration, moisture levels, temperature and complexing agents such as lipids all affect the extent of retrogradation (Rooney and Pflugfelder 1986). Reassociation of starch polymers can result in starch that is even more resistant to digestive enzymes than the raw starch before the steam flaking treatment.

Steam flaking vs dry rolling

Steam flaking of corn and sorghum typically results in a much greater proportion of starch being fermented in the rumen than when these grains are dry rolled. A 30% improvement in rumen starch digestibility was reported when corn was steam flaked rather than dry rolled (Cole *et al.* 1976; Zinn *et al.* 1995) whilst Huntington (1997) reported that steam flaking increased rumen digestibility of starch from 75 to 85% in corn and from 52 to 78% in sorghum. When steam flaking increases starch digestibility in the rumen, this results in a lower pH,

higher VFA concentrations (Plascencia and Zinn 1996) and higher proportion of propionate (Zinn 1987; Zinn *et al.* 1995; Joy *et al.* 1997).

Theurer *et al.* (1999b) compared dry rolled and steam flaked sorghum and Table 2.4 shows how the site of starch digestion changed when processing method was varied.

Table 2.4 Effect of steam flaked (SF) vs dry rolled (DR) sorghum on site of starch digestion in cattle. From Theurer *et al.* (1999b).

	Starch Digestibility (% of starch intake)			
	Rumen	**Small Intestine**	**Large Intestine**	**Whole Tract**
SF Sorghum	82	16	0.5	98.9
DR Sorghum	67	28	1.2	96.5

Table 2.4 shows that steam flaking significantly improved the whole tract digestibility of sorghum-starch with the majority of digestion taking place in the rumen (Theurer *et al.* 1999b). The digestibility of starch in the small intestine (expressed in relation to duodenal starch flow) was also higher for steam flaked sorghum than dry rolled sorghum (91 vs 85%).

The post-ruminal digestibility of steam flaked corn was found to be 40% higher than dry rolled corn (Cole *et al.* 1976; Theurer 1986; Zinn *et al.* 1995). It is clear for sorghum (Theurer *et al.* 1999a; Swingle *et al.* 1999) and corn (Theurer 1986; Theurer *et al.* 1999a) that, whilst a smaller amount of starch will reach the small intestine with steam flaked grain, the small intestinal digestibility of this starch is higher than dry rolled grain. As a result, whole tract starch digestibility of sorghum and corn is increased by steam flaking (Theurer 1986; Poore *et al.* 1993).

Improvements in rumen starch digestibility due to steam flaking are much smaller for wheat and barley than for corn and sorghum as both dry rolled wheat and barley are relatively well-digested. The full benefits of steam flaking wheat may be realised in the small intestine. Zinn (1994) established that the *in vitro* enzymatic digestibility of steam rolled wheat was higher than dry rolled wheat when the grains were finely ground. This shows that steam flaking of wheat improved digestibility in ways beyond changes in grain particle size and this is also true of corn and sorghum (Galyean *et al.* 1981). Zinn (1994) reported that cattle had a higher average daily gain (ADG) and improved feed conversion ratio (FCR) when they were fed diets based on steam rolled rather than dry rolled wheat. DMI did not vary between the processing treatments. These improvements in feed efficiency observed by Zinn (1994) occurred despite

no significant differences in rumen starch digestibility between dry rolled and steam rolled wheat whilst post-ruminal and whole tract starch digestibility was higher for steam rolled wheat. This information suggests that the steam flaking of wheat improves starch digestion in the small intestines and that the steam flaking of wheat could be useful, provided that the benefit of the improvement in the efficiency of feed utilisation outweighs the cost of processing.

In contrast, steam rolled barley was found to have a higher rumen starch digestibility than dry rolled barley (Zinn 1993) in cattle fed a 90% concentrate diet *ad libitum*. The steam rolling of barley also reduced DMI and improved FCR in this experiment. Hale *et al.* (1966) had previously concluded that the steam rolling of barley did not improve feed efficiency yet increased DMI. The inconsistency of these results makes it impossible to draw definite conclusions about the effect of steam flaking barley.

2.7.4 The importance of flake density

Once the decision has been made to steam flake grain, the optimal flake thickness needs to be defined. A range of factors must be taken into consideration in order to assess the value of any particular steam flaked grain. Different flake thicknesses can have dramatic effects on the costs of processing, starch and nitrogen digestibility, the efficiency of feed conversion, DMI and the propensity to cause acidosis.

Processing costs

The cost of steam flaked grain decreases with increasing flake density (Xiong *et al.* 1991; Reinhardt *et al.* 1997) as less energy is required to produce a coarser flake with the added benefit of increasing mill throughput (Swingle *et al.* 1999).

Starch digestion

Starch digestion generally improves with decreasing flake density. Thinner steam flakes have higher levels of starch gelatinisation and greater disruption to the protein matrix. However, an increase in the digestibility of starch may not be observed in situations where rapid starch fermentation causes acidosis.

Osman *et al.* (1970) concluded that the degree of flaking was the principal factor governing starch availability after examining the *in vitro* enzymatic digestibility of starch in ground samples of steam flaked barley and sorghum. Interestingly, steamed grain that was not flaked had a lower starch digestibility than untreated grain (Osman *et al.* 1970). Similarly, the *in vitro* fermentation characteristics of barley and sorghum were not improved when grain was only

steamed (Trei *et al.* 1970). The reason for this remains unclear but Osman *et al.* (1970) suggested that heat and moisture may increase the strength of the bonding within starch granules, making starch less susceptible to enzymatic degradation.

Decreasing the flake density of corn from 437 kg/L to 283 kg/L was reported to increase *in vitro* enzymatic digestibility of starch from 40% to 69% (Theurer *et al.* 1999b). Zinn (1990a) showed that decreasing flake densities of steam flaked corn increased rumen, post-ruminal and whole tract starch digestibility. Joy *et al.* (1997) reported similar findings to Zinn (1990a) but Theurer *et al.* (1999b) found that decreasing flake density of corn did not affect starch digestibility in either the small or large intestine. Differences in experimental conditions and results make it hard to clarify the effect of decreasing flake density on the post-ruminal digestion of corn-starch.

Rumen starch digestibility of steam flaked barley has been reported to be unaffected by flake thickness (Zinn 1993; Yang *et al.* 2000; Beauchemin *et al.* 2001). In contrast, Koenig *et al.* (2003) reported rumen starch digestibility was higher for more extensively steam rolled barley. Whole tract starch digestion has been shown to linearly increase with decreasing flake densities of barley (Yang *et al.* 2000; Beauchemin *et al.* 2001; Koenig *et al.* 2003) whilst Zinn (1994) reported no difference in the *in vivo* starch digestibility of high (0.39 kg/L) and low (0.30 kg/L) flake density wheat.

In vitro digestibility of sorghum-starch has been shown to increase linearly with decreasing flake density (Theurer *et al.* 1999b; Swingle *et al.* 1999). Decreasing flake density of sorghum has been reported to increase rumen and whole tract starch digestibility but post-ruminal digestibility remained unchanged (Swingle *et al.* 1999; Theurer *et al.* 1999b). This is inconsistent with the view (Owens *et al.* 1986) that processing treatments that increase rumen starch digestibility also increase starch digestibility in the small intestine. It could be speculated that the bacteria in the rumen are digesting the most available starch, leaving the least digestible material to pass into the small intestine. For this reason, there may be little difference in the quality of the starch leaving the rumen for high and low flake density grain.

Nitrogen digestibility

The digestibility of crude protein in the rumen, small intestine and whole tract has been shown to increase linearly with decreasing flake density of sorghum (Swingle *et al.* 1999). Microbial nitrogen flow to the duodenum has been shown to be similar for equal intakes of steam flaked and dry rolled sorghum (Theurer *et al.* 1999b) and for decreasing flake densities of sorghum (Theurer *et al.* 1999b) and corn (Zinn 1990a; Joy *et al.* 1997; Theurer *et al.* 1999b). This

suggests that cattle fed grain of lower starch digestibility in the rumen will not necessarily have less microbial protein flowing from their rumen to the intestines.

Feed conversion efficiency

Although starch digestibility tends to increase with decreasing flake thickness, this is not always reflected by improved animal performance. In certain circumstances (Zinn 1994), cattle fed diets based on well flaked grain may have the same feed efficiency as cattle fed diets based on poorly flaked grain whilst heavily steam flaked grain may even reduce overall efficiency (Zinn 1990a). This is most likely because of rapidly fermenting starch causing acidic conditions and general digestive upset.

Similar feed efficiency has been observed in cattle fed coarsely flaked wheat (0.39 kg/L) or thinly flaked wheat (0.30 kg/L) (Zinn 1994). Rumen and whole tract starch digestion were similar for these two grains although cattle fed thinly flaked wheat tended to have a higher incidence of liver abscesses (Zinn 1994). Zinn (1993) reported that the efficiency of feed utilisation tended to improve when the flake thickness of barley was reduced from 0.39 to 0.19 kg/L. This improvement occurred despite the fact that there were no differences between the grains in whole tract starch digestibility. Zinn (1993) suggested that the benefits of thinly flaked barley arose through a greater use of rumen nitrogen (reflected by an increased passage of non-ammonia nitrogen from the rumen) and a reduction in methane energy loss as the thin flake produced 48% more rumen propionate than the coarse flake. For these reasons, it may still be beneficial to process barley to a lower flake density, even if whole tract starch digestibility is not improved. In contrast to barley, the results of Zinn (1994) suggested that processing wheat to a lower flake density may have no benefit and may also be highly undesirable with an increased risk of acidosis in the rumen.

Zinn (1990a) reported cattle fed a diet based on poorly flaked corn (0.42 kg/L) out-performed cattle fed a diet based on well flaked corn (0.30 kg/L) even though whole tract starch digestibility was marginally higher for the well flaked corn (98.5 vs 99.6%). Zinn (1990a) suggested that animal performance was not improved for thinly flaked corn due to a lower rumen pH in cattle fed this diet (6.08 vs 6.29, measured 4 h posprandial). The assumption of digestive upset caused by the well flaked corn was supported by the observation of much greater variability in the performance of animals fed this diet. The results of Zinn (1990a) demonstrate that maximising *in vitro* and *in vivo* starch digestibility does not automatically maximise the efficiency of feed utilisation.

The available evidence is variable but tends to suggest that sorghum should be flaked to a relatively high flake density to maximise the efficiency of production: 0.39 kg/L (Theurer *et al.* 1999c); 0.36 kg/L (Reinhardt *et al.* 1997); and 0.36 kg/L (Swingle *et al.* 1999). In contrast, Xiong *et al.* (1991) reported that sorghum was most efficiently utilised when the grain was more extensively processed (0.28 kg/L).

Reinhardt *et al.* (1997) concluded that sorghum should not be over-processed as cattle fed low flake density (0.28 kg/L) sorghum had lower DMI, ADG and efficiency of gain than cattle fed high flake density sorghum (0.36 kg/L). Cattle fed the high density flake also had heavier carcass weights. This result disagrees with earlier findings for steam flaked sorghum of the same flake densities. Xiong *et al.* (1991) found that while low flake density sorghum (0.28 kg/L) depressed DMI, ADG was not depressed and feed conversion was improved. The reason for the differences between these studies is almost certainly due to the overall extent of starch availability. Reinhardt *et al.* (1997) suggested that grains of similar bulk densities may still vary in the level of starch gelatinisation, degree of partial solubilisation of starch and extent of disruption to the protein-starch matrix. It is possible that starch availability of steam flaked grain may not be adequately described by one measurement and this difficulty in describing the degree of steam flaking contributes to the problem identifying the optimal level of grain processing.

Dry matter intake

The effects of more rapidly fermented starch sources on DMI are variable. DMI increased with lowering flake thickness of barley (Yang *et al.* 2000) and corn (Theurer *et al.* 1999b), yet decreased with lowering flake density of steam flaked sorghum (Xiong *et al.* 1991; Reinhardt *et al.* 1997; Theurer *et al.* 1999c; Swingle *et al.* 1999). Theurer *et al.* (1999c) suggested that the reason for a decrease in DMI was the higher digestibility of well flaked grain. Hence, cattle can eat less grain but still meet their energy requirements. The accumulation of acid is also a possible reason for reduced DMI (Knowlton 2001) as low rumen pH is known to reduce fibre digestibility (Slyter 1976) which in turn may increase rumen fill.

Propensity to cause acidosis

A high degree of steam flaking is of little value when starch digestibility is already high and in such situations, further processing can be undesirable if rate of fermentation exceeds the buffering and absorptive capacity of the animal. Beauchemin *et al.* (2001) reported that rumination time decreased when steers were fed diets based on decreasing flake thickness of barley. A decrease in rumination time with an associated reduction in saliva secretion could

accelerate rumen acidosis. Reinhardt *et al.* (1997) found that lowering the flake density of sorghum increased both the risk and severity of acidosis and rumen pH has been reported to decrease linearly with flake density of corn (Zinn 1990a) and steam rolled barley (Zinn 1993). In the latter study, rumen pH 4 h postprandial was 5.35 for thinly flaked barley (0.19 kg/L). This pH would almost certainly be adversely affecting the microbial population and normal gut function.

2.8 Genetic Variation in Animals Affecting the Extent and Site of Starch Digestion

2.8.1 Introduction

Additional to diet manipulation, it may be possible to improve starch digestion by taking advantage of any individual differences between animals in their capacity to digest starch. Being able to identify and select for cattle that are more efficient at digesting starch is of potential importance to the feedlot industry. In addition to economic and environmental benefits, identification of animals that are more efficient at starch digestion may also improve the safety of grain-feeding through reduced incidence of acidosis. This section develops the idea that variation in the efficiency of starch digestion exists and highlights the possible mechanisms causing this between-animal variation.

2.8.2 Does variation in starch digestion exist in cattle?

There is always variation in any biological system and differences will always occur between animals. The process of natural and human selection of ruminants has traditionally occurred under grazing conditions based largely on pasture and/or supplements of hay or silage. Therefore, it is not surprising that the digestive tract of ruminants is ideally suited to utilising low quality fibrous material and is not particularly well-adapted to digesting cereal grain. The pancreatic and duodenal digestive enzyme systems and the absorptive mechanisms of the small intestine have never evolved to handle high levels of starch.

There has been no genetic selection for efficient starch digestion in cattle even though over 700,000 head were being fed in Australian feedlots in December 2002 (ALFA 2003). The fact that a feedlot steer still spends over half of its life in a paddock may have contributed to the lack of interest in this area of selection. Breeding females must also still be productive on a forage-based diet.

Conversely, poultry and pigs have been more intensively selected for feed conversion efficiency on grain-based diets. Despite this selection pressure, high phenotypic variability in starch digestibility has been shown to exist in young broilers eating wheat-based diets (Rogel 1985). In that instance, there was no difference in weight gain between birds because birds with compromised starch digestion ate more to compensate for poor energy utilisation. Svihus (2003) also reported that poor starch digestibility was a problem for a small percentage of birds and suggested that digestion in these birds was impaired due to a high feed intake and passage rate. Evidence of variation in starch digestion in other species gives reason to believe that it may exist in cattle.

2.8.3 Likely mechanisms behind variation in starch digestion

There are many factors likely to vary between animals that may effect the site, rate and extent of starch digestion in the gastrointestinal tract. Animal factors that could possibly be under genetic control are discussed in this section.

Feeding and intake

Feeding behaviour, DMI, dentition and the quantity and composition of saliva production (Slyter 1976) may all vary between animals. It is known that younger cattle chew feed more thoroughly than older animals (Owens *et al.* 1986) which might reflect relative intake differences (calves vs compensating yearlings). The level of mastication/rumination influences feed particle size and saliva production and ruminants eating grain-based diets are particularly dependent on the buffering capacity of saliva (Argenzio 1993c; Beauchemin 2000).

DMI is dependent on animal, environmental and dietary factors. Sheep selected for clean fleece weight have been shown to have a higher DMI than sheep selected against fleece weight (Thompson *et al.* 1989; Kahn *et al.* 2000). Eating rate (within a meal) has also been reported to be highly variable between animals (Frisch and Vercoe 1977) but the effect that this might have on DMI and rate of passage is not known. Steers selected for high efficiency of feed utilisation (low residual feed intake; RFI) had a lower DMI and ate less often during the day than steers with a low efficiency of feed utilisation (high RFI) (Richardson *et al.* 2000). The implications of this feeding behaviour on digestive function and starch digestion are unclear.

The rumen

The frequency and efficiency of rumination, rumen motility and residence time of particles may differ between similar individuals given the same diet (Hegarty 2000). Differences in

rumen dilution rate (rumen turnover or retention time) may affect starch digestion in a number of ways:

- changes in the microbial species within the rumen
- changes in the pattern of VFA and methane production
- changes in microbial efficiency
- changes in the amount of undegraded starch and dietary protein supplied to the small intestine

There is evidence that selection for and against wool growth in Merino sheep has resulted in concomitant changes in rumen function. Kahn (1996) found that sheep selected for high wool yield produced significantly more microbial protein per kg DMI, than those selected for low wool yield. Selection for wool yield had evidently caused changes in the rumen environment that stimulated microbial growth, most probably due to changes in rumen dilution rate.

Orskov *et al.* (1971a) reported large differences in the digestibility of a diet containing 93% pelleted corn between two sheep. Around 97% of available starch was fermented in the rumen of one sheep compared with 57% in the other sheep. This sheep produced substantially less rumen microbial protein and more starch entered the small intestine and hindgut. The authors suggested these differences were due to variation in rumen outflow rate between the sheep.

Variation in rumen outflow rate in cattle was reported by Orskov *et al.* (1988). In particular, they found that differences in rumen outflow rates between cows persisted with both *ad libitum* and restricted intakes of high and low roughage diets. The consistency in the differences between cows under controlled experimental conditions indicates that rumen outflow rate is under some genetic influence.

Oesophageal (reticular) groove physiology and activation stimuli (Orskov *et al.* 1970a) are other factors that may influence the efficiency of rumen starch digestion. There may also be physiological differences between animals in the structural integrity of the rumen, small intestine and hindgut wall. For example, considerable variation in the rate of parakeratosis development in the rumen was reported in similar steers fed pelleted lucerne diets (Hinders and Owen 1965).

The abomasum

Residence time of digesta and the rate of HCl secretion in the abomasum may vary between animals. Protein digestion is initiated in the abomasum with the secretion of pepsinogen

(Argenzio 1993c) which is converted to its active form, pepsin, in the presence of acid in the lumen. Similarly, the pancreas secretes inactive trypsinogen into the small intestine, which is then activated to the protease, trypsin (Argenzio 1993c). Trypsin then activates the remaining trypsinogen as well as the other proteolytic proenzymes, chymotrypsin and elastase (Argenzio 1993a). Proteases are important in breaking down cell walls and structural protein and the extent of starch-protein interactions in cereal grain are known to affect starch digestibility (Rooney and Pflugfelder 1986). The implication of this association is that animals that are more efficient at digesting protein may indirectly be more efficient at starch digestion.

The small intestine

The ability to adapt to diets delivering more starch to the small intestine may vary between individuals. Orskov *et al.* (1971b) demonstrated considerable differences between two sheep in their capacity to absorb glucose from the small intestine, as shown in Figure 2.8.

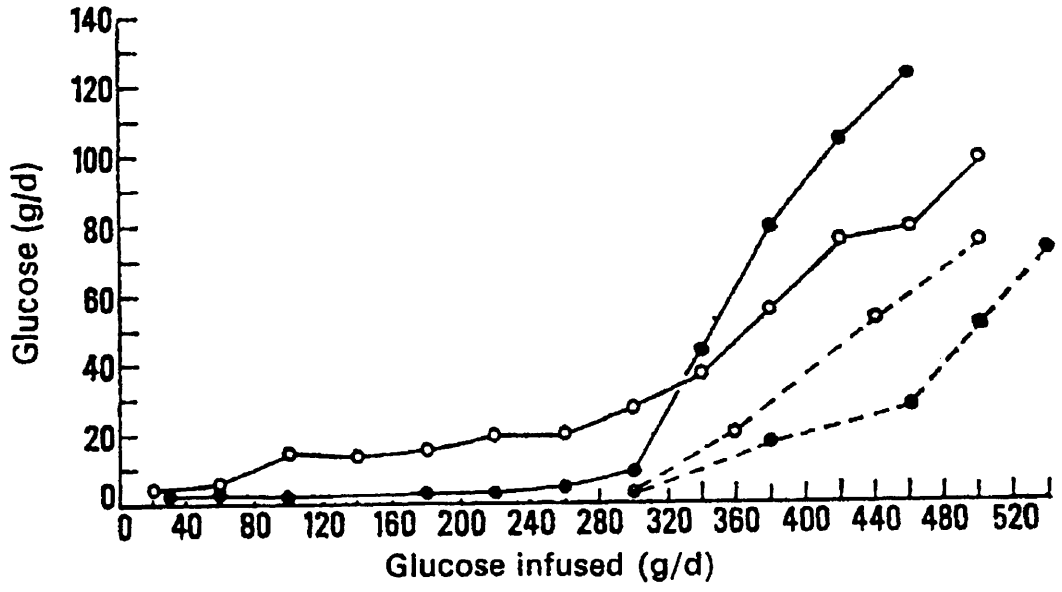


Figure 2.8 Effect of the level of glucose infused into the abomasum on glucose passing the terminal ileum (-) and excreted in faeces (---) (g/d) of sheep 1 (●) and sheep 2 (○). From Orskov *et al.* (1971b).

It could be assumed that an animal with more starch or free glucose leaving the small intestine would be more susceptible to hindgut acidosis. In the experiment by Orskov *et al.* (1971b), an increased supply of glucose to the hindgut resulted in watery faeces and reduced faecal pH.

The variation depicted in Figure 2.8 may provide some explanation for the differences that exist between animals in their susceptibility to acidosis.

Small intestinal motility influences the time that substrate is available for enzymatic digestion and this may vary between individuals. Provided that DMI is not limited, a slow rate of passage through the small intestine is ideal to ensure more complete digestion of carbohydrate before the terminal ileum.

The hindgut

The efficiency of digestion in the rumen and small intestine indirectly affects the quantity and composition of digesta reaching the hindgut. Siciliano-Jones and Murphy (1989b) observed between-animal differences in total caecal VFA, acetate and propionate concentrations and although it was not known what caused this variation, the authors suggested that differences in DMI and starch flow to the hindgut may be involved.

2.8.4 Evidence of genetic variation in starch digestion

It is already known that the overall efficiency of feed utilisation in cattle is determined partly by genotype (Herd *et al.* 1997; Richardson *et al.* 1998). Whilst it could be expected that FCR is associated with the efficiency of starch digestion, the two do not necessarily correlate. For example, two animals may have a similar capacity to digest feed; however other factors (metabolism, maintenance requirements, disease status or activity levels) may indirectly cause one animal to exhibit a better FCR (Oddy and Herd 2000).

In relation to between-breed differences, it is not known if *Bos indicus* content influences the capacity for starch digestion. Warwick and Cobb (1975) reviewed genetic variation in cattle in their ability to digest nutrients but did not focus on starch. These researchers reported inconsistent results but reached the general conclusion that cattle with *Bos indicus* content were slightly more efficient than their *Bos taurus* counterparts in digesting DM and crude protein. For starch-based diets, there were no differences in DMI, ADG and FCR between *Bos indicus* crossbred and *Bos taurus* cattle (Boyles and Riley 1991; Krehbiel *et al.* 2000). Beaver *et al.* (1989) fed a finishing diet of corn silage and whole shelled corn to Angus and Brangus steers over two years. The Angus cattle had a greater DMI for the first year but were no more efficient in digesting starch than Brangus steers. In year 2, the Angus steers had higher DM and starch digestibilities than Brangus steers. This suggests that *Bos indicus* cattle did not increase starch digestibility, as did the *Bos taurus* animals, possibly indicating the existence of genetic differences in long-term adaptation to high-starch diets.

Richardson *et al.* (1996) reported cattle with high net feed conversion efficiency had a 1% higher DM digestibility than cattle with low net feed conversion efficiency when fed a pelleted 70% lucerne: 30% wheat mixture. Likewise, low RFI (high efficiency) Angus steers tended to digest about 2% more DM from a feedlot ration than high RFI (low efficiency) Angus steers (calculated DM digestibility = 78%) (R.M. Herd *et al.,* unpublished data). Herd *et al.* (1993) reported genetic variation in whole tract OM digestibility (OMD) in sheep, over and above systematic variation due to the level of feed intake.

Meissner *et al.* (1996) observed large differences between 8 corn-fed steers in the amount of starch flowing to the duodenum which ranged from 340 to 1000 g/d. In these steers, the percentage of OM digested in the rumen was not related to DMI but was negatively associated (R^2=0.77) with postruminal starch flow. Consequently, the observed variation in OM digestibility in the rumen and the passage of starch to the small intestine appeared to be due to inherent variability in rumen retention time but may also be related to differences in digestion rate.

In the feedlot, perhaps the most obvious sign that animals vary in their capacity to digest starch efficiently is a noticeable variation in faecal consistency and pH. While faecal DM content is not a useful predictor of starch digestion, it may possibly indicate an animal's susceptibility to acidosis. It is difficult to predict which animals will develop acidosis upon entry into the feedlot and the variability between animals has also made it difficult to study the condition (Slyter 1976; Rowe and Pethick 1994). Considerable variation in the ability of animals to cope with a 'carbohydrate challenge' has been reported by Brown *et al.* (2000) and Dougherty *et al.* (1975b). In the latter study, 70 g grain (75:25 whole corn:whole oats)/kg LW was administered directly into the rumen of three steers. The rumen pH in one steer did not fall below 5.5 and diarrhoea was the only visible sign of acidosis in this animal. The rumen pH in the other two steers decreased to 4.0 and both experienced acute acidosis.

The question remains why some animals experience acute acidosis, yet others maintain good health and feed intake when fed a diet high in fermentable starch. The reasons for this variation are not clear but could involve differences in salivary buffering, the microbial population (before and after exposure to a large amount of fermentable carbohydrate), differences in the immune response to tissue damage/disease or variable changes in the gut wall characteristics and the extent of inflammation and leakage. The ability to digest starch efficiently and safely is likely to be involved and there may be scope for genetic selection for 'resistance to acidosis' on this basis. In particular, if an animal has the ability to extensively digest starch in the small intestine, there is less chance of delivering fermentable starch to the hindgut.

2.9 Conclusions from the Literature Review and Directions for Experimental Work

The diet and the animal are the two main areas of interest that may potentially be targeted to improve starch digestion in cattle.

- Diet formulation, grain processing and feeding practices are the traditional tools to manage starch digestion and there is still scope for improving current grain selection and processing techniques. Steam flaking is particularly expensive and it is difficult to objectively determine the most desirable level of processing. A conflict remains between maximising whole tract starch digestibility and avoiding acidosis, particularly at sub-clinical levels.
- Animals that are potentially susceptible to acidosis cannot be identified and the significance of hindgut acidosis in grain-fed cattle is unknown.
- There are theoretical advantages of shifting the site of starch digestion from the rumen to the small intestine. Starch digestion in the small intestine is important for three reasons:
  - Higher energetic efficiency than fermentation.
  - No risk of acidosis at this site.
  - Provides a direct supply of glucose that may have potential benefits in marbling.
- Supplying a readily digestible source of starch to the small intestine that is not extensively digested in the rumen is a practical problem. Increasing the supply of starch to the small intestine may also increase the supply of starch to the hindgut due to limitations to digestion in the small intestine.
- Starch structure appears to have an important influence over digestion in the small intestine although there is conjecture as to what is the first limiting factor for starch digestion. It appears that α-amylase concentration in the small intestine increases with energy intake, however, there is a need to properly identify mechanisms regulating α-amylase production to enhance secretion of this enzyme.
- Genetic variation in starch digestion almost certainly exists in cattle and there are numerous factors that may differ between animals that can influence the extent and site of starch digestion. It is important to establish the extent and cause of this variation.

Chapter 3. General Materials and Methods

3.1 Introduction

This chapter describes procedures that are common to two or more of the experiments reported in the following chapters. Any variation from these procedures is described within the relevant sections of future chapters.

3.2 *In vitro* Fermentation Assay

Two slightly different *in vitro* fermentation methods were used to simulate fermentation in the rumen and specifically, to rank grains in terms of fermentation rate. 'System 1' was based on the original method described by Bird *et al.* (1999) where 30 g of grain was incubated for 5 h.

'System 2' was a modification of this method where the entire size of system 1 was reduced by 2/3 to allow for a larger number of samples to be tested over 5 h. In their earlier work, Bird *et al.* (1999) hammer-milled grains through a 0.5 mm screen. However, for the majority of work reported in this thesis, grains were fermented in the 'as-fed' form which has been previously examined and shown to give consistent results (S. Bird, pers. comm).

3.2.1 System 1

Grains were fermented in 1 L, glass bottles (incubation vessels). Each vessel was sealed with a rubber cork fitted with a 3-way plastic tap allowing the sampling of liquid and the collection of fermentation gas. Gas was collected in inverted measuring cylinders initially filled with water in water baths.

Rumen fluid (2 L/animal) was collected from two steers (Low-line, 340 kg) fitted with permanent rumen cannulas and adapted to a diet of 50% mixed, dry rolled grain (20% each of barley, oats, wheat, corn and sorghum) and 50% oaten chaff. The steers were always fed in the morning and rumen liquor was collected just before feeding. The collected liquor was strained through nylon mesh and then through two layers of cheese-cloth before being immediately taken to the laboratory for use in the fermentation assay. The liquor from the two animals was combined to create a single source of rumen fluid.

Thirty grams of each treatment grain ('as-fed' weight) was added to an incubation vessel with 375 mL of pre-warmed, McDougalls buffer and urea (1.2 g/L) and 125 mL of rumen fluid. Control vessels (blanks) containing only buffer and rumen fluid were also fermented. Each

bottle was then flushed with CO_2 gas to maintain aerobic conditions before corks were replaced. Fermentation of the grain was continued over 5 h in a shaking water bath (39°C), according to the protocol described by Bird *et al.* (1999).

Cumulative gas production was measured every 30 min as the volume (mL) of water displaced in the inverted measuring cylinders. The fastest rate of gas production generally appeared to be over the last half of the fermentation period (presumably after the rumen microbial population had fully colonised the substrate). Rate (mL/min) was calculated between 2.5 and 5 h.

At the conclusion of the fermentation period, 15 mL of liquor was removed from each incubation vessel, acidified and frozen for later VFA and lactate analysis. The final pH of the fluid was taken before 15 mL of 20% sulfuric acid was added to the vessels to stop fermentation.

Gas, VFA and lactic acid data was corrected for the results of the blanks.

3.2.2 System 2

The *in vitro* fermentation procedure for system 2 was the same as system 1 with some important modifications. Plastic flasks (250 mL) with screw top lids were used as incubation vessels. Gas production was not measured and the lids had a small hole to allow fermentation gases to escape. The volume of rumen fluid added to the fermentation flask was reduced to 42 mL and used with 10 g of grain and 125 mL of buffer. Fermentation was stopped with the addition of 5 mL of 20% sulfuric acid to the flasks.

In the experiment reported in chapter 9, particulate residue left in the flasks following fermentation was transferred into pre-weighed aluminium trays and dried for three days in an 80°C draught oven before being dried to a constant weight for one day in a 105°C draught oven. Starch concentrations of the original grains, grain residues and blank residues were determined (section 3.5.1) to calculate starch digestibility.

Starch digestibility was not usually measured in the fermentation systems because total acid production (VFA + lactic acid) was believed to provide a reliable measure of the rate of starch fermentation (Bird *et al.* 1999). Given that most cereal grains are over 60% starch, acid production and starch digestibility are likely to be closely associated. However, results need to be interpreted with a degree of caution as other grain fractions, such as protein, also contribute to total acid production when fermented. Similarly, fermented fibre will influence acid production and be particularly important in low-starch grains such as oats.

3.3 *In sacco* fermentation

In sacco fermentation studies reported in this thesis used bags (10 x 15 cm) made of a precision-woven monofilament polyester cloth with a pore size of 44 μm (Perdok 1986).

Grains were weighed (5 g; 'as-fed') into the pre-weighed nylon bags which were then tied with fishing line. Groups of nylon bags to be removed at each time were placed in separate onion bags tied to a short length of chain to ensure that these remained below the raft of the rumen and to facilitate bag removal (Perdok 1986).

Upon removal, bags were washed under running tap water until the effluent was clear and then washed using a normal cold water cycle in an automatic tub washing machine. Bags were then dried at 50°C for 72 h before being re-weighed to calculate DM disappearance for each incubation time. It was considered that DM disappearance would be closely related to starch digestion, however, caution must be used when interpreting these results.

3.4 *In vitro* Enzyme Assay

The *in vitro* enzyme assay used in this thesis was described by Bird *et al.* (1999) and is designed to simulate the digestion of starch by amylolytic enzymes in the small intestine. The assay is a modification of the Megazyme total starch determination method (section 3.5.1).

Ground (0.5 mm screen) grain was weighed (0.1 g) into duplicate culture tubes (30 mL). Ethanol (80%) was added (0.2 mL) to wet and disperse the sample before the addition of 3 mL of thermostable α-amylase solution (1 mL of amylase in 30 mL of 50 mM MOPS buffer, pH 7). Amyloglucosidase was added (0.1 mL) before a 1 h incubation period in a 39°C shaking water bath. The digestion mixture in each tube was then made up to 10 g using deionised H_2O and centrifuged (Beckman centrifuge, Model TJ-6, Beckman Instruments Inc., USA) at 3000 rpm for 10 min.

Subsequent steps for sample dilution, incubation and calorimetric determination of glucose were as described for the total starch determination method (section 3.5.1). *In vitro* enzymatic starch digestibility was expressed as the proportion of total starch in the original sample that was solubilized as glucose.

Replication is generally not required to obtain a reliable result from this diagnostic test. Previous studies have shown that this assay is highly repeatable when using finely ground grains (S. Bird, pers. comm) and there is little value in performing more than one test for a grain. The assay is regarded in a similar way to other chemical measurements.

3.5 Analytical Procedures

3.5.1 Starch

Starch analysis was performed according to the Megazyme total starch determination method (AA/AMG 6/98). This procedure is based on the method developed by McCleary *et al.* (1994) and modified by McCleary *et al.* (1997) and is approved by the AOAC (1990) (AOAC Method 996.11). Details of the chemical reagents used are described in the Megazyme starch assay kit.

Samples were ground (0.5 mm screen) and weighed (0.1 g) into duplicate culture tubes (30 mL). Ethanol (80%) was added (0.2 mL) to wet and disperse the sample before the addition of 2 mL of dimethyl sulfoxide (DMSO) to break-up any residual starch. Starch was gelatinised by placing the tubes in a 98-100°C heating block for 10 min. Thermostable α-amylase solution (1 mL of amylase in 30 mL of 50 mM MOPS buffer, pH 7) was then added (3 mL) before tubes were returned to the heating block for a further 30 min. Each tube then received 4 mL of sodium acetate buffer (pH 4.5) and 0.1 mL of amyloglucosidase before 1 h of incubation in a 50°C shaking water bath. The digestion mixture in each tube was then made up to 10 g using deionised H_2O and centrifuged (Beckman centrifuge, Model TJ-6, Beckman Instruments Inc., USA) at 3000 rpm for 10 min.

Samples of the supernatant (0.5 mL) were diluted with deionised H_2O (4.5 mL). Duplicate aliquot samples (0.1 mL) were then added to a test tube with 3 mL of GOPOD solution (glucose determination reagent) and incubated for 20 min in a 50°C shaking water bath. Control blanks (H_2O) and glucose standard solutions (50 and 100% glucose) were also incubated with GOPOD.

Upon cooling for 15 min, the absorbance of the sample mixture (glucose) was read at 510 ηm against deionised H_2O using a Hitachi 150-20 Spectrophotometer (Hitachi Limited, Japan).

Starch (%) that was solubilized to glucose = $\Delta A \times \frac{F}{W} \times 90$

Where,

ΔA = absorbance of sample – absorbance of reagent blank;

F = 100/Absorbance for 100 μg glucose;

W = Weight (mg) of original sample; and

90 = adjustment from free glucose to anhydrous glucose (as occurs in starch).

3.5.2 Volatile fatty acid analysis

For the *in vitro* fermentation assay (system 1) reported in chapter 5, VFA concentrations in liquor samples were determined by gas-liquid chromatography (Erwin *et al.* 1961). Isocaproic acid (400 μL) was added as an internal standard (Geissler *et al.* 1976) to 700 μL of supernatant. The gas-liquid chromatograph used was a Packard Model 427 (Packard Instrument Company, Illinois, USA). This instrument was connected to a data recorder and processor (Packard Model 604, Packard Instrument Company, Illinois, USA). Samples were injected (1 μL) into a packed column with an injection and flame ionisation detector temperature of 200°C and an oven temperature of 150°C. Run-time was 20 min.

For all subsequent VFA analysis, measurement was performed using a Varian CP 3800 gas chromatograph (Varian Analytical Instruments, California, USA) with a Varian CP 8400 autosampler (Varian Analytical Instruments, California, USA). The procedure was similar to that described for the Packard Model 407 however, the injection temperature was 150°C and oven temperature was 60°C. The packed column gas flow rate was 5 mL/min at 2.5 psi.

3.5.3 Lactic acid analysis

The concentration of D- and L-lactic acid was measured using a Cobas Bio (Roche Diagnostic Systems) and a Boehringer Mannheim D-Lactic acid/L-Lactic acid kit (catalogue number 1112821). The D-Lactic Acid/L-Lactic Acid UV method was used which involved the stochiometric measurement of NADH formation at 340 ηm and the indirect calculation of lactic acid concentration using a 1:1 ratio.

3.5.4 pH

A portable Ecoscan pH 5/6 pH meter (Eutech, Singapore) was used to determine pH.

The pH of rumen fluid was determined immediately after sampling. Faecal and caecal pH (digesta obtained using caecal cannula – chapter 9) were determined after thoroughly mixing 4 g of sample with 4 mL of deionized water. This method of pH measurement has previously been used for faeces by other researchers (Russell *et al.* 1980; Haaland *et al.* 1982; Barajas and Zinn 1998) and is considered to be accurate and unbiased for comparative analysis.

3.5.5 Faecal nitrogen

Faecal nitrogen content (%) was determined on sub-samples of oven-dried faeces. Nitrogen loss under oven drying conditions was unaccounted for but assumed to be negligible. The analysis for total nitrogen was performed using the Leco FP 2000 system (Leco corporation,

USA). Finely ground samples were weighed (1 g) into pre-weighed ceramic crucibles before being combusted at 1000°C. Nitrogenous compounds were converted to N_2 and NO_x gases which were subsequently measured by a thermal conductivity detector. This procedure is approved by the AOAC (1990) (AOAC Method 990.03).

3.5.6 Dry matter determination

Fresh faeces, grain/feed samples and fermentation residues were added to a pre-weighed container (cont wt), weighed (wet + cont wt) and dried to a constant weight (dry + cont wt) in an 80^0C oven for 3 d.