1. CHAPTER 1: GENERAL INTRODUCTION

Low-quality roughages are major feed resources for ruminant livestock in most parts of the tropical and sub-tropical regions of the world, whereas in most temperate areas their use is mainly restricted to times of crisis, especially during prolonged droughts (Leng 2003). The tropical areas are also the areas with a rapid increase in human population and a large deficit in animal protein supply relative to demand (Jasiorowski 1975). The increase in population in that region has resulted in intense pressure on the use of available land resources with the land being devoted mainly to human food production with very little reserved for animal feed production (Sundstol *et al.* 1993). As a result crop residues form the main, and in some case the only feed resource available in many areas to sustain ruminants such as cattle, buffalo, sheep, and goats (Jalaludin *et al.* 1992).

The main roughages include; crop residues such as cereal straw and stover, over-mature natural pastures, and some fibrous agro-industrial by-products such as sugar cane bagasse. Globally about one billion metric tonnes of crop residues are produced annually (Kosila 1984), with most being used in developing countries (Wanapat and Devendra 1985; Wedin and Hoveland 1987; Owen and Jayasurya 1989). It is estimated that the yield of cereal grain to straw is in the ratio of 1:1 and therefore, any expansion in grain production is likely to result in an equally large output of straw (Leng 1992). This also poses a major challenge in the disposal of crop residues due to the environmental pollution associated with the traditional burning approach widely practised in many countries in the world. In Australia alone, an estimated 30 million tonnes of cereal straw is produced annually with most of it either being burned or retained on the farm as cereal stubble (Dryden 1986; Leng 1992).

The use of low quality roughage such as cereal straw as a feed resource for ruminants and the nutritional constraints associated with its use, and the effect these constraints have on animal production performance has been reviewed by O'Donovan (1983). These nutritional constraints include deficiency in protein, energy, minerals and vitamins which tend to limit full utilisation of crop residues as a feed resource in ruminant livestock (Makkar 2004). Though the nutritive value

of crop residues is generally low, it is also highly variable and influenced by many factors such as genotype, stage of maturity or the time at which the grain is harvested (Bolsen 1977).

According to Leng (1990), low-quality roughages are forages with the following characteristics: a digestibility of less than 55%; less than 80 g crude protein (N x 6.25).kg^{-1}DM and a low content of soluble sugars (<100 g/kgDM). Balch (1977) suggested that all forages that provide less that 7.5 MJ ME/kgDM should be considered as low-quality roughage, a classification that would include most of the crop residues. The primary factor limiting voluntary intake of high fibre, low protein roughage in ruminants is mainly their low nitrogen (N) content (Weston 1967; Topps 1972; Obara *et al.* 1991), though in some cases S can also be important. In spite of these limitations, crop residues can be utilised by ruminants provided that they undergo physical processing and/or chemical treatment, and are supplemented with deficient rumen degradable nutrients, and by-pass protein to improve digestibility and intake (Leng 2003). The poor production performance of animals given low-quality roughage as basal diets has been attributed to the low digestibility; this leads to gut-fill limitations resulting in very low dietary intake (Silva *et al.* 1989). The low digestibility has been attributed to several factors that include: high cell wall content (CWC); low N content; low level of readily fermentable carbohydrates; low and imbalanced mineral content, low palatability, and even the presence of anti-nutritive factors such as silica in some crop residues such as rice straw (Kamastra *et al.* 1980; Hennessy *et al.* 1983; Hamada 1989). Owing to the low digestibility and intake, the intake of ME and also other digestible nutrients tends to be low and at a level that is hardly adequate to meet the maintenance requirements of the animal (Erickson 1981). Therefore, low quality roughages are of little use as ruminant feed for any production level above maintenance, unless modified by some form of treatment and/or supplemented with deficient nutrients so as to improve on their digestibility and intake.

The studies reported in this thesis were conducted to investigate the role of N, protein and energy supplementation as a means of enhancing digestibility and intake of low quality roughage in ruminants. The main objective of the study was to further understand the fundamental changes in the rumen and tissue metabolism that are associated with the supplementation, and the effect of those changes in improving utilisation efficiency of low quality roughage by ruminants.

2. CHAPTER 2: LITERATURE REVIEW

2.1. Background information

Low quality roughages that include mainly cereal crop residues are generally poorly utilised by ruminants mainly due to their low rate and extent of digestion in the rumen (Preston and Leng 1987; Sundstol *et al.* 1993). This is brought about by a number of factors that include: nutrient deficiencies associated with these roughages, especially N and minerals such as sulphur, and a high content of CWC with a high degree of lignification (Minson 1967a; Weston 1967; Yates and Moir 1983). The inadequate supply of rumen degradable nutrients generally contributes to the low rate of microbial growth and therefore results in a low critical pool size of rumen microbes; this then impacts negatively on fermentation activity leading to a low rate and extent of fibre digestion in the rumen (Weston 1984; Leng 1990, 2003). As a result, rumen microbes are unable to ferment the potentially digestible OM in the basal roughage to obtain enough energy for their own maintenance, and the volatile fatty acid (VFA) supply to the host animal is also likely to be sub-maintenance. Furthermore, the low microbial growth in the rumen means that the quantity of microbial biomass flowing into the small intestines will not provide sufficient protein to meet the protein requirements of the animal. Supplying the rumen microbes with the requisite critical rumen degradable nutrients is a strategy that can be used to stimulate rumen microbial growth; as their numbers increase, they are then able to degrade the potentially digestible OM in the roughage and obtain additional energy.

In ruminants fed low quality basal roughage the main fermentation products (VFAs absorbed mainly from the rumen and microbial cells that are digested and absorbed in the small intestines) are often inadequate in both quantity and quality. The absorbed fermentation products also tend to be unbalanced in protein relative to energy (P/E) (Illius and Jessop 1996), as well as in the ratio of glucogenic to acetogenic substrates (Leng 1990). Moreover, the low microbial fermentation activity in the rumen and the high content of lignified CWC in the roughage leads to a low rate of digesta clearance from the rumen, and ultimately a low voluntary intake. This is due to the fact that the refractory material needs to be retained in the rumen longer for it to be fully comminuted to particle sizes that are small enough to pass through the reticulo-omasal orifice (*see* Lechner-Doll *et al.* 1991). The comminuting process is accomplished by rumination

and complemented by microbial degradation, which in high-fibre, low-protein basal roughage normally takes a long time to accomplish leading to low voluntary intake and therefore inadequate intake of digestible nutrients by the animal.

Long mean retention time (MRT) is a critical factor influencing voluntary intake in ruminants and especially, those subsisting on poor quality roughage where rumen fill (gut distension) is a major constraint to adequate intake of OM (Van Soest 1975; McLeod and Minson 1988a; Lechner-Doll *et al.* 1991). Thornton and Minson (1972) established that there was an inverse relationship between voluntary intakeof forages and the retention time of digesta in the rumen. They concluded that fibre content was a major factor limiting dry matter (DM) intake in ruminants mainly because of its positive influence on retention time. Low voluntary intake results in inadequate absorption of digestible nutrients (fermentation products) from both the rumen and the lower parts of the gastro-intestinal tract. This affects mainly energy and protein that are hardly adequate to meet the maintenance requirements of the animal, let alone production. The inevitable consequence is that animals subsisting on low-quality basal roughages have to mobilize their own endogenous body reserves to meet the maintenance requirements especially for energy, leading to a loss in body weight. This situation can be remedied if the fundamental factors limiting digestion of fibre in the animal's digestive system are clearly understood, and attempts made to improve digestibility and intake of high-fibre low-protein basal roughage through treatment, and/or supplementing low quality basal roughage with the deficient nutrients (Preston and Leng 1987).

2.2. Factors limiting digestion and intake of low quality roughage

2.2.1. Fibre content

Low quality roughages generally tend to be high in fibre content and this is a major factor hindering their effective digestion in the rumen. The CWC essentially comprises of lignin and structural polysaccharides with the latter including cellulose, hemicellulose and pectin (Paterson *et al.* 1994; Van Soest 1994). In the early stages of a plant growth cycle the CWC mainly consists of cellulose, hemicellulose and pectin. As the plant grows to maturity and attains senescence there is an extensive deposition of lignin on the primary cell wall and middle lamella resulting in secondary thickening (Theander 1989; Minson 1990). It is this lignification that ends

up forming a formidable physical and/or chemical barrier that hinders effective digestion of structural polysaccharides in ruminants as the un-lignified structural polysaccharides are generally fairly digested in the rumen by rumen microbes provided they are supplied with rumen degradable nutrients (Leng 2003). It is therefore, the high content of lignin that has a major influence on the rate and extent of fibre digestion in ruminants, mainly due to its effect in reducing the hydrophobic nature of the polysaccharides, and accessibility by microbes and their enzymes (Theander 1989; Van Soest 1994).

2.2.2. Cellulose, hemicellulose and pectin

Most structural polysaccharides are complex polymers made up of a large number of mainly pentose and hexose monomers. They are often found in association with lignin where they provide support to the plant. Cellulose is a complex polymer consisting of a large number of repeating ß-D glucose units linked together by ß (1, 4)-glucosidic bonds. Depending on the source of cellulose, a typical cellulose molecule may contain as many as 10,000 to 15,000 glucose units (Bailey 1973; Colvin 1981). The molecular structure of cellulose is in the form of straight ß-D glucopyranosyl chains that are strongly linked together by hydrogen bonds forming a highly ordered three-dimensional rigid formation (Greulach 1973; Darvill *et al.* 1980), with interchanging crystalline and amorphous sections whose proportions vary with source of cellulose, and especially the plant species (Theander 1989). The difference between the crystalline and amorphous section is thought to be important in cellulose digestion in the rumen because microbial attack tends to be mainly concentrated on the amorphous sections (Wood 1981). As a result most refractory plant material whose cellulose is high in the proportion of crystalline sections tends to be degraded very slowly in the rumen, with cellulose in grasses containing a higher proportion of the amorphous sections (Beveridge and Richards 1975).

Unlike cellulose that occurs in straight chains, hemicellulose and pectin are both commonly referred to as matrix carbohydrates mainly because they occur in an amorphous form rather than the straight chains. Both are also easily extracted from fibrous plant material using common solvents such as hot water or ammonium oxalate for pectin (Bailey 1973). Further treatment of the residue with 10% NaOH tends to solubilise most of the hemicellulose (Dehority 1973). Pectins are polymers of uronic and galacturonic acids or their residues and are normally closely associated with the middle lamella (Darvill *et al.* 1980), where they play a vital cementing role

between the cell walls. In addition, the pectinic substances may also contain highly branched polymers of other carbohydrates such as galactose, arabinose, xylose, fucose and rhamnose (Darvill *et al.* 1980). Hemicellulose is a polymer of several five or six carbon sugars (pentoses and hexoses) that include xylose, arabinose, mannose, galactose, rhamnose, uronides and galacturonic acids (Gaillard and Bailey 1968), with xylan and araban being the most common in monocotyledonous plants (cereal straws and grasses). It consists mainly of ß (1-4) linkage though others such as ß (1, 2) and α (1, 2) linkages may also be present (Kato 1981). In most cases the ß (1-4) linkage in structural polysaccharides is readily hydrolyzed by the cellulase enzyme produced by rumen microbes.

2.2.3. Lignin, cutin and silica

Lignin, cutin and silica occur in plants in variable proportions and tend to be closely associated with structural polysaccharides and their proportion may vary widely with genotype, part and age of the plant. Unlike structural polysaccharides that can be digested to supply rumen microbes with energy, and by extension, fermentation products to the host animal, lignin, cutin and silica are largely anti-nutritive. Most plant materials that are highly indigestible in ruminants are also quite high in these anti-nutritive plant components, especially lignin (Mertens and Ely 1982). The molecular structure of lignin and its association with structural polysaccharides has been reviewed by Hartley (1973) and Chesson and Travis (1997). Lignin is a complex non-carbohydrate organic material that is normally associated with structural polysaccharides. It is added to the primary cell wall as the plant grows towards maturity mainly to provide structural support (Theander 1989). It is basically an inert polymeric material that is highly resistant to microbial attack, especially in the anaerobic rumen environment (Theander 1989; Zadrazil *et al.* 1995). Lignin tends to influence fibre digestion in the rumen mainly through its close association with cell wall polysaccharides (Waldo and Smith 1972; Chesson and Travis 1997). Most cell wall polysaccharides are generally readily degradable by rumen microbes, but their encrustation by lignin tends to greatly hinder their digestion in the rumen (Smith *et al.* 1971). The potential digestibility of cellulose increases from 14 to 72% following delignification (Belyea *et al.* 1983).

The mechanism through which lignin inhibits microbial digestion of structural carbohydrates in the rumen is not well understood though a number of theories have been proposed. They include: reduction in rate of colonization and microbial attachment in the heavily lignified cellular

component of plant tissues such as xylem, even when high in structural polysaccharides (Latham *et al.* 1978; Czerkawski 1986b). Limited colonization of the highly lignified tissues by rumen microbes may be due to either lack of a suitable surface for microbial attachment and/or lack of accessible nutrients to nourish the rumen microbes (Chesson and Travis 1997). This situation may be ameliorated by any treatment, physical or chemical, that solubilises the lignin (Kerley 1985). Hydrolysis of the covalently bonded linkages releases the lignin-carbohydrate complexes into the rumen liquor (Conchie *et al.* 1993), a process that can also be effectively accomplished by the rumen anaerobic fungi (Orpin 1984; Joblin and Naylor 1989). This makes the structural polysaccharides in the lignified material more susceptible to further attack by the other rumen microbes, especially the cellulolytic bacteria (Lam *et al.* 1992; Lowry and Kennedy 1996).

Cutin and silica also variably reduce digestion of carbohydrates in the rumen, with cutin being found mainly on the surface of the epidermal layer of leaves and stems where it affords protection to the more delicate tissues against physical damage and attack by pathogens and insects. It also has an important role in reducing excessive moisture loss by evaporation. Cutin is basically a complex polymer consisting of a mixture of esters of long-chain fatty acids (C_{24-36}) and equally long-chain alcohols, and may also contain some free fatty acids, alcohols, large ketones and other long-chain hydrocarbons (Brown 1960). It is synthesized in the protoplasm of epidermal cells and then extruded to the outside surface where it undergoes further oxidation forming a water repellant waxy cuticle that is also highly resistant to degradation by rumen microbes. Because of the way the cuticle affords protection to the generally un-lignified but more readily digestible mesophyll tissue, it has to be physically disrupted during mastication to allow rumen microbes and their enzymes to access the cell wall polysaccharides to enable digestion to take place. The role and distribution of silica in plant tissues has been reviewed extensively by Jones and Handreck (1967). Silica occurs both in lignified and un-lignified parts of plants and its content varies with plant genotype and plant part. Rice straw in particular, is well known for its high silica content, especially in the leaf and seed hulls. Its low digestibility is generally attributed to silica rather than lignin (Jackson 1978). Though legumes generally contain more silica than grasses, the silica in legumes is more easily solubilised in the animal's gut and therefore tends to have less effect in depressing fibre digestion (Hartley 1970). While there is an apparent negative correlation between the silica content and DM digestibility, it is not clear exactly how silica depresses fibre digestion in ruminants (Van Soest 1982). A combination of both silica and lignin content is a better predicator of DM digestibility in an additive way than

either of them alone (Van Soest and Jones 1968). This strongly indicates that silica, like lignin, may affect fibre digestion in different ways including encrustation of cell wall polysaccharides. Their presence *per se* in plant material does not necessarily translate into low digestibility (Van Soest 1970b, pp 103, cited in Van Soest 1982).

2.3. Rumen microbial growth, fermentation and yield

2.3.1. Ruminant digestive system

The ruminant animal has to ingest large quantities of low quality roughage, and extract as much energy and nutrients as possible to meet its nutritional requirements for maintenance and production. To be able to cope with this challenge, ruminants have evolved a highly sophisticated and versatile digestive system characterized by two main features. These include: a voluminous and highly compartmentalized fore-stomach consisting of the rumen and reticulum (the rumen), and omasum, and the hindgut consisting of the colon and the caecum. Both the fore- and the hindgut are inhabited by a large population of anaerobic microorganisms, including bacteria, protozoa and anaerobic fungi that form a symbiotic relationship with the host animal (Hungate 1966; Brockman 1993; Theorodou and France 1993). The rumen and the hindgut are both capable of carrying out extensive fermentative digestion, with the rumen in particular having a capacity to retain digesta for a considerable period (Hungate 1966; Theorodou and France 1993).

2.3.2. Rumen microbial growth and environment

The growth of rumen bacteria and protozoa is rapid and is largely exponential (Russell and Hespell 1981). This rapid growth rate, however, cannot be sustained for long mainly due to severe competition for space and the depletion of fermentable substrates in the media, and also the possible accumulation of microbial waste metabolites (Czerkawski 1986a). The rumen environment generally has a stable temperature that varies within a very narrow range of 38-42^{0}C. It is characterized by low redox potential (-300 to -350 mV), except probably for relatively short periods such as during ingestion of food or the chewing and swallowing of the cud, and drinking of water. The anaerobic conditions are quite conducive to rumen microbes and most are either obligate or facultative anaerobes (Theorodou and France 1993). The pH of the rumen fluid ranges from 5.5 to 7.0 though for most roughage diets it is fairly stable at about 6.0-7.0. This is

also the range that is within the pH optima for most microbial enzymes including cellulases (Pearce and Bauchop 1985; Wilson and Wood 1992a). The maintenance of a stable rumen pH depends mainly on the balance between rate of production of VFA, the moderating effect of buffers contained in saliva, the removal of VFA by absorption across the rumen epithelium and outflow to the omasum (Stevens 1970). Because the rate of VFA absorption from the rumen is normally slow relative to production, the rumen pH is mainly stabilized by the combined buffering effect of saliva and liquid outflow to lower parts of the gut (Sutherland 1976).

The rumen is also continuously replenished with water and a wide range of substrates/nutrients from both exogenous (dietary) and endogenous sources that are mixed at regular intervals by the rhythmic contraction of the rumen. The rumen environment is also unique in that, under normal circumstances, the microbial waste metabolites such as VFA and fermentation gases rarely accumulate to critical levels that would be considered detrimental to the well-being of the rumen microbes. The net effect of such a stable rumen environment that is also well charged with nutrients and water is the existence of a highly complex and very stable microbial ecosystem (Wolin 1981; Theorodou and France 1993).

2.3.3. The rumen microbial population

The rumen microbial population consists of over 200 species of bacteria, over 20 species of protozoa, several species of anaerobic fungi and a relatively small number of other species such as bacteriophages and mycoplasma (Theorodou and France 1993). It is conservatively estimated that the microbial population in the rumen ecosystem consists of about 10^{10} bacteria, 10^{6} ciliate protozoa and 10^{6} phycomycetes per ml of rumen fluid (Dehority 1998). In terms of microbial biomass it is estimated that bacteria and protozoa make up about 50 and 40% respectively, and fungi 5-8% (Orphin 1981). Bacteria therefore have the highest proportional representation both in terms of numbers and biomass, followed by protozoa and fungi.

There exists a highly dynamic relationship and interaction between these microbial species leading to a continuous and sometimes very wide variation in their relative proportions within the rumen of one animal and also between animals. The relative proportion of each group in the rumen at any one time is influenced by many factors that include form and composition of diet, animal internal factors, dilution and outflow rates and the competitive pressure between different

microbial species (Hungate 1966). The latter can take various forms such as competition for nutrients and even space, predation of one group by another and secretion of inhibitory substances (toxins) that can inhibit the growth of a potential competitor. In most cases there is also complementary association between the microbes. These microbes, through their competitive and /or complementary roles can influence in various ways the feed digestion and metabolism in the rumen and whose net effect to the host animal may be either positive or negative. Of these three groups of rumen microbes (i.e. bacteria, protozoa and fungi), cellulolytic bacteria and anaerobic fungi are the most important as far as fibre digestion is concerned (Demeyer 1989; Hogan 1996, p. 35). Besides the critical role that rumen microbes generally play in the digestion of fibrous roughage, rumen microbial populations have long been known to be an important source of protein to the host animal upon digestion in the small intestines (Virtanen 1966).

The specific roles of bacteria, protozoa and fungi in the digestion process in the gut have been the subject of several reviews (e.g. Hobson and Summers 1967; Demeyer 1981; Akin 1986; Hobson *et al.* 1988, Nolan *et al.* 1989c). Hence only the major highlights of these roles will be mentioned in this review.

2.3.3.1. Bacteria

Because of their dominant presence, in the rumen both in numbers and total biomass, bacteria represent the most diverse group of microbes in the rumen (Hungate 1966). They also make the largest contribution to both fibre digestion and microbial protein supply to the animal. Bacteria have also been identified as being essential to the survival of the ruminant animal, unlike other rumen microbes such as protozoa. They generally form consortia whose complementary effect can be quite effective in fibre degradation in the rumen. Rumen bacteria also tend to vary widely in morphology, substrate preference and utilisation, niche selection and ability to grow under aerobic or anaerobic conditions (Hungate 1966).

Based on substrate preference and utilisation bacteria have been classified into several groups that include celluloytic, amylolytic and proteolytic bacteria, though some can be placed in more than one category. Among these groups, the cellulolytic bacteria are the most important as far as fibre degradation in the rumen is concerned. The main species in this group include:

Ruminococcus albus, Ruminococcus flavefaciens, Bacteroides (Fibrobacter) succinogenes and *Eubacterium cellulosolvens* that are considered to be some of the most cellulolytic bacteria in the rumen (Hungate 1966; Bryant 1973; Dehority 1991). Other bacterial species that play an important t(hough secondary) role in fibre digestion include *Butyrivibrio fibrisolvens, Clostridium longisporium and Clostridium locheadii.* While most of these bacteria will readily degrade structural polysaccharides, especially cellulose and hemicellose, they also readily utilise a variety of monosaccharides and disaccharides (Hungate 1966, pp 533). Others such as *Butyrivibrio fibrisolvens, Bacteroides ruminicola, Selenomonas ruminantium* and *Streptococcus bovis* tend to be more prevalent in the rumen only when concentrates or starch are included in the diet in significant proportions (Hobson *et al.* 1988). Besides *Streptococcus bovis,* other well-known starch fermenting bacteria in the rumen includes *Bacteroides amylophilus* and *Succinomonas amylotica* (Prins 1977). There are also some other important bacteria species in the rumen such as methanogenic bacteria that play an important complementary role in fibre digestion. Methanogens play this role by utilizing hydrogen, an intermediate metabolite of microbial fermentation, and their high numbers can contribute to efficient functioning of the rumen fermentation process.

2.3.3.2. Protozoa

Protozoa form a significant group of rumen microbes that are widely distributed in most domesticated and wild herbivores including the camelids (Williams and Coleman 1991, pp 1). It is estimated that protozoa could account for anything from zero to 80% of total microbial biomass in the rumen (Harrison and MacAllan 1980). This wide range may be due to the fact that their number is highly influenced by the substrate availability (diet), especially the proportion of concentrates, feeding pattern and feed fermentation (Bergen 2004). Their accurate enumeration is also partly complicated by the fact that they are widely distributed within a range of ecological niches in the rumen that includes sequestration in the rumen epithelium and also within the digesta. Morphologically their most important feature is the cilia (in ciliates) and flagella (in flagellates) which act as the locomotory apparatus, with ciliates forming the largest proportion among all protozoal groups in the rumen. The flagellates or the non-ciliated protozoa normally constitute only a small proportion of less than 10^5 cells.ml^{-1} of rumen fluid (Williams and Coleman 1991, pp 133).

The main protozoa genera that have been identified in the gut of domestic and wild herbivores include: *Entodinium, polyplastron, Eudiplodinium, Isotricha* and *Holotrich* (Fonty *et al.* 1984), with the *Holotrich* and *Polyplastron* normally representing the large protozoa. Entodiniomorphids are the majority representing about 33% of the microbial biomass in the rumen. The proportion of various protozoa species in the rumen depends on the ruminal pH, and this in turn is influenced by the type of diet eaten by the animal (Newbold *et al.* 1986b)

Among the ciliates, the entodiniomorphs constitute the largest numbers in the rumen with the small ciliates being dominant especially in animals fed high-concentrate diets. The large protozoa represented mainly by the holotrich are much lower in number, though they occupy a significantly large space in terms of cellular volume. For example, when the *Isotrichid* holotrichs account for only 5% of total ciliate population in the rumen, they nevertheless account for some 35% of protozoal volume, and nearly 40% of protozoal N (Abe *et al.* 1981). This suggests that population alone is not the only important factor when it comes to the contribution of the various species of protozoa to the rumen metabolism and therefore nutrition of the host animal, as biomass in terms of both cell size and numbers is probably as important (Clarke *et al.* 1982; Dehority *et al.* 1983).

Protozoa survive in the rumen mainly by engulfing feed particles such as starch grains and other microbes, especially bacteria (Coleman 1967, 1989), a feeding behaviour that may be beneficial as well as harmful to the nutrition of the host animal. The engulfment of feed particles by protozoa has been reported as being quite beneficial especially in animals feeding on diets that are high in starch and sugars. The rapid engulfment of the starch grains by the entodiniomorphs, and uptake of soluble sugars by the holotrich makes the substrate less available to the amylolytic bacteria that would otherwise ferment such substrates very rapidly precipitating the onset of lactic acidosis (Williams and Dinusson 1973; Mackie *et al.* 1978; Kariya *et al.* 1989). The removal of fermentable carbohydrate from the bacterial medium slows down the rate at which the starch is fermented. This prevents the drastic fall in rumen pH that would otherwise be detrimental to optimal functioning of the protozoa and cellulolytic bacteria given their sensitivity to low pH (Ørskov and Frazer 1975; Russell *et al.* 1979; Newbold *et al.* 1986b). However, the engulfment of rumen microbes by protozoa, especially bacteria and fungal zoospores reduces their number and could be detrimental to both fibre digestion and microbial protein production efficiency in the rumen. In particular, predation is likely to reduce the amount of total protein reaching the

small intestines (Bird *et al.* 1979), which lowers the protein to energy ratio and could contribute to lower animal productivity (Leng *et al.* 1984; Nolan 1989a, p. 211).

Protozoa tend to be present in several ecological niches that include rumen fluid, the wall of the reticulo-rumen (Abe *et al.* 1981; Leng *et al.* 1986) and sequestration within the fibre particles (Orpin 1985). The proportion in each niche is dependent on the time lapse after feeding (Leng 1989b). Because of their unequal distribution in the rumen, it has been proposed that if the rumen fluid is to be used to determine the ciliate population, the most ideal time to sample would be 3 h post-feeding (Dehority and Tirabasso 1989). At this time the protozoal population in the rumen fluid tends to reach a peak as they take advantage of the nutrients available following ingestion of the feed. Attempts have also been made to explore other more reliable methods of enumerating protozoa in the rumen such as use of [^{14}C]-choline to label protozoa (Coleman *et al.* 1980; leng 1989b) and more recently the use of Real-Time PCR technology (Bergen 2004; Sylvester *et al.* 2004).

While the role of protozoa in the digestion of starch is well understood, their role in fibre digestion in the rumen is equivocal. *In vitro* studies have demonstrated that protozoal fibre digestion may account for as much as 19-28% of total cellulase activity in the rumen (Gijzen *et al.* 1988), further strengthening the view that they may have a role in fibre degradation. However, it has not been elucidated whether the cellulase activity is from the protozoa or the cellulolytic bacteria that have been engulfed (Coleman 1989). Demeyer (1981) conducted a series of *in vivo* experiments and concluded that about 34% of the digestion of fibrous materials in the rumen could be attributed to protozoa. In a review of a large number of experiments that have been conducted with both defaunated and faunated animals (Williams and Coleman 1991, p. 317) concluded that the rate of cellulose digestion in the rumen was reduced by 20% when protozoa were absent. However, the whole body (total tract) digestion of cellulose was not significantly affected by defaunation as some of the fibre that may have escaped ruminal degradation was apparently digested in the hindgut. The absence of protozoa from the rumen, however, brings a wide range of other changes, including a shift in numbers of the remaining microbes which may have far-reaching effects on fibre digestion in the gut and other aspects of rumen metabolism. The removal of protozoa from the rumen removes a critical predatory pressure on the other rumen microbes which may respond by increasing in number (Soetanto 1985). Increase in fungal population was observed following defaunation (Romulo *et al.* 1986,

1989; Newbold and Hillman 1990). Such an increase in the biomass of cellulolytic microbes in the rumen is expected to have a far-reaching beneficial effect on fibre digestion in the rumen and microbial protein flow to the intestines, provided that nutrients such as ammonia are not limiting.

Although most diets would support high numbers of protozoa 50% of the microbial biomass in the rumen, their numbers in the omasal fluid is on average only about 20-30% of the level in the rumen (Bird *et al.* 1978; Imai *et al.* 1981; Steinh and Clarke 1982; Michalowski *et al.* 1986; Towne and Nagaraja 1990). This has lead to the conclusion that their contribution to the microbial biomass leaving the rumen is generally minimal (Hungate *et al.* 1971; Weller and Pilgrim 1974; Nolan 1975; Ulyatt *et al.* 1975b). The exact proportion of protozoal N in the total microbial N supply to the small intestines, however, is still an area of much contention with some reports estimating it to be only about one-quarter of the total (Coleman 1979a). The inability to correctly quantify the protozoa N in the total microbial N both in the rumen and in digesta flowing to the small intestines is mainly due to lack of a proper marker that clearly and distinctly identifies protozoal N separately from bacterial and other microbial N. However, this is likely to change following recent development of real-time PCR technology that specifically marks and quantifies protozoal N (Bergen 2004; Sylvester *et al.* 2004).

2.3.3.3. The anaerobic fungi

The presence of fungi in the rumen was not discovered until the 1970s, due mainly to the pioneering work of Orphin (1975, 1976) and later by Bauchop (1979b). This delay in the identification of rumen anaerobic fungi was mainly due to the flagelleted fungal zoospores being mistaken for the flagellated protozoa. The anaerobic rumen fungi are classified together with other primitive aquatic fungi in the phycomycetes group. The anaerobic phycomycete fungi have already been isolated from the compartmentalized part of the digestive system of most domesticated and wild herbivores (Trinci *et al.* 1994; Gordon and Phillips 1995). The existence of fungi in compartments such as the rumen is mainly due to the presence of copious quantities of digesta which are also retained for a prolonged period of time (Bauchop 1989). The main genera that have been isolated from the rumen of domestic and wild herbivores include mainly those in the Neocallimastaceae family: *Neocallimastix spp, Anaeromyces spp* and *Orphinomyces*. The most important mainly due to their high cellulase activity are: -*Neocallimastix frontalis, Piromonas communis* and *Sphaeromonas communis* (Akin 1986).

The growth cycle of rumen anaerobic phycomycetes fungi has been reported by several workers (Orpin 1975; Bauchop 1981; Lowe *et al.* 1987a; Gold *et al.* 1988), and consists of two notable stages that appear to alternate: the motile and non-motile stages. The motile phase consists of the flagellated zoospores found mainly in the rumen, and also germinating on fibre particles. They are normally released in large quantities after the rupture of mature sporangia and form the main inoculum that inoculates the freshly ingested feed in the rumen (Orpin 1974, 1976, 1977a). The non-motile vegetative phase commences with the germination of the zoospore and results in the formation of a thallus or a rhizoidal-mycelial growth that deeply and extensively penetrates the entire plant material. Upon maturity this vegetative thallus forms fruiting bodies called sporangia that are located on the exterior part of the plant fragments and are approximately 100 μm in length (Bauchop 1989). Besides these two stages, there are also reports on the existence of a third phase that occurs mainly outside the gut environment and which is thought to be latent (Wubah *et al.* 1991a, 1991b, 1991c; Davies *et al.* 1993). Being comprised of resistant sporangia, this resting stage may be important in enabling the anaerobic fungi to survive in the hostile environment outside the gut (Wubah *et al.* 1991a). The life cycle of anaerobic fungi in the rumen from germination of zoospores to formation of mature vegetative reproductive stage ready to discharge spores takes about 24-32 h (Bauchop 1979b, 1989; Joblin 1981). As a result, rumen anaerobic fungi generally tend to be associated with the more refractory fibrous plant material with a relatively long MRT in the rumen (Bauchop 1989).

Although rumen anaerobic fungi constitute a relatively smaller proportion of rumen microbial biomass compared to either bacteria or protozoa, they nevertheless play a very critical role in the degradation of structural polysaccharides (Orphin and Letcher 1979; Pearce and Bauchop 1985; Williams and Orphin 1987; Hebraud and Fevre 1988; Williams *et al.* 1994; Hogan 1996 pp 33). Fungi have been reported to preferentially colonize the more lignified parts of plant tissues, especially the sclerenchyma (Akin *et al.* 1983; Akin 1989; Grenet *et al.* 1989). This colonization has been identified as being quite effective in weakening the most recalcitrant plant tissues, and may even assist in the penetration of the cuticle (Kolattukudy 1985; Pearce and Bauchop 1985; Akin and Rigsby 1987; Akin *et al.* 1989). The rumen anaerobic fungi therefore contribute to the degradation of the refractory plant materials, a task that cannot be easily accomplished by bacteria or protozoa (Akin *et al.* 1983; Akin and Rigsby 1987). Furthermore, fungal activity

helps to pulverize the refractory plant material opening it up to secondary invasion by bacteria and protozoa, thus accelerating its degradation in the rumen (Bauchop 1989).

2.3.4. Factors influencing microbial growth in the rumen

For the growth of rumen microbes to be optimized they have to be provided with all the required nutrients. The specific nutritional requirement of major microbial groups has been a subject of several reviews (e.g. Hungate, 1966; Bryant 1973; Demeyer 1981). Besides energy, the main nutrients that influence microbial growth in the rumen include ammonia, amino acids, branched-chain fatty acids and minerals.

2.3.4.1. Nitrogen

It has long been known that ruminants can survive on NPN as the sole source of N and even produce some milk (Virtanen 1966). This is possible due to the fact that NPN sources are readily hydrolyzed to ammonia, and most rumen microbes have the capacity to grow optimally using ammonia as their preferred source of N. About 60-70% of the N in microbial cells is derived from ammonia (Sauer *et al.* 1975), which indicates that a substantial proportion of ammonia-N is used in the synthesis of bacterial amino acids (Mathison and Milligan 1971; Nolan and Leng 1972). Cellulolytic bacteria have a specific requirement for ammonia for optimal growth (Bryant 1973), due to a lack of an efficient mechanism to transfer amino acid N across their cell membranes into the cell protoplasm (Bryant 1973; Russell *et al.* 1990). Cellulolytic bacteria have evolved the ability to survive on ammonia mainly because they are normally associated with the degradation of fibrous materials that are low in protein.

The main source of ammonia in the rumen is the degradation of dietary protein and NPN sources including urea. This is augmented with endogenous N sources, especially endogenous urea secreted in the saliva and through the rumen epithelium from the blood (Houpt 1970). The endogenous N source is critical in animals subsisting on poor quality roughage basal diets such as cereal straw and stover that are quite low in N. The reliance of cellulolytic bacteria on ammonia as the main source of N does not preclude the fact that they may still have a requirement for a preformed protein source (Nolan and Leng 1972). Up to 50% of the N in the rumen bacteria of sheep fed roughage diets is from amino acids or peptides (Nolan 1975). Since such diets

ordinarily tend to be very low in protein N, it can only be concluded that the amino acids may have been sourced from cross-feeding on other rumen microbes, amino acids secreted by the microbes into the rumen fluid and sloughing from the rumen wall.

Inadequate ammonia in the rumen tends to have a negative effect on the growth of rumen microbes, especially the cellulolytic bacteria and this may in turn reduce their capacity to digest structural polysaccharides (Tamminga 1982a; Ferrell *et al.* 1999). Deficiency of ammonia in the rumen besides depressing microbial fermentation activity, also results in high microbial turnover due to the microbial lysis (Smith and Smith 1977). This has a net effect of reducing both the absorbed VFA energy and microbial protein flow to the small intestines of the host animal (Leng 1986). Deficiency of NH_3-N in the rumen is likely to occur when the diet is low in N content or when the dietary protein has been excessively protected from degradation in the rumen (Ferrell *et al.* 1999). Rumen ammonia occurs in two forms: the unionized or molecular (NH_3) and the ionic form (NH_4^+). The absorption of ammonia from the rumen into the blood or up take by the microbes is largely in the molecular form (NH_3) rather than in ionic form. The NH_3: NH_4^+ equilibrium is influenced by ruminal pH and ammonia concentration in the rumen (Siddons *et al.* 1984).

The ammonia concentration in the rumen at any one time is a net balance between production from various exogenous and endogenous sources and removal through various physiological and metabolic processes in both the animal and the microbes (Nolan 1993). Rumen microbes are confronted with an environment where a ammonia concentration is constantly changing and as such must adapt accordingly to survive (Brown *et al.* 1974). Bacteria have been reported as being able to grow quite well over a wide range of ammonia conctrations, i.e. from 50 to over 200 mg /L of rumen fluid (Satter and Slyter 1974; Mehrez *et al.* 1977; Boniface *et al.* 1986). To cope with wide fluctuation in ammonia in the rumen, bacteria have evolved with two-systems for assimilating ammonia (Hespell 1984). However, when the ammonia concentration is adequate, the bacteria relies more on a passive mechanism of assimilation that does not require any ATP energy (Hespell 1984). However, when the ammonia conctration in the rumen is low, as is likely to occur in animals subsisting on low quality roughage diets, or during fasting, a two-step ATP consuming mechanism is activated. The later involves two key enzymes, namely the glutamate synthase and glutamine synthetase (Erfle *et al.* 1977). Because the reaction involved with the

latter enzyme requires ATP input, there are negative implications for microbial growth efficiency when rumen ammonia is low, i.e. below 20 mg/L (Schaefer *et al.* 1980).

The optimal ammonia concentration in the rumen depends on the fermentable energy supply and the aspect of the rumen metabolism being considered. For example, different levels of ammonia in the rumen are required to support maximum microbial growth; optimize OM digestibility and stimulate high levels of DM intake. On the basis of an *in vitro* study, Satter and Slyter (1974) suggested that about 50-80 mg ammonia/L in rumen fluid was required for maximum microbial cell growth. However, under *in vivo* conditions similar growth was achieved with a much lower level at only 22 mg ammonia /L (Salter *et al.* 1979). Maximum utilisation of low quality roughage such as straw also appears to require a relatively high ammonia concentration in the rumen. Preston and Leng (1987) reported that digestibility of straw DM in cattle increased as rumen ammonia level increased up to 80 mg ammonia/L, whereas DM intake continued to increase until ammonia concentration reached 200 mg/L. An even higher concentration (250 mg/L) was reported as being required for maximum fermentation in the rumen and maximum feed intake in sheep and cattle (Boniface *et al.* 1986; Perdok 1987; Perdok and Leng 1989). The high NH_3-N requirement in ruminants subsisting on low quality roughage may be attributed to the large population of cellulolytic bacteria that are known to rely on ammonia for optimal growth (Bryant 1973). There are also suggestions that the increased rate of microbial fermentation associated with high ruminal ammonia concentration may be partly due to rumen pH being increased due to the formation of $(NH_4)_2CO_3$ (Hespell 1979; Hespell and Bryant 1979). The pH around neutrality is the optimal level for cellulolytic bacteria. A readily fermentable energy source may also increase ammonia requirements in the rumen mainly due to enhanced bacteria capacity to assimilate more ammonia in microbial protein synthesis. Mehrez *et al.* (1977) reported that 200-270 mg/L was required in the rumen for maximum disappearance of DM from the barley grain.

2.3.4.2. Amino acids and peptides

It has been established that for optimal growth rumen microbes including cellulolytic bacteria amino acids and peptides may be required (Nolan and Leng 1972; Hespell and Bryant 1979). Salter *et al.* (1979) indicated that rumen bacteria might use up to 80% N as α-amino-N rather than ammonia and that the proportion may increase even further with the protein content of the diet.

In their study of N dynamics in the rumen of sheep fed lucerne chaff diet, Nolan and Leng (1972) reported that while 80% of the N incorporated in microbial cells was sourced from ammonia, the remaining 20% came from peptides, amino acids or both. Most amylolytic bacteria species such as *Selenomonas ruminantium, Streptococcus bovis* and *Bacteroides amylophilus* appear to have a preference for amino acid N (Hobson *et al.* 1968; Russell 1983; Maeng *et al.* 1989, Leng 1990). An increase in microbial cell yield (Y_{ATP}) was realized when part of the urea-N in the starch-based growth medium was replaced by a preformed protein (Maeng *et al.* 1976; Stern and Hoover 1979; Maeng *et al.* 1989). However, no such increase in Y_{ATP} was noted when the growth medium was based on cellulose as the basal energy source where celllulolytic bacteria were presumably predominant in the microbial biomass (Maeng *et al.* 1989).

There are indications that cellulolytic bacteria may have a requirement for amino acids, but only in very small or catalytic amounts which under *in vivo* conditions can easily be met from a variety of dietary and endogenous sources. The later mainly include salivary protein, secretions from the gut and sloughing from the rumen epithelium (Buttery 1977; Kennedy and Milligan 1980a). In addition, to these there is also the microbial lysis, which is an integral part of intra-ruminal recycling of nitrogen (Nolan and Stachiw 1979). It is estimated that endogenous non-urea N inputs from sloughed gut wall tissues and salivary proteins may supply about 5-10 g N/d to the rumen of a sheep fed roughage diets (Kennedy and Milligan 1980a; MacRae and Reeds 1980). There are indications that under some conditions branched-chain carbon skeleton amino acids such as methionine and cysteine, or the complex benzene ring containing amino acids such as phenylalanine may be limiting for microbial growth in the rumen (Smith 1979; Hespell 1984). The synthesis of such rather complex core molecular structure in the microbe requires more energy and takes time, and presumably the microbe would be better off acquiring an already assembled core molecular structure for faster microbial synthesis and growth (Nolan and Leng 1972). It has therefore been suggested that rumen microbes may be using these branched-chain amino acids as precursors of branched- or higher-chain carbon skeleton that they require for the synthesis of protein required for maintenance and growth (Allison 1970; Hume 1970c).

2.3.4.3. Branched-chain volatile fatty acids

Hemsley and Moir (1963) reported that microbial growth in the rumen was stimuled, and increased feed intake when animals were supplemented with branched-chain VFA. Later Kay

and Phillipson (1964) also demonstrated that there was an increased flow of microbial protein to the small intestines when sheep fed low quality roughage were supplemented with branched-chain volatile fatty acids. Further work by Hume (1970b) showed that supplementing sheep with branched-chain VFA resulted in increased microbial protein production. In spite of these results, the response to branched-chain volatile fatty acids supplementation has been equivocal, and this may be attributed to animals' ability to mobilize endogenous sources of branched-chain amino acids (Allison 1970; Hogan 1996), which presumably are able to meet the animal's requirements for these VFAs. In well-nourished animals, rumen microbes are unlikely to suffer any deficiency of amino acids/peptides or even branched-chain VFAs as these are easily derived from protein catabolism in the rumen (Mackie and White 1990). Such protein may be from a variety of dietary or endogenous sources (Hogan 1975; Kennedy and Milligan 1980b), including microbial lysis (Allison 1970; Nolan and Leng 1972).

2.3.4.4. Fermentable energy

Energy is probably the most important input that is required by all rumen microbes for both maintenance and growth and energy is a major factor limiting microbial growth under both *in vitro* and *in vivo* situations (Kaufmann and Lupping 1982; Tamminga 1978, 1982a). Microbial growth is required to maintain a viable population in the rumen and if the doubling time of a particular species is longer than the MRT of that species, then that species is likely to face depletion and eventual disappearance. Rumen microbes probably require energy in the form of high-energy phosphate substrates such as adenosine triphosphate (ATP). Most sources of digestible OM, especially those high in carbohydrates and to some extent also protein are readily fermented by the rumen microbes to supply them with both ATP energy and the necessary organic carbon to support microbial synthesis (Bergen and Yokohama 1979). Therefore, the fermentability of the energy source (OM) is a major determinant of the rate of generation of ATP to the microbes, which in turn influences microbial growth efficiency (Y_{ATP}).

Carbohydrate $\longrightarrow$ VFAs + Microbial cells + Gases (CH_4, CO_2) + ATP + Heat

Though carbohydrates are the major sources of fermentable energy, the rumen microbes do also have a capacity to ferment protein/amino acids as a source of ATP and organic carbon (Cotta and Hespell 1986). However, the energy from protein is much lower, yielding only 12-15 moles ATP per Kg protein compared to 25-30 moles per Kg carbohydrate (Demeyer and van Nevel 1979; Tamminga 1979, 1982a). Carbohydrates therefore, provide the highest yield of energy available to the rumen microbes, not only because of their higher ATP yield potential, but also because they normally constitute the highest proportion (usually 70-80 %) of the degraded substrate in the rumen (Tamminga 1982a).

The rumen microbes depend entirely on anaerobic respiration where some of the substrate carbon being oxidized is also simultaneously utilised as the final electron acceptor (i.e. dismutation), a process that severely limits ATP energy yield (France and Siddons 1993; Kelly *et al.* 1993). In spite of the apparent limitations in available energy, Czerkawski (1986a, pp 127) has argued that microbial synthesis of organic polymers such as lipids may not require much energy, as there is an adequate supply of reduced co-factors within the highly reduced rumen environment. The absolute supply of fermentable energy notwithstanding, its availability also needs to be synchronized with that of other nutrients, especially N and S that are critical in determining microbial growth efficiency in the rumen (Bartley and Deyoe 1977; Sinclair *et al.* 1993; Hogan 1996; Tevaskis *et al.* 2001). Asynchrony between the supply of fermentable energy and other nutrients may result in a substantial proportion of the energy being diverted to 'energy spilling reactions' (Russell and Strobel 1993).

While most basal and supplemental carbohydrates and protein sources can be variably fermented in the rumen to provide both energy and organic carbon to support microbial synthesis, the same cannot be said of lipids and some organic acids (AFRC 1993). The majority of lipids, including those that are high in long chain fatty acids, are not readily fermented by rumen microbes, and besides depressing fibre digestion in the rumen, such lipids may even be toxic to the microbes (Devendra and Lewis 1974; Tesfa 1992; Doreau and Chillard 1997). Similarly, with a few exceptions such as lactic acid and a few other intermediate organic acids, rumen microbes are generally unable to effectively utilise most of the short chain organic acids energy source for maintenance or growth, mainly because these organic acids are themselves waste metabolites of microbial activity (AFRC 1993).

2.3.4.5. Minerals

Like multi-cellular animals, rumen microbes a requirement for both macro- and microelements for optimal growth. The minerals that microbes require include: Ca, P, K, S, Cl and Mg, and a range of trace elements including Cu, Co, Fe, I, Mn, Se, and Zn (Durand and Kawashima 1980; Mackie and Therion 1984). Among the minerals required by the microbes in the rumen S, P, Mg and Co are considered as being the most critical (Durand and Komisarczuk 1988). Phosphorus is required for nucleic acid synthesis and is therefore instrumental in cell multiplication, a major factor influencing microbial growth in the rumen. Most forage in the tropics tends to be deficient in P (McDowell *et al.* 1984), and therefore, its addition to the basal diet has been shown to enhance microbial protein synthesis by rumen microbes (Leng 1989a).

After N, S is the second most important element required by rumen microbes and is essential for the synthesis of sulphur amino acids (Bray and Till 1975). Initial studies on S requirements by rumen anaerobic fungi showed a dramatic increase in fungal biomass in the rumen, and enhanced fibre degradation when S was supplemented either directly to animals feeding on hay (Gordon 1985) or indirectly through fertilization of growing forage such as *Digitaria pentzii* (Akin *et al.* 1983, Akin and Hogan 1983). Recommendations from the ARC (1980) indicate that ruminant diets need to have a minimum of 1g S /kg DM of feed for the rumen microbes to be considered well supplied with the element. Furthermore, due to the close relationship between N and S, especially in protein synthesis, the two nutrients have to be well balanced in relation to each other with N: S ratio of 10 to 15:1 for both sheep and cattle (Kandylis 1984). The ratio for the sheep will normally be slightly higher (10:1) than that for cattle (15:1), mainly due to the higher requirement of S amino acids to support wool growth.

Rumen microbes can utilise both inorganic (elemental S and SO_4^{2-}) and organic forms of dietary S, though there are indications from *in vitro* studies that the reduced forms, including sulphide(S^{-2}), and that provided by protein may be better utilised (Orphin 1988; Phillip and Gordon 1991). There are also indications that substantial quantities of S (up to 300 mg/day) could be transferred to the rumen of a sheep as a component of endogenous protein (Kennedy and Milligan 1978b; MacRae and Reeds 1979). Rumen microbes, especially protozoa do play a major role in facilitating the metabolism and recycling of S within the rumen through their proteolytic activity, including making reduced forms of S available to the anaerobic fungi (Hegarty *et al.* 1989; Hogan 1996, pp 33). Magnesium has also been reported as being essential to all rumen microbes

especially to cellulolytic bacteria (Pettipher and Latham 1979). Generally the quantities of the various minerals offered to satisfy the animal's requirements also tend to be much higher than the requirements of the rumen microbes (Durand and Kwashima 1980). Rumen microbes besides benefiting from recycling of minerals in the animal's body through the saliva (Yano *et al.* 1991) are unlikely to suffer from any mineral deficiency so long as the animal is adequately supplied with a well-balanced mineral supplement (Durand and Kwashima 1980; Hogan 1996, p. 33).

2.3.5. Microbial protein supply to the host animal

The microbial biomass is basically an assembly of organic polymers synthesized using carbon skeletons from the fermented OM, with addition of various minerals (Bergen and Yokohama 1977). Microbial cells contain polysaccharides, polypeptides, microbial lipids and polynucleotides which are potentially available to the host ruminant mainly as a protein and other energy-rich organic materials (Williams and Coleman 1991, p. 348). The microbial protein is mainly of bacterial and protozoal origin and is the main source of amino acids available to the ruminant animal (Purser and Buechler 1966); it accounts for 40-80% of the total protein flowing to the duodenum (Sniffen and Robinson 1987; Merchen and Titgmeyer 1992).

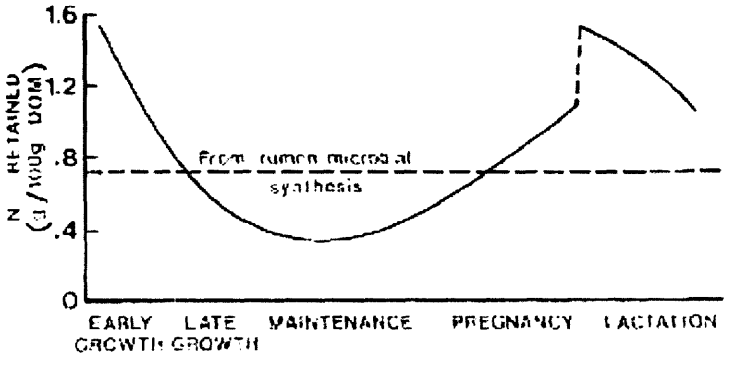


Figure 2.1. The effect of different physiological states of ruminants on the N retention in relation to digestible organic matter intake (adapted from Ørskov 1970)

Ørskov (1970) suggested that, under some physiological states such as non-pregnant and non-lactating, the microbial protein alone could be adequate to meet the ruminant animal's requirements for protein. In addition, when microbial protein production is optimized, it can meet the protein requirements for maintenance, early and mid pregnancy and the late lactation

period, but it is inadequate to meet the requirements of animals in more demanding physiological states, i.e. during early stages of life when growth is most rapid, the late gestation period and in early lactation (Ørskov 1970) (**Figure 2.1**). Kaufmann and Lupping (1982) also estimated that in well-fed high yielding dairy cows 60-70% of the protein requirements could be met from the rumen microbial biomass alone.

Animals in more demanding physiological states therefore require an additional source of dietary protein that escapes degradation in the rumen to augment microbial protein supply, so as to completely meet their protein requirements (Ørskov 1970; Loerch *et al.* 1983; Leng 1986, Hussein and Jordan 1991). There is therefore a need to enhance microbial growth efficiency in the rumen and to also correctly determine the microbial protein flow to the small intestines, so that any deficit can be covered through supplementation with by-pass protein (Chen *et al.* 1995b).

2.3.5.1. Microbial growth efficiency and protein yield in the rumen

The efficiency of microbial growth in the rumen has been expressed as Y_{ATP}, i.e. g microbial cell DM produced per mole of ATP available from substrate fermentation. Bauchop and Elsden (1960) suggested that Y_{ATP} could be constant and even proposed a value of 10.5. However, Isaacson *et al.* (1975) and Hespell and Bryant (1979) suggested that the actual Y_{ATP} value may be higher than 10.5. In practice, the microbial growth efficiency has been expressed as yield of microbial crude protein (MCP) per kg of organic matter apparently digested in the rumen (OMADR) (Tamminga 1978). Microbial growth efficiency is essentially dependent on the quantity of OM fermented in the rumen and the efficiency with which such energy is utilised for growth rather than maintenance (Owens and Bergen 1983). Irrespective of the approach used to express microbial growth efficiency in the rumen, both methods appear to indicate a wide range of values. Under *in vitro* conditions Y_{ATP} values ranging from 21 to 26 were reported by Hespell and Bryant (1979), while Harrison and MacAllan (1980) reported values ranging from 4 to 21. In a number of studies involving *in vitro* incubation of microbes in various anaerobic media, theoretical calculations produced Y_{ATP} values that ranged from 27 to 32 (Stouthamer 1979). However, it has been suggested that under *in vivo* conditions the Y_{ATP} values are likely to be much higher (Hogan and Weston 1970; Russell and Hespell 1981). Contrary to the views of Bauchop and Elsden (1960), it seems that Y_{ATP} is unlikely to be a constant value, and Hogan and

Weston (1970) suggested that the value is likely to vary with many factors including the type of microbes considered and dilution rates. Equally a wide range of values have been reported when microbial growth efficiency in the rumen was expressed on the basis of g MCP/kg OMTDR (Stern and Hoover 1979; Smith 1979). ARC (1984) has recommended that values within the range of 14 to 49 g N/kg OMADR would be acceptable.

The large variation in Y_{ATP} values is mainly due to a significant proportion of the energy realized from the fermentation of OM being utilised for maintenance by the rumen microbes rather than for growth, especially at low growth rates (Hespell and Bryant 1979; Stern and Hoover 1979). The proportion of ATP energy diverted to maintenance (i.e. M_{ATP}) as opposed to growth is variable and may be influenced by a number of factors such as the imbalance in the supply of energy, and key nutrients including N, branched-chain VFA and minerals that are required for microbial growth. The M_{ATP}, which in turn determines Y_{ATP}, is dependent on the period of time the microbes spend in the rumen before washout. However, such a period is influenced by rumen dilution and outflow rates. Leng (1982c) argued that in an anaerobic environment such as that in the rumen a large proportion of energy in fermented OM is retained in the VFA and other products of microbial fermentation. According to the stoichiometry of fermentation in the rumen, OM is apportioned to three main end products that include VFA, microbial cells, and fermentation gases (Preston and Leng 1987; Nolan 1989b).

Given that the proportion of OM converted to combustible gases is normally small and fairly constant at 6-8%, it follows that the conversion to either VFA or microbial cells is inversely proportional, with one product being produced at the expense of the other (Leng 1982c). Therefore, production of high amounts of VFA (and heat), which reflects high energy expenditure on maintenance, is normally accompanied by low microbial cell yield. Leng (1982c) attempted to compute the relationship between Y_{ATP} and the carbohydrate fermented in the rumen to VFA or conserved in microbial cells and proposed a model to predict total efficiency for the animal. According to the model, the highest total efficiency delivering the most balanced fermentation products in terms of protein to energy (P/E) ratio occurred when the Y_{ATP} was within the range of 20-30. This was achieved when about 40-60% of energy in OM was conserved as microbial biomass and VFA respectively (Leng 1982c) (**Figure 2.2**).

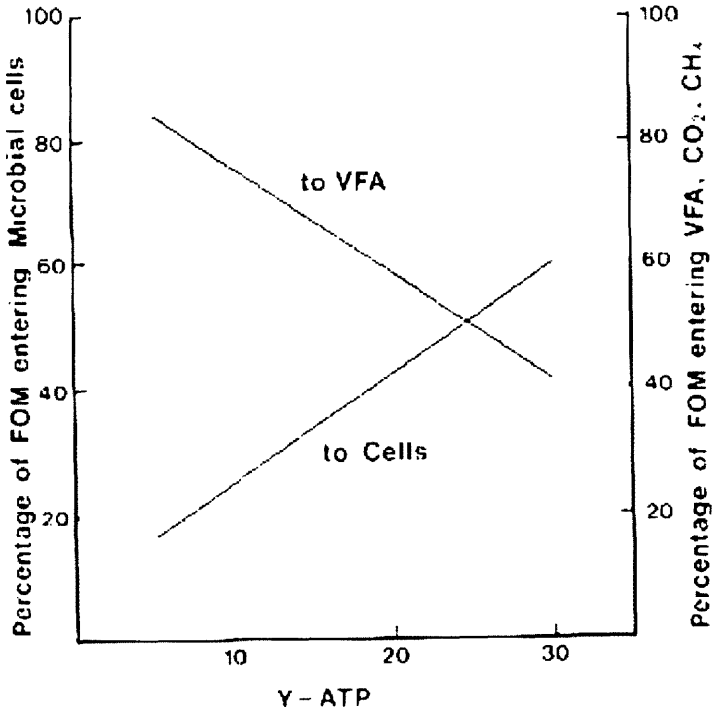


Figure 2.2. The relationship between microbial growth efficiency (Y_{ATP}) and the proportion of fermented OM partitioned to VFA, microbial cells and gases (adapted from Leng 1982c).

2.3.5.2. Factors affecting microbial protein yield

As much as the microbial growth efficiency (Y_{ATP}) is a useful measure of microbial protein synthesis in the rumen, it does have some limitations. Firstly, it fails to take into account the fact that some of the microbes grow and die in the rumen, and therefore never flow out of the rumen to the small intestines to supply amino acids to the host animal (Allison 1970). This is in spite of the fact that such microbes still use a significant proportion of energy and other nutrients in the rumen for their maintenance. Secondly, some of the rumen microbes such as protozoa also prey on other rumen microbes, yet their proportional contribution to the microbial biomass flowing out of the rumen to the small intestines is low (Coleman 1979a). As such, it has long been recognized that microbial biomass flowing out of the rumen reflects only the net growth (yield) rather than the total biomass synthesized in the rumen (Allison 1970). The three major factors that directly influence yield of microbial cells leaving the rumen are: availability of nutrients to the microbial population, dilution rates, and the turnover of microbial cells within the rumen (i.e. recycling of N in the rumen) (Ørskov 1994).

2.3.5.2.1. Availability of nutrients

For optimal microbial growth in the rumen a range of nutrients that include fermentable OM as an energy source, ammonia, amino acids, branched-chain VFA, and minerals, especially S, and other precursors are required (Leng 1982c). In particular, microbial output and hence microbial growth efficiency in the rumen is highly influenced by the availability and utilisation of energy-yielding substrates (Tamminga 1982a; Kelly *et al.* 1993). Furthermore, because fermentable energy and N are the two most critical factors influencing microbial growth, they need to be available in a synchronized way to optimize microbial growth efficiency (Sinclair *et al.* 1993; Tevaskis *et al.* 2001). The balance between the rate of carbohydrate fermentation in the rumen and the availability of ammonia, together with peptides/amino acids and other essential nutrients that are utilised for microbial growth, has a major influence on microbial protein yield in the rumen (Nocek and Russell 1988; Russell *et al.* 1991). When there is an extreme mismatch between the fermentable energy and those other nutrients, fermentation of carbohydrate continues but with practically no net cell growth in the rumen (Wallace and Cotta 1988). The specific role of the various nutrients was reviewed in details earlier in **2.3.4.**

2.3.5.2.2. Rumen dilution rates

Increasing dilution rates increases microbial growth efficiency in both batch (Stouthamer and Bettenhaussen 1973) and continuous cultures (Isaacson *et al.* 1975). This is attributed mainly to a lower proportion of ATP energy being diverted to maintenance (M_{ATP}) of microbial cells. However, the dilution rate that optimizes microbial growth efficiency depends on the rate of growth or cell division of the microbial species concerned. The microbial adaptability to a given dilution rate is influenced by the maintenance energy requirements. The maintenance energy (M_{ATP}) requirements per unit of microbial biomass tend to fall with increase in dilution rate (Sutherland 1976). As a result those microbes with the lowest M_{ATP} for a given dilution rate will be selectively retained over time leading to their high proportional representation in the rumen (Hobson and Summers 1967; Sutherland 1976).

Under *in vivo* conditions enhanced microbial growth efficiency has been reported following increase in dilution rates (Potter *et al.* 1972; Harrison *et al.* 1975, 1976; Kennedy and Milligan 1978b; Zin and Owen 1983; Robinson *et al.* 1985). This is due to lower microbial lysis and predation, and therefore a higher proportion of rumen microbes leaving the rumen to the small intestines. The net effect of dilution rate on microbial growth efficiency may therefore depend on

diet and rumen conditions such as ruminal pH (Russell 1984). Under *in vitro* conditions, dilution rate has been manipulated with ease to determine its influence on microbial growth efficiency, especially with batch (Stouthamer and Bettenhaussen 1973) and continuous cultures (Issacson *et al.* 1975). However, under *in vivo* conditions the scope for increasing dilution rates appears to be rather limited and mainly restricted to increasing saliva outflow, though the results are not always consistent. Djaouvinov and Todorov (1994) tried to increase dilution rates in the rumen by feeding pelleted diets that were similar in DE and CP but with a variable ratio of barley straw and barley grain (10:90, 30:70 and 50:50), but did not detect any difference in microbial growth efficiency. However, when the straw fraction was replaced with polyvinyl chloride flakes (PVC) and dehydrated lucerne, there was a notable increase in microbial growth efficiency (Djaouvinov and Todorov 1994), presumably due to a higher saliva output from increased rumination associated with the flakes.

Any change in the rumen, dietary or otherwise, that increases osmolality is likely to draw more fluid from the blood into the rumen resulting in an increase in dilution rate and this has the potential to increase Y_{ATP}. Inclusion of sodium bicarbonate ($NaHCO_3$) and other inorganic buffers in high grain diets fed to dairy cows ostensibly to neutralize acidity was reported to increase milk yield and maintained butterfat content (Knowlton 2001). Besides neutralizing acidity in the rumen, such buffers also bring about changes in the osmotic pressure in the rumen leading to an inflow of more fluid from the blood and also a higher water intake, both of which may lead to an increase in dilution rates (Russell and Chow 1993). Such an increase in dilution rates in the rumen of animals fed basal diets high in starch can result in more starch and microbial N flowing to the small intestines (Knowlton 2001). However, practical attempts to manipulate dilution rates to enhance microbial flow to the small intestines directly by inducing higher water intake through the use of salts (Hemsley 1975) or indirectly by stimulating higher saliva output with chemical stimulants (slaframine) have not yielded positive results (Jacques *et al.* 1989).

2.3.5.2.3. Turnover of microbial biomass in the rumen

Generally the longer the microbes stay in the rumen, the more the M_{ATP} that they utilise without contributing to the microbial biomass flowing to the small intestines (Leng 1982c). The probability of their being engulfed by protozoa or being subjected to microbial lysis leading to a higher microbial N turnover in the rumen is also increased (Sniffen and Robinson 1987). During microbial lysis, the cellular components will most likely be degraded or fermented to VFA,

ammonia, CH_4 and CO_2 but not much ATP energy is realized from such fermentation, mainly due to the high content of protein and NPN in the lysed products. While some of the products such as branched-chain VFA, amino acids and ammonia may be salvaged and re-used for microbial synthesis, they represent only about 10% of lysed microbial biomass (Leng 1982b). The other fermentation products including most of the VFA, heat and fermentation gases cannot be utilised for microbial synthesis, and are therefore largely wasted. As a result, a high turnover of microbial biomass in the rumen is considered wasteful and undesirable from microbial growth efficiency point of view.

Any change in rumen digesta turnover rate that ensures prompt removal of microbial biomass before it is extensively recycled or turned over may enhance microbial growth efficiency in the rumen. This can be achieved by increasing the turnover rate of rumen digesta, which also corresponds to a higher outflow rate (thus reducing both microbial cell turnover and M_{ATP} requirements in the rumen) while at the same time increasing microbial synthesis (Hemsley 1975; Christopherson and Kennedy 1983). There are also suggestions that a high turnover rate decreases the concentration of potential growth inhibitory substances in the rumen, which could indirectly increasing Y_{ATP} (Hespell and Bryant 1979). Increased feed intake, besides supplying more fermentable substrates, also increases fractional turnover rate of liquid in the rumen (Bull *et al.* 1979) which may increase microbial growth efficiency. However, increase in digesta turnover rates has not always consistently resulted in higher microbial yield efficiency (Kempton *et al.* 1979).

2.3.5.3. Measurements of microbial protein supply

Microbial protein production in ruminants has been determined in the past using natural and internally synthesized microbial markers such as 2, 6-diaminopimelic acid (DAPA) or external markers such as radioisotopes (Ørskov 1982; Jayasurya 1998). The use of these methods, however, generally requires that the experimental animals undergo complicated and invasive post-ruminal surgical procedures to allow periodic sampling of digesta (Chen and Gomez 1992; Balcells *et al.* 1993). Furthermore, the method is generally tedious and complex, especially the sampling procedure making it prone to errors. In recent years an alternative approach for estimating microbial protein supply in ruminants that uses urinary purine derivative (PD) excretion has received much attention, mainly due to its inherent simplicity and also its non-

invasive nature (Chen *et al.* 1990 a, b, c; Chen *et al.* 1991; Chen and Gomez 1992; Balcells *et al.* 1993). Because this technique will be applied in this study to compare the microbial protein production between the different dietary treatments, it will be reviewed further in the next section.

2.3.5.4. The use of purine derivatives to determine microbial protein synthesis

The determination of microbial protein supply using the urinary purine derivatives (PD) technique is based on a basic principle that most ruminant feed sources, especially those of plant origin are generally low in purines and that the amount present is extensively degraded in the rumen by microbial enzymes (McAllan and Smith 1973). It follows therefore, that any nucleic acid N that leaves the rumen and reaches the small intestines will have been predominantly synthesized in the rumen by microorganisms (McAllan and Smith 1973). The microbial nucleic acid (consisting mainly of RNA and small amounts of DNA) is then extensively digested in the duodenum by the host animal's endogenous enzymes (ribonucleases and diesterases) to oligonucleotides and then to mononucleotides, nucleosides (adenosine and guanosine) and finally to their respective free bases (adenine and guanine) (McAllan and Smith 1973). Both the nucleosides and free bases are subsequently absorbed into through the intestinal mucosa and pass into the blood before being transferred into the liver for further metabolism and excretion mostly in urine. However, depending on the level and activity of xanthine oxidase which varies with animal species, the nucleosides and free purine bases may undergo catabolic metabolism during their absorption across the intestinal epithelium where they are metabolised to their respective intermediate metabolites namely xanthine and hypoxanthine (Chen *et al.* 1990a).

The xanthine and hypoxanthine may still undergo further metabolism in the blood and the liver where they are converted to uric acid and finally to allantoin by the action of endogenous uricase. Xanthine and hypoxanthine, uric acid and allantoin are collectively referred to as purine derivatives (PD). Any unconverted nucleosides and free bases in the systemic circulation, together with xanthine and hypoxanthine can be salvaged and therefore utilised for body *de novo* synthesis of tissue nucleic acids (Chen *et al.* 1990a). Uric acid and allantoin are considered as final waste metabolites that cannot be re-used. They have to be excreted mostly through the urinary route, though a relatively very small proportion is excreted through non-urinary routes such as milk (Susmel *et al.* 1995), and saliva (Chen 1989; Chen *et al.* 1989).

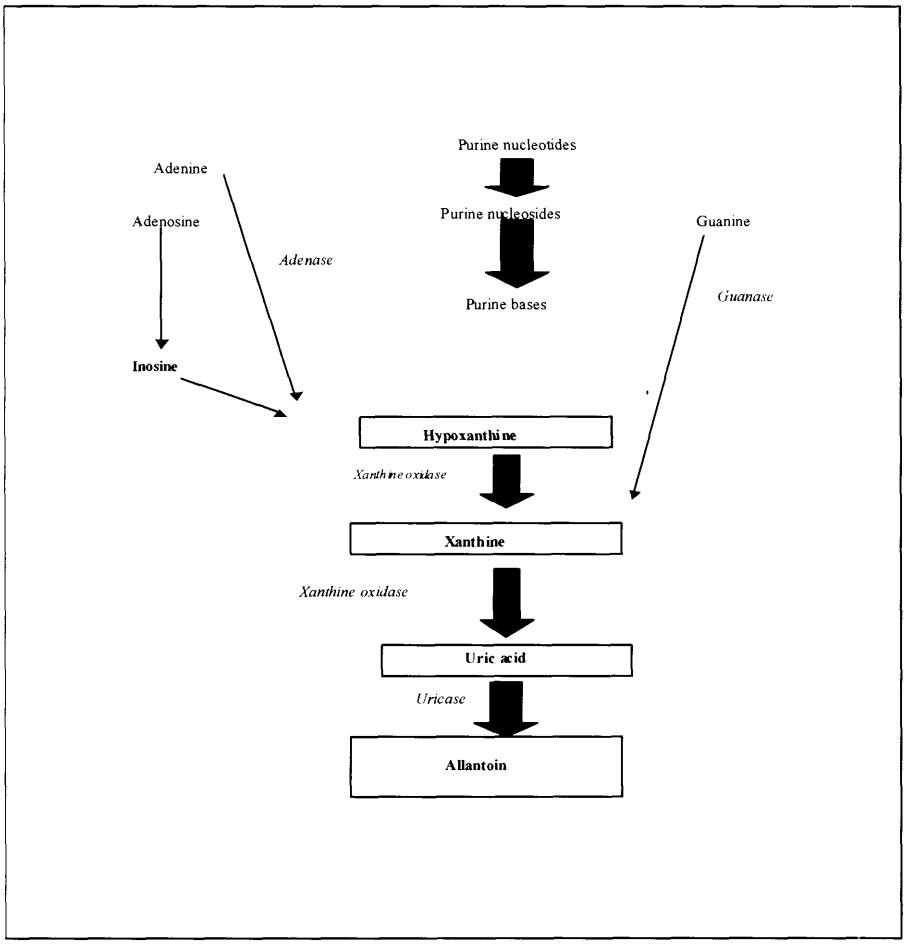


Figure 2.3. The catabolism of purine nucleotides and formation of purine derivatives (PD) (Chen and Gomez 1992).

The amount of PD excreted in urine daily is proportional to the amount of microbial nucleic acid or microbial protein flowing to the duodenum from the rumen (Chen *et al.* 1990a, b, c, d; Verbic *et al.* 1990; Balcells *et al.* 1991; Chen and Gomez 1992) so the daily urinary excretion of PD can be used to estimate the microbial purine flow to the small intestines in ruminants (Chen *et al.* 1990a; Chen *et al.* 1992b). A mathematical relationship between urinary PD excretion and intestinally absorbed exogenous purine is used (Verbic *et al.* 1990; Balcells *et al.* 1991). However, the relationship appears to be species or even breed specific mainly due to differences in the activity of key enzymes such as xanthine oxidase and uricase (Al-Khalidi and Chaglassian

1965; Chen *et al.* 1990b). The metabolic pathways of purine metabolism are summarized in **Figure 2.3.**

Large ruminants (cattle and buffalo) tend to have a very high xanthine oxidase activity in their intestinal mucosa and blood plasma and therefore catabolise most of the absorbed purine to uric acid and finally to allantoin in the liver (Verbic *et al.* 1990). In contrast, sheep generally have low xanthine oxidase activity in the intestinal mucosa which results in a significant proportion of the absorbed purine either being retained or converted to xanthine and hypoxanthine. This difference in purine metabolism between sheep and cattle has lead to different prediction models being applied to estimate microbial protein flow to the duodenum. The models developed so far are for European breeds of cattle (Vebic *et al.* 1990), sheep (Chen *et al.* 1990a) and more recently for indigenous breeds of cattle and sheep normally found in the tropical areas (see Makkar 2004). The prediction models of microbial protein supply from purine excretion in cattle and sheep are given below.

Cattle: $Y=0.85X + 0.385W^{0.75}$ $\Rightarrow X = (Y-0.385W^{0.75})/0.85$ (Verbic *et al.* 1990)

Sheep: $Y= (0.385W^{0.75}e^{-0.25x}) + 0.84X$ $\Rightarrow X= Y - (0.150W^{0.75}e^{-0.25x})/0.85$

$X_{n+1}=X_n - [f'(x_n)/f'(X_n)]$

$f(x_n) = (0.84X+0.150W^{0.75}e^{-0.25x})-Y$

$f'(x_n)= 0.84X-(0.038W^{0.75}e^{-0.25x})$ (Chen *et al.* 1990a).

where: -

Y= Determined daily PD excretion in urine (mmol/d) in cattle or sheep

X = predicted PD absorption in the small intestines (mmol/d)

$W^{0.75}$= Metabolic live weight of the animal.

Other vital information that is required includes: -

The digestibility of microbial biomass in the duodenum, the N content and ratio of purine N: total N of microbial biomass to enable calculation of microbial N supply in ruminants. For both sheep and cattle these variables have been estimated at 0.83, 70 and 11.6:100 respectively. Based on these assumptions the microbial protein reaching the small intestines can be predicted as follows-

:

Microbial N supply (g/day) = (X x 70)/ (0.83 x 0.116 x 1000).

In this study the equation proposed by Balcells *et al.* (1991) that relates microbial protein supply to allantoin excretion only, will be applied. This is given below: -

$Y = 0.8015X - 43.7 \quad \Rightarrow X=(Y+43.7)/0.8015$

Where: -

Y= Allantoin excretion (µmol/kg $BW^{0.75}$) per day, and

X= Purine infused duodenally (µmol/$kg^{0.75}$BW) per day.

2.4. Digestion and absorption of nutrients in ruminants

2.4.1. Carbohydrates

The carbohydrates in the OM ingested by ruminant herbivores fall into two main categories: -

1. The structural polysaccharides consisting mainly of cellulose, hemicelluloses and pectin that sometimes occur in combination with lignin.
2. The storage polysaccharides comprising mainly of starch, and some non-starch polysaccharides such as fructosan and inulin.

Structural polysaccharides normally constitute the largest proportion of a typical diet of ruminants, though energy concentrates consisting mainly of starch may also be used as supplements to boost energy density, especially for some classes of ruminant livestock.

2.4.2. Digestion of structural polysaccharides in the rumen

Because structural polysaccharides are largely insoluble, the cellulolytic rumen microbes carry out the digestion process initially by secreting complex extracellular cellulases that include among others the exo-ß (1, 4) - and endo-ß (1, 4) glucanases (Wood *et al.* 1988; Borneman *et al.* 1989). These enzymes are quite effective in hydrolyzing structural polysaccharides to simple 5-C and 6-C simple sugars that are immediately assimilated into the protoplasm of the rumen microbes where they are fermented first to pyruvate through either the Embden-Meyerhof pathway (glycolysis) or the pentose-phosphate pathway (Threodorou and France 1993). The pyruvate is then further metabolised to generate 2-3 ATP and therefore producing a net of about 4

ATP per mole of hexose fermented by the rumen. A major waste metabolite of this process is the VFA comprising of acetate, propionate and butyrate that account for 95% of the molar proportion of all VFA produced in the rumen (Czerkawski 1986a, pp 45). The remainder consists of smaller quantities of other higher- and branched-chain VFA such as isobutyrate, valerate and isovalerate (Wolin 1981; Preston and Leng 1987; Russell and Strobel 1993; Brockman 1993). In some cases, depending on dietary factors and rumen conditions of the host animal, substantial quantities of lactic acid may also be produced (Rowe 1997).

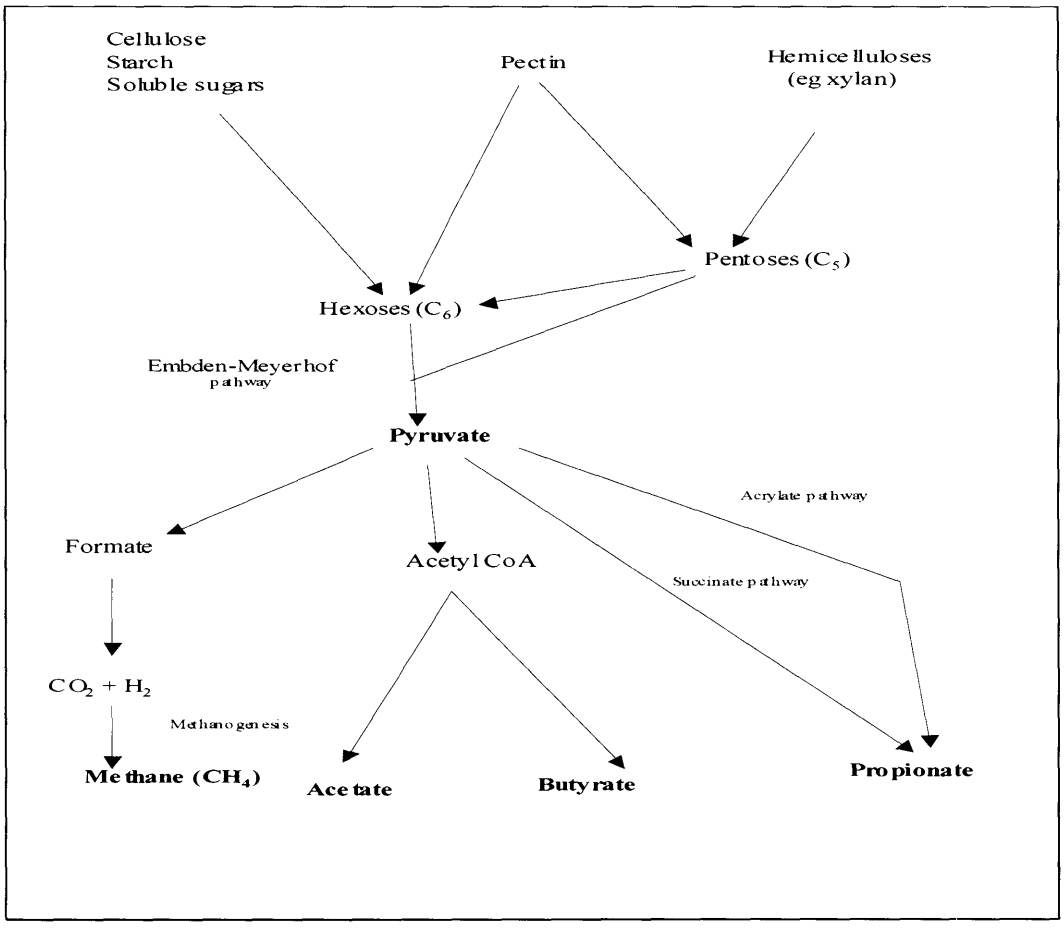


Figure 2.4. Carbohydrate metabolism pathways in the rumen (France and Siddons 1993).

The molar proportions of the main VFA in the rumen, especially the glucogenic (propionate) to lipogenic (acetate and butyrate) vary widely and are influenced by a wide range of factors such as type and physical form of the diet, level of intake, frequency of feeding, microbial profile in the rumen, outflow/ dilution rates, and in some cases use of chemical additives such as ionophores (e.g. monensin) (France and Siddons 1993). Generally most roughage diets tend to generate a

high acetate: propionate ratio, while concentrates tend to promote a fermentation pattern with more propionate and less acetate. Both acetate and butyrate are considered as lipogenic and can be converted to fat, while propionate and some of the 5-C branched-chain VFA (e.g. valeric) are glucogenic and can be utilised for glucose synthesis and conserved as glycogen in the liver and muscles. The ratio of propionate to the total VFA production in the rumen has been reported as one of the main attributes considered as important in the ranking of nutritive value of forages (Norton and Poppi 1995). Although the largest proportion of VFA is absorbed across the rumen epithelium into the blood, mainly by a combination of simple and facilitated diffusion, an estimated 10-20% is thought to flow out of the rumen with the digesta and is absorbed post-ruminally, mainly in the omasum and abomasum (Weston and Hogan 1968). The two major factors having the greatest influence on the rate of absorption are the molar concentration of VFA in the rumen and the pH (Stevens 1970). The main pathways of carbohydrate digestion and metabolism in the rumen are summarized in **Figure 2.4.**

2.4.3. Digestion of sugars and storage polysaccharides in the rumen

The digestion of starch in ruminants has been reviewed extensively by Waldo (1973) and Ørskov (1986). Unlike structural polysacchrrides that are fermented very slowly and over a prolonged period of time, most storage polysaccharides are readily fermented in the rumen. Their fermentation also influences the molar proportions of VFAs in the rumen (McCarthy *et al.* 1989; Moore *et al.* 1992; Aldrich *et al.* 1993; Knowlton *et al.* 1996b; Plascencia and Zin 1996) and microbial profile (Nocek 1997; Knowlton 2001).

The rapid fermentation of sugars and starch in the rumen produces a large quantity of VFA that may overwhelm the buffering capacity of the saliva, absorption across the rumen epithelium and outflow to lower parts of the gut leading to acidosis. In some cases lactic acid which is an intermediate organic acid can also accumulate to high levels, and given its lower p^{ka} of 3.86 compared to that of VFAs at 4.80, it has a powerful effect of lowering rumen pH (Rowe and Pethick 1994; Knowlton 2001). This ultimately creates a very highly acidic environment mostly within the range of 5.0-5.8. While such a low pH has little effect on amlyolytic bacteria, it is lower than the optimal pH for cellulolytic bacteria and other fibrolytic microbes in the rumen and so fibre digestion is impaired (Weston 1988; Russell and Wilson 1996). Fibre digestion in the rumen is normally depressed when the rumen pH falls below 6.0 (Mould and Ørskov 1983;

Miller and Muntifering 1985; Hoover 1986; Russell and Wilson 1996). The intake of roughage is also depressed when pH falls below 6.0 (Robinson and Kennelly 1988; McCarthy *et al.* 1989). However, the magnitude of the depression depends to a large extent on the amount of time that the pH is below the optimal level for cellulolysis (Royes *et al.* 2001). The depression of fibre digestion in the rumen combined with reduced intake of roughage leads to what is often referred to as a "*negative associative effect*" between the storage polysaccharides and the roughage (Dixon and Stockdale 1999).

2.4.4. Mechanisms involved in the depression of digestion and intake

It has been demonstrated in many *in vitro* (Terry *et al.* 1969; Sterwart 1977; Hiltner and Dehority 1983) and *in vivo* (Mould *et al.* 1983; Hoover 1986; Ørskov 1986; Sanson and Clanton 1989; Tamminga 1993) studies that the use of energy-rich carbohydrates tends to depress digestion of basal roughage. The so-called "*negative associative effect*" is more pronounced when the rapidly fermentable supplement forms a large proportion the total diet DM (Dixon and Stockdale 1999). Similarly, the reduction in the intake of the basal roughage following carbohydrate energy supplementation has lead to what is often referred to as "*substitution effect*" (Mulholland *et al.* 1978; Mathers and Miller 1981; Dixon and Stockdale 1999). While the consequences of energy-rich carbohydrate supplements in depressing fibre digestion both *in vitro* and *in vivo* and intake of roughage are well known, the mechanism through which they exert this effect has been a subject of many studies and speculations. A review of work done in this area shows that several mechanisms have been proposed.

The preferential utilisation of the supplement instead of the basal roughage by some highly cellulolytic rumen bacteria such as *Bacteroides succinogenes* and *Ruminococcus flavifaciens* that pre-dominate roughage diets (Mackie *et al.* 1978) is one of the mechanisms through which digestion and intake of basal is depressed. Though these rumen bacterial species are basically fibre fermenters, they do also have a capacity to ferment and utilise starch as an alternative energy source when it becomes available, especially when the basal roughage is supplemented with grain (Hungate 1966). As a result the microbes may be able to meet most their energy requirements from the supplement and therefore dispense with the need to utilise the refractory plant material. The supplement does also have an effect on the profile of the rumen microbial population with the slow-growing cellulolytic bacteria and anaerobic fungi that dominate

roughage diets being replaced by the fast-growing amylolytic bacteria, especially the *Selenomonads, Streptococci,* and *Lactobacilli* whose numbers increase rapidly following intake of the supplement. Populations of the large protozoa species (Holotrichs) may also increase appreciably, especially with soluble sugar supplementation (Bird *et al.* 1979; Ffoulkes 1984; Habib 1988). Supplementation of roughage with readily fermentable carbohydrates also causes a substantial fall in rumen fluid pH that depresses fibre digestion in the rumen. However, the magnitude of the fall in ruminal pH is dependant on the quantity and fermentability of carbohydrate used as the supplement (Fonseca *et al.* 2001; Royes *et al.* 2001). Following supplementation of roughage with energy-rich carbohydrates, opportunities are created in the rumen for intense competition among the major cellulolytic and amylolytic groups of microbes for the scarce nutrients/substrates such as ammonia, amino acids and sulphur (Dixon and Stockdale 1999). Those such as the amlolytic bacteria that are present in large numbers due to the presence of their preferred substrate will have immense competitive advantage in utilizing any nutrient(s) that may be limiting microbial growth (Kaneko *et al.* 1989). Based on *in vitro* studies, starch has been found to extend the lag time and therefore increasing the time taken by the microbes to fully colonize fibre particles (Mertens and Loften 1980). If the same is replicated under *in vivo* conditions, then delay in microbial attachment could be yet another mechanism through which energy-rich carbohydrate supplements may depress fibre digestion in the rumen.

2.4.5. Rumen digestion of digestible fibre carbohydrates

In contrast to soluble sugars and storage and structural polysaccharides, digestible fibre carbohydrates that include cotton trash, soybean hulls and un-molassed sugar beet pulp tend to occupy a unique intermediate position. Although they contain cellulose, hemicellulose and pectin, they are largely un-lignified which makes them almost completely degradable in the rumen (Van Soest 1982). Furthermore, they are also fermented gradually than sugars and starch (Royes *et al.* 2001). Their digestion in the rumen therefore occurs without causing wide fluctuation of pH in the rumen (Mould and Ørskov 1983).

2.4.6. Digestion of carbohydrates in the small intestines

The endogenous enzymes in the small intestines cannot digest any structural carbohydrates that escape microbial fermentation in the rumen. These carbohydrates must wait until they reach the

large intestines before they undergo further microbial fermentation. Although a large proportion of cereal grain starch will normally be readily fermented in the rumen, when dietary levels of starch are very high, some may escape into the small intestines where it is digested to glucose by the animal's endogenous enzymes (Herrera-Saldana and Hubert 1989; Herrera-Saldana *et al.* 1990b; Fonseca *et al.* 2001). When the diet contains high proportions of starch, even the small intestines are overwhelmed and some of the starch passes to the hindgut where it is fermented to VFA and gases by microbes (DeGregorio *et al.* 1982; Siciliano-Jones and Murphy 1989a, 1989b).

Starch that escapes ruminal fermentation is digested in the duodenum by the pancreatic α-amylase and brush border carbohydrases like amylo-glucosidase (AMG) initially to malto-dextrose and maltose, and finally to glucose. This glucose is then variably transported across the intestinal mucosa into the blood through a combination of two complementary systems that include the apical Na^+-dependent glucose transporter (**SGLT1** (Wright 1993) and the basolateral facilitative transporter (**GLUT2**) (Cheeseman, 1992, 1993; Takata *et al.* 1993). Although the animals' own digestive enzymes are the main agents that drive starch digestion in the small intestines, there is some evidence of low microbial activity as well (Allison 1993) that may also contribute in a small way to the disappearance of starch in that part of the gut (Kreikmeier *et al.* 1991). Ruminants generally are able to effectively handle low to moderate quantities of starch in the small intestines mainly by increasing secretion of pancreatic α-amylase (Armstrong and Beever 1969; Owens *et al.* 1986; Russell *et al.* 1981) and possibly by increasing the gut absorptive capacity for glucose (Bauer *et al.* 1995; Shirazi-Beechy *et al.* 1991; Zhao *et al.* 1998). In contrast to the situation in monogastrics, the small intestines of ruminants may be unable to cope with large inflows of starch, mainly due to lower amylase activity (Ørskov *et al.* 1971b; Owens *et al.* 1986; Ørskov 1986). There are also reports that the glucose absorptive capacity of the ileum is rather limited (Ørskov *et al.* 1971b; McAllan and Lewis 1981). Similarly, there is also ample evidence that the sucrase (invertase) activity in the small intestines of ruminants is low (Walker 1959b; Siddons 1968). As a result any sucrose or sucrose-rich supplements such as molasses that bypass or escape rumen fermentation may end up in the hindgut causing extensive fermentation (Ørskov *et al.* 1972). Although the small intestines is the main centre of glucose absorption in ruminants, there are indications from DNA studies that the fermentative parts of GIT (i.e. rumen, omasum, and the caecum) may have functional and nutritional roles in the active transport of glucose through the gut epithelium (Zhao *et al.* 1998).

2.4.7. Volatile fatty acids as an energy source

Because the VFA are only partially oxidized waste metabolites of anaerobic respiration in the rumen, they still retain much of the energy that was contained in the initial fermentable substrate. They are an important energy and substrate carbon source for the host ruminant animal (Wolin 1981; Preston and Leng 1987). The importance of VFA as an energy source to the ruminant is emphasized by the fact that they not only supply most of the animal's energy requirements, they also represent about 70-80% of all digestible energy in the rumen and nearly 50-70% of digestible energy intake (Brockman 1993; France and Siddons 1993). Though the rumen is the main site of VFA production and absorption (Hungate 1966), some blood VFA is derived from fermentation in the hindgut (Ulyatt *et al.* 1975a).

The VFA and the microbial cells are the two most important end products of microbial fermentation as they are the major sources of energy and protein respectively for the host ruminant animal (Brockman 1993; Nolan 1993; Russell and Strobel 1993). Nevertheless the energy yield from these end products is lower than that in the initial OM mainly because some of the energy is utilised by the rumen microbes for maintenance (M_{ATP}) and is released as heat. It is also estimated that CH_4 gas energy loss may account for about 6-17% of the digestible energy (Ørskov 1975) or about 6-8% of the gross energy contained in OM at high intake levels (ARC 1980). The loss of energy when the OM is fermented in the rumen to VFA, fermentation gases and heat by the microbes represents a significant energetic cost of rumen microbial digestion (Preston and Leng 1987; Van Soest 1982). The energy loss associated with digestion of fibrous roughage in the rumen is a direct consequence of the inefficiency of microbial fermentation in a very highly reducing environment. There are, however, concerns when similar energy losses occur when high quality feeds that are intestinally digestible such as energy concentrates are extensively fermented in the rumen. This is because such high quality energy sources, especially starch would be more efficiently utilised if they were digested in the small intestines by the host animal's endogenous enzymes rather than undergoing microbial fermentation in the rumen (Leng 1982c; Harmon and McLeod 2001).

2.4.8. Digestion of protein

2.4.8.1. General

Dietary protein and NPN, like carbohydrates, are also subjected to extensive microbial degradation in the rumen and on average about 60-70% of protein fed to ruminants is degraded in the rumen. A large proportion of the remainder is digested in the small intestines (Satter and Roffler 1977; Kaufmann 1982). As a result the protein reaching the small intestines in most cases has no resemblance to the dietary protein from which it was derived. The extent of the transformation varies with the degradability of the dietary protein source with the readily soluble sources such as forage proteins and NPN undergoing the greatest transformation (ARC 1980; Demeyer and Van Nevel 1980; Nugent and Mangan 1981). Protein sources that are less degradable in the rumen such as fishmeal (ARC 1984; Ganev *et al.* 1979) and blood meal (Loerch 1983) tend to undergo the least transformation (Hume 1974; Broderick *et al.* 1991).

Various factors influence the rate and extent of degradation of protein in the rumen (Broderick *et al.* 1991). Protein solubility is thought to be important, with the more soluble proteins generally being degraded at a faster rate (Wohlt *et al.* 1973). However, there are some proteins that are fairly soluble but nevertheless resist degradation in the rumen (Nugent *et al.* 1983; Wallace 1983b). Apparently, it may not be necessary for an insoluble protein to enter the soluble protein pool for it to be attacked by microbial proteases (Broderick *et al.* 1991). The chemical structure, particularly the presence of disulphide bonding (e.g. albumin and immunoglobins) and the cross-linking that develop following chemical treatment, tends to reduce degradability of protein in the rumen (Mahadevan *et al.* 1980; Nugent *et al.* 1983). The extent to which a protein is degraded is also influenced by its residence time in the rumen (Ørskov and McDonald 1979), which in turn is affected by level of intake and particle size (Elimam and Ørskov 1984a, b, 1985).

The degradation of dietary protein by rumen microbes to NH_3 followed by microbial protein synthesis ensures that irrespective of the variation in the initial quality, the final protein reaching the small intestines is of fairly uniform quality (Hogan and Weston 1970). Besides this leveling of the dietary protein quality, microbial synthesis also ensures that low quality dietary N sources such as NPN are upgraded to high quality microbial protein (Wolin 1981). It also enables ruminants to survive on low quality roughage with very low N content by relying mainly on the endogenous N sources, particulary recycled urea (Satter and Roffler 1977; Kennedy and Milligan

1980a; Broderick *et al.* 1991). Influencing the amino acid content in the intestinally absorbed protein can only be achieved through manipulation of undegraded dietary protein (Broderick *et al.* 1991).

During microbial digestion of dietary protein in the rumen, the protein is initially hydrolysed to shorter polypeptides (oligopeptides) and peptides by proteolytic enzymes that usually act extracellularly (Kopency and Wallace 1982; Nugent and Mangan 1982; Wallace *et al.* 1990). However, in protozoa, intracellular enzymes are thought to be more important because most of the proteolysis of insoluble protein in these microbes normally takes place after engulfment of the particulate protein sources including other microbial cells (Bartley and Deyoe 1977; Broderick *et al.* 1991). In green forages plant proteases that are released after initial mastication during also complement microbial enzymes in the hydrolysis of dietary protein. The resultant peptides and amino acids are subsequently assimilated into the protoplasm of the microbial cells (Wallace *et al.* 1990), either for direct incorporation or after further degradation (deamination) to volatile fatty acids, gases and ammonia (Prins 1977; Nolan and Leng 1972). Fermentation of amino acids therefore yields ATP energy that the microbes can utilise for both maintenance and growth (Broderick *et al.* 1991). However, protein yields only 1.2-1.5 moles ATP per 100 g protein fermented compared to 2.5-3.0 per 100 g carbohydrate (Demeyer and Van Nevel 1979; Tamminga 1979).

The microbial protein together with any undegraded dietary protein (UDP) enters the abomasum where protein digestion is initiated by the pepsin resulting in the formation of shorter polypeptides (Argenzio 1993d). These polypeptides are hydrolysed by pancreatic trypsin, chymotrypsin and elastase to peptides and amino acids that are finally absorbed into the blood (Argenzio 1993a). Microbial protein consists of 75-86% amino acid N (true protein), and about 15-25% as non-amino acid N, mainly as nucleic acid (Purser and Buechler 1966). With a digestion coefficient of 0.7-0.8 or even higher it is considered to be highly digestible (Hagemeister *et al.* 1980; Storm *et al.* 1983; Wallace 1983a) and the protozoal portion has a higher digestibility than bacterial (Purser and Buechler 1966). The amino acid profile of microbial protein is also relatively high in most essential amino acids (Czerkawski 1976; Owens and Bergen 1983; Storm and Ørskov 1983, 1984), particularly lysine and isoleucine (Hogan and Weston 1970). There are, however, reports that some essential amino acids may be limiting,

especially methionine (Storm and Ørskov 1984). Overall the metabolisable protein content in microbial protein is estimated to be about 64% (AFRC 1993).

2.4.8.2. The inefficiencies associated with protein digestion in ruminants

Rumen microbial degradation of high quality protein in the rumen is considered to be inefficient for a number of reasons. Firstly, the microbes degrade high quality protein to amino acids/peptides and then utilise some of the amino acids, in the process degrading them to VFA, gases and ammonia. While some of the ammonia-N may be utilised for microbial synthesis, the microbial protein may be of lower quality than the dietary protein (Stern *et al.* 1985). Secondly, due to certain limitations such as insufficient supply of fermentable energy to the rumen microbes, the asynchrony between the supply of energy and of ammonia to the rumen microbes (Sinclair 1993; Trevaskis 2001) or insufficiencies of other essential nutrients such as S, not all the available ammonia is effectively incorporated into microbial biomass. Any excess ammonia not incorporated into microbial synthesis is absorbed across the rumen epithelium and transported to the liver where it is converted to urea. Though some of the urea can be salvaged by being recycled through the saliva and diffusion into the rumen from the blood, the largest proportion is excreted in urine and therefore represents a net loss of N to the animal (McDonald 1948). As much as 40% of total urea synthesized in the liver under conditions of adequate fermentable energy supply in the body can be from excess rumen ammonia. The percentage can rise to 60% or more in animals with inadequate digestible energy intake (Kaufman and Lupping 1982). Thirdly, the absorption of excess rumen ammonia tends to strain the animal's physiological and metabolic processes with reports of excess ammonia being implicated in reduced reproductive performance in some dairy cows (Jordan and Swanson 1979; Folman *et al.* 1981). This wasteful degradation of protein in the rumen tends to increase an animal's protein requirement per unit of ME (Jenness 1985). This problem can however, be minimized by protecting high quality dietary protein from extensive degradation in the rumen using various methods to maximize delivery of amino acids to the body tissues (Hogan and Weston 1970; Siddons *et al.* 1984; Lynch *et al.* 1987; Waghorn *et al.* 1987; Faldet *et al.* 1988; Broderick *et al.* 1991).

2.4.9. Digestion and absorption of lipids

Lipids rarely form a substantial proportion of the ruminant diet and most typical forages contain between 3-8% lipids (MAFF 1975; Pethick and Dunshea 1993). When dietary lipids are ingested, they undergo three major transformations in the rumen that contrast sharply to what happens in monogastric animals. The lipids are rapidly hydrolysed to free fatty acids and other moieties through the activities of microbial and plant lipases, followed by hydrogenation, especially for those long-chain fatty acids that are largely unsaturated (Fernadez 1999; Scollan and Huws 2005). There is also the *de novo* synthesis of microbial lipids utilising acetate and H_2 in the rumen (Emmanuel 1974) and direct incorporation of some dietary fatty acids into microbial cells (Guttierez *et al.* 1962). As a result the lipid entering the duodenal portion of the small intestines is a complex mixture of microbial lipids, hydrogenated dietary lipids and lipid that may have escaped ruminal digestion. All these lipids are then emulsified by the bile salts and subsequently digested by pancreatic lipase, producing free fatty acids, mono- and di-acylglycerides that are absorbed into intestinal mucosa (Noble 1981).

2.5. Voluntary feed intake in ruminants

Voluntary feed intake is considered important in livestock because it is a major factor influencing animal production efficiency and health (Adams 2001). It is of particular importance in ruminants because, even when given access to feed *ad lib* they are still unable to consume enough feed to satisfy nutrient requirements needed to realize their full production potential (Forbes 1993). Numerous attempts have been made to understand the factors that limit feed intake (Baumgardt 1970; Campling 1970, Bines 1971; Forbes 1971; Weston 1988), with several reviews being written on the mechanisms involved (e.g. Forbes 1986a; NRC 1987).

2.5.1. Factors regulating voluntary feed intake

When animals are offered feed, they rarely eat it continuously but will take it in small meals spread over a certain period until their daily intake is met. Most ruminants take 5-20 such discrete meals over the 24 h period (Forbes 1986a; 1993). Meal sizes are influenced by changes in blood hormones and circulating metabolites and distension and movement of the digestive tract (Forbes 1993). Voluntary feed intake in ruminants is, however, regulated by many factors that are related to the diet, animal, and the environment (Forbes 1993).

2.5.1.1. Physical regulation of feed intake

When simple-stomach animals are offered high quality feed in terms of both nutrient density and digestibility, they are able to adjust their intake so as to maintain a uniform caloric intake (Adolph 1947). When ruminants started being supplemented with concentrates in increasing amounts, it was realized that they too are able to regulate their intake based on digestible energy (DE). However, it was soon discovered that this adjustment in intake with changing DE concentration was not always perfect, as there are cases of over-consumption of energy when the animals were offered high energy density feeds, and inadequate caloric intake at low energy density or high caloric dilution (Dinius and Baumgardt 1970). When the DE concentration of the feed is higher than 10.5 MJ DE/Kg DM, the animal is unable to satisfactorily reduce feed intake to a lower level that can satisfy energy requirements for maintenance and production without consuming excess calories (Baumgardt 1970; Dinius and Baumgardt 1970; Grovum 1987).

Most of the basal roughages available to ruminants are high in cell wall constituents (CWC), and therefore generally of low digestibility. When such roughages are ingested in substantial quantities, gut distension causes the stretch receptors located in the gut wall to send neural signals to the hypothalamus. The existence of such a control mechanism that is based on physical presence of feed in the rumen along side chemical stimulation by metabolites of the rumen fermentation was demonstrated by Anil *et al.* (1993). To relieve the ruminal pressure the ingested feed has to be subjected to a combination of intense physical breakdown during rumination and microbial fermentation in the rumen until it is comminuted to particle sizes that are small and dense enough to be cleared from the rumen through the reticulo -omasal orifice (Ulyatt *et al.* 1986; Fahey and Merchen 1987; Lechner-Doll *et al.* 1991).

In ruminants intake of roughage is determined not so much by the rate at which the digestible fraction is digested, but by the rate at which the indigestible fraction is reduced to a particle size that can be cleared from the rumen (Poppi *et al.* 1981a, b). As a result the rate of forage digestion in the rumen and the subsequent passage of indigestible particles through the digestive tract is a major factor likely to limit food intake in ruminants (Van Soest 1975; Forbes 1993). Accordingly, any processing that increases the rate and extent of degradation of the potentially digestible fraction and/or the rate at which the indigestible fraction is physically broken down is

likely to enhance voluntary intake (Ellis *et al.* 1988; Forbes 1993). Digestibility is a major factor in determining whether the voluntary intake will ultimately be regulated by 'physical' or 'metabolic' factors (Baile and Forbes 1974; Forbes 1993).

Because the rate of physical breakdown of the herbage in the rumen (or degradation) and passage rates are normally positively correlated, it follows that the rate of degradation is a major factor in the determination of intake (Conrad *et al.* 1964). Other workers have similarly noted a positive relationship between voluntary intake and degradability (Hovell *et al.* 1986; Ørskov *et al.* 1988). On the other hand, there are also reports of voluntary intake being positively correlated to the soluble fraction in roughage and the rate of degradation of DM and NDF (Carro *et al.* 1991). Presumably, this occurs because of the soluble fraction stimulating higher microbial activity in the rumen (Mertens 1977). Forages that are high in refractory material tend to offer higher resistance to physical breakdown. Such forages will have a longer mean retention time in the rumen which in turn leads to lower voluntary intake (Troelsen and Bigby 1964; Hogan *et al.* 1969). In contrast, those forages that offer least resistance to degradation and/or stimulate higher microbial growth and fermentation activity in the rumen will result in higher intake (Hemsley and Moir 1963; Carro *et al.* 1991).

The principle of the physical limitation of the gut is, however, unable to explain why lactating cows tend to have a higher DM intake than non-lactating cows of the same body weight, even though both would be expected to have almost similar gut capacity (Forbes 1993). Lactation imposes a large increase in demand for nutrients to support milk production, and this is likely to stimulate high voluntary intake subject to physical limitations of the gut (Forbes 1993). There are, however, indications that oxytocin secreted during lactation to stimulate milk let down may also be involved in stimulating higher gut motility. This increases the rate of passage leading to increased feed intake (Godwin and Zaenuri 2000).

2.5.1.2. Metabolic regulation of feed intake

It has been established that beyond a certain DE density, a positive relationship exists between digestibility of forage and daily intake (Baile and Forbes 1974). Beyond a certain level of DE concentration (proportional to digestibility) the physical limits of the gut ceases to be a major factor constraining intake. At this point intake is then metabolically controlled. At that level,

intake is negatively related to digestibility and the animal's energy requirement determines intake (Conrad *et al.* 1964). The level of DE concentration at which metabolic regulation controls intake has been reported to be 10.5 MJ DE/Kg DM. This corresponds to a digestion coefficient of about 0.67 (Conrad *et al.* 1964). Ruminants rely mainly on the fermentative digestion in the rumen where most of the feed energy is released and absorbed as VFA. Therefore, it is conceivable that the presence of fermentation products, especially VFA in the gut and/or their absorption into the blood, will have a major influence on the regulation of feed intake.

2.5.1.2.1. Role of volatile fatty acids in intake regulation

In ruminants the concentrations of VFAs or their metabolites in the blood may play a major role in short term chemostatic control of feed intake (Dowden and Jacobson 1960). Unlike in monogastrics where intestinally absorbed glucose is a major substrate regulating intake, the fermentative nature of the ruminant gut rarely allows any significant quantity of glucose to be absorbed. Following feeding, the molar concentrations of VFA both in the rumen and in the blood are expected to increase appreciably. Any accumulation of VFA in the rumen and the blood to excessively high levels can lead to a disruption of the body's physiological and metabolic processes. Such a situation is normally avoided by cessation of eating until the animal has metabolised the already absorbed load of VFA, which may be the reason why the animal normally takes its daily intake in meals.

Increase in the concentration of acetate and propionate tend to have a greater effect in depressing intake than butyrate and the specific receptors of these two VFAs are most likely located in the rumen epithelium where neural signals or humoral agents are transmitted to the feeding centre in the hypothalamus (Baile and Mayer 1969, 1970). Acetate and propionate but not glucose are quite effective in depressing feed intake. Lactic acid together with minor VFAs such as valeric acid causes only a small and inconsistent depression of intake (Dowden and Jacobson 1960). Egan (1977) also found that intra-ruminal infusion of acetate depressed feed intake in sheep. The work of Dowden and Jacobson (1960) established that butyrate played only a very minor role in regulating intake. This is probably because, during absorption butyrate is extensively metabolised in the epithelium to ketones, acetate and CO_2 leaving only a small amount to enter the general circulation (Annison and Armstrong 1970).

2.5.1.2.2. Glucostatic regulation of feed intake

In simple-stomach animals, feed intake is inversely related to plasma glucose concentration which leads to the proposition that glucoreceptors existed in the hypothalamus whose role was to monitor blood glucose levels and to regulate feed intake to ensure a sustained supply of glucose to the body tissues (Mayer and Bates 1952). This is the glucostatic theory of food intake regulation in animals. It was argued that feeding would be initiated by the hypothalamus when the glucoreceptors detected lower than normal blood glucose and ultimately would stop once normal glucose level was restored. It was later established that glucoreceptors were also located in the epithelium of the gastro-intestinal tract (Oomura *et al.* 1969).

It is however, widely acknowledged that in ruminants glucose is unlikely to play a major role in regulation of feed intake (Baile and Forbes 1974), mainly because very little glucose is absorbed directly from the gut (Dowden and Jacobson 1960). However, DE intake is correlated with glucose production rate and therefore glucose entry rate (GER) may be more important in regulating feed intake than blood glucose concentration (Houpt 1974).

2.5.1.3. The role of feed chemical factors in intake regulation

Chemical factors associated with feed can regulate voluntary intake in animals in different ways including the organoleptic influence and the effect on microbial fermentation in the rumen and tissue metabolism. Palatability is one of the most important factors that influences feed intake in animals at least on a short-term basis (Ralphs *et al.* 1995; Adams 2001, pp 47; McDonald *et al.* 2002, pp 467). Some of the factors affecting palatability may do so either directly through organoleptic stimulation (smell, flavour, taste etc) or indirectly by affecting other factors associated with feeding such as digestibility or toxicity (Barry 1989; Barry and McNabb 2000). Animals are likely to associate certain nutritional attributes of a feed with specific flavours and will eat such food in anticipation of favourable post-ingestive outcomes (Villalba and Provenza 1996; Francis *et al.* 2003). More recent studies have shown that palatability alone is not adequate to sustain high voluntary intake unless such feed is also fairly digestible so as to release nutrients that satisfy the animal's total requirements (Pain *et al.* 2005).

Voluntary feed intake may also be constrained by nutrient deficiencies or imbalance at the rumen or tissue metabolism level. When a roughage diet is deficient in certain key nutrients that are

required by the rumen microbes, microbial growth and fermentation activity will be impaired and consequently intake will be depressed (Leng 2003). Supplementing the diet with the deficient nutrients has been shown to stimulate microbial growth and voluntary intake (Minson 1982; Leng 2003). However, it has also been proposed that deficiency and imbalance of protein relative to energy (P/E) at the tissue metabolism level rather than low digestibility is a major factor constraining voluntary intake in ruminants (Preston and Leng 1987). As a result increased microbial protein supply and by-pass protein supplementation, have been shown to stimulate feed intake (Leng *et al.* 1977), presumably by correcting the imbalance between the supply of amino acid relative to energy at the tissue level (Egan 1965b, 1977; Lindsay and Loxton 1981; Illius and Jessop 1996).

2.5.1.4. Energy balance in intake regulation

Animals are able to maintain a constant live weight (or fatness) by regulating their feed intake in such a way that a balance is maintained between energy intake and utilisation. Inability to increase feed intake to meet increasing demand for energy on a short-term basis may lead to mobilisation of stored reserves and hence loss in body weight (Forbes 1993). Because such mobilisation is not sustainable in the longer term, the depleted reserves need be replenished through higher intake when feed becomes available.

Protein and fat are the two most important energy reserves in the body (Butterfield 1966; Lindsay and Buttery 1980). For mature animals that have stopped growing, fat deposition is the main avenue through which they are able to maintain a constant live weight (Meyer and Clawson 1964). Fat content is also the most variable component in the body of animals, which further supports the view that energy balance in the body may be maintained by adjusting the amount of fat stored. As the animals grow to maturity they tend to deposit more fat relative to other body tissue components and generally the fatter the animal the lower the feed intake for any body size (Forbes 1993). The lipostatic theory postulates that some signals originating from the adipose tissue may be directly or indirectly conveyed to the hypothalamus, which in turn regulates feed intake and body activities in such as a way that fat reserves are maintained (Scharrer and Langerhans 1990).

In recent times focus has turned to a possible role of the polypeptide hormone, leptin, secreted by the white adipose tissues. It has been found to stimulate or suppress the release neuropeptides from the hypothalamus (McDonald *et al.* 2002, pp 466). It is these neuropeptides that have been implicated in increasing feed intake and thermogenesis through enhanced physical activity (Baile and McLaughlin 1987), presumably in an attempt by the animal to maintain the desired energy balance. The plasma concentration of leptin is also correlated to the percentage of body fat and may be lowered by fasting (Ahima *et al.* 1996; Nagatani *et al.* 2000; Henry 2003)

2.5.1.5. Thermostatic regulation of intake and effect of climate

Ruminants are homeotherms and so rely mainly on metabolic heat generated from within the body for the homeostatic regulation of their body temperature. A significant proportion of this heat is generated from activities associated with digestion of food and absorption and metabolism of nutrients in the body tissues. As a result there is always an enhanced heat production in the animal's body following feeding, especially during the absorptive phase. Because of the intense rumination and microbial fermentation such heat production is even more pronounced in ruminants fed high-fibre low-protein roughages (Leng 1990).

Animals generally will adjust their feed intake to a level that will allow them to have an effective homeostatic regulation of their body temperature without risking either hyperthermia or hypothermia. High ambient temperature generally will result in a decrease in feed intake by ruminants (Moose *et al.* 1969), apparently in response to the reduced capacity to dissipate the excess body heat that may predispose the animal to hyperthermia. In contrast, animals exposed to low ambient temperatures tend to have a higher voluntary feed intake (Leng 1990). Animals subjected to cold stress normally increase their feed intake, essentially through increased rate of passage of digesta through the rumen (i.e. higher rumen turnover) and intestinal tract (Kennedy and Milligan 1978a; Orton *et al.* 1985b; Kennedy *et al.* 1986).

2.5.1.6. Neural and hormonal regulation of feed intake

The fermentative digestion in the rumen and hindgut, intestinal digestion, and tissue metabolism are all linked in a highly integrated and regulated manner that is controlled by the hypothalamus. The hypothalamus then sends signals that are associated with initiation or cessation of feeding (Baile *et al.* 1968), in the process controlling the meal sizes that ultimately determine voluntary

intake. Though the signals reaching the hypothalamus are either neural or hormonal, the source of the stimulus may be from either endogenous or exogenous origin. For example, stimulation of the central nervous system through the administration of various adrenergic/cholinergic agonists stimulates feeding in ruminants (Baile and McLaughlin 1987). Similarly, opioid peptides that are mostly secreted by the hypothalamus have also been reported to stimulate feeding in ruminants (Baile and McLaughlin 1987). More recently, ghrelin (an endogenous peptide hormone secreted mainly by the oxyntic glands in the stomach) has been implicated in triggering the onset of feeding, especially in programmed feeding compared to continuous feeding, with the plasma levels of the hormone increasing just before the scheduled feeding time (Sugino *et al.* 2004).

Following ingestion of feed there are notable changes in hormonal levels in the blood that may be involved in the determination of meal sizes and ultimately voluntary feed intake (Forbes 1993). Bray (1978) has reviewed in detail the role of the major hormones in voluntary feed intake. These hormones include: the intestinal hormones (cholecystokinin, enteroglucagon and neurotensin) whose secretion tends to induce satiety in animals. The two steroids, progesterone and estrogen, have antagonistic effects on feed intake in animals, with progesterone generally inducing a dose-related positive response in intake in rodents (Hervey and Hervey 1967), while oestrogen (estradiol) depresses feed intake (Bray 1978; Forbes 1986b, 1993). Thyroid hormones that include triiodothryronine (T3) and thyroxine (T4) tend to increase feed intake in ruminants, possibly because they increase basal metabolic rate (Bray 1964). Similarly, administration of insulin and glucagon either singly or in combination depress feed intake, while insulin and propionate or glucagon and propionate also depressed intake (Deetz and Wangsness 1981). The effect of growth hormone on feed intake is however not clear, but may depend on the physiological state of the animal. Feeding activity in sheep is suppressed when growth hormone level is high (Driver and Forbes 1981). However, the secretion of growth hormone during the feeding process is itself preceded by high plasma levels of ghrelin (Sugino *et al.* 2002a, b). Thus both growth hormone and ghrelin have a role in energy homeostasis in the ruminant.

2.6. Increasing digestibility and intake of low quality roughage in ruminants

Animal production performance in terms of milk yield or live weight gain is closely related to forage composition, digestibility and intake (AFRC 1992; Sniffen *et al.* 1992; Beever 1997). With increases in digestibility, forage OM intake is also increased mainly because the rate of

reduction of the roughage to particle size that allow faster clearance from the rumen to the lower parts of the gastro-intestinal tract is also increased (Lechner-Doll *et al.* 1995). However, most low quality roughages such as cereal straw are high in CWC and low in rumen degradable nutrients, especially N and S. As a result they support very low microbial growth and fermentation activity in the rumen, leading to long MRT and therefore low voluntary intake (Dixon 1987; Kennedy and Murphy 1988). Most roughage after ingestion has an average MRT of about 40-60 h in the rumen, during which the potentially digestible OM has to be degraded to release nutrients (Ørskov 1994).

There are basically four main ways to enhance digestion and intake of low quality basal roughage in ruminants-:

1. Modify the refractory roughage by physical, chemical or biological treatment, to make the material more susceptible to microbial degradation and therefore, more readily reduced to smaller particle sizes during mastication;
2. Supplement the animal with deficient rumen degradable nutrients to enhance microbial growth and fermentation activity in the rumen;
3. Manipulate the rumen microbes and their ecosystem (e.g. inoculating ruminants with microbes from other herbivores) to promote growth of more effective fibre digesting microbes;
4. Ensure that absorbed nutrients are balanced, especially in the P: E ratio to remove any amino acid deficiency that may impair the metabolism of acetogenic substrates in the body tissues. It is anticipated that this will have a stimulative effect on DM intake.

The scope of this review is restricted mainly to the first two, particularly the use of supplements because of its relevance to the work reported in this thesis.

2.6.1. Treatment of roughages

Various forms of physical, chemical, physico-chemical or biological treatments of the high-fibre, low-protein roughage can be effective in modifying lignin-cellulose linkages and thus increasing digestibility and/or intake of low quality roughage. The treatments that can be applied to low quality roughage to enhance digestibility and intake are reviewed in the following sections.

2.6.1.1. Physical treatment or processing

Physical processing methods that have been used include: simple soaking the roughage in water, shredding, chopping and grinding with or without pelleting, boiling or pressure cooking/steaming (Hart *et al.* 1980). With few exceptions, such as boiling and steam treatment that appear to be quite effective, most physical treatments tend to increase intake without significantly improving digestibility. Fine grinding and pelleting of refractory roughage to particle sizes with a diameter of less than 1 mm will result in a large surface area/volume ratio that facilitates faster colonization of feed particles by rumen microbes (Mertens 1977; Chesson and Travis 1997). Such processing also increases the rate of passage, thereby reducing the MRT of the fibrous material in the rumen which incidentally may reduce fibre digestion. In some cases, however, the reduction in digestibility may be more than compensated by the higher intake of OM, thereby resulting in a net increase in the intake of DE (Jackson 1978; Ribeiro 1989). The effect of physical processing, however, appears to vary between animal species, as chopping has been reported to increase intake of sorghum stover in sheep but not in cattle (Osafo *et al.* 1997).

Another potential benefit of chopping and grinding of refractory roughage prior to feeding is the increased efficiency in use of ME by the animal mainly because "less work" is done in the digestion of the food. It has been variably reported that the high heat increment that is associated with consumption of highly fibrous roughage diets compared to concentrates, is due to the "high energetic cost of digestion" (Leng 1990). This arises from the high ATP requirements to perform the muscular work associated with feeding and digestion of the roughage (Ørskov and MacLeod 1990; 1993; Ørskov 1994).

2.6.1.2. Biological treatment

White-rot fungi that belong to the group of aerobic basidiomycetes are quite effective *in vitro* in hydrolysing ligno-cellulosic material. Growing these fungi on the roughage is the most widely applied form of biological treatment (Jung *et al.* 1992). However, the improvement in digestibility of DM has been associated with a loss in DM of up to 42%, which makes the practical application of this method very unlikely (Sundstol *et al.* 1993). Lignin itself is very resistant to microbial hydrolysis especially in the highly anaerobic rumen environment. So far no fungi have been isolated that can effectively utilise lignin as the sole energy source (Zadrazil *et*

al. 1995). Owing to this limitation, fungi under aerobic or anaerobic conditions normally utilise structural polysaccharides rather than lignin as the major energy source. Their growth reduces the carbohydrate energy available for use by the animal. The scope for using biological treatment as a means of improving the nutritive value of poor quality roughage appears to be rather limited (Zadrazil *et al.* 1995).

2.6.1.3. Chemical treatment

Various chemicals have been used to treat crop residues to increase digestibility and intake. Most of these chemicals act by solubilising hemicellulose making it more accessible to microbial enzymes (Sundstol *et al.* 1978; Naseer *et al.* 1988). Some forms of chemical treatments have the additional benefit of supplying rumen microbes with N or S, both of which can be limiting microbial growth.

Among the chemical treatments are strong alkalis such as sodium hydroxide, potassium hydroxide and calcium hydroxide or oxide (Lesoing *et al.* 1980). Strong alkalis are particularly effective in improving digestibility of the most refractory fibrous material. Other chemicals that have been used include alkaline hydrogen peroxide and anhydrous NH_3 or NH_3 generated from urea (Sundstol *et al.* 1978; Hadjipanayiotou 1982; Cloete *et al.* 1983). There are also some acids and gases that have been used such as hydrochloric acid (Israelides *et al.* 1979), peroxy-acetic acid (Kamastra *et al.* 1980), chlorine (Yu *et al.* 1970), SO_2 as a gas (Fahmey and Ørskov 1984; Dryden 1986) or dissolved in water to form sulphurous acid (Ben-Ghedalia and Miron 1981; 1984a, b) or sulphuric acid (Arndt *et al.* 1980). Ozone has also been used in the treatment of wheat straw *in vitro* (Ben Ghedalia and Miron 1981).

Chemical treatment is thought to improve digestibility of lignified fibrous forage material by weakening the inter-molecular ester and/or ether covalent linkages and hydrogen bonds between lignin and the structural polysaccharides (Bacon 1979; Chesson 1981; Chesson *et al.* 1986). This solubilises the phenolic acid-carbohydrate complex in the plant cell walls releasing the cellulose and hemicellulose and making them more accessible to microbial enzymes (Lam *et al.* 1992; Lowry and Kennedy 1996). Some chemical treatments such as anhydrous NH_3/ urea, sulphur dioxide and calcium hydroxide or oxide may provide specific nutrients that limit microbial growth in the rumen. The increase in both rate and extent of digestion of crop residue in

ruminants following urea-NH_3 treatment is attributed to both the alkali (or treatment) effect and the improvement of N status of the treated straw (Cloete and Kritzler 1984; Owens and Goetsch 1988; Djajanegara and Doyle 1989; Mgheni *et al.* 1993).

In spite of the apparent effectiveness of chemical treatments, in improving digestibility, especially the strong alkalis, the practical application of the technology has been hampered by a range of factors. These include the high cost of the chemicals relative to the expected benefit, the risk to the health of both human and animals occasioned by the corrosive nature of the alkali, inappropriate application techniques and the possible detrimental effect of some of the chemicals on machinery and the environment (Owen and Jayasuria 1989; Devendra 1991; Sansoucy 1995). Only ammoniation with anhydrous NH_3 or urea-NH_3 has so far been widely used in the treatment of crop residues (Cloete *et al.* 1983; Dias-da–Silva and Sundstol 1986; Djajanegara and Doyle 1989; Sansoucy 1995). But even after ammoniation, the roughage only allows the animals to maintain weight and achieve very low weight gains (O'Donnovan 1978). This suggests that even with chemical treatment, the nutritive value of low quality roughages is still low (Ibbotson *et al.* 1984; Djajanegara and Doyle 1989). Supplementation may be used to complement chemical and other forms of treatment and to further enhance the nutritive value of diets based on low quality roughages.

2.6.2. Supplementation of low quality roughage

2.6.2.1. Protein/nitrogen supplementation

When ruminants are fed on low quality roughage supplementation with NPN or protein has been shown to stimulate microbial growth and fermentation rate in the rumen with beneficial effects on digestion, intake and efficiency of feed utilisation (Silva *et al.* 1989; Ferrell *et al.* 1999; Fonseca *et al.* 2001). The supplement increases the availability of ammonia to the rumen microbes, including the cellulolytic bacteria and anaerobic fungi that are more actively involved in the digestion of structural polysaccharides (Kellaway and Leibholz 1983). Enhanced microbial growth and fermentation activity in the rumen, besides supplying the host animal with VFA and microbial protein also facilitates faster reduction of the ingested feed to particle sizes and density enabling them to be cleared more quickly from the rumen (Ulyatt *et al.* 1986; Lechner-Doll *et al.* 1991; Dixon and Stockdale 1999).

2.6.2.2. Non-protein N supplementation

Adult ruminants (e.g. cows) are able to survive on NPN as the sole source of N and even produce some milk (Virtanen 1966). Though the production of milk was low, it was however, improved further by addition of a preformed protein source (Virtanen and Ettala 1969). Furthermore, the microbial protein produced in the rumen of animals fed NPN and N-free roughage was found to contain all the essential amino acids ruminants need (Owens and Bergen 1983). While it is quite possible for the majority of celluloytic bacteria in the rumen to grow with ammonia as the sole source of N, the growth of some other rumen bacteria is stimulated by the presence of small amounts of amino acids/peptides (Russell *et al.* 1992) and branched- and higher-chain fatty acids (Russell and Sniffen 1984). Most rumen bacteria do indeed have a specific requirement for amino acids (Maeng and Baldwin 1976), with nearly 50% of the amino acid N in microbial protein being sourced from direct incorporation of preformed amino acid N (Nolan *et al.* 1976). A wide range of NPN sources including urea can be used as a source of N for rumen microbes because they are readily hydrolysed to ammonia in the rumen (Tamminga 1982a). Ammonia is efficiently used by the rumen microbes for microbial synthesis provided that a complementary source of fermentable energy is also available (Romero *et al.* 1976).

Cereal straws that have very low digestibility in the range of 40-45% rarely respond to urea supplementation because they do not provide adequate fermentable substrates to support microbial growth (Hogan 1996, pp 47). However, when such straws are treated with alkali to increase OM digestibility, the response to urea supplementation is likely to be increased (Hogan 1996, pp 47). It therefore appears that part of the beneficial effects of a protein besides supplying ammonia may be to boost the level of potentially digestible OM available to the rumen microbes. One of the major drawbacks to an efficient utilisation of most NPN sources in low quality roughage diets is their rapid rate of degradation in the rumen to NH_3 which may not be synchronized with a slower rate of release of fermentable energy from the basal roughage. The inefficiencies associated with the utilisation of such N source were reviewed earlier (**2.4.8.2**). In contrast, some protein sources are degraded at much lower rate than NPN supplying the rumen microbes with peptides, amino acids, ATP, as well as ammonia (Kropp *et al.* 1977; Redman *et al.* 1980; Peterson *et al.* 1985; Ortigues *et al.* 1990). The slow release of amino acids is thought to increase efficiency of microbial synthesis (Maeng *et a*l. 1976).

2.6.2.3. Role of protein in fibre digestion in the rumen

The beneficial effects of protein on digestibility and intake of basal roughage are associated with favourable changes in the rumen environment, and also at the tissue metabolism level (Siebert *et al.* 1976; Kellaway and Leibholz 1983). However, there are conflicting reports on the beneficial effects of protein on roughage digestion. For example, protein does not always increase roughage DM digestibility beyond the level normally achieved with urea supplement alone (Kropp *et al.* 1977; Redman *et al.* 1980; Peterson *et al.* 1985; Iwanyanwu *et al.* 1990). With low quality roughage the source of N is not important, so long as it can readily supply the rumen microbes with adequate NH_3-N. Cellulolytic bacteria rely mainly on this ammonia as the sole source of N to sustain maximum growth in the rumen (Mathison and Milligan 1971). It is these bacteria and anaerobic fungi that are the most important fibre digesters in the rumen. Nevertheless, these cellulolytic microbes may still require small amounts of amino acid N that are easily met from endogenous sources or by 'cross-feeding'.

The extra energy supplied by the protein in the rumen may increase the total number of bacteria by boosting the population of amylolytic bacteria creating a situation whereby the total microbial biomass in the rumen increases but without any net increase in cellulose digestion. Protein supplementation has resulted in higher dietary DM intake while depressing that of the basal roughage (Kropp *et al.* 1977; Redman *et al.* 1980; Peterson *et al.* 1985; Iwanyanwu *et al.* 1990), i.e. producing a substitution effect.

2.6.2.4. Metabolic influence of protein at the tissue level on intake

The efficiency of utilisation of low quality roughage diets in ruminants is increased more by protein supplementation than by NPN (Egan 1965b, 1977). The increase in voluntary intake was achieved, even without any increase in OM digestibility (Leng 1982c), suggesting that the beneficial effects of the protein may be mediated within the tissues rather than the rumen fermentation environment. When an undegradable dietary protein is used as a supplement to low quality roughage, the undegraded component together with microbial biomass flows to the small intestines where it is digested by the host animal's endogenous enzymes (Leng 1990). This "undegraded dietary protein" (UDP), (SCA 1990; AFRC 1993) or "by-pass protein" (Kempton *et al.* 1976; Preston and Leng 1987) increases the availability of amino acids to the body tissues thereby stimulating higher metabolic activity at the tissue level including conservation of energy

as fat. This creates a demand for more dietary energy and therefore an increase in voluntary intake (Egan 1965a; 1977).

In animals subsisting on low quality roughages of low digestibility, satisfying this demand for extra dietary energy at the tissue metabolism level can present a great challenge. The scope for increasing dietary intake is constrained by the low rate of digesta clearance from the rumen (Lechner-Doll *et al.* 1991). Studies conducted with duodenally infused casein showed that an increase in voluntary intake of roughage can occur, even without any significant increase in digestibility (Egan 1965a). The extra DM intake is capable of delivering more DE to the tissues, particularly when combined with longer MRT in the rumen that can increase the extent of digestion, even without any increase in digestibility (Egan 1977). When the amino acid supply at the tissue level is favourable, the glucogenic potential is also greatly enhanced enabling the animal to utilise higher dietary energy intake (Kempton *et al.* 1976; Kempton and Leng 1983; Cronje 1991). However, taking a higher digesta load is not without cost as the animal may have to tolerate some degree of discomfort from a slight increase in gut distention.

2.6.3. Energy-rich carbohydrate supplements in urea-treated roughage

2.6.3.1. General

Energy-rich carbohydrate supplements such as those from cereal grains and other agro-industrial by-products have a potential to enhance utilisation of roughage in ruminants (Devendra 1991; Obara *et al.* 1991). Opportunities for optimizing the use of energy-rich supplements are even more critical in those parts of the world where use of protein sources is constrained by unavailability and cost (Elliot and Topps 1963; Leng and Preston 1983; Devendra. 1991). Supplementation of low quality roughage diets with carbohydrates will in most cases result in a positive response in intake, increased digestion of OM and even increase in live weight (Obara *et al.* 1991). However, it is widely acknowledged that the fundamental problem associated with low quality roughage is their low N content (Obara *et al.* 1991).

The energy supplement is mainly used to enhance the fermentable energy content of the slowly fermented high-fibre low-protein roughage so as to enhance microbial growth in the rumen, provided that rumen ammonia is not limiting (Obara *et al.* 1991). This enables the rumen microbes to digest the roughage in the rumen more efficiently and also to fully utilise most of the

available ammonia. The more efficient digestion provides the host animal with more energy from VFA and also enhances microbial protein synthesis in the rumen which then supplies the animal with fermentation products that are well balanced in protein to energy (Illius and Jessop 1996; Leng 2003). However, the proportion of energy-rich carbohydrates should not exceed 25% of the total diet DM, as higher levels can depress fibre digestion in the rumen (Mulholland *et al.* 1978; Mathers and Miller 1981).

Depending on many factors such as level of intake and form of processing used, some of the energy-rich carbohydrate in supplements, especially cereal grains, may escape rumen fermentation and be intestinally digested, supplying the animal with absorbed glucose (Obara *et al.* 1991; Fonseca *et al.* 2001). Such intestinally absorbed glucose could augment the glucose synthesized in the liver mainly from propionate (Brockman, 1993). A combined supply of glucose from these two sources may provide adequate quantities to meet the body's glucose requirements and therefore dispense with the need to catabolise tissue proteins. Furthermore, this may minimize the need to divert the more expensive dietary protein from other essential physiological and metabolic functions that specifically require protein such as tissue synthesis (Kempton and Leng 1979; Kempton and Leng 1983).

2.6.3.2. The effects of energy-rich carbohydrates on digestion and intake

Energy-rich carbohydrate supplements can be classified into three categories depending on their fermentative behaviour in the rumen, viz. soluble sugars, starch and digestible fibre (Royes *et al.* 2001). The differences in their fermentation in the rumen were reviewed earlier in **2.4.4** and **2.4.5.** The use of energy-rich carbohydrate supplements in roughage diets has evoked quite varied responses in digestibility, intake and production. In some cases quite favourable responses have been obtained. A number of factors have been implicated in such varied response and include: the type of supplement and its rate of fermentation; the proportion of the supplement relative to the basal roughage; other factors dependent on diet such as ruminal ammonia levels; and the mode of feeding the supplement (McLennan *et al.* 1995).

Carbohydrate supplementation increases cellulose digestion mainly in those instances which ammonia is not limiting. Starch increased cellulose digestion from 52 to 79% and was more effective than dextrose (Belasco 1956). Ruminal digestion of neutral detergent fibre (NDF) was

not adversely affected when steers fed Bermuda grass hay were supplemented with maize at a rate of 0.3% BWT. In a different trial, a group of steers fed orchard hay, had their digestion of NDF improved by the same level of supplementation (Jones *et al.* 1988). Similarly, supplementing steers fed alkali-treated oat straw with 700 g/day of barley grain did not significantly affect either ruminal or total-tract OM digestion (Spragg *et al.* 1986). Supplementing lactose at 42% of daily OM intake in sheep fed lucerne hay did not affect the percentage of digestible OM disappearing from the rumen (Poncet and Rayssguiser 1980). Fermentation of energy-rich carbohydrate supplements can also have other beneficial effects on rumen metabolism such as increased recycling of urea N (Engelhardt *et al.* 1978; Obara and Simbayashi 1988; Obara *et al.* 1991). The recycled N is likely to increase microbial synthesis in the rumen and therefore, higher microbial biomass output. Carbohydrate fermentation may also be useful in maintaining a relatively low rate of ammonia absorption from the rumen into the blood because most of the ammonia will exist in the ammonium ion (NH_4^+) form at low pH rather than in molecular form (NH_3) in which form it is less readily absorbed (Bartley and Deyoe 1977; Siddons *et al.* 1984).

When young cattle grazing poor quality pastures were supplemented with low-protein, high-energy sorghum pellets, no change in live weight gain was noted (Hennessy *et al.* 1981). Similarly, when young cattle fed on poor-quality roughage were supplemented with the same sorghum pellets or protein meal, the protein supplement resulted in the highest response in basal roughage intake (Hennessy *et al.* 1983). In these two cases, very poor quality roughage was used as the basal diet and presumably the rumen of these animals also had very low ammonia levels. In a study with heifers fed harvested low quality hay, supplementation with urea improved DM digestibility and intake compared to the control (Iwanyanwu 1990). However, in the same study no further improvement in digestibility or intake was noted when urea and 100-300 g/day of molasses was used. Cellulolytic bacteria together with anaerobic fungi are the major fibre-digesting microbes in the rumen and any deficiency of ammonia or S is likely to reduce their capacity to degrade fibre (Leng and Nolan 1984; Perdock and Leng 1989). It can therefore be argued that an efficient utilisation of available energy (basal and supplement) at both the rumen and tissue metabolism level is possible only when there is a balance of protein and energy sources.

2.7. Glucose metabolism in ruminants

2.7.1. Glucose requirements in the body

Leng (1970b) and Bergman (1973) have outlined the main metabolic activities in the body that require glucose as an energy or carbon source. Briefly these include: the oxidative cellular metabolism that supplies ATP to the body cells including those which for some reasons cannot effectively utilise VFA as an energy source such as erythrocytes and the uterus/fetal tissue (Bergman 1983; Pell and Bergman 1983). The body also normally utilises glucose to meet the immediate energy requirements of muscle, especially when under intense activity where as much as 80% of the glucose undergoes anaerobic oxidation to lactate through the glycolytic pathway (Reitzer *et al.* 1979). During pregnancy, and particularly in the last trimester, glucose requirements increase considerably (Leng 1970a), and can increase even further during early lactation. Most of the glucose utilised for lactation in mammary tissue is diverted to the synthesis of lactose, and glucose is the main precursor of this important milk component (Neville *et al.* 1983). Some of the earliest reports on the role of glucose in lactation indicate that up to 80% of total glucose available in a lactating animal may be diverted to milk synthesis in the mammary gland (Annison and Linzell 1964). Other reports have indicated a lower figure of 50-60% of the glucose being destined for lactose synthesis (Bickerstaffe *et al.* 1974; Baird *et al.* 1983). The total demand for glucose to meet lactation requirements is likely to be even higher in high-yielding dairy cows (Beever 2003; Rulquin *et al.* 2004). There are even indications from *in vitro* studies with isolated enterocytes of dairy cattle that the rate of glucose absorption from the gut may be adjusted to match glucose requirements at the tissue metabolism level, especially during early lactation (Okine *et al.* 1994, 1995). During periods of peak lactation when glucose is in high demand for milk synthesis, it is highly likely that the passive transfer of glucose from the blood to the mammary tissue (Delaquis *et al.* 1993) may be complemented by the active sodium-dependent glucose transportation (**SGLT1**) system (Zhao *et al.* 1999).

Glucose is also involved *albeit* indirectly, in the synthesis of fatty acids conserved as fat (Yang and Baldwin 1973). For this to be accomplished, glucose is oxidized through the pentose-phosphate pathway to provide the hydrogen required for the production of reduced nicotinamide adenine dinucleotide phosphate (NADPH), the reducing equivalent that is utilised in the stepwise elongation of acetate to long chain fatty acids (LCFA) (Bickerstaffe *et al.* 1974). Some glucose is also required for the formation of glycerol for the esterification of LCFA to form mono-, di-

and tri-acylglycerides that are then stored in the adipose tissue as fat (Baldwin *et al.* 1973). Glucose is also used as a carbon source to support synthesis of ribose and de-oxyribose moieties that are required in the formation nucleotides (RNA and DNA) for cell multiplication (Zielke *et al.* 1984). The extensive degeneration and death of cells that normally accompanies prolonged shortage of glucose supply in the body is caused not so much by lack of glucose as a source of energy, but by inadequate supply of glucose carbon required for the anabolic reactions in the synthesis of nucleotides for cell multiplication (Zielke *et al.* 1984). When glucose supply is constrained, metabolic processes tend to mobilize whatever glucose source is available for cell multiplication, while resorting to the use of amino acids (glutamine), fatty acids and ketones as energy source (Zielke *et al.* 1984). There are reports that under *in vitro* conditions, isolated enterocytes from dairy cattle can metabolise glutamine via glutaminolysis to supply them with energy, and in the process producing CO_2 and various nitrogenous metabolites such as ammonia, alanine, aspartate and glutamate (Okine *et al.* 1995).

The glucose requirement in a ruminant animal is high and there are suggestions that the whole-body glucose turnover is almost similar to that of simple-stomached animals (Ballard *et al.* 1969). In ruminants, glucose plays a more critical role as a carbon source than as an energy source (Zielke *et al.* 1984). Whereas monogastric animals rely mainly on glucose for their energy, ruminants absorb most their energy as VFA, which can be utilised by most body tissues (Bergman 1973; Van Soest 1982; Preston and Leng 1987).

2.7.2. Sources of glucose in ruminants

A typical ruminant diet consists mainly of roughage that often contains only relatively small quantities of starch. Moreover, fermentative digestion in the rumen ensures that most of the dietary sugars and starch are fermented to VFA (Bergman 1973; Bassett 1975; Brockman 1993; Lescale-Matys *et al.* 1993). Ruminants therefore, rely mostly on gluconeogenesis to meet their requirements for this vital substrate (Bergman *et al.* 1970; Bergman 1973). There are, however, exceptions, such as the case in North America where lactating dairy cows and feedlot beef cattle receive basal diets that are high in cereal grains (starch) in the bid to stimulate high lactation performance or finish the cattle quickly for the premium meat market (Zhao *et al.* 1998). Under those feeding regime as much as 30-50% of dietary starch may escape fermentation in the rumen, with significant amounts entering the small intestines (McCarthy *et al.* 1989; Hill *et al.* 1991).

2.7.3. Gluconeogenesis

Most of the gluconeogenesis in a ruminant takes place in the liver. The liver usually accounts for 85-90% of all glucose synthesized in the body, while the kidneys account for only 9% of total body glucose production (Bergman *et al.* 1970; Bergman 1973; Bergman *et al.* 1974). Although the kidney plays a relatively minor role in gluconeogenesis (Kaufman and Bergman 1971), the situation can change during fasting/starvation or early lactation when its role as an alternative organ for glucose synthesis can increase dramatically to account for up to 40% of glucose synthesis (Bergman *et al.* 1968).

The gut may contribute as much as 30% of whole-body glucose turnover, especially in animals ingesting high concentrate diets (van der Walt *et al.* 1983). Most of this glucose comes from the dietary starch that escapes ruminal fermentation rather than from actual glucose synthesis (Brockman, 1993). However, as ruminant basal diets are generally low in starch, the microbial storage polysaccharides reaching the small intestines are the other possible source of alimentary glucose. There are many reports of protozoa engulfing starch and therefore reducing its rate of fermentation in the rumen, thus allowing it to reach the small intestines within the confines of cells (Mendoza *et al.* 1993). Some species of bacteria are also known to accumulate reserves of α (1-4) linked glucans (Cheng *et al.* 1977; Wallace 1980), with *Selenomonas ruminantium* containing as much as 40% polysaccharides in their cellular DM (Czerkawski 1986a, pp 107).

2.7.4. Glucogenic substrates

The main glucogenic substrates include: volatile fatty acids (mainly propionate, and some branched-chain 5-carbon VFA such as valerate and isobutyrate), protein (glucogenic amino acids), lactate/pyruvate and glycerol (Kaufman and Bergman 1974; Huntington and Eisemann 1988). These substrates are sourced from both exogenous and endogenous sources, with the relative contributions being influenced by dietary as well as the physiological state of the animal (Leng 1970b; Houtert 1991).

2.7.4.1. Propionate and amino acids

Propionate is the only major VFA that can be utilised for glucose synthesis in the liver (Judson and Leng 1973b; Cronje *et al.* 1991). While valerate and isobutyrate can also be utilised for glucose synthesis, their production in the rumen is normally very low. Propionate normally constitutes about 20-30% of total VFA, with higher percentage when soluble sugars and storage polysaccharides are included in the diet (Tamminga 1982b; Czerkawski 1986a, pp 107). The role of propionate in gluconeogenesis is emphasized by the fact that 40-55% (Huntington and Eisemann 1988) or 50-60% (Leng *et al.* 1967; Judson *et al.* 1968) of all glucose synthesized in the body is derived from this substrate. Cridland (1983) has even indicated that the contribution from propionate may be more than 90%.

Propionate and amino acids together may account for as much as 80% of net glucose production (Kelly *et al.* 1993). While some essential amino acids such as threonine are glucogenic, their effective contribution to gluconeogenesis is considered to be low (Egan *et al.* 1983). It is mainly the non-essential amino acids such as glycine, glutamate, alanine and aspartate that play a major role (Black *et al.* 1968; Bergman 1983). Alanine and the glutamine-glutamate couplet are reported to account for about 40% of the glucogenic potential of all amino acids (Wolff and Bergman 1972b; Brockman *et al.* 1975). Glutamine can be a significant source of energy to the actively lactating cow in early lactation, especially when glucose is in short supply (Okine *et al.* 1995).

2.7.4.2. Lactate

Lactate is also a major glucogenic substrate (Brockman 1993). The lactate available in the body circulation which can be utilised for gluconeogenesis may originate by direct absorption from the gut or from various metabolic processes in the body. Lactate may accumulate in the rumen of animals fed high-cereal grain diets pass into the portal blood and contribute to the lactate pool in the body (Weigand *et al.* 1972a).

Some propionate is metabolised to lactate within the rumen epithelial tissue during the absorption process (Leng *et al.* 1967; Bergman and Wolff 1971). About 50-70% of the ruminal propionate converted to glucose in fed sheep normally passes through the lactate pool (Leng *et al.* 1967). It is also possible that some of the lactate in the blood plasma may have originated from amino acid

metabolism, as there are instances when the tissue lactate is higher than can be accounted for by the available glucose-carbon (Fodge and Rubin 1975; Zielke *et al.* 1976). Though glutamine is mainly oxidized to CO_2, it is estimated that about 13% is oxidized to lactate (Zielke *et al.* 1976; Reitzer *et al.* 1979). Also when glucose is metabolised in adipose tissue to provide glycerol for esterification of fatty acids, any glucose in excess is exported from the adipocytes as lactate (Yang and Baldwin 1973).

The largest source of endogenous lactate, however, is the metabolism of glucose through glycolysis under anaerobic conditions in active skeletal muscle.

2.7.4.3. Glycerol

Most of glucose synthesis from glycerol takes place in the kidneys rather than in the liver, and only about 5% of glucose carbon is thought to originate from glycerol in fed sheep. However, the percentage can increase significantly to 20-30% during fasting and even further to 40% in pregnant ketotic ewes when fatty acids from the body adipose tissue are undergoing hydrolysis to supply energy (Bergman *et al.* 1968; Bergman 1973). Besides fasting, late pregnancy and early lactation are the two other most important physiological states when glycerol plays a more significant role in gluconeogenesis (Wilson *et al.* 1983).

2.7.5. Efficiency of propionate-derived versus intestinally-absorbed glucose

It has been argued that absorbed glucose from the carbohydrate digested in the small intestines is used more efficiently than glucose synthesized from propionate derived from the rumen fermentation of glucose (Lindsay 1970). The two pathways for glucose delivery to the body tissues are shown in **Figure 2.5**. Owens *et al.* (1986) reported that starch digested in the small intestines was used 42% more efficiently than when fermented in the rumen. Similarly, Dixon and Stockdale (1999) noted that digestion of cereal grain starch in the rumen through fermentation reduced the energy value by as much as 30-50%, although some of this energy could be recovered through post ruminal digestion of the microbial biomass and the oxidation of the absorbed amino acids. Leng (1982c) also indicated that digestion of carbohydrate post-ruminally yields 11-30% more energy than when fermented in the rumen.

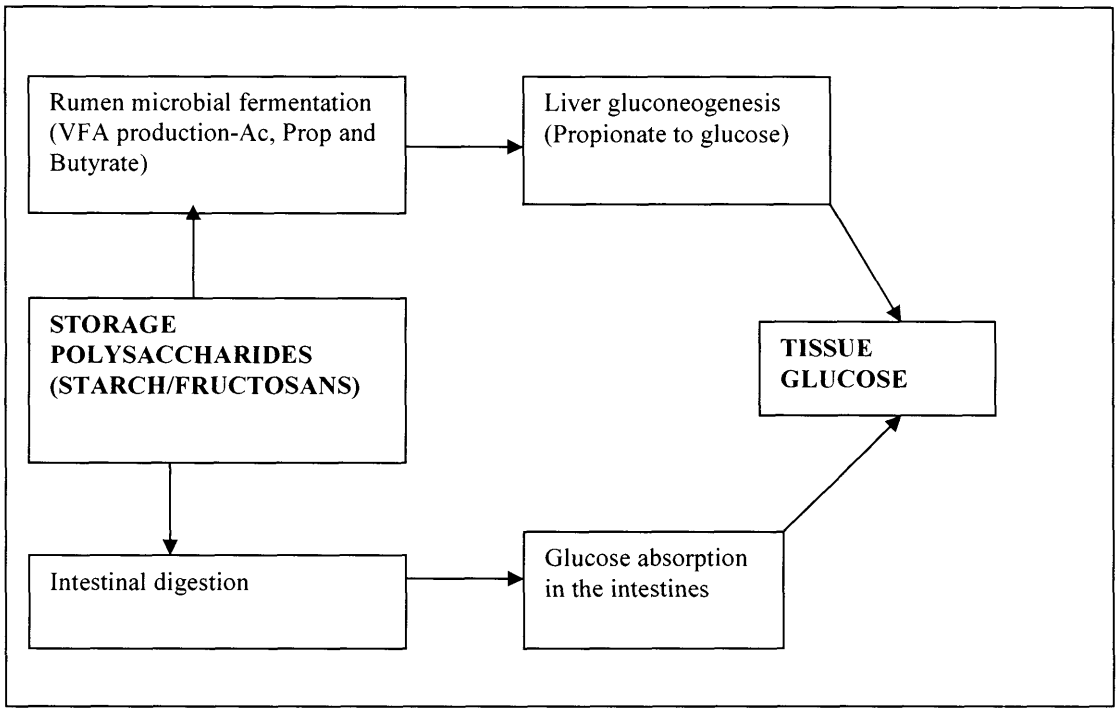


Figure 2.5. Glucose production and supply to body tissues in ruminants.

More recently, Harmon and McLeod (2001) also pointed out that fermentation of starch in the rumen and hindgut was 25-30% less energetically efficient than starch digestion in the intestines. Besides the energetic losses associated with fermentation, gluconeogenesis in the liver generally involves substantial energy expenditure (Leng 1982c). However, the energetic efficiency of intestinal digestion of starch and soluble sugars over fermentation can only be realized when other nutrients, especially essential amino acids are not limiting (Leng 1982c). Furthermore, the animal should also be able to efficiently digest and absorb all dietary starch reaching the small intestines (Harmon and McLeod 2001). The perceived benefit of supplying starchy concentrates in the diet must also be weighed against possible depression of fibre digestion by the carbohydrate supplement in the rumen.

This thesis reports on studies of the effect of intraruminal or abomasal supplementation of carbohydrate energy (sucrose) on rumen and tissue metabolism in sheep fed urea-treated low quality basal roughage.

2.8. Metabolism of acetogenic volatile fatty acids

2.8.1. Acetate metabolism: implications for intake of low quality roughage

Owing to the fermentative nature of the ruminant digestive system, 50-80% of DE intake is absorbed as VFA (Bergman *et al.* 1965; Ballard *et al.* 1969). For concentrate and roughage diets, acetate accounts for over half of the absorbed VFA (Tyrell *et al.* 1979; Pethick *et al.* 1981; Brockman 1993). With roughage diets, the molar proportion of acetate in rumen contents can be 60-70% or more (Ørskov and MacLeod 1990; Beever 1993, France and Siddons 1993; Dijstra 1994). There is also considerable inter-conversion of acetate to butyrate and vice versa mainly by rumen bacteria. This interconversion is highly in favour of acetate (Gray *et al.* 1952; Ramsey and Davis 1965).

Most of the acetate produced in the rumen during microbial fermentation appears to be readily absorbed and transported across the epithelium into the portal blood. The absorption occurs mainly through facilitated diffusion and both rumen pH and gut epithelial tissue metabolism appears to modify absorption rate (Stevens 1970). However, when the molar proportion of acetate in the rumen is high, only about 67-75% is effectively absorbed across the rumen wall, while the rest flows to the lower parts of the digestive tract (Peters *et al.* 1992). While a relatively small proportion of the absorbed acetate may be utilised by the gut epithelial tissue either as energy source or metabolised to ketones, the largest proportion is transferred to the portal blood and to the plasma acetate pool (Stevens 1970; Bergman and Wolff 1971; Bergman 1975). The whole-body production of acetate in sheep on a maintenance diet is about 120-150 mmol/h (Annison *et al.* 1967a; Bergman and Wolff 1971). About 25% of this turnover is derived from acetate absorbed from the portal drained viscera (Bergman and Wolff 1971). A small but significant quantity of acetate in the blood is from endogenous sources, especially from *de novo* synthesis in the liver (Annison and White 1962). Although the liver is known to produce about 20% of the acetate pool, it also utilises almost a similar amount with the net production being only about 5% of total acetate flux (Brockman 1993).

Acetate is widely distributed to most body tissues where it is utilised both as an energy source, and as a carbon substrate to support various anabolic processes. Unlike propionate that is glucogenic, acetate is a non-glucogenic substrate and any acetate in excess of that required for immediate ATP requirements has to be either converted to long chain fatty acid (LCFA) and

stored as fat in the adipose tissues or alternatively be wastefully oxidized to heat through 'futile cycles'. However, the synthesis of LCFA from the excess acetate requires a source of reducing equivalents mainly in the form of NADPH and is mostly obtained from oxidation of glucose through the pentose-phosphate pathway.

2.8.2. Oxidation of acetate for ATP production

Acetate is a major energy-yielding substrate in ruminants and readily undergoes oxidative metabolism in the body to provide ATP to fuel maintenance and production functions (Brockman 1993). About 26% of the respiratory CO_2 in ruminants is derived from acetate oxidation (Pethick *et al.* 1981) and this can increase to 40% if all the available acetate in the body is oxidized (Brockman 1993). Thus only about two-thirds of acetate is oxidized, with the remaining one-third being diverted to lipogenesis (Ballard *et al.* 1969), although the partitioning is likely to depend on the physiological state of the animal.

When completely oxidized to CO_2 and H_2O in the TCA cycle, one mole of acetate yields a total of 18 moles of ATP (Preston and Leng 1987, p. 49). However, the metabolism of acetate through oxidative phosphorylation is only feasible when there is a high demand for ATP energy in the body. The oxidation of acetate in the TCA cycle is also dependent on a continuous supply and regeneration of oxaloacetate (OAA), a 4-carbon intermediate product, produced from glucose or its precursors (Ørskov and MacLeod 1990). Sufficient quantities of propionate or glucogenic amino acids are necessary to guarantee a steady supply of OAA for an efficient oxidation of acetate. Any acetate not required for ATP and not conserved as fat, must be disposed of otherwise its continued presence in the general circulation will ultimately depress voluntary food intake. This can occur through the accumulation of ketones formed from the circulating acetate and/ or through the heat generated when the excess acetate is oxidized via the 'futile cycles'.

2.8.3. Substrate cycle oxidation of acetate

Compared to concentrates diets, roughage diets often generate higher heat production (heat increment) mainly because of the high energetic cost associated with their digestion (see Von Mering and Zuntz 1877; Kellner 1905, cited in Bull *et al.* 1970). According to the proponents of this "work of digestion" hypothesis, the process of mastication of roughages and rumination, gut

motility (peristalsis), and absorption involved "more work", and therefore higher energy demand, that resulted in high heat production in gut tissues.

The pioneering work of McClymont (1952), and later by Armstrong and Blaxter (1956, 1957a, b, 1961) and Armstrong *et al.* (1957a, b, 1961) provided the first evidence that the high heat production in ruminants fed low quality roughage diets rather than concentrates, was attributable not just to the "more work" being done during digestion, but also due to the inefficient metabolism of the fermentation products (mainly acetate) arising from the fermentation of roughage in the rumen (Bull *et al.* 1970). Later the terms "futile" or "substrate cycle" were coined to describe this "wasteful oxidation" of acetate (Blaxter 1962). It was viewed as the body's attempt to dispose of the surplus acetate when glucose or glucogenic precursors were inadequate to facilitate acetate conversion to LCFA (MacRae and Lobley 1982; MacRae *et al.* 1985).

While the exact mechanism by which acetate is oxidized to CO_2, and H_2O and heat produced is still not well understood, several 'cycles' have been proposed. One possibility is the conversion of acetate to acetyl-CoA through an ATP consuming activation process, and then Acetyl-CoA to acetate again, in the process generating some ATP that is dissipated as heat (Berg *et al.* 2002). According to this theory, the amplification of the forward reaction that involves acetylation could generate even more heat energy from the oxidation of ATP (Berg *et al.* 2002). Sheep fed roughage (high acetate output) tend to have in their adipose tissue a higher activity of enzymes similar to those involved in putative futile cycle, when compared to those fed concentrates (low acetate output) (Scollan *et al.* 1988). Similarly, the hepatocytes of sheep fed roughage diets were reported to have a high substrate cycle activity (Jessop *et al.* 1990), perhaps due to a need to dispose of surplus acetate as heat.

The substrate cycle theory has, however, been challenged by some workers who have argued that it cannot be entirely responsible for all the heat produced from acetate metabolism. Crabtree *et al.* (1987) reported that only about 0.5% of total heat production in the metabolism of the hind limb of a sheep could be attributed to substrate cycles, and that in the mitochondria and cytoplasm of liver cells of a rat substrate cycle accounted for only 1% of total heat production (Crabtree *et al.* 1990). The results from these two studies, though not entirely conclusive, appear to suggest that the role of substrate cycle in heat production from acetate may have been

overestimated, thereby de-emphasising other possible sources of metabolic heat. An imbalance of absorbed nutrients has also been suggested as a possible avenue for higher heat production. Excessive acetate availability in the body tissues relative to glucogenic substrates could result in changes in cell membrane permeability to Na^+ and K^+ ions which results in elevation of ATPase activity in cells to restore the ionic balance, with the extra ATP generated from acetate metabolism being dissipated as heat (Jessop *et al.* 1991; Jessop and Leng 1993).

Oxidation of acetate through substrate cycles and the associated heat production may be quite useful for ruminants in cold climates (Preston and Leng 1987; Leng 1990). However, for animals in a warm climate where ambient temperatures are normally higher than the comfort zone, the disposal of acetate via futile cycles would overburden the animal with heat that would be difficult to dissipate (Preston and Leng 1987). To minimize the risk of hyperthermia, animals in warm climates tend to respond by reducing feed intake (Young 1987; Leng 1990; McDonald *et al.* 2002), which has a negative effect on productivity. This is one possible reason for the low feed intake in ruminants fed low quality roughages in tropical environments (Preston and Leng 1987). These arguments also help to resolve a paradox whereby ruminants in cold and hot climates fed similar low quality diets tend to respond differently in intake to protein supplementation. Those in the tropics mostly show a positive response, whereas those in temperate climates show a lesser response or no response (Leng 1990). Finally, there are reports that the high heat production and the associated energetic inefficiency in the utilisation of acetate in ruminants may be part of a short-term adaptation to diets that are high in acetogenic substrates relative to glucogenic precursors, and that in the longterm, differences in heat production is not significant (Elliot *et al.* 1965; Bull *et al.* 1970).

2.8.4. Utilisation of acetate in lipogenesis

Ruminants have evolved over time a complex metabolic process that enables them to conserve as fat any acetogenic substrate in excess of that required for immediate energy needs. The conservation of surplus acetate in the form of fat seems to make sense in an environment characterized by seasonal fluctuations in feed supply and it may be in the interest of the species' long-term survival (Van Soest 1982). In ruminants, acetate is a major substrate that is used in LCFA synthesis both in the adipose tissue and also in the udder, whereas in monogastrics, glucose is the major substrate (Hanson and Ballard 1967; Ballard *et al.* 1969). These LCFA in

tissues and milk consist of stearic, palmitic and oleic acids, and may constitute up to 85% of the plasma non-esterified fatty acids in the general circulation (Pethick *et al.* 1987; Pethick and Dunshea 1993). There are also reports that acetate is used widely by the mammary tissue in the synthesis of milk fat and that fatty acids with up to 16-C atoms can be synthesized in the udder (Popjack *et al.* 1951a, Annison *et al.* 1967b). About 20-40% of all milk fat is synthesized *de novo* from acetate in mammary tissue and consists mainly of short-chain fatty acids. The remainder (60-80%) of the milk fat mainly comprises of LCFA from dietary sources and/or from the adipose tissues (Annison *et al.* 1967b, 1968).

Lipogenesis is a complex process that involves stepwise elongation of acetate to LCFA, and for the process to proceed as proposed by Wakil (1961), there has to be an obligatory participation of NADPH (Armstrong and Blaxter 1961). The biosynthesis of palmitate (C_{16}) from acetate is summarized by the stoichiometric equation below -:

$$8\text{AcetylCoA} + 7\text{ATP} + 14\text{NADPH} \rightarrow \text{Palmitate} + 7\text{ADP} + 7P_i + 14\text{NADP}^+ + 8\text{CoA-SH}$$

The NADPH is produced mainly from oxidation of glucose through the pentose-phosphate pathway (Armstrong 1965; Berg *et al.* 2002). This oxidation of glucose occurs in the cytosol and is catalysed by two key enzymes, namely glucose-6-phosphate dehydrogenase and 6-phospogluconate dehydrogenase, whose existence in ruminant liver was confirmed by Hanson and Ballard (1967). The complete oxidation of glucose via this pathway can be summarized by the stoichiometric equation below-:

$$\text{Glucose-6-phosphate} + 12\mathbf{NADP^+} + H_2O \longrightarrow \mathbf{12NADPH} + 12H^+ + CO_2$$

As discussed earlier the glucose for this reaction is mostly sourced from glucogenic precursors through gluconeogenesis. Some glucose is also required for the production of glycerol that is utilised in the esterification reaction of LCFA to form mono-, di- and tri-acylglycerides (Yang and Baldwin 1973). The adipose tissue (tail fat pad) of a sheep extracted 10% of the glucose presented to it, and most of this glucose was used as a source of NADPH for fatty acid synthesis and glycerol for the esterification processes (Khachadurian *et al.* 1966).

2.8.5. Acetate as an alternative source of NADPH

Although the oxidation of glucose through the pentose-phosphate pathway is the main source of NADPH (Hanson and Ballard 1967), some tissues such as mammary gland apparently synthesized more fatty acids than could be accounted for by the available glucose or precursors. This leads to the suggestion that an alternative mechanism for sourcing NADPH could exist in some tissues. Acetate was identified as the most probable substrate, generating NADPH when it was oxidized through the isocitrate dehydrogenase pathway (Bauman *et al.* 1970; Ingle *et al.* 1972a). However, this pathway also generates α-ketoglutarate that has to be disposed of by oxidation in the TCA cycle, which generates ATP, or used to synthesize amino acids (Bauman and Davis 1975). It was therefore, postulated that NADPH generated via oxidation of isocitrate would be feasible in those tissues with a simultaneous requirement for both NADPH and ATP, such as the mammary gland (Bauman *et al.* 1970) where the NADPH could be utilised in the synthesis of milk fatty acids, while the ATP generated was used to fuel the synthesis of both the fatty acids and other milk components such as protein/amino acids.

It would be energetically efficient for adipose tissue to rely on glucose as the main source of NADPH (Bauman and Davis 1975), since the relatively low ATP generated by the pentose-phosphate pathway would be adequate to fuel fatty acid synthesis (Hovel *et al.* 1976). According to estimates made by Bauman (1976), the oxidation of acetate through the isocitrate dehydrogenase pathway may provide about 25% of the required NADPH in the adipose tissue, with the pentose-phosphate pathway contributing 75%. However, in mammary tissue the contribution from the two pathways in the supply of NADPH was estimated to be 50:50 (Bauman and Davis 1975). Therefore, the relative role of glucose and acetate as potential sources of NADPH for lipogenesis in different tissues may be dependent on the magnitude of their ATP requirements.

For efficient fatty acid synthesis in ruminants to occur, a simultaneous supply of both acetate and glucose is necessary (Tyrell *et al.* 1979; Preston and Leng 1987; Cronje *et al.* 1991). Because most of the glucose in ruminants has to come from gluconeogenesis, with propionate as the main precursor, it has been estimated that 1 mole of propionate would be required for every 4.1 moles of acetate when the pentose-phosphate pathway is the only source of NADPH (Ørskov *et al.* 1979). For an animal without any other immediate use for the ATP, acetate cannot be used as an

alternative to glucose and its ability to dispose of acetate will be constrained by shortage of glucose leading to an inefficient utilisation of the basal roughage (Black *et al.* 1984). When an animal is forced by circumstances to rely almost entirely on acetate as the source of reducing equivalents, then the resultant ATP has to be wastefully oxidized to heat, leading to the high heat increment reported by Armstrong and Blaxter 1957a; Blaxter 1962; Hovel *et al.* 1976). It follows that when an animal fed a roughage diets that promotes high acetate: propionate ratio is supplemented with a glucose source (or its precursors), the supply of NADPH is potentially increased and so the conversion of acetate to lipid is also enhanced (Bull *et al.* 1970). At the same time, the need to dispose of such acetate via futile cycles is reduced with the result that acetate is more efficiently utilised (MacRae and Lobley 1982; Black *et al.* 1984). For ruminants in the tropics faced with roughage diets that are high in CWC, and also contending with high ambient temperatures, additional glucose sources can increase intake and utilize the available feed resources more efficiently (Preston and Leng 1987; Leng 1990).

2.8.6. Role of protein in acetate metabolism: implications on roughage intake

Protein is a multi-use nutrient that plays a major role in many metabolic processes both in the rumen and in the tissues. When ruminants subsisting on low quality roughage are supplemented with a source of UDP, there is often a higher intake of basal roughage, a lower heat increment and a higher efficiency in feed utilisation (Perdock 1987; Silva *et al.* 1989). While some of the benefits of the protein supplementation may be mediated through favourable changes in the rumen fermentation environment (Ortigues *et al.* 1990), there are also strong indications that changes at the tissue level, especially in P/E ratio are also important (**2.6.2.3 & 2.6.2.4**).

Protein supplementation is reported to enhance acetate utilization in the body, which in turn increases voluntary intake of the basal roughage (Leng 1990). However, the mechanism by which enhanced acetate use is achieved has been a subject of speculation and intense research, with suggestions that the mechanism could be enhanced conversion of acetate to LCFA. Enhanced microbial protein synthesis in the rumen and by-pass protein supplementation lead to a higher rate of absorption of amino acids from intestines and some of the amino acids are available to supply glucose that can in turn provide the NADPH required to reduce acetate to LCFA.

Protein supplementation may also stimulate higher energy expenditure by the body through catabolism of excess amino acids. Whenever the protein supplementation results in an amino acid supply to the body tissues that is above immediate body requirements, the excess amino acids have to be deaminated. The resultant carbon skeleton is utilised for other metabolic functions. This leads to an increase in protein turnover in the body, a process that is associated with higher energy expenditure (Reeds *et al.* 1982; Lobley *et al.* 1987; Harris *et al.* 1992). The extra demand in ATP to support protein degradation and synthesis is met from the oxidation of acetate through the isocitrate dehydrogenase pathway, leaving less acetate for disposal through futile cycles. This may partly explain the apparent reduction in heat production in ruminants fed low quality roughages when supplemented with by-pass protein (Preston and Leng 1987; Leng 1990). It also provides the basis of the rationale for recommending by-pass protein supplementation to high producing animals experiencing heat stress such as fast-growing lambs (Bunting *et al.* 1992), finishing cattle (White *et al.* 1992), and lactating dairy cows (Higginbotham *et al.* 1989; Huber *et al.* 1994).

2.8.7. Acetate clearance rate as an index of glucogenic potential

In animals fed on roughage diets, the production of acetogenic substrates tends to be in excess of that required by the body to provide ATP for maintenance. The surplus acetate has to be disposed of in the most efficient way, while avoiding disruption in the physiological and metabolic processes in the body. The conversion of acetate to fat through the synthesis of LCFA fulfills this requirement. Availability of glucose (or its precursors) increases conservation of excess acetate as fat, thereby increasing acetate clearance rate (Cronje *et al.* 1991).

The earliest studies of acetate clearance in ruminants were reported in the 1950s and 60s with most of them attributing high acetate clearance rates to the general quality of feed, rather than any specific substrate or metabolite (Jarrett *et al.* 1952; Pugh and Scarisbrick 1952; Reid 1958; Jarrett and Filsell 1960). Egan (1965b) was the first to suggest that acetate clearance reflected the rate at which acetate was removed from circulation by oxidation or utilised for the synthesis of fatty acids, and which in turn was influenced by the availability of glucose (or its precursors), and the N status of the animal. Later reports showed there was a linear relationship (response) between

acetate clearance rate and feed intake, which was explained in terms of an increased availability of digestible nutrients (Weston 1966).

The first to study the effect of specific substrates on acetate clearance rate in ruminants fed low quality roughage was Cronje (1987). His results established that when sodium propionate was used to replace acetate in increasing proportions, both glucose entry rate (GER) and acetate clearance rate were increased in the same proportion (Cronje 1987; Cronje *et al.* 1991). A positive response in basal roughage intake to by-pass protein supplementation was also noted (Cronje 1987). It was therefore concluded that acetate clearance rate could be used reliably as an index of glucogenic potential in ruminants (Cronje *et al.* 1991).

2.9. Summary and research approach

The major constraint to improved efficiency of utilisation of low quality roughage by ruminants is the low microbial growth and fermentation activity in the rumen. This is further exacerbated by the low rate of physical breakdown of the roughage in the rumen caused by low microbial numbers and high CWC. This leads to a slow rate of passage through the gastro-intestinal tract and hence low voluntary intake. The net effect of the low rate and extent of digestion in the rumen and low voluntary intake is inadequate energy supply to the microbes, and by extension the host animal. Besides the poor release of the VFA energy to the host animal, low microbial growth in the rumen also leads to inadequate supply of microbial protein for digestion in the small intestine. Low microbial protein supply can lead to an imbalance in P/E ratio in substrate supply to the body tissues. Moreover, the fermentation pattern of roughages is characterized by low propionate: acetate ratio leading to absorption of fermentation products that are also unbalanced in glucogenic: acetogenic substrates. The combined result is an insufficient metabolism of VFA in the body tissues, manifested as a high heat increment, with further negative feedback on voluntary intake and ultimately low animal productivity.

Any attempt to improve on the efficiency of utilisation of low quality roughage in ruminants must therefore address the fundamental factors hindering optimal voluntary intake of roughages. The main areas that may require intervention include-:

1. Improving the microbial growth and fermentation rate in the rumen by correcting the nutritional deficiencies associated with high-fibre, low protein forages, and especially of especially of N and S or other key nutrients
2. Physically and/or chemically treating the roughage in order to increase the susceptibly of the refractory plant fibre material to microbial degradation in the rumen; this will increase the provision of ruminally fermentable substrates , enhancing microbial growth rate and the supplies of VFAs and microbial materials for the hoost tissues
3. Supplementing the animals with a source of by-pass protein to correct the P/E imbalance at the tissue level.
4. Supplementing the animal with some readily fermentable carbohydrate (RFC) source to enhance the energy supply to the rumen microbes, or to the host animal; at the same time ensuring that there is sufficient ammonia and minerals in the rumen at all times to prevent deficiencies that could impair fibre digestion in the rumen, and also that there are sufficient supplies of amino acids available to the tissues to enable the supplementary carbohydrate to be efficiently utilised.

Broad objective

The broad objective of the present study was to investigate the effects of N and energy supplementation as ways of maximizing ammonia and fermentable energy in the rumen and supplying substrates to correct the imbalanced supplies of microbial end-products for the tissues that reduce the efficiency of acetate utilization by ruminants on low quality roughage.

A feeding trial was conducted where animals were offered low digestibility basal roughage considered to be limiting in rumen degradable nutrients, especially N. The basal diet was supplemented with N, energy or both, and the response in intake determined. Then a more detailed trial was undertaken in which animals fed urea-treated low quality roughage as the basal diet were supplemented with carbohydrate source (sucrose) via ruminal or abomasal routes. This trial was undertaken to test the hypothesis that there would be no difference in the levels of intake, digestibility, and performance of various rumen and tissue metabolism parameters when the supplements were administered.

Specific objectives

1. To investigate the effect of urea-N or protein supplementation on the voluntary intake in ruminants (sheep) fed low digestibility basal roughage.
2. To investigate the effect of ruminal or abomasal routes of supplementation of sucrose (a readily fermentable CHO source) on rumen metabolism in animals fed urea-treated low quality roughage (parameters monitored included: voluntary intake, *in vivo* digestibility, *in sacco* degradation, molar quantity and proportions of VFA production, changes in pH, protozoa count, rumen dilution rates.
3. To determine the efficiency of microbial protein production in the rumen, and N balance in animals fed urea-treated low digestibility roughage without or with sucrose supplementation via ruminal or abomasal routes.
4. To study the effect of intraruminal and abomasal supplementation of sucrose on tissue metabolism of acetate and specifically the glucogenic potential in body tissues. An acetate clearance test was used an index of the glucogenic potential.

Hypothesis

The study was undertaken and designed to test the following hypotheses-:

1. That there is no difference in the basal roughage intake in animals fed low quality roughage supplemented with NPN or protein N, with and without additional ME.
2. That, in animals fed urea-treated low quality roughage and supplemented with RFC energy source through the ruminal (fermentative) or abomasal (intestinally digested) routes, there is no difference in major ruminal and tissue metabolism measures-:
    i. Intake and digestibility of dietary and basal DM/OM,
    ii. Rumen metabolism as described by characteristics such as VFA (molar and proportion) production, rumen NH_3, pH, protozoa counts and rumen dilution rates,
    iii. Glucogenic potential indexed on propionate: acetate ratio & acetate clearance rate,
    iv. N balance and microbial protein yield as determined by urinary excretion of PD.

3. CHAPTER THREE - EXPERIMENT 1: THE EFFECT OF NITROGEN, PROTEIN AND ENERGY SUPPLEMENTATION ON VOLUNTARY INTAKE IN SHEEP FED LOW QUALITY ROUGHAGE

3.1. Introduction

Crop residues and dry over-mature pasture form an important feed resource for ruminants in most parts of the world, especially in Africa and Asia (Preston & Leng 1987; Sundstol *et al.* 1993). In spite of their enormous potential as a feed resource, hays and straws generally are poorly utilised by ruminants. This is mainly due to their low rate and extent of digestion in the rumen. This leads to low rate of digesta clearance from the gut and therefore low intake of OM and of digestible nutrients. As a result, the animal is unable to ingest enough ME and nutrients to meet its maintenance and production requirements and so there is mobilisation of fat and other body reserves leading to loss of body condition and body weight. As the fat depots are depleted, there is increased mobilisation of tissue protein and serious deterioration of body condition which impairs the animal's reproduction and production capacity. This is a frequent occurrence in cases of prolonged dry periods in most tropical and sub-tropical areas.

It has been reported that the fundamental reason for the low utilisation of low quality roughages in ruminants is their low rate and extent of digestion in the gut and this is associated with low microbial growth and fermentation activity in the rumen (Ferrell *et al.* 1999). The low microbial growth and fermentation activity in the rumen is due to many factors, the major ones being: the high content of lignified fibre and nutrient deficiency inherent to low quality roughage, especially the low content of protein-N and minerals such as S. Supplementation of the low quality roughage with moderate levels of a non-protein N or protein source has been known to stimulate higher digestibility and therefore improved feed intake, mainly through enhanced microbial growth and fermentation activity in the rumen (Coombe 1985; Silva *et al.* 1989; Ferrell *et al.* 1999; Fonseca *et al.* 2001). Furthermore, rumen microbes do have a capacity to digest cellulose and more refractory structural polysaccharides such as hemicellulose and pectin provided that nutrient deficiencies that inhibit their growth in the rumen are alleviated. In some studies, energy concentrates have been included as well, even though they may depress cellulose digestion and/or

intake of basal roughage, especially when they are included at high proportion in the basal roughage diets. The value of such energy supplements is therefore questionable when they are given with low quality roughages where protein-N sources are the main supplements and N is the main limiting factor constraining growth of rumen microbes.

The objective of this study was therefore to establish whether supplements providing additional energy affect DM intake when low quality basal roughages are supplemented with NPN or protein supplements.

3.2. Materials and methods

Animals and the diet

Thirty (30) Border Leicester x Merino crossbred wether lambs weighing about 25± 2.5 kg (SD) were brought into the animal house one week before the commencement of the trial and kept in individual pens on a slatted floor. They were fed basal roughage consisting of chaffed oaten straw (11.8 g/kgDM or 7% CP) for one week to background them. This was done to enable the animals to become accustomed to the animal house conditions and the low quality roughage. Any animal that was not eating the roughage well during the first week was replaced. After one-week the animals were weighed and allocated to the five (5) treatment groups in a stratified randomized procedure using liveweight as the blocking factor. This was done in a way that ensured that each treatment group had six (6) animals with at least one animal from each weight category, so that the initial mean weight of the animals in the 5 treatment groups did not differ significantly. The animals were weighed again at the start and end of the 5-day measurement period. The mean weight was used in the calculation of intake based on metabolic body weight ($g/kgW^{0.75}$).

The treatments were made in such a way as to provide the animal with basal roughage alone or basal roughage supplemented with NPN, protein, NPN and energy or protein and energy.
The treatments were as follows: -

T1- Basal diet (control)

T2- Basal + Urea

T3- Basal + CSM

T4- Basal + Urea + Bran/Molasses

T5- Basal + CSM + Bran/Molasses

Urea and cottonseed meal (CSM) were used as the sources of NPN and protein respectively, and wheat bran and molasses (3:1 DM basis) were used as the energy sources.

Feeding management

After the one-week adaptation period, the animals were offered their respective supplements in addition to the basal roughage during a further two-week adaptation period followed by a 5-d measurement period. The supplement was given first, after which the animals were offered their roughage allocation. However, some modification in the mode of supplement offer was necessary where urea (and molasses) were involved, as these could not be fed separately without risking the welfare of the animal (*see below*). To ensure that the intake of basal roughage was not restricted, the roughage was offered at about 120% of the previous three days' mean daily intake if the animals cleared the previous day's ration. The composition of the treatments is given in **Table 3.1**.

Table 3.1 Composition of the treatments used in the feeding trial.

	Treatments				
Ingredients	**T1**	**T2**	**T3**	**T4**	**T5**
Oaten straw (*ad lib*)	+	+	+	+	+
Urea (g/d)	-	15	-	12	-
Cottonseed meal (g DM/d)	-	-	102	-	81
Wheat bran (g DM/d)	-	-	-	75	75
Molasses (g DM/d)	-	-	-	25	25
Supplemental N (g/d) (Estimated)	-	7.0	7.0	7.0	7.0
Energy supplement (g DM)	-	-	-	100	100
Total supplement (g DM/d)	0	15	102	112	181

The quantity of supplements offered was calculated to provide each animal with approximately **7 gN** for each of the treatments **T2, T3, T4** and **T5.** The dietary treatments (except control) were therefore essentially iso-nitrogenous, differing only in the form in which the N supplement was offered. Where urea was used (**T2**), it was dissolved in water to make 150 ml of solution that was sprinkled on approximately one-third of the estimated daily roughage allocation. This

portion was mixed thoroughly and offered to the animals. In the treatments where both urea and molasses were involved, they were dissolved in 150 ml of water and sprinkled on the wheat bran together with about one-third of the estimated daily allocation of basal roughage, mixed thoroughly and then offered to the animals. In the case of protein and energy concentrates, the protein source (CSM) was fed separately, and molasses dissolved in water to make 150 ml solution that was mixed with a portion of the roughage and wheat bran that was and then offered to the animals. Only after the animals had eaten the supplements was the remaining basal roughage made available.

Animals were offered fresh supplement and roughage daily at 09.00 h. Refusals were collected every morning before the animals were offered their daily ration. The refusals from the 5-day measurement period were bulked, mixed thoroughly, and then sub-sampled for the determination of DM and other analyses. The animals were allowed unrestricted access to water during the entire period of the trial.

Chemical analyses

The DM content of the basal roughage and the refusals was determined by drying the fresh samples in a forced-draught oven at 65°C. The dried samples were then ground to pass through 1mm screen followed by a further drying at 105°C for 12 h (AOAC 1990). Total N was also determined by Kjeldahl method (AOAC 1990).

Statistical analysis

The basal and total DM intake data for each of the 6 individual animals in each of the 5 treatments were analysed by the General Linear model procedure of SAS. Where there was significance difference between the treatments, the separation of means was done by the method least significant difference (LSD) at 5% significance level.

3.3. Results

The intakes of total diet DM and basal DM are shown in **Table 3.2**. The total N intake by the animals was increased from 9.2 g/d for the basal diet to 16-18 g/d in the supplemented dietary treatments. There was no difference ($P>0.05$) in basal roughage DM intake between the five dietary treatments. However, total dietary DM intake was increased ($P<0.05$) by protein with or

without energy supplements, and also by N (urea) and energy. Overall the dietary DM intake in the five dietary treatments ranged from 3.2-3.6% BWT

Table 3.2 The mean N and DM intakes by sheep fed oaten chaff basal roughage supplemented with NPN, protein-N and energy (means over 5 d).

	Nitrogen intake (g/d)			Daily intake (g DM)		
Treatment	**Basal N**	**Supplemental N**	**Total N**	**Basal Hay**	**Total Diet**	**% BWT**
Basal roughage	9.2	-	9.2	785 (73.2)	785[a] (73.2)	3.2
Basal + Urea	9.4	6.7	16.1	799 (73.2)	814[a] (74.6)	3.3
Basal + CSM	9.5	8.3	17.8	809 (72.4)	911[b](81.5)	3.5
Basal + Urea + Bran/Molasses	8.7	7.5	16.2	741 (68.4)	853[ab] (78.8)	3.4
Basal + CSM + Bran/Molasses	8.7	8.5	17.2	735 (67.0)	916[b] (83.6)	3.6
SEM	-	-	-	37.9 (3.48) ns (P>0.05)	33.9 (3.11) * (P<0.05)	-

SEM refers to standard error of means. Figures in parentheses are on metabolic body weight basis, g. $(kgW^{0.75})^{-1}$. Different superscripts in the same column indicate that means differ significantly (P<0.05).

3.4. Discussion

Following CSM supplementation of the basal low quality roughage there was an increase in daily total dietary DM intake from 785 to 911 g/d, representing an increase of about 16%, but no detectable change in the intake of basal hay. This indicated that there was an effect of CSM (a source of bypass protein, ME and minerals) on the total DM intake which did not occur when an isonitrogenous amount of urea (a ruminally degradable NPN source) was used as a supplement for the hay. However, there was no further increase in daily dietary DM intake following supplementation with molasses (a source of ME and minerals). In contrast, N supplementation in the form of urea, a readily degradable form of N did not increase the intake of both basal and total dietary DM compared to the control diet. Furthermore, the intake was also not improved any further by the ME supplied by the molasses supplementation. The increase in total dietary DM intake following protein supplementation was due to the supplement rather than the basal roughage, i.e. there was a substitution effect. The fact that there was no response in basal

roughage intake to protein N or NPN supplementation indicates that intake of the basal roughage was not N was not constrained by insufficiency of rumen degradable N. Though the DM digestibility of the basal roughage was not determined, it was presumably at medium to high level. This may have resulted in the relatively high intake of the basal roughage at 785 g/d which is equivalent to 3.2% BWT. Preformed protein was not superior to NPN (urea) in stimulating higher basal DM intake in studies by other workers (Kropp *et al.* 1977; Peterson *et al.* 1985; Redman *et al.* 1985; Iwanyanwu *et al.* 1990). Mathison and Milligan (1971) have suggested that this is mainly because cellulolytic microbes rely solely on NH_3 as the sole source of N for growth, and NH_3 can be sourced from NPN sources such as urea. When the potentially digestible OM in the basal roughage is low or the supplement is highly digestible, then intake of the OM from the protein (or energy) may result in depression of roughage intake (Kropp *et al.* 1977; Peterson *et al.* 1985; Redman *et al.* 1985; Iwanyanwu *et al.* 1990) i.e. a substitution effect. When the protein supplement has a high proportion of rumen undegradable fraction (UDP), then that will deliver amino acids to the body tissues. A supply of amino acids in the body tissues will improve the P: E ratio and to attenuate the negative feedback from gut distension, allowing a higher intake of basal roughage DM (Egan 1977). However, such adjustments normally take time to take effect, which perhaps explains the lack of response in intake in feeding trials conducted for relatively short periods of time, as was the case in this study.

The lack of response to energy supplementation in this study confirms earlier reports that energy is not the main factor constraining intake of low quality roughage (Iwuanyanwu *et al.* 1990), especially in animals in a state of low to medium levels of production. Therefore, supplementing low quality roughage with readily fermentable carbohydrate sources may increase total dietary intake but not necessarily improve digestibility and/or intake of basal roughage. For example, when *Bos indicus* cattle given free access to low quality basal hay (3.1% CP) were supplemented with urea or urea and graded levels of molasses, there was no additional response in DM digestibility or DM intake to molasses supplementation over that obtained with urea alone (Iwuanyanwu *et al.* 1990). In steers grazing on mature sub-tropical pastures, supplementation with protein meal alone, or with energy-rich sorghum pellets and protein meal, improved DM intake and also LWT gain, while supplementation with sorghum pellets without a protein-N source resulted in loss of body weight (Hennessy *et al.* 1983). This suggests that supplementing animals on low quality basal roughage with an energy source when N is limiting only worsens the imbalance between P/E ratio leading to reduced feed intake and loss in body weight.

Forages containing less than 10 gN/kgDM (6.25% CP) are likely to supply insufficient rumen ammonia, as cellulolytic bacteria require a minimum of about 50 mg ammonia-N /L to meet their N requirements and therefore digest fibre in the rumen effectively (Satter and Slyter 1974; Hogan 1996). This suggests that N was not a critical factor limiting the intake of basal roughage in this study. It is quite possible that a combination of this moderate level of N (11.8 $g.kg^{-1}DM$ (7% CP), and probably a relatively low degree of lignification in basal roughage may have enabled the animals to achieve a relatively high rate of particle comminution and to ingest an appreciable quantity of hay. This is partly supported by the relatively high daily DM intake of the unsupplemented basal roughage at 785 g/d (73.2 g/ ($kgW^{0.75}$)) equivalent to 3.2% BWT which can be considered to be close to maximum for this type of basal diet. However, rumen microbes do still require ammonia for their growth and synthesis of protein (Bryant 1973) which is necessary to enable them degrade fibre in the rumen more effectively. Responses in forage intake to NPN or protein supplementation can be expected if the intake without supplementation is very low (Ferrell *et al.* 1999); however, if the intake is already relatively high, then a positive response is unlikely. A critical factor that is likely to influence the response to protein/N supplementation is the ratio of digestible OM to crude protein in the basal forage (DOM: CP). A positive response can be expected if the ratio is greater than 7:1 (temperate forages) or 5:1 (tropical forages) as is common with most digestibility cereal straws (Hogan 1996). For example, roughage with 50% digestibility or more would require a minimum N content of 11.4 gN/kgDM (7% CP) to support optimal digestion of OM in the rumen.

The lack of response in basal DM intake to N supplementation has been reported before. Jordon *et al.* (2002) did not obtain responses in either basal or total diet OM intake in cows grazing low quality native range pastures (10.9 gN/kgDM or 6.8% CP, 485 g/kgDM *in vitro* OM disappearance) that were supplemented with the following forms of protein/N-: urea, dried poultry waste (DPW) + urea, soybean meal (SBM), DPW + SBM or DPW. Similarly, Hollingsworth-Jenkins *et al.* (1996) and Lamb *et al.* (1997) found no response in OM intake in animals grazing poor quality winter range pastures (4.5-6.0% CP) when they were supplemented with degradable intake protein (DIP), again suggesting that the N was not limiting intake. However, Körster *et al.* (1996) reported an increase of 150% in the intake of digestible OM when animals fed low quality roughage containing very low N content (3.2 g/kgDM or 2% CP) were supplemented with DIP, but obtained only a modest increase in intake when the same supplement

was used in animals fed roughage containing 6-7% CP. It is apparent from these reports that the response in intake to protein/N supplementation depends in part on the N content of the basal roughage; the greatest intake response will be achieved in basal roughage with the lowest N content and low intake in the absence of supplements. Moreover, the potential digestibility of the range pastures may be low due to high CWC especially, when the lignin content is high.

3.5. Conclusion

It is concluded that N did not limit intake of the oaten chaff used as the basal roughage in this study, and that supplementation with protein concentrate, or protein and energy concentrate did not stimulate hay intake above that offered by the combinations of N already contained in the basal roughage and from the urea. However, by-pass protein supplementation may have a positive effect on basal roughage intake by ensuring a balance of protein: energy (P/E) ratio in the body tissues provided animals are allowed adequate adjustment period.

4. CHAPTER FOUR - EXPERIMENT 2 - THE EFFECT OF ENERGY SUPPLEMENTATION ON FEED INTAKE AND RUMEN DIGESTION IN SHEEP FED UREA-TREATED LOW-QUALITY ROUGHAGE

4.1. Introduction

Low quality roughages such as cereal straw and stover are generally high in fibre but low in N and minerals such as sulphur. As a result, they are poorly utilised by ruminants mainly due to a combination of high CWC, low microbial growth and fermentation activity in the rumen. This low microbial growth in the rumen mainly arises due to limited availability of key nutrients such as N and S. These nutrients are required by the rumen microbes to synthesise protein and enable them to digest fibre effectively to obtain energy for maintenance and growth (Preston and Leng 1987; Ferrell *et al.* 1999).

Urea or ammonia-treatment of low quality roughage has been reported to increase OM digestibility, though not as effectively as stronger alkalis such as NaOH (Cloete *et al.* 1983; Cloete and Kritzler 1984; Mgheni *et al.* 1993). In spite of this, ammoniation with urea is preferred by most ruminant livestock farmers in developing countries mainly due to its availability and convenience of use (Cloete *et al.* 1983; Dias-da–Silva and Sundstol 1986; Djajanegara and Doyle 1989; Sansoucy 1995). Supplementation of low quality roughage with moderate levels of protein or NPN stimulates higher digestibility and intake mainly due to the enhanced growth of rumen microbes, and increased fermentation activity in the rumen (Silva *et al.* 1989). Most cellulolytic bacteria that are associated with fibre digestion in the rumen have a specific requirement for ammonia (Bryant 1973) which can be obtained from most NPN sources such as urea, or from preformed protein. Microbes are able to digest the structural polysaccharides composed mainly of cellulose and hemicellulose that constitute a high proportion of OM in high-fibre, low-protein roughage.

In **Exp. 1** the supplementation of basal oaten chaff straw (11.8 gN/kgDM or 7% CP) with urea alone or urea and energy concentrates (wheat bran/molasses 3:1 DM) did not increase ($P>0.05$)

intake of either basal roughage or dietary DM. However, total dietary DM intake was increased ($P<0.05$) by protein or protein and energy supplementation. The lack of response in intake of basal roughage in response to urea supplementation was partly attributed to the adequacy of N content of the basal roughage at 11.8 g/kgDM (7% CP), and the relatively high level of fibre or cell wall constituents (CWC). When the basal roughage N content is higher than 10 gN/kg (6.3% CP), supplementation with N is unlikely to result in any significant response (Hogan 1971, 1996). This may apply to roughage that is high in refractory plant material where fermentable energy insufficiency can also limit microbial growth. However, for roughage of higher potential digestibility, the level of N required may have to be increased to match the higher supply of fermentable energy (Hogan 1996). There are also situations where the low quality roughage is very high in refractory fibre fraction (highly lignified fibre), which results in long mean retention time (MRT) in the rumen (Lechner-Doll *et al.* 1991). This is mainly because such fibre normally takes a long time to be reduced to particles that are small and dense enough to allow them to be cleared from the rumen through the reticulo-omasal orifice (Poppi *et al.* 1981a, b). Supplementation of roughage with N or fermentable energy sources is unlikely to evoke a response in either digestibility or intake unless the roughage is physically or chemically treated so as to increase its susceptibility to microbial attack in the rumen. Alkali treatment of low quality roughage using urea-ammonia may be one such process that may complement N and energy supplementation.

Energy supplementation has been reported as being variably successful in enhancing digestibility and intake of basal roughage (Ørskov 1986; Doyle 1987; Fonseca *et al.* 2001). Lee *et al.* (1987) supplemented steers fed poor-quality basal roughage (4.3 g $N.kg^{-1}DM$ or about 2.7% CP) (N x 6.25) with crushed or whole maize as an energy source and noted an increase in both digestibility and intake of roughage such that the digestible OM intake was almost doubled. However, there are indications that the responses in digestibility and intake of poor quality basal roughage to energy supplementation have been variable and appear to be influenced by a wide range of factors that include; the type, form and amount of energy supplement used and the relative proportion and quality of the basal roughage (Obara *et al.* 1991). Some workers have used readily digestible carbohydrate supplements to increase the potential fermentable energy available to ruminants given roughage (Obara *et al.* 1991). This strategy, however, poses a risk of lower cellulolytic activity (as a result of higher VFA production lowering pH) in the rumen. The consequence is that digestibility of the roughage in the diet will be reduced.

The main objective of the present study was to investigate the effect *in vivo* of supplementation with sucrose as a means of improving rumen fermentation, and voluntary intake so as to maximize the absorption and balance of fermentation and digestion products from the gut to optimise tissue metabolism. The study involving both *in vivo* and *in sacco* experiments was undertaken using sheep fed urea-ammonia treated, low quality basal roughage.

4.2. Materials and methods

(a) Animals and their management

Four (4) Border Leicester x Merino crossbred wethers weighing 45.0±4.38 kg (SD), each fitted with a permanent rumen cannula and an abomasal cannula were re-located to the animal house and housed in individual pens on slatted floor for one week before the commencement of the feeding experiment. They remained in these pens for the entire duration of the experiment, except for one week in each period during which they were transferred to metabolism crates when the digestibility and N balance trial was being conducted.

(b) Experimental design and treatments

A feeding trial involving four wethers and four treatments was carried out in four periods in a balanced 4 x 4 Latin square design. The four dietary treatments are shown in **Table 4.1**.

Table 4.1 The four dietary treatments in the trial

	Treatments	
T1	Basal diet (11% CP) **(Control)**	$\mathbf{E_0}$
T2	Basal diet + Energy* (100% intraruminal)	$\mathbf{E_R}$
T3	Basal diet + Energy (100% intra-abomasal)	$\mathbf{E_A}$
T4	Basal diet + Energy (50% intraruminal + 50% intra-abomasal)	$\mathbf{E_{RA}}$

*Energy supplied as sucrose dissolved in water

Sheep were allocated randomly to the treatments as shown in **Table 4.2**. Each animal completed a schedule of activities as shown in **Table 4.3**, with each period taking about 4 weeks. In total it took about 5 months to complete the trial.

Table 4.2. The allocation of animals to the treatments during each of the 4 periods of the trial.

	Animals			
PERIOD	**Animal 915**	**Animal 916**	**Animal 3498**	**Animal 3492**
1	E_A	E_R	E_{RA}	E_0
2	E_{RA}	E_0	E_A	E_R
3	E_R	E_{RA}	E_0	E_A
4	E_0*	E_A	E_R	E_{RA}

*Animal replaced at the end of 3rd period.

Table 4.3. Schedule of activity undertaken and data collection.

PERIOD	ACTIVITY UNDERTAKEN
(7 d)	All animals were brought into the animal house for backgrounding and fed on basal diets for one week (only in period 1).
D1-D14 (14 d)	Animals were weighed and allocated to their respective treatments. Sugar (sucrose) dosage commenced on day one. Basal roughage diet was offered *ad lib* and the intake data collected during the 2nd week were used to determine the voluntary intake. Animals were kept in slatted floor pens.
D15-D22 (7 d)	Collection of refusals, faeces, urine was done for 7 d in the metabolism crates. Animals were offered a restricted amount of basal diet (95% of *ad lib* intake).
D23-D24 (2 d)	Ruminal fluid sampling for liquid kinetics in the rumen using Cr-EDTA as liquid phase marker, and determination of pH, molar concentration and proportion of VFA, NH_3 and protozoa. Sampling at 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 21 and 24 h.
D25-D30 (5 d)	*In sacco* degradability where duplicate samples of barley straw were incubated in the rumen of the four animals for 6, 12, 24, 48, 72 and 96 h.
D30-D34 (4 d)	Acetate clearance test with single dose of sodium acetate into the jugular vein of catheterized animals. Blood samples collected before injection and 30, 60, 90, 120 and 150 min post-injection. Animals were catheterized a day earlier.
D35 (2 d)	Animals rested for two days in readiness for the next period.

(c) Diets and the application of the treatments to animals

The basal diet consisted of wheaten chaff/ barley straw roughage (3:1 DM basis). The basal diet was then supplemented with urea at 2.5% DM to bring the crude protein content (**N x 6.25**) of the

basal roughage diet to about 11% (**Table 4.1**). Batches of 100 kgDM of the basal diet were prepared, enough to last the four animals for 2-3 weeks. A mixture of wheaten chaff (91% DM, 0.71% N) and barley straw (93% DM, 0.55% N) was mixed using the Feed mill batch mixture and during the mixing process urea solution (2.5 kg urea in 20 L warm water) was added.

The mixing process was done for about 10-15 min to ensure that the basal roughage was mixed thoroughly. The mixture was then put in 100 kg synthetic gunny bags, compacted and stored at room temperature until it was removed for use on a daily basis. Sampling of the urea-treated basal roughage was done twice a week and bulked for later analysis.

Table 4.4. The composition of the diets and dietary treatments by ingredients.

	Treatments			
Ingredients	**Basal + E_0**	**Basal +E_R**	**Basal + E_A**	**Basal + E_{RA}**
Wheaten chaff (%)	73.1	73.1	73.1	73.1
Barley straw (%)	24.4	24.4	24.4	24.4
Urea (%)	2.50	2.50	2.50	2.50
Total basal diet (%)	100	100	100	100
Sucrose (g/d)	0	112.5	112.5	112.5
Cottonseed meal (g/d)	50	50	50	50
Complete mineral mix (g/d)	5	5	5	5

E_0, no sucrose supplementation (control), or sucrose supplement administered through the rumen (**E_R**), abomasum (**E_A**) or both routes in equal amounts (**E_{RA}**).

The basal diet was offered to all four animals in the pens for one week before the experiment was started to accustom them to their surroundings and also make them familiar with the feed (1^{st} period only). Thereafter, the animals were weighed and allocated to the four treatments in a randomized manner (see *b*) in readiness for the two-week adaptation period. The animals were offered the basal diet *ad lib* and supplemented with supplements providing equal amounts of ME

in the form of a sucrose solution administered intraruminally, intra-abomasally or by both routes simultaneously (50:50). The energy supplement was prepared by dissolving 375 g in 750 ml of warm tap water to make about 1 L of sugar solution each day. About 300 ml of this solution was administered to each animal in two doses of 150 ml at 09.00 and 16.00 h each day using a 50 ml syringe. Therefore, in total each of the three experimental animals, with the exception of control ($\mathbf{E_0}$) received approximately equal amounts of sucrose, i.e. about 112.5 g/d administered intraruminally, abomasally or through both routes (50:50). In addition, animals in each of the four treatments received daily a mixture of 50 g cottonseed meal (CSM) and 5 g complete mineral supplement. The mixture was spread on top of the basal roughage given each morning. Animals tended to give the CSM-mineral mixture first preference and therefore eating all of it very quickly, and then spreading the eating of the rest of the basal roughage diet over the 24 h period. Clean drinking water was available to the animals at all times. The composition of the diets/treatments is shown in **Table 4.4**.

(d) Determination of voluntary feed intake

Voluntary intake of the basal roughage and total diet was determined for the four treatments during the 14 d adaptation period when the animals were in individual pens and offered the basal diet and the sucrose supplements. During this period the animals were offered the basal diet in quantities so that daily refusals were approximately 15-20% of that offered. This ensured that intake was not restricted by unavailability of feed. The daily ration of roughage was offered at 09.00 h, while the energy supplement (sucrose dosage) was given in two equal portions at 09.00 and 16.00 h. The refusals were collected every morning before the daily feeding, weighed and 10% retained for analysis. To determine the voluntary intake of both the *basal roughage* and the *total diet*, the data collected on the 2nd week of the two-week adaptation period was used. The daily voluntary intake was expressed on the basis of metabolic body weight (i.e. gDM or OM/kgW$^{0.75}$), to facilitate comparison between animals of different weights.

(e) Digestibility and N balance

The digestibility and N balance trial was conducted during the 3rd week of each period immediately after the 14-day adaptation period, and involved total collection of faeces and urine. The animals were transferred to the metabolism crates in a room with 24 h lighting. During the N balance trial, animals were offered a restricted quantity of roughage equal to 95% of the voluntary intake determined during the 2nd week of the adaptation period. Refusals were

collected each morning before new feed was put out. The refusals were weighed and a 10% aliquot retained. The aliquots were bulked at the end of the collection period (7 d) and stored at 4°C.

Urine was collected into 5 L plastic buckets containing 50 ml of 10% (v/v) H_2SO_4. A pH of below 3 was needed to stop any microbiological or enzymatic activity that might have degraded the purine metabolites in the urine, and also to prevent loss of ammonia. Every morning before feeding, the urine was transferred from the plastic bucket to a 2-litre measuring cylinder and the volume recorded, after which the contents of the cylinder was made up with cold tap water to 2 L. Immediately a 50 ml sub-sample was drawn for bulking and stored at –20°C. After the 7-day collection period the bulked urine was thawed, mixed thoroughly and a 20 ml sub-sample (triplicate) transferred into McCartney bottles and kept at –20°C until analysis for total N and purine derivatives (PD). The daily faecal output was also collected in the morning and mixed thoroughly and then a 10% aliquot taken and bulked for the entire collection period of 7 d. The faecal samples stored at 4°C were thawed and dried in forced draught oven at 60°C for 72 h, ground in Wiley Mill (1 mm) and the samples stored for later chemical analysis. The N balance was determined by subtracting the combined faecal and urinary N losses from the total N intake, taking into account the N content in the refusals. For the determination of faecal pH, freshly voided faeces were weighed, mixed with water (1:2 fresh weight basis) and then pulverized with a glass rod to make a slurry. The pH was determined with a glass electrode pH meter.

(f) Estimation of microbial N supply

The amount of microbial purine absorbed from the intestines (X, mmol/d) was predicted from the quantity of purine derivatives (PD) excreted in urine (Y, mmol/d) using the relationship for sheep derived by Chen *et al.* (1990a) and Chen and Gomez (1992);

$$Y = 0.84X + (0.150\ kg^{0.75}e^{-0.25x}). \quad [1]$$

The supply of microbial N (MN g/d) based on total PD excreted in urine was then estimated as follows: -

$$MN/day = 70X/ (0.116) (0.83) (1000) = 0.727X. \quad [2]$$

Where X is the intestinally absorbed PD and Y is the excretion of PD in mmol/d. The validity of this relationship is based on the assumptions that:

- Digestibility of infused purine is about 0.83
- The N content of purine is about 70 mg/mmol and
- The ratio of purine N: total N of mixed rumen bacteria of 11.6:100.

In this study, estimation of the microbial N supply was based on daily excretion of *allantoin* only. In sheep ideally all four PD (hypoxanthine, xanthine, uric acid and allantoin) should be determined, but allantoin normally forms the highest proportion, and there is also a strong and positive correlation between allantoin and total PD excreted in urine of sheep (Khan *et al.* 2001). The relationship first proposed by Balcells *et al.* (1991) was therefore used in the estimation in the total daily excretion of PD. This relationship indicates that the excretion of allantoin (Y, $\mu mol/kg^{0.75}$) and the purine infused duodenally (X, $\mu mol/kg^{0.75}$) can be described by the following equation-:

$Y = 0.8015X - 43.7$, and therefore $X = (Y+43.7)/0.8015$. [3]

The urinary allantoin concentration was determined by the colorimetric method described by Chen and Gomez (1992).

The microbial N production efficiency was expressed as g N per kg OM apparently digested in the rumen (OMADR) to enable comparisons to be made between dietary treatments.

(g) *In sacco* degradability

Because of the potential risk that the presence of sucrose in the rumen might impair cellulolytic activity, it was decided that the effect of sucrose supplementation on rumen degradation parameters be evaluated by means of an *in sacco* experiment. The objective was to compare the differences (as affected by the treatments) in the *rate* and *extent* of degradation of a common substrate (*barley straw*) incubated in the rumen of sheep fed urea-treated basal roughage diet, and supplemented with energy as sugar (sucrose) administered intraruminally, abomasally or by both routes. Air dried samples of barley straw (2 g) were ground to 2 mm particle size in a Wiley Mill were weighed into nylon bags (size 150 x 80 mm, pore size 44 micron) together with a marble (5 g) to ensure that the bags remained submerged in the rumen digesta during incubation. The bags were then tied firmly with fishing line before being suspended in the rumen of the four animals

through the rumen fistula for 6, 12, 24, 48, 72 and 96 h. Duplicate bags were used for each time period.

After each incubation time, bags were removed from the rumen and washed thoroughly with running warm tap water while being squeezed gently until no more visible colour (turbidity) came from the bags and their contents. The bags together with their contents were dried in a forced draught oven at 65°C for 72 h, cooled in a desiccator, weighed and the loss in weight determined.

The estimate of DM degradability (%) of the barley straw was based on the model proposed by Ørskov and McDonald (1979)-:

DM degradability (P) = $a + b\ (1\text{-}e^{-ct})$ [1]

where-:

P = DM degradability, a = DM disappearance at time zero, and determined as washing loss, b = the fraction potentially degraded in the rumen in time *t*, c is the degradation rate constant representing the rate at which the potentially degradable fraction (b) is degraded (h^{-1}), and t = incubation time (h). The potential degradability (PD) was calculated as a + b, for all the incubation times used in the study as follows:

$P = a$ (up to time t_0) [2]

$P = a + b\ (1\text{-}e^{-ct})$ (from t_0 onwards) [3]

Where a, b, and c are as described above and t_0 is the lag phase (h). The effective extent of degradation (ED) was calculated hourly using the equation:

$$P = a + \{[bc/(c + k)]\ [1\text{-}e^{-(c+k)t}]\} \quad [4]$$

Where *k* is the fractional outflow rate of solids from the rumen. For components that contained a lag phase, degradation was calculated as:

$P = a$ (up to lag) [5]

$$P = a + \{[(bc)/(c + k)]\ [1\text{-}e^{-(c+k)\,(t\text{-}lag)}]\ (e^{-k*lag})\} \quad [6]$$

from lag time onwards.

In this study the passage rate was not determined and therefore, a passage rate (k) of 0.03 h^{-1} was assumed in order to calculate the effective degradability (ED) of the DM as per equation [4] above (Bonsi *et al.* 1994). The variables a, b, PD, c, ED and lag time (LT) were determined for all the four treatments with duplicate bags being used for each time period.

(h) Rumen liquid volume and liquid outflow rate (rumen liquid kinetics)

Rumen fluid volume, outflow rate and $T_{1/2}$ (i.e. time taken for half of the rumen fluid volume to be removed and replaced) were determined using Cr ethylenediamine tetra-acetic acid (Cr-EDTA) complex as the marker for the liquid phase (Downes and McDonald 1964).

The Cr-EDTA complex solution containing 2.77 mg Cr/ml was delivered into the rumen through the fistula as a single injection (dosage 0.5 ml/kg live weight) immediately before the animals were offered their day's ration. As all the animals were 40-50 kg live weight, about 20 ml of CrEDTA solution per animal was required. Samples of rumen fluid (25 ml) were collected before the Cr-EDTA injection and thereafter at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 21 and 24 h.

Samples of approximately 25 ml of rumen fluid per sampling were withdrawn using a syringe connected to a rumen fluid sampling probe that was fitted with a fine-stocking gauge material (pore size <200μ). About 5 ml of the fresh rumen fluid was used to determine the pH immediately after collection. Another portion (5 ml) of the rumen fluid was reserved for protozoa counting and was processed as outlined in part *(i)*. The last portion of approximately 15 ml was transferred to a 20 ml McCartney bottle and 0.4 ml 18 M H_2SO_4 was added to preserve the sample contents, especially the ammonia. The acidified samples were stored at –20°C for later analysis of Cr, ammonia and VFA. However, prior to doing these analyses, the samples were thawed and centrifuged at 3000 x g and the clear supernatant decanted into clean McCartney bottles in readiness for chemical analyses.

The Cr concentration in the supernatant of the centrifuged rumen fluid samples was determined after the samples were digested with perchloric acid ($HCLO_4$)/ H_2O_2 (7:3 v/v). The Cr concentration in the digested samples was analyzed using an Inductively Coupled Plasma Optical Spectrometer (ICP-OES) with wavelength range covering 175-785 nm. The readings were

interpolated from the standard curve generated from various dilutions prepared from the Cr standard (Ajax Finechem, Seven Hills Australia).

In order to validate this method and therefore ascertain its reliability, some of the rumen fluid samples were spiked with standard Cr solution, and subjected to the same digestion process as the other samples, and the recovery determined. The recovery levels varied from 93.7-97.0% with a mean of 95% (**Appendix III**).

The dilution of Cr over time in the rumen was assumed to obey first order kinetics described by the standard equation;

$C_t = C_0 e^{-kt}$ [1]

where:

C_t is the concentration of Cr at time (t), C_0 is the concentration of Cr (mg/L) at zero time (obtained by finding the y-intercept of the regression line: ln [Cr] = a + b*t*), and *k* is the rate constant (slope of the regression line).

The pre-injection concentration of Cr was subtracted from all the other subsequent hourly concentrations to give the net concentration, which was then transformed to a natural logarithm (*ln*) scale. The ln [Cr] was regressed against time (h). The coeffient of determination ® of the regression line was used to assess the goodness of fit of the fitted line.

Rumen fluid volume (RV L), outflow rate (L/D) and $T_{1/2}$ (h) were then calculated as follows:

Rumen fluid volume (RV, L) = Dose injected (mg Cr)/C_o

Outflow rate (L/d) = RV x slope of regression line

$T_{1/2}$ = 0.693/k or ln (2)/k.

(i) Protozoa numbers in the rumen fluid

The protozoa were enumerated in the rumen fluid as described by Bird *et al.* (1979). In brief about 4 ml of the fresh rumen fluid was added to 16 ml of 4% (v/v) formal-saline solution in McCartney bottles, shaken thoroughly to mix and kept at 3-4°C for later determination of the protozoa numbers. The 4% formal-saline was prepared by combining 40% (v/v) formaldehyde and normal saline solution (9 g NaCl/L) in a 1:9 ratio. However, because formaldehyde was only

available in 38% instead of 40% formulation, it was necessary to make adjustments. The 4% formal-saline was therefore prepared by dissolving 9 g NaCl in approximately 900 ml of de-ionised water, and then 42 ml of 38% (v/v) formaldehyde added before finally topping up with more water to 1 L which was adequate to process samples collected in one period at a time.

During counting the diluted rumen fluid was shaken well and pipetted into a counting chamber (C. A. Hanser & Sons, Maxley) of 0.0625 square mm and 0.2 mm depth, and covered with a cover slip. Counting was done at x 40 magnification using a light microscope. At least 3 separate unit blocks each covering 12 out of the 16-cell sub-unit were counted. The protozoa were assessed as total count only without attempting to differentiate them between the various species, though it was apparent that in some samples a number of protozoa species were present mainly the entidiniomorphs and Holotrich.

(j) Acetate clearance rate

Acetate clearance rate was determined using the method first proposed by Weston (1966) and later modified by Cronje (1987). The animals were catheterized in the jugular vein a day before acetate clearance was determined, in readiness for injection of pre-warmed (37-40°C) sodium acetate adjusted to pH 7.40 with 1M NaOH. A single dose (4 mmol/ kg BWT) in 50 ml of de-ionised water was injected into the jugular vein over a period of 2-3 min. During the 3rd period one apparently quite healthy animal (**No 915**) died within a very short period of time post injection (2 min) from what appeared to be a reaction to the acetate solution. Postmortem examination indicated that there was extensive clotting of blood in the heart. It was subsequently replaced with an animal of similar weight to complete the rest of the trial. Because of the general risk of acetate clearance test it was always scheduled to be the last intervention in each period (**Table 4.3**).

A 5 ml blood sample from the jugular vein was drawn into a heparinised tube before acetate injection, and 30, 60, 90, 120 and 150 min post injection to monitor the changes in the level of acetate in the blood with time. The blood sample drawn before acetate injection was to establish the background (basal) concentration of acetate in the animals prior to intravenous acetate loading. During the 2.5-h collection period the blood samples were kept in a cooled ice-box and then centrifuged at 3000 x g for 15 min to separate the blood cells from the plasma. The cellular portion was discarded while the plasma supernatant (3 ml) was decanted and then deproteinised

by adding 0.3 ml of 50% (w/v) sulphosalicyclic acid, mixed thoroughly and centrifuged. The clear deproteinised plasma was decanted and stored at –20°C for later analysis of acetate. The plasma acetate concentration was determined using gas liquid chromatography (Model CP-3800GC) with iso-caproic acid as the internal standard.

Calculations

The acetate concentration (mmol/L) in the plasma samples collected post injection was corrected for the pre-injection concentration of acetate, and then transformed to a natural logarithm scale. The natural logarithm (ln) of acetate concentration was then regressed against sampling time, i.e. $\ln(Ac_t-Ac_o)$ vs t.

where: Ac_t is the concentration of acetate at time t, Ac_o is the plasma acetate concentration pre-injection, and t is the sampling time.

The rate of decline in acetate concentration in the plasma with time was determined from the slope of the regression line, i.e. the acetate clearance rate constant (k) (min^{-1}). The coefficient of determination (r) of the regression was also determined so as to establish what proportion of the variance in acetate in the blood was attributed to the time factor. The time required for the injected acetate dose in the blood of the sheep to be halved ($T_{1/2}$) was calculated as follows-:
$T_{1/2}$= ln(2)/2 or 0.693/k where k is the slope of the regression line of the plot ln (Ac_t-Ac_o) versus t.
The volume of distribution of the acetate load was given by-:
Volume (L) = Ac load (mol)/(Ac_o mol/L) and acetate clearance rate (mol/min) was calculated as the product of compartment size (mol) and the rate constant (/min).

(k) Analytical methods

Dry matter, organic matter and ash

The DM (DM) content of feed, refusals and faeces was estimated by drying samples in triplicate from each animal in each period in a forced draught oven at 60°C for a minimum of 72 h, or until constant weight was achieved. Thereafter, the samples were bulked and milled in a Wiley Mill to pass through a 1mm screen, and dried overnight in crucibles at 105°C to determine the final DM content. The DM was then combusted in a muffle furnace at 600°C for 4 h to determine both the organic matter (OM) and ash content (AOAC 1990).

Total N

The total N content in the feed ingredients, feed refusals, faeces and urine was determined using the automated semi microkjeldahl system (AOAC 1990).

Ammonia N

The concentration of ammonia N in the rumen fluid supernatant (see part *a*) was estimated using an autoanalyser (Technicon), according to the method described by Crook and Simpson (1971) and modified by Beitz (1974). The proportion of un-ionised ammonia in the total ammonia concentration was calculated using the Henderson-Hasselbach equation (Siddons *et al.* 1984) taking into account total ammonia concentration and rumen fluid pH:

Un-ionised $[NH_3] = 1-(1/ (1 + \text{antilog } [pH-pK'_a]$, where $pK'_a = 9.02$

Volatile fatty acids

The total molar concentration (mmol/L) of all VFAs, and molar percentages of the major VFA, acetic, propionic, butyric, and the minor VFAs (isobutyric, iso-valeric and valeric acid) were estimated in the rumen fluid supernatants by the methods of Erwin *et al.* (1961), using gas liquid chromatography (GLC) (Model CP 3800GC), and iso-caproic acid used as an internal standard. The ratio of glucogenic to acetogenic substrates in the rumen was determined by the percentage of propionic to acetic acid. The percentage of propionic acid in total VFA energy was used to calculate the glucogenic ratio according to the method proposed by Blaxter (1967). The ratio was expressed as follows-:

G/E = Propionate/(Propionate + 0.6 Acetate + 1.4 Butyrate), where the VFAs were expressed as molar percentages. The ratio of energy supplied by propionate to that of total VFA was used as an index for expressing the *glucogenic potential* of the nutrients (fermentation products) available to the host animal (Preston and Leng 1987).

(l) Statistical analysis

The data were analyzed by analysis of variance (ANOVA) for a 4 x 4 Latin square experimental design using Minitab computer statistical software (Ryan *et al.* 1985). Where significant differences between the treatments means were detected, separation of means was done using the Tukey test at 5%.

4.3. Results

4.3.1. Chemical composition of the basal roughage and the dietary ingredients

The composition of the basal roughage and the ingredients used in the basal diet and dietary treatments are shown in **Table 4.5**.

Table 4.5. The composition of basal roughage and the ingredients used in the basal diet.

Parameter	Ingredients					
	Basal roughage	**Wheaten chaff**	**Barley straw**	**Cottonseed meal**	**Mineral supplement**	**Sucrose**
DM(g/kg)	772	910	932	918	963	999
OM(g/kgDM)	931	927	924	924	67	1000
N(g/kgDM)	22.2 (139)	7.1(44.6)	5.5(34.6)	73.6(460)	nd	nd
Ash(g/kgDM)	69.5	73.4	75.7	75.9	932.8	0.0

DM, dry matter; OM, organic matter; N, nitrogen; CP, crude protein (N x 6.25); Figures in parentheses refer to the CP (g/kgDM); nd, not determined. The basal roughage is as it was fed to the animals.

4.3.2. Feed intake and digestibility

The results of dietary and basal roughage intake and digestibility of DM and OM are shown in **Table 4.6**. Animals that were supplemented with sucrose entirely through the rumen ($\mathbf{E_R}$) or abomasum ($\mathbf{E_A}$) had significantly higher ($P<0.05$) dietary and basal roughage intake of both DM and OM. However, there were no differences ($P>0.05$) in total and basal diet intakes for both DM and OM between the unsupplemented animals (control) ($\mathbf{E_0}$) and those supplemented with sucrose via both the rumen and abomasum in equal proportions ($\mathbf{E_{RA}}$). The N intake also followed similar trend where animals receiving the sucrose supplement wholly through the rumen ($\mathbf{E_R}$) or abomasum ($\mathbf{E_A}$) had a higher ($P<0.05$) N intake than those on the control diet ($\mathbf{E_0}$) or those supplemented with sucrose through both ruminal and abomasal routes ($\mathbf{E_{RA}}$).

Table 4.6. Dietary intake and digestibility of DM and OM in sheep fed urea-treated roughage (E_0) and supplemented with sucrose intraruminally (E_R), abomasally (E_A) or via both routes (50:50) (E_{RA}).

Constituent	Dietary treatments				
Daily intake	E_0	E_R	E_A	E_{RA}	SEM
Diet DM(g/kg$^{0.75}$)	61.4^a	72.3^b	67.9^{ab}	61.4^a	2.06 (***)
Diet OM(g/kg$^{0.75}$)	57.0^a	67.6^b	63.5^{ab}	57.3^a	1.91 (***)
Basal DM(g/kg$^{0.75}$)	58.4^a	63.3^b	58.8^{ab}	52.2^a	2.08 (**)
Basal OM(g/kg$^{0.75}$)	54.4^a	59.0^b	54.8^{ab}	48.6^a	1.94 (**)
Nitrogen(g/d)	23.0^a	26.6^b	23.9^{ab}	22.4^a	0.89 (**)
Total apparent digestibility					
DM(g/kg)	595^a	627^{ab}	639^b	605^a	27.83 (***)
OM(g/kgDM)	612^a	640^a	653^b	620^a	7.74 (**)
Nitrogen(g/kg)	737^c	721^{bc}	702^b	670^a	8.48 (***)

E_0, no sucrose supplementation (control), or sucrose supplement administered through the rumen (E_R), abomasum (E_A) or both routes in equal amounts (E_{RA}); SEM, standard error of mean; ns, not significant, * P<0.05, ** P<0.01, *** P<0.001; means within row with different superscripts differ at P<0.05.

The DM digestibility was higher (P<0.05) in animals supplemented with sucrose wholly through the rumen (E_R) or abomasum (E_A) than for animals on the control diet or those that received the sucrose supplement by both routes. However, the OM digestibility was significantly higher (P<0.01) for animals that were abomasally supplemented with sucrose (E_A) (653 g/kgDM) than for those on the other three dietary treatments. Organic matter digestibility in the unsupplemented animals (E_0) and those supplemented with sucrose entirely through the rumen (E_R) or through both the rumen and abomasum (E_{RA}) did not differ significantly (P>0.05). The N digestibility was highest in animals on the control diet (E_0) and those supplemented with

sucrose entirely through the rumen ($\mathbf{E_R}$) at 737 and 721 g/kg respectively, and lowest in those animals that received sucrose supplement through both the ruminal and abomasal routes ($\mathbf{E_{RA}}$)(670 g/kg).

4.3.3. *In sacco* degradation

The results of the *in sacco* degradation characteristics of barley straw in the rumen of animals in each of the 4 dietary treatments are shown in **Table 4.7**. There was no difference (P>0.05) in the soluble fraction (a), slowly degradable fraction (b), potential degradability (PD) and effective degradability (ED) between the four treatments. Sucrose supplementation did not significantly (P>0.05) affect the rate of degradation (*c*) or the lag time. The *in sacco* results also show that barley straw attained 96 h PD and ED levels of 615-645 and 368-415 g/kg DM, respectively, in the four dietary treatments.

Table 4.7. The degradation characteristics of barley straw incubated in the rumen of sheep fed urea-treated low quality basal roughage and supplemented with sucrose through the rumen or abomasum*.

	Dietary treatments				
Parameter	$\mathbf{E_0}$	$\mathbf{E_R}$	$\mathbf{E_A}$	$\mathbf{E_{RA}}$	**SEM**
Soluble fraction(a)(g/kgDM)	91.0	87.6	92.2	97.6	5.8 (ns)
Degradable fraction(b)(g/kgDM)	539	533	523	548	20.7 (ns)
PD (a + b)(g/kgDM)	630	621	615	646	19.6 (ns)
Effective degradability(g/kgDM)	415	382	368	368	29.6 (ns)
Rate of degradation(c)(/h)	0.05	0.04	0.03	0.03	0.006 (ns)
Lag time(h)	1.67	1.90	2.58	1.93	0.76 (ns)

$\mathbf{E_0}$, no sucrose supplementation (control), or sucrose supplement administered through the rumen ($\mathbf{E_R}$), abomasum ($\mathbf{E_A}$) or both routes in equal amounts ($\mathbf{E_{RA}}$); PD, potential degradability.
*All the primary data were fitted well by equation except for one animal (**916**) (see **appendix I** in detail).

4.3.4. Nitrogen retention, excretion of purine derivatives and microbial protein supply

The results for N retention, excretion of allantoin and predicted microbial protein synthesis in the sheep given the four dietary treatments are presented in **Table 4.8.**

Table 4.8. The N retention and microbial protein production in sheep fed urea-treated basal roughage (E_0) and supplemented with sucrose intraruminally (E_R), abomasally (E_A) or by both routes (E_{RA}).

	Dietary treatments				
N-value	**E_0**	**E_R**	**E_A**	**E_{RA}**	**SEM**
Intake (g/d)	23.0[a]	26.6[b]	23.9[ab]	22.4[a]	0.89 (**)
Excretion (g/d)					
In urine	15.1	15.6	14.3	13.0	1.53 (ns, P = 0.08)
In faeces	6.20[a]	7.36[b]	7.04[ab]	7.38[b]	0.30 (*)
Balance (g/d)	1.78	3.68	2.56	2.07	1.64 (ns)
Urinary allantoin (mmol/($kg^{0.75}$per d)	0.38	0.43	0.44	0.43	0.103 (ns)
Microbial N (g/d)	6.39	7.67	7.75	7.58	1.67 (ns)
Microbial N (g/kgOMADR)	18.8	18.1	18.5	18.9	4.34 (ns)

N, nitrogen; OMADR, organic matter apparently digested in the rumen (0.65DOM); ns, not significant, * P<0.05, ** P<0.01; means within row with different superscripts differ at P<0.05.

The N intake of animals supplemented with sucrose entirely through the rumen (E_R) or abomasum (E_A) was higher (P<0.05) than that of control animals (E_0) or those that received the sucrose supplement through both rumen and abomasum (E_{RA}). The faecal N excretion was higher (P<0.05) for the sucrose supplemented animals than for the unsupplemented group. There was however, no difference (P>0.05) in faecal N excretion between the three supplemented

dietary treatments (i.e. $\mathbf{E_R}$, $\mathbf{E_A}$ & $\mathbf{E_{RA}}$). Urinary N excretion did not differ (P>0.05) between treatments.

The average daily N balance of the sucrose supplemented animals tended to be higher than the unsupplemented (control) animals but the difference between the four treatments was not significant (P>0.05). Although the mean values for daily urinary allantoin excretion and microbial N production (g/d) in the animals supplemented with sucrose wholly through the ruminal ($\mathbf{E_R}$), abomasal ($\mathbf{E_A}$) or both routes ($\mathbf{E_{RA}}$) were generally higher than in the control group, the differences were not significant (P>0.05). The lack of difference (P>0.05) between dietary treatments was maintained even when the microbial N synthesis was expressed on the basis of organic matter apparently digested in the rumen (OMADR).

4.3.5. Rumen pH, concentration of VFA and ammonia, and protozoa count

The results on the variation in pH, VFA and ammonia concentration, and total protozoa count in the rumen of the sheep on the four dietary treatments are presented in **Table 4.9** and **Figures 4.1-4.11.**

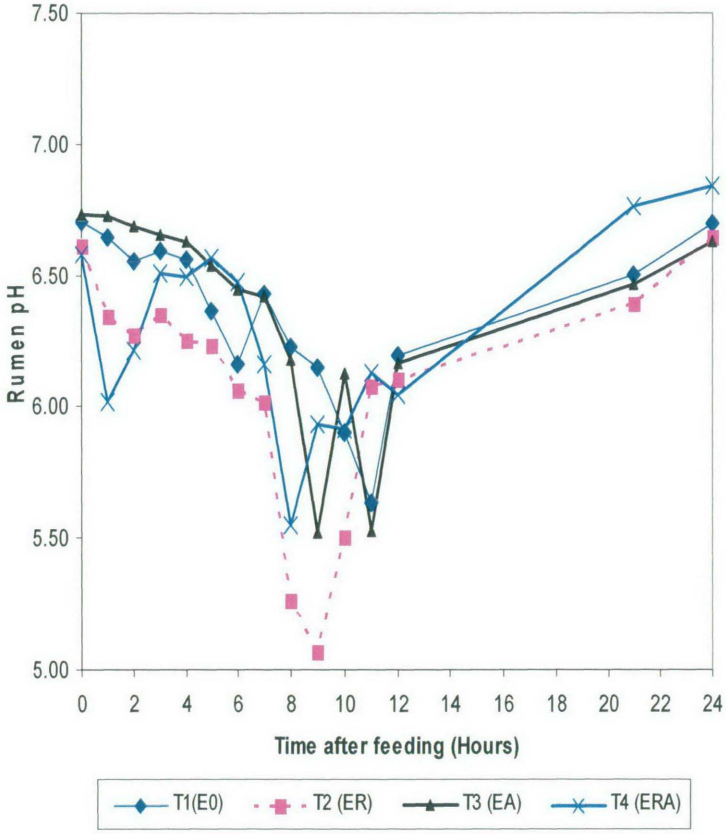
Rumen fluid pH

Animals that received the sucrose supplement wholly through the rumen ($\mathbf{E_R}$) had a significantly lower (P<0.001) mean rumen fluid pH (6.13) than the animals in the other treatments. Although the pH in the rumen of animals receiving dietary treatment $\mathbf{E_{RA}}$ at 6.32 was lower compared to that of animals on the control diet (6.40) or those supplemented sucrose through the abomasum (6.44), the difference was not significant (P>0.05) (**Table 4.9**). The results of the dietary and temporal variation in rumen pH shows that following introduction of the feed to the animals in the morning, the rumen fluid pH of the animals in all the four dietary treatments, decreased reaching the lowest levels at about 8-12 h post-feeding (**Figure 4.1**). In particular, there was a marked decrease in the pH immediately after feeding, and again after the 8^{th} h (2^{nd} feeding) in animals receiving dietary treatment $\mathbf{E_R}$ and to some extent those on $\mathbf{E_{RA}}$. However, such a marked decrease in pH was not apparent in animals on the dietary treatments $\mathbf{E_0}$ or $\mathbf{E_A}$. The 0 and 8 h also coincided with the times of the day (09.00 and 16.00 h) when the 1^{st} and 2^{nd} dosage of the sucrose supplement were administered to the supplemented animals.

Table 4.9. The variation with diet in rumen pH, concentration of VFA and ammonia, and protozoa in the rumen of sheep fed urea-treated low quality basal roughage ($\mathbf{E_0}$) and supplemented with sucrose intraruminally ($\mathbf{E_R}$) abomasally ($\mathbf{E_A}$) or by both routes (50:50)($\mathbf{E_{RA}}$).

Constituent	Dietary treatments				
	$\mathbf{E_0}$	$\mathbf{E_R}$	$\mathbf{E_A}$	$\mathbf{E_{RA}}$	SEM
Rumen pH	6.40^{b}	6.13^{a}	6.44^{b}	6.32^{b}	0.06 (***)
Total VFA (mmol/L)	84.3^{b}	98.2^{c}	65.4^{a}	78.2^{ab}	3.60 (***)
Acetate (%)	68.8^{c}	61.3^{a}	70.9^{d}	63.5^{b}	0.51 (***)
Propionate (%)	20.7^{a}	27.8^{b}	19.7^{a}	26.8^{b}	0.60 (***)
Butyrate (%)	8.3^{c}	9.1^{c}	6.4^{a}	7.4^{b}	0.24 (***)
Other VFA (%)	2.25^{a}	1.82^{a}	3.07^{b}	2.31^{a}	0.18 (***)
Propionate/acetate	0.30^{a}	0.46^{b}	0.28^{a}	0.43^{b}	0.01 (***)
G/E	0.28^{a}	0.39^{b}	0.28^{a}	0.36^{b}	0.01 (***)
Total ammonia (mg/L)	235	244	225	208	18.1 (ns)
Un-ionised ammonia (mg/L)	0.72^{b}	0.40^{a}	0.74^{b}	0.55^{ab}	0.08 (**)
Protozoa ($x10^5$)/ml	5.0^{b}	7.5^{c}	2.8^{a}	3.8^{ab}	0.44 (***)

$\mathbf{E_0}$, no sucrose supplementation (control), or sucrose supplemented through the rumen ($\mathbf{E_R}$), abomasum ($\mathbf{E_A}$) or both routes in equal amounts ($\mathbf{E_{RA}}$); G/E refer to 4.2 (*k*); SEM, standard error of mean; ns, not significant, * $P<0.05$, ** $P<0.01$, *** $P<0.001$; means within row with different superscripts differ ($P<0.05$).



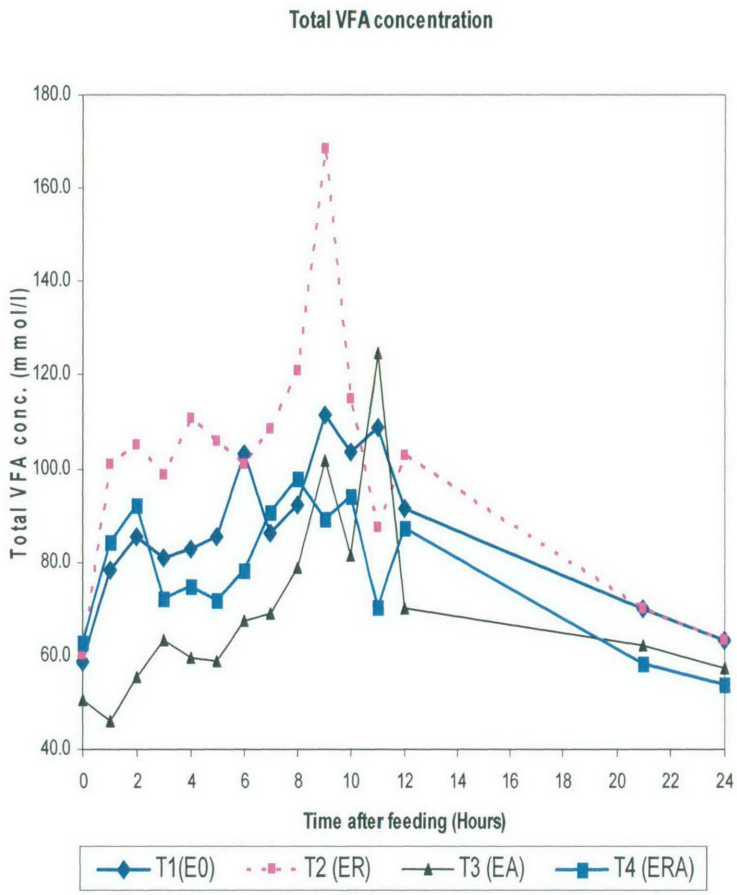
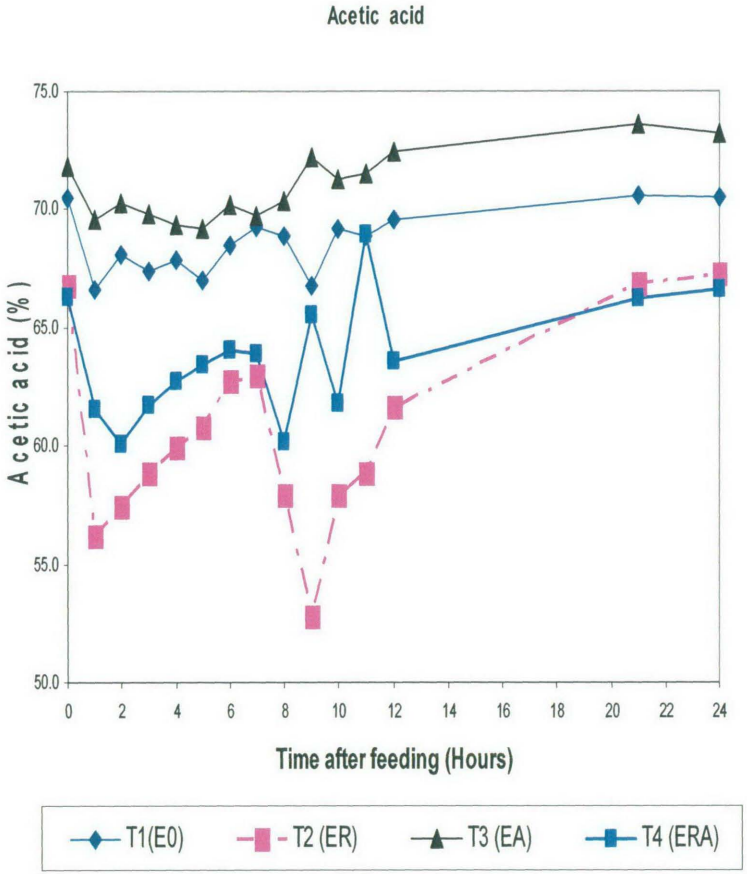


Figure 4.1 & 4.2. The diurnal variation and variation with diet in pH and total VFA concentration in the rumen of sheep fed urea-treated basal roughage ($\mathbf{E_0}$) and supplemented with sucrose intraruminally ($\mathbf{E_R}$), abomasally ($\mathbf{E_A}$) or by both routes ($\mathbf{E_{RA}}$).



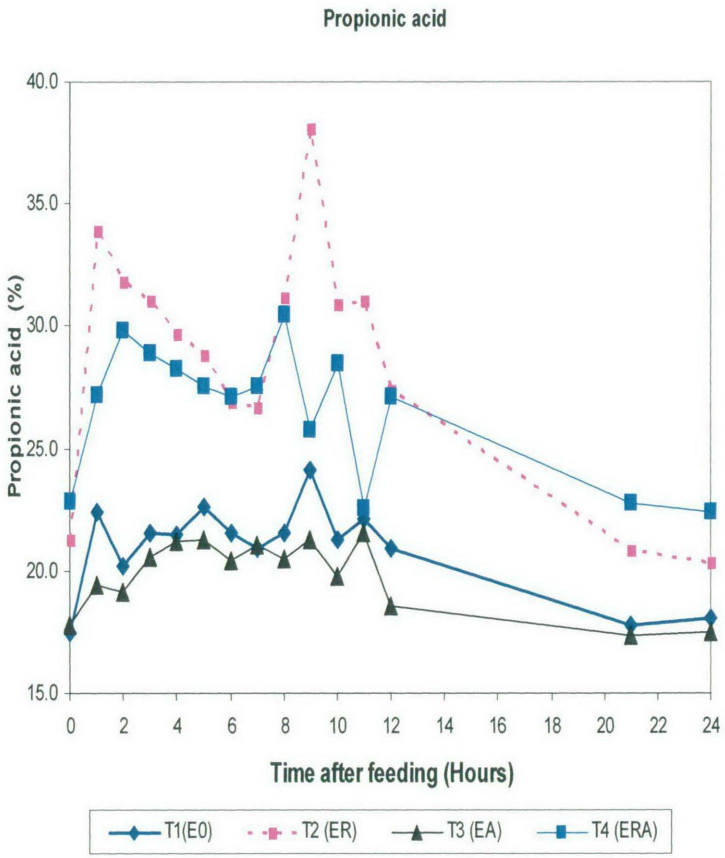


Figure 4.3 & 4.4. The temporal variation and variation with diet in the molar proportion (%) of acetic and propionic acid in the rumen of sheep fed urea-treated basal roughage and supplemented with sucrose intraruminally or abomasally.

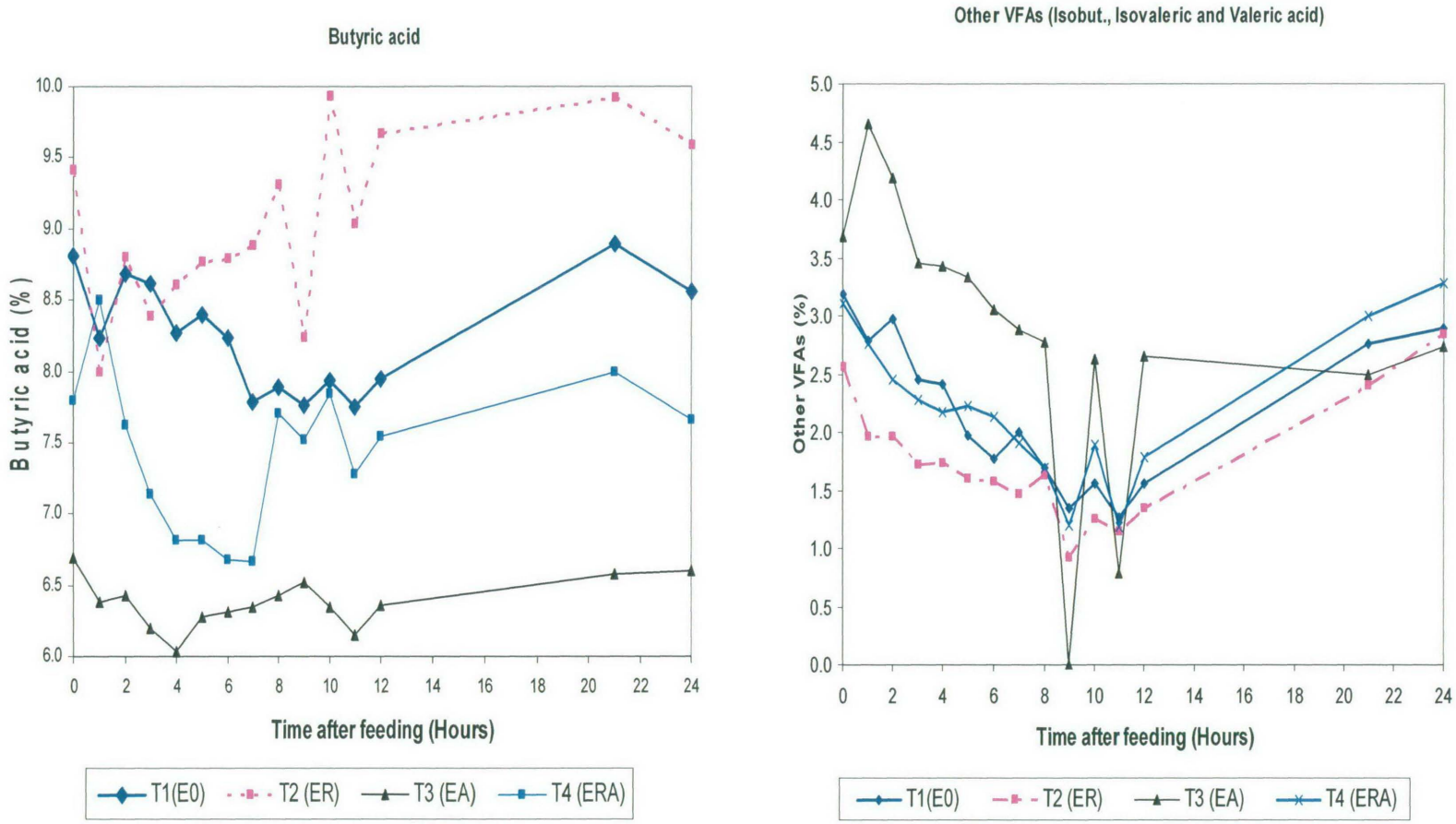


Figure 4.5 & 4.6. The temporal variation and variation with diet in the molar proportion of butyric acid and other VFAs (isobutyric, isovaleric and valeric) in the rumen of sheep fed urea-treated basal roughage and supplemented with sucrose ruminally or abomasally.

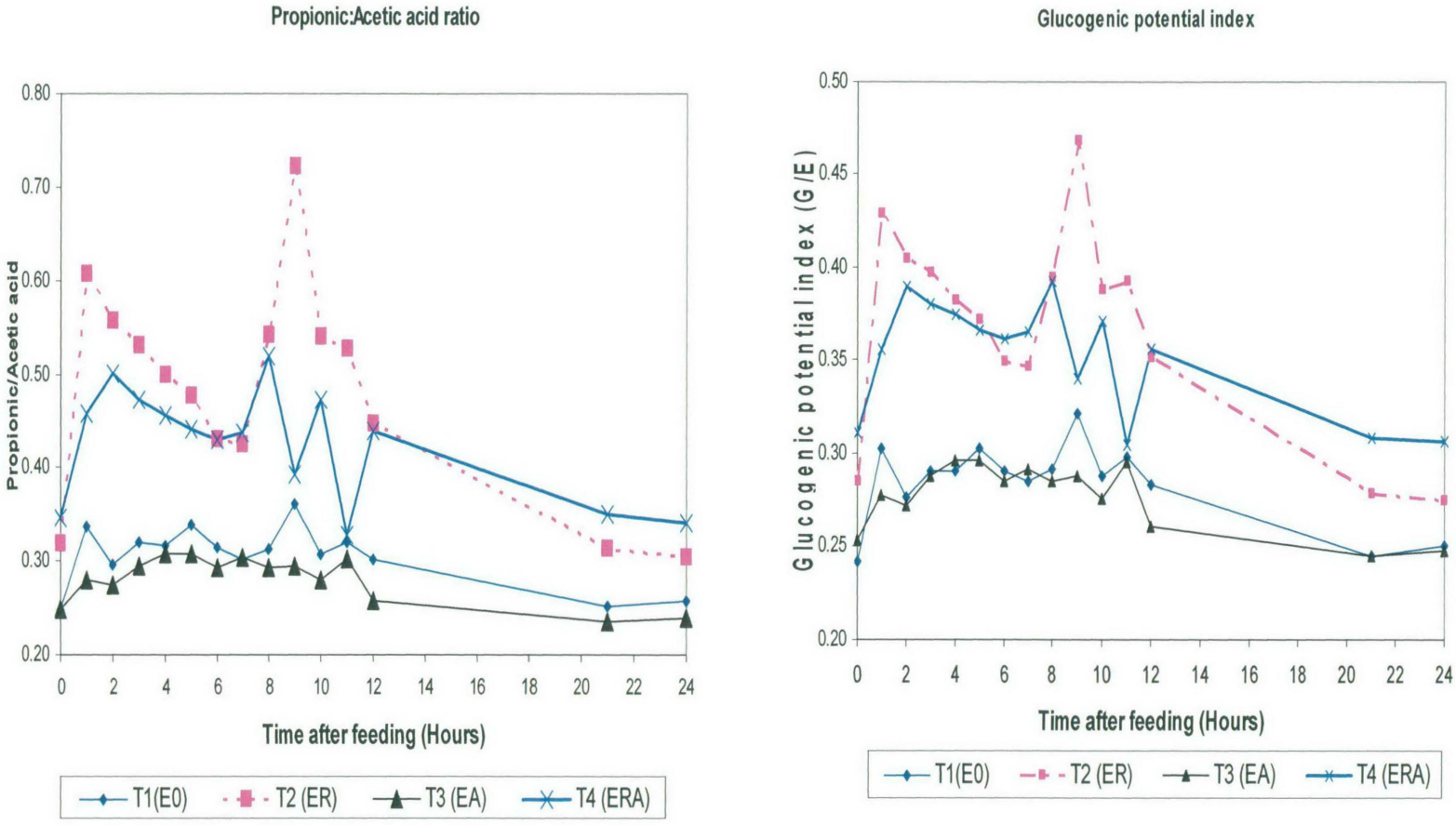


Figure 4.7 & 4.8. The diurnal variation and variation with diet in the molar proportion of propionic to acetic acid ratio and the glucogenic potential index (G/E) in the rumen of sheep fed urea-treated basal roughage supplemented with sucrose ruminally or abomasally.

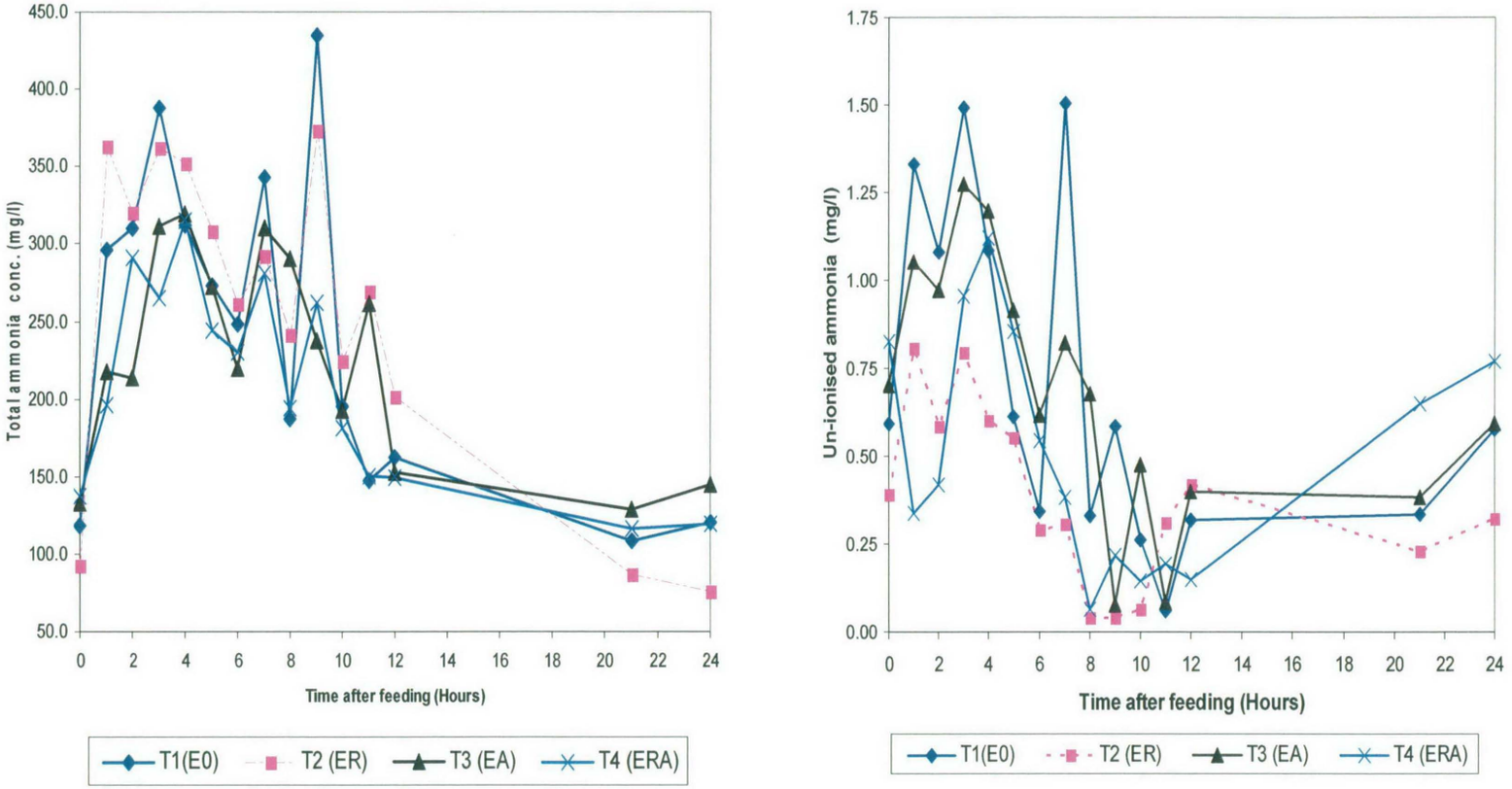


Figure 4.9 & 4.10. The diurnal variation and variation with diet in total NH_3 and un-ionized NH_3 concentration in the rumen of sheep fed urea-treated low quality basal roughage and supplemented with sucrose through the rumen or abomasum.

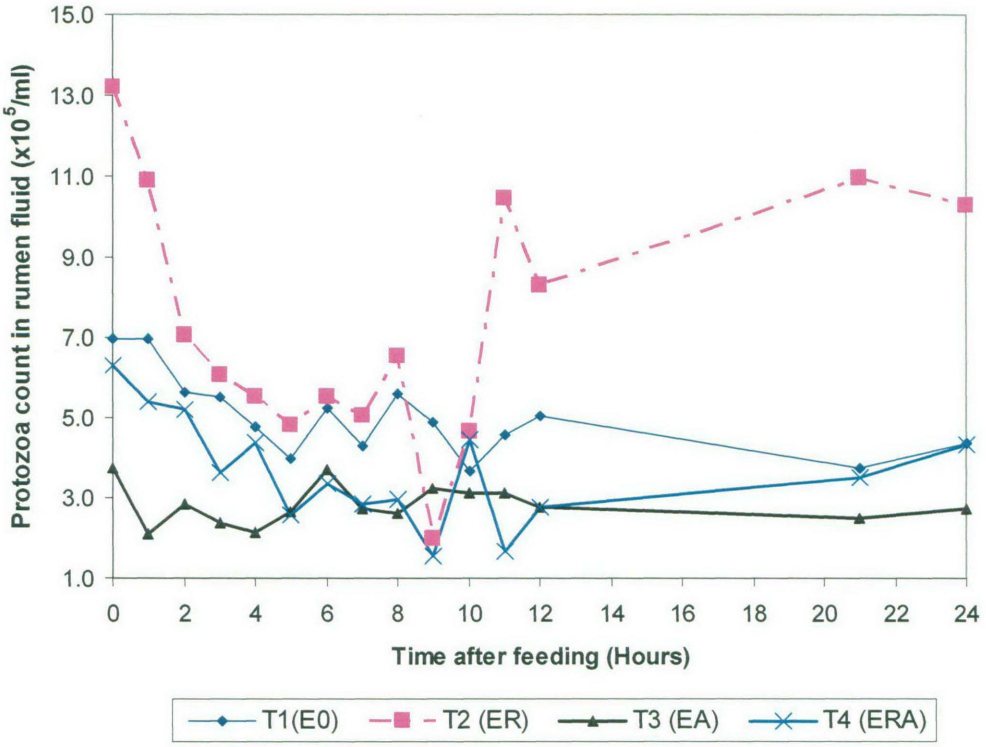


Figure 4.11. The diurnal pattern of the change in total protozoa with diet in the rumen fluid of sheep fed urea-treated low quality basal roughage ($\mathbf{E_0}$) and supplemented with sucrose via the rumen ($\mathbf{E_R}$), abomasum ($\mathbf{E_A}$) or both routes ($\mathbf{E_{RA}}$).

Thereafter, the pH started to increase gradually, and by 21-24 h post-feeding, it was at the level that existed prior to the morning feeding time. The diurnal variation also shows clearly that those animals that received the sucrose supplement entirely through the rumen ($\mathbf{E_R}$) generally had the lowest pH on an hourly basis and also the minimum for the day, followed by those receiving the supplement through both routes ($\mathbf{E_{RA}}$). The period for which the rumen pH was below 6.0 also seems to be longest (3-4 h) in animals receiving dietary treatment $\mathbf{E_R}$, followed by those on $\mathbf{E_{RA}}$ and shortest in those on the control $\mathbf{E_0}$ (**Figure 4.1**).

Total volatile fatty acids concentration and individual VFA proportions in the rumen

There was a very highly significant ($P<0.001$) difference in total VFA concentration and also the molar proportions of the various VFAs (acetate, propionate, butyrate and other VFAs) in the rumen between the sheep receiving the four dietary treatments (**Table 4.9**). The total VFA

concentration in the rumen was highest in those animals that received the sucrose supplement entirely through the rumen (98.2 mmol/L) and lowest in those that received the sucrose supplement wholly through the abomasum (65.4 mmol/L). The unsupplemented animals (control) and those that received the sucrose supplement through both ruminal and abomasal routes had intermediate total VFA concentration at 84.3 and 78.2 mmol/L respectively. The molar proportion of acetate in the total VFA produced in the rumen was highest in those animals that received the sucrose supplement through the abomasal route (70.9%) and lowest in the intraruminally supplemented group (61.3%). The control group and those that received the supplement through both routes were intermediate (68.8 & 63.5%). The temporal variation in total VFA concentration in the in the rumen of animals receiving the four dietary treatments showed a general trend where there was an increase immediately following the onset of feeding reaching the maximum levels at about 8-12 h post-feeding before starting to decrease to the lowest level of about 60-80 mmol/L by 21-24 h from the start of feeding (**Figure 4.2**). The diurnal variation also showed that the molar proportion of acetate in the rumen of animals on the control dietary treatment, and those supplemented with sucrose wholly through the abomasum were consistently higher and also showed lower hourly fluctuations compared to those of animals supplemented with sucrose wholly or partly through the rumen ($\mathbf{E_R}$ & $\mathbf{E_{RA}}$) (**Figure 4.3**).

The molar percentage of propionate in rumen fluid was significantly higher ($P<0.001$) in those animals that received the sucrose supplement wholly ($\mathbf{E_R}$) or partly ($\mathbf{E_{RA}}$) into the rumen compared to those that received no supplement ($\mathbf{E_0}$) or received the sucrose entirely through the abomasal route ($\mathbf{E_A}$). There was, however, no difference ($P>0.05$) in the percentage of propionate between the animals on the control dietary treatment and those receiving the sucrose supplement abomasally, or between the intraruminally supplemented animals and those receiving the sucrose supplement through both intraruminal and abomasal routes. The diurnal variation in the molar proportion of propionate in the rumen showed that the hourly fluctuation in animals supplemented with sucrose wholly through the rumen or both ruminal and abomasal routes were similar but greater in magnitude than the control or abomasally supplemented animals (**Figure 4.4**).

The molar percentage of butyrate in the rumen of animals on the four dietary treatments ranged from 6.4-9.1% and the difference between dietary treatments was very highly significant ($P<0.001$) (**Table 4.9**). The proportion of butyrate in the control animals (8.3%) and those

supplemented sucrose wholly through the rumen (9.1%) was generally higher ($P<0.05$) than for the animals that received the sucrose supplement through both the rumen and abomasum (7.4%). Those animals that received the sucrose supplement through the abomasum had the lowest (6.4%) molar proportion of butyrate in the rumen. There was a much greater hourly fluctuation in the molar proportion of butyrate in both dietary treatments $\mathbf{E_R}$ and $\mathbf{E_{RA}}$ compared to the control ($\mathbf{E_0}$) or the abomasally supplemented animals ($\mathbf{E_A}$) (**Figure 4.5**)

The percentage of the other VFAs (mainly the branched-chain VFAs) in the rumen of animals in all the treatments was generally low and accounted for only about 1.8-3.1% of total VFA concentration. Animals supplemented with sucrose wholly through the abomasum had higher ($P<0.001$) molar proportion of other VFAs in the rumen (3.1%) compared to controls ($\mathbf{E_0}$) or those supplemented with sucrose wholly through the rumen ($\mathbf{E_R}$) or through both the rumen and the abomasum ($\mathbf{E_{RA}}$). There was, however, no difference ($P>0.05$) in the molar proportion between the three treatments (i.e. $\mathbf{E_0}$, $\mathbf{E_R}$ & $\mathbf{E_{RA}}$). The diurnal variation in the molar percentage of other VFAs showed a continuous decrease in all four treatments as the post-feeding time increased, culminating with the lowest percentage at about 8-12 h post-feeding, which incidentally also coincided with highest molar percentage of total VFA (**Figures 4.6 & 4.2**).

There was a very highly significant ($P<0.001$) difference in both propionate/acetate and G/E ratio between animals receiving sucrose supplement entirely or partly through the rumen ($\mathbf{E_R}$ & $\mathbf{E_{RA}}$) and the unsupplemented (control) ($\mathbf{E_0}$) or the abomasally supplemented animals ($\mathbf{E_A}$). However, there was no difference ($P>0.05$) in both propionate/acetate and G/E between the animals on dietary treatments $\mathbf{E_R}$ and $\mathbf{E_{RA}}$ or even between those on control diet ($\mathbf{E_0}$) and the abomasally supplemented animals ($\mathbf{E_A}$) (**Table 4.9**). The diurnal variation in propionate/acetate and G/E ratio among animals on the four treatments showed a general trend that was very similar (**Figure 4.7 & 4.8**). The temporal variation also showed clearly that on an hourly basis, the propionate/acetate and G/E ratio were highest in animals on treatment $\mathbf{E_R}$ followed closely by those on $\mathbf{E_{RA}}$ with values for each treatment being characterized by large fluctuations that attained clearly defined peaks approximately 1 h and again 8-12 h post-feeding compared to the control $\mathbf{E_0}$ and $\mathbf{E_A}$ treatments, whose hourly fluctuations were relatively low and fairly stable.

Ammonia concentration

The total ammonia concentration in the rumen of animals on all four treatments ranged from 208 in $\mathbf{E_{RA}}$ to 244 mg/L in $\mathbf{E_R}$, but concentrations were not significantly different ($P>0.05$) between the treatments. However, the concentration of un-ionized ammonia (NH_3) was lower ($P<0.05$) in those animals that received sucrose supplement entirely or partly through the rumen, i.e. 0.40 in $\mathbf{E_R}$ and 0.55 mg/L in $\mathbf{E_{RA}}$. There was no difference ($P>0.05$) between the concentrations of un-ionized NH_3 in the rumen of animals on the control diet and those that received the sucrose supplement wholly through the abomasum or through both routes (**Table 4.9**). Total ammonia concentration tended to increase quite rapidly following the onset of feeding, reaching peak levels in 2-3 h (**Figure 4.9**). Thereafter, there was a continuous decrease in total ammonia concentration and by 21-24 h post-feeding, it was about 100 mg/L. The diurnal variation of un-ionized NH_3 concentration in the rumen showed much greater variation between the four treatments and also higher hourly fluctuation (**Figure 4.10**). The temporal variation also showed that immediately following the onset of feeding, there was an abrupt and sharp increase in un-ionized ammonia concentration but soon after there was a steady decrease reaching the lowest level at about 8-12 h post feeding in all treatments. The lowest concentration of un-ionised ammonia also coincided with the highest total VFA concentration, and also the lowest rumen fluid pH. Thereafter, the concentration of un-ionised ammonia continued to increase, *albeit* gradually, reaching pre-feeding levels by 21-24 h post-feeding in all treatments (**Figure 4.10** and **4.1**). Overall the concentration of un-ionised ammonia in the rumen of the animals in all treatments was generally low (<2 mg/L) relative to total ammonia.

Total protozoa count

The difference in the total protozoa count in the rumen fluid of the animals receiving the four dietary treatments was very highly significant ($P<0.001$) (**Table 4.9**). The total protozoa count was highest in animals that received the sucrose supplement entirely through the rumen (7.5 x 10^5/ml) and lowest in those supplemented with sucrose entirely through the abomasum (2.8 x 10^5/ml). The unsupplemented (control) animals and those receiving the sucrose supplement through both ruminal and abomasal routes had an intermediate count of 5.0 x 10^5 and 3.8 x 10^5/ml respectively, and were not significantly different ($P>0.05$). The diurnal variation in the total protozoa count showed a *marked decrease* within the first 4-5 h of feeding, and again immediately after the 8^{th} h in those animals that received sucrose supplement entirely or partly through the rumen ($\mathbf{E_R}$ & $\mathbf{E_{RA}}$) (**Figure 4.11**). No sharp decrease in total protozoa count was

apparent in the rumen of animals on the control diet or those supplemented sucrose entirely through the abomasum. The hourly fluctuation in total protozoa count was highest in animals on dietary treatment $\mathbf{E_R}$ followed by those on treatment $\mathbf{E_{RA}}$, but it was much lower in animals on the control diet and those on treatment $\mathbf{E_A}$ (**Figure 4.11**).

4.3.6. Liquid kinetics in the rumen

The results of the liquid kinetics in the rumen of sheep on the four dietary treatments are shown in **Table 4.10**.

Table 4.10. The liquid kinetics in the rumen of sheep fed urea-treated low quality basal roughage and supplemented with sucrose through the rumen or abomasum.

	Dietary treatments				
Parameter	$\mathbf{E_0}$	$\mathbf{E_R}$	$\mathbf{E_A}$	$\mathbf{E_{RA}}$	**SEM**
Rumen fluid volume (L)	11.3	13.0	8.4	9.6	1.84 (ns)
Rate constant (x 10^{-2})/(h)	5.51	6.99	4.15	5.08	0.00997 (ns)
Outflow rate (L/d)	11.3	14.7	8.9	11.4	2.37 (ns)
T½ (h)	13.1	9.9	14.2	11.5	3.08 (ns)

$\mathbf{E_0}$, no sucrose supplementation (control), or sucrose supplement administered through the rumen ($\mathbf{E_R}$), abomasum ($\mathbf{E_A}$) or both routes in equal amounts ($\mathbf{E_{RA}}$).

There was no significant difference ($P>0.05$) in any measures of the rumen kinetics; rumen fluid volume, dilution rate constant, outflow rate, and T½, between the animals receiving the four treatments. However, the mean values tended to be higher in animals supplemented with sucrose entirely through the rumen ($\mathbf{E_R}$), and followed closely by those supplemented with sucrose through both routes ($\mathbf{E_{RA}}$).

4.3.7. Acetate clearance

The acetate clearance results of the sheep fed urea-treated basal roughage and supplemented with sucrose intraruminally or abomasally are presented in **Table 4.11.**

Table 4.11. Acetate clearance in the body of sheep fed urea-treated low quality basal roughage and supplemented with sucrose energy.

Parameter	Dietary treatments				
	E_0	E_R	E_A	E_{RA}	SEM
Clearance rate constant (x 10^{-3})/min	9.00	8.67	13.0	6.50	0.00280 (ns)
Acetate clearance rate (x 10^{-2})(mol)/h)	9.78	8.97	16.0	6.95	0.0306 (ns)
Acetate Half-life (T½)(h)	1.30	2.61	0.94	1.93	0.55 (ns)

E_0, no sucrose supplementation (control), or sucrose supplement administered through the rumen (E_R), abomasum (E_A) or both routes in equal amounts (E_{RA}).

There was no significant difference (P>0.05) in clearance rate constant (*k*), acetate clearance rate ($molh^{-1}$), and clearance half-life (T½) between the treatments. It was however, noted that animals supplemented with sucrose entirely through the abomasum generally had the highest mean clearance rate constant (13 x 10^{-3} /min) and acetate clearance rate (16.0 x 10^{-2} mol/h), and also the shortest clearance half-life (0.94 h), even though they were not significantly different (P>0.05) from those of the other three dietary treatments (i.e. E_0, E_R & E_{RA}).

4.3.8. Faecal characteristics

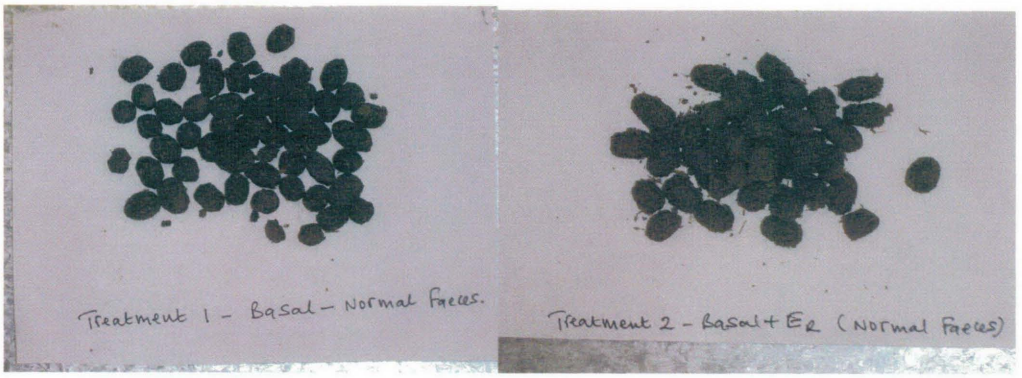
The faecal characteristics of the animals are shown in **Table 4.12** and **Figure 4.12**. The faecal DM content of animals supplemented with sucrose entirely through the abomasum (E_A) was lower (P<0.05) than that of control animals or those supplemented with sucrose through the rumen (E_R). The faecal DM content of animals supplemented with sucrose through both ruminal and abomasal routes (E_{RA}) was intermediate but not significantly different (P>0.05) from that of animals supplemented with sucrose through the abomasum (E_A). The faecal pH of the animals on the four treatments was 8.69, 8.70, 4.91 and 6.96 for E_0, E_R, E_A and E_{RA} respectively. Visual appraisal also showed that the faeces from animals on dietary treatment E_A did not have the normal consistency and besides being quite moist also tended to form lumps rather than normal faecal pellets. However, control animals and those supplemented with sucrose wholly through

the rumen generally had normal faeces with well-formed pellets. The faecal consistency of animals on dietary treatment $\mathbf{E_{RA}}$ was intermediate (**Figure 4.12**).

Table 4.12. The faecal characteristics of sheep fed urea-treated low quality basal roughage ($\mathbf{E_0}$) and supplemented with sucrose through the rumen ($\mathbf{E_R}$), abomasum ($\mathbf{E_A}$) or both routes 50:50 ($\mathbf{E_{RA}}$).

	Dietary treatments				
Parameter	$\mathbf{E_0}$	$\mathbf{E_R}$	$\mathbf{E_A}$	$\mathbf{E_{RA}}$	**SEM**
Faecal DM (g/kgDM)	339^{b}	322^{b}	273^{a}	290^{ab}	12.7 (***)
Faecal pH*	8.69	8.70	4.91	6.96	NA
Visual appraisal	Normal	Normal	Very moist	Intermediate	-

*Means for period 1 only and hence data not analysed statistically (NA). means within row with different superscripts differ ($P<0.05$).



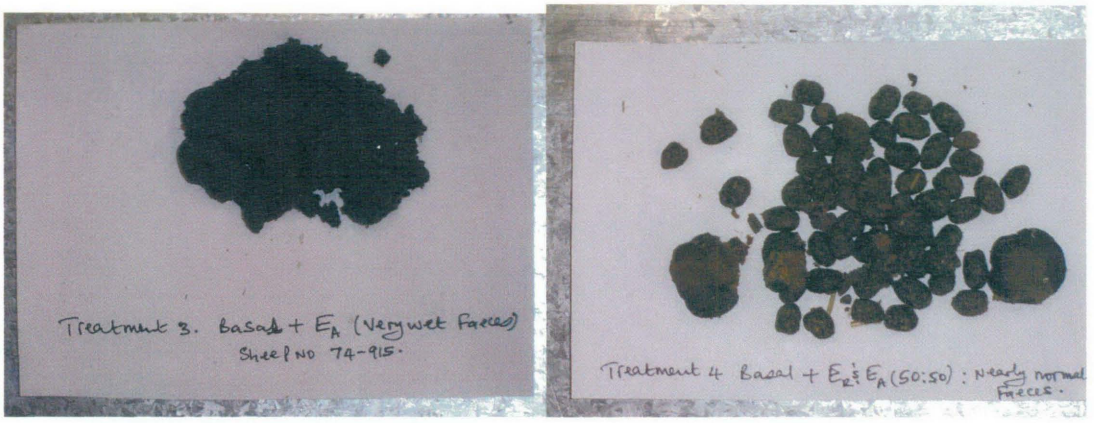


Figure 4.12. The faeces of sheep fed urea-treated basal roughage ($\mathbf{E_0}$) and supplemented with sucrose intraruminally ($\mathbf{E_R}$), abomasally ($\mathbf{E_A}$) or both routes 50:50 ($\mathbf{E_{RA}}$).

4.4. Discussion

4.4.1. Feed intake and digestibility

Nitrogen is considered to be a major nutrient limiting efficient utilisation of low quality basal roughage in ruminants (Preston and Leng 1987), as it is required for growth by cellulolytic microbes in the rumen (Bryant 1973; Russell *et al.* 1990). Its content in forages also is one of the most widely used criteria in classifying the nutritive value of forages, with roughage containing 8-12 gN/kgDM (5-8% CP) generally being categorized as low quality roughage (Leng 1990).
The N content of the basal roughage (22.2 g/kgDM or 13.9 % CP) was quite high due to the addition of urea. The role of urea in enhancing the N content of low quality roughage is appreciated when it is considered that the two main ingredients used in constituting the basal roughage, wheaten chaff and barley straw with 7.1 and 5.5 gN/kgDM respectively, were quite low in N. Mixing the wheaten chaff and barley straw together in the ratio of 3:1 (DM basis) to constitute the basal roughage without incorporating any urea would have produced a basal roughage containing 6.7 gN/kgDM (4.2% CP) which is low compared to the 22.2 gN/kgDM (13.9% CP) that was attained after treatment 2.5% urea. All animals were therefore considered to be adequately supplied with more than the minimum requirements of N for optimal microbial growth in the rumen. This was evident from the high total ammonia concentration in the rumen of the animals on all the four treatments, i.e. 208-244 mg/L and which remained above 100 mg/L for most of the feeding period. The increase in N content following urea-treatment is in agreement with results reported by other workers (Cloete *et al.* 1983; Cloete and Kritzler 1984; Djajanegara and Doyle 1989; Iwanyanwu *et al.* 1990).

The dietary and basal roughage intake of both DM and OM, and DM digestibility were highest in animals supplemented with sucrose entirely through the rumen ($\mathbf{E_R}$) or abomasum ($\mathbf{E_A}$). The high intake of roughage and total DM and OM may be attributed to favourable conditions for microbial growth in the rumen and/or hindgut, and possibly also the enhanced nutrient balance, especially the protein: energy ratio (P/E) at the tissue metabolism level (Egan 1965a, 1977), as influenced by sucrose supplementation. This, however, does not preclude a possible role of other factors in influencing intake. Voluntary intake in ruminants, especially of roughage is highly complex and is determined by the rate at which ingested feed material is broken down to particle sizes that are small and dense enough to be cleared from the rumen to the lower parts of the gut

through the reticluo-omasal orifice (Poppi *et al.* 1981a, b; Lechner-Doll *et al.* 1991). The process of fibre breakdown in the rumen is mainly accomplished through rumination and complemented by microbial degradation (Ulyatt *et al.* 1986), with the effectiveness of the latter being highly dependant on the rumen microbial fermentation environment, which in turn is influenced by composition of the diet eaten by the animal (Chen and Hovell 1996).

In this study, the *in sacco* degradation of barley straw did not show any significant difference in major degradation characteristics between the sucrose supplemented and unsupplemented animals. Furthermore, the 96-h potential degradability (PD) level attained for the barley straw (615-645 g/kgDM) was fairly high for roughage. This suggests that the roughage diet contained adequate amounts of nutrients to meet microbial growth requirements, and that the presence of sucrose in the rumen did not adversely affect cellulolytic activity. That sucrose supplementation to the animals entirely through the rumen or abomasum resulted in a higher voluntary intake of DM (dietary and basal), and total apparent digestibility of DM (and also OM in $\mathbf{E_A}$), even when *in sacco* results showed no significant difference in the main degradation characteristics in the rumen, suggests that a significant proportion of DM may have been digested post-ruminally.

Besides the physical barriers such as lignin and silica that may interfere with OM digestion in the rumen, a poor microbial fermentation environment as influenced by the diet can also hinder achievement of 100% potential degradability (asymptote). Such an environment may be manifested in the form of insufficient NH_3, low rumen pH, and the fermentation of large amount of soluble sugars and starch in the rumen following dietary intake (Chen and Hovell 1996). These factors are likely to affect microbial growth in the rumen by increasing lag time and in the process lead to a reduction in rate of fibre degradation in the rumen, even without affecting the extent of degradation (Chen and Hovell 1996). Carbohydrates (soluble sugars and starch) are generally fermented very rapidly in the rumen, depressing pH and this can adversely affect growth and cellulolytic activity in the rumen (Ørskov and Fraser 1975; Russell *et al.* 1979; Mould *et al.* 1983; Mould and Ørskov 1983). The magnitude of the depression in fibre digestion can be quite pronounced, especially if the pH is depressed to a level below 6.0 for a prolonged period of time (Miller and Muntifering 1985; Hoover 1986; Royes *et al.* 2001). In this study it was only in animals that were supplemented with sucrose entirely through the rumen ($\mathbf{E_R}$) that attained mean rumen fluid pH as low as 6.13, which is still higher than values thought to inhibit cellulolytic activity (Mold and Ørskov 1983; Hoover 1986; Russell and Wilson 1996).

Furthermore, the rumen pH remained below 6.00 for no more than 3 h each day. Taken together with the relatively high rumen ammonia levels, this may explain why fibre degradation in the rumen was not adversely affected by intraruminal sucrose supplementation.

Any difference in apparent digestibility between the dietary treatments cannot be attributed to the rumen fermentation environment. However, differences in digestion certainly occurred in the large intestines and possibly other parts of the gut. This is supported by the results on total and roughage intake that showed that intake of both DM and OM were highest in the intraruminally ($\mathbf{E_R}$) or abomasally ($\mathbf{E_A}$) supplemented animals. This is in spite of the intraruminally supplemented animals having the lowest mean rumen pH. This provides further evidence that sucrose supplementation entirely through the rumen or abomasum did not adversely affect digestibility or intake of the roughage. Total intakes of roughage were indeed even improved by sucrose supplementation relative to the control sheep or those supplemented through the ruminal and abomasal routes. Given that the rumen degradation characteristics between the four treatments were not significantly different, and that the total apparent digestibility and intake of DM in animals on both $\mathbf{E_R}$ and $\mathbf{E_A}$ were not significantly different, it follows that the higher apparent DM digestibility in these two dietary treatments ($\mathbf{E_R}$ & $\mathbf{E_A}$) may be attributed to higher hindgut digestion and/or longer retention of digesta in the gastrointestinal tract as a whole. Longer mean retention time (MRT) of digesta in the gut can lead to greater *extent* of digestion (without change in the *rate* of degradation) and therefore higher total apparent digestibility of DM, thus extracting more VFA from the roughage (Egan 1965a, 1977).

There was ample evidence of extensive hindgut fermentation activity in animals supplemented with sucrose entirely through the abomasum ($\mathbf{E_A}$), and also, to lesser extent, those supplemented via the ruminal and abomasal routes ($\mathbf{E_{RA}}$). Animals supplemented with sucrose entirely through the abomasum produced very moist faeces with low DM content (273 g/kgDM), poor consistency and devoid of any pellet formation besides being moderately acidic (pH 4.91). The same was noted in animals on dietary treatment $\mathbf{E_{RA}}$ though to a lesser extent. All these changes in faecal characteristics point to a possible impairment in fluid exchange in the hindgut between the digesta and the body tissues. These changes were attributed to the presence of soluble sugars in the hindgut that had passed through the small intestines without being effectively digested and absorbed. The fermentation of these sugars produced acidity from the VFA resulting in the low faecal pH, while some of the unfermented sugars, and VFAs caused osmotic changes that lead to

an influx of water into the lumen of the hindgut resulting in moist faeces with abnormal consistency. Ørskov *et al.* (1972) attributed hindgut fermentation of sucrose to low sucrase (invertase) activity in the small intestines of ruminants, and this has also been reported by others (Walker 1959b; Siddons 1968).

There are reports that in ruminants up to 25-30% of cellulose or even dietary DM may be digested in the hindgut depending on dietary factors, such as composition, particle size and intake levels (Armstrong and Beeever 1969; Beever *et al.* 1971, 1972; Ulyatt *et al.* 1975a). While the microbial fermentation of OM in the hindgut may still benefit the host animal mainly through production and absorption of VFA (Ulyatt *et al.* 1975a), it confers no benefit to the host animal as far as microbial N synthesis is concerned with most of the microbial biomass N being voided in faeces (Ørskov *et al.* 1971b; Ulyatt *et al.* 1975b). In this study high faecal N was noted in all sucrose supplemented animals, and more so those supplemented through the abomasum (more in **4.4.2**).

4.4.2. Nitrogen digestibility, retention and microbial protein supply

The higher N intake in animals supplemented with sucrose entirely through the rumen or abomasum was wholly attributed to their higher total intake of DM. Given that the basal roughage had a relatively high N content (22.2 g/kgDM) with a large proportion of the N being in NPN due to the presence of urea, it was anticipated that a higher intake of roughage would boost N intake as well. Moreover, the total apparent digestibility of N was also fairly high mainly because a large fraction of the NPN was absorbed as ammonia. All animals that received sucrose supplementation, irrespective of the route of administration had a higher faecal N excretion than the controls. The relatively high faecal N excretion in animals supplemented with sucrose abomasally, and in those supplemented through both rumen and abomasum was attributed to the extensive hindgut microbial growth and fermentation activity (Ørskov *et al.* 1972) (**4.4.1**). Extensive microbial fermentation in the hindgut produces VFAs that are absorbed into the blood. Most of the microbial biomass is voided in faeces, thus contributing to the high faecal N excretion. Although the DM intake and DM digestibility, and therefore N intake of the animals on the control ($\mathbf{E_0}$) or dietary treatment $\mathbf{E_{RA}}$ were not different, the latter had a significantly higher faecal N excretion. This indicated that microbial N from the hindgut fermentation contributed significantly to faecal N excretion. It is not clear as to why there was a rather high

faecal N excretion in the animals supplemented with sucrose entirely through the rumen ($\mathbf{E_R}$), yet these animals did not appear to have had extensive hindgut fermentation like those on treatment $\mathbf{E_A}$ or $\mathbf{E_{RA}}$. It can only be presumed that this high faecal N in these animals was as a result of their high DM intake. Faecal N is known to include a significant endogenous component whose content is proportional to DM intake (AFRC 1993). NRC (1985) suggests that the metabolic faecal protein N be estimated as 33.44 g N/kg DMI, while NRC (1988) suggested a mean value of 30 (with a range of 21-40) g N/ kgDMI.

A high faecal N implies a depressed apparent digestibility of N as was evident in dietary treatments $\mathbf{E_0}$ and $\mathbf{E_{RA}}$. Control animals ($\mathbf{E_0}$) had the highest N digestibility (737 g/kg), while those supplemented sucrose through both ruminal and abomasal routes had the lowest (670 g/kg), in spite of the fact that the N intake (23 and 22.4 g/d) of animals receiving these two dietary treatments were not significantly different though lower compared to the other two treatments ($\mathbf{E_R}$ & $\mathbf{E_A}$). However, the animals on the control diet had a significantly lower faecal N excretion (6.20) than those on $\mathbf{E_{RA}}$ (7.38 $g.d^{-1}$) which contributed to the large difference in the N digestibility values between treatments $\mathbf{E_0}$ and $\mathbf{E_{RA}}$. The amount of fermentable carbohydrates reaching the hindgut and fermented by microbes can increase fecal N significantly and therefore depress apparent N digestibility even without having any effect on the amino acid N absorbed in the body (Kay 1969). The relatively high N digestibility of urea-treated basal roughage observed in this study is in agreement with reports from other workers (Ferrell *et al.* 1999), and is attributed to the form of N in the diet.

It is, however recognized that high N digestibility *per se* may not necessarily translate to a higher metabolisable protein (amino acid) supply to the body tissues, especially when a high proportion of the N disappearing or absorbed from the gut is in form of NH_3 that is not directly useful to the animal, unless it is incorporated into microbial biomass in the rumen. The roughage used in this study was quite high in N, but this N consisted mostly of RDN from the urea. Urea is highly soluble in the rumen and degraded rapidly to ammonia leading to high ruminal ammonia concentration as was evident in this study where concentrations ranged from 208-244 mg/L. Ammonia is absorbed into the blood mainly in its un-ionised (NH_3) rather than ionic (NH_4^+) form and converted to urea in the liver. Although some of the blood urea is recycled back into the gut (mainly through saliva or in secretions) the largest proportion is excreted in urine (McDonald 1948). A low rumen fluid pH such as that occurring in the intraruminally supplemented animals

can be expected to shift the equilibrium between NH_3 and NH_4^+ more towards the ionic form, and therefore slowing down the rate of absorption across the rumen epithelium (Bartley and Deyoe 1977; Siddons *et al.* 1984), which may reduce the amount of urea N excreted in urine. Animals normally can tolerate high ruminal ammonia concentrations without suffering toxicity effects provided the pH is low enough to ensure that most of the ammonia is in the ionic rather than molecular form (Bartley and Deyoe 1977).

Though the urinary N excretion of the four dietary treatments did not differ significantly, this avenue of N excretion represented about 60% of total N intake in the animals. A high excretion of N in urine can be expected when dietary N intake results in a relatively high concentration of rumen ammonia, especially when dietary energy intake is either inadequate or not well synchronized with ammonia N release in the rumen (Bartley and Deyoe 1977; Sinclair *et al.* 1993, Trevaskis *et al.* 2001). Because urinary N excretion between the four treatments was not significantly different, the differences in net N retention were mostly influenced by the faecal N loss, with all the dietary treatments returning a positive value that did not differ statistically.

The supplementation with sucrose was meant to boost the fermentable OM content in the rumen, and to stimulate higher uptake of ammonia by the microbes, thereby reducing the total ammonia concentration in the rumen. However, the additional energy apparently did not significantly stimulate additional ammonia uptake by the rumen microbes for microbial synthesis. There was no evidence of reduction in total ammonia concentration in the rumen fluid, or increase in microbial protein synthesis based on daily allantoin excretion between the unsupplemented (control) animals and those supplemented with sucrose through the rumen, abomasum or by both routes. However, the microbial N production in all the four treatments that averaged 18.1-18.9 g per kg OMADR is satisfactory for this type of basal diet. These values are also within the 14-49 g MN per kg OMADR that have been achieved with a wide range of diets (ARC 1984). The fact that intra-ruminal supplementation with sucrose did not significantly depress rumen ammonia concentration or increase microbial protein synthesis beyond the level achieved with the control diet or even treatment $\mathbf{E_A}$ suggests that microbial growth efficiency was not being limited by energy or N. Rumen microbes were therefore able to fully exploit the energy contained in the urea-treated roughage given the adequate supply of ammonia (and S) in the rumen. This is supported by the results of this study that showed that the 96-h PD of barley straw (615-645 g/kgDM) and total apparent OM digestibility (612-653 g/kgDM) are within the medium range

indicative of good quality hay. It is therefore, possible that digestion of the urea-treated basal roughage in the rumen was able to provide adequate fermentable OM to meet the energy requirements of rumen microbes even without sucrose supplementation. Energy-rich supplements can improve the efficiency of ammonia utilisation in the rumen, but the results generally vary with the type of supplement used. When comparing three carbohydrates of different fermentation rates (sucrose, starch and cellulose), sucrose was found to be the most efficient in increasing N utilisation efficiency in the rumen (Syrjala 1972), presumably when it was also matched with a readily degradable N source.

In the present study, it is likely that factors other than ammonia concentration such as the composition and metabolic efficiency of microbial groups dominant in the rumen or rumen liquid turnover rates of (dilution) and solid fractions may have limited microbial growth. Microbial N output realized in this study (18-19 g/kg OMADR) is less than a half of the maximum possible output in the range (14-49 g/kg OMADR) reported by ARC (1984). When sheep fed fresh lucerne (high in RDN) were infused with sucrose intraruminally (190 g/d), there was also no increase in incorporation of ammonia N into microbial N synthesis compared to the controls (Obara *et al.* 1991), in spite of an apparently well synchronised energy and ammonia supply in the rumen. The lack of response in microbial N synthesis was partly attributed to a possible substitution of rumen microbes with less efficient groups in terms of energetics of microbial growth in the rumen (Obara *et al.* 1991).

4.4.3. Rumen fluid pH and VFA production

Animals supplemented with sucrose entirely through the rumen had the lowest pH followed by those supplemented via the rumen and abomasum, and higher and almost equal levels in the control and the abomasally supplemented animals. Sucrose is highly soluble and rapidly fermented by rumen microbes, especially by soluble sugar utilising bacteria such as *Megasphaera elsdenii* (and protozoa). This rapid fermentation leads to a rapid build up in the concentration of short-chain fatty acids (mainly VFAs). This will depress rumen fluid pH and may reduce cellulolytic activity in the rumen, unless the acidity is neutralized by the various processes in operation in the rumen. These include the buffering effect of saliva arising from the extensive rumination normally associated with roughage diets, particularly those high in CWC such as the ones used in this study (Wolin 1981), high ruminal NH_3 concentration, and the absorption of the

VFAs across the rumen epithelium or their removal by outflow to the lower parts of the gut (Stevens 1970; Sutherland 1976). A high population of protozoa in the rumen may also somehow assist in ameliorating rumen pH through sequestration of carbohydrates, and therefore making the substrate unavailable for rapid microbial fermentation (Ørskov and Frazer 1975; Russell *et al.* 1979; Newbold *et al.* 1986b; Kariya *et al.* 1989.

Among the four dietary treatments, the lowest mean rumen fluid pH in the 24 h feeding period (6.13) occurred in animals supplemented with sucrose entirely intraruminally. Although the rumen pH of the animals on the four dietary treatments was at times lower than 6.0, especially in those supplemented with sucrose through the rumen, the low pH was relatively short-term (<3 h) before increasing again to levels higher than 6.0. This may explain why microbial degradation was not adversely affected by sucrose supplementation in any of the treatments as supported by *in sacco* degradation results. These results suggest that the mechanisms for neutralizing the excess ruminal acidity arising from sucrose fermentation were quite effective, especially the buffering effect of saliva, and the high rumen ammonia concentration. Extensive rumination of a high fibre diet has been reported to result in high saliva output that buffers ruminal acidity, and stabilizes rumen pH (Mould *et al.* 1983). Similarly, high NH_3 concentration can result the formation of $(NH_4)_2CO_3$ which also buffers ruminal pH towards neutrality (Hespell 1979; Hespell and Byrant 1979). Depression of rumen pH from 6.6 to 6.2 reduces fibrolytic activity in the rumen and activity is markedly inhibited when pH falls below 6.0 (Mould *et al.* 1983; Mould and Ørskov 1983). However, the time for which the pH remains below the threshold level (6.0) is more important in determining the magnitude of the depression in fibre digestion than the absolute minimum pH in the rumen (Royes *et al.* 2001). This is influenced by the type and amount of carbohydrate used, and the mode of supplementation (Mulholland *et al.* 1978), as the rate of fermentation tends to vary between supplements such as soluble sugars, starch and digestible fibre (Royes *et al.* 2001).

The high total VFA concentration in the rumen of animals supplemented with sucrose wholly through the rumen ($\mathbf{E_R}$) was attributed to the high intake of DM and the presence of extra fermentable substrate in the rumen from the sucrose. The total VFA concentration in control animals (84.3 mmol/L) was higher than that of animals supplemented with sucrose through both rumen and abomasum (78.2 mmol/L) though the two concentrations were not significantly different. The relatively high total VFA concentration in the rumen of animals that received no

sucrose supplement (control) suggests that the basal roughage was well fermented in the rumen, and this was attributed to the treatment of the roughage with urea, and the high ruminal ammonia levels which increased the digestibility of the roughage and provided adequate ammonia to meet microbial requirements. Perdok and Leng (1989) suggested that for optimal microbial function, the rumen ammonia concentration of sheep fed roughage has to be maintained at about 100 to 200 mg/L. Although the total VFA concentration in the rumen of the animals receiving the sucrose (energy) supplement through both rumen and abomasum was lower than that of the controls (78.2 vs 84.3 mmol/L), it was not significantly different, and this can be explained by the fact that animals on the two dietary treatments had similar intakes of dietary and basal DM (also OM), and therefore almost similar amount of OM fermented in the rumen. The concentration of total VFA in the rumen is positively correlated to rate of fermentation and therefore production of VFA (Leng 1970a).

The total VFA concentration in the rumen of an animal at any particular time is the result of a highly dynamic process reflecting a balance between VFA production (from microbial fermentation) and the removal through absorption into the blood and outflow postruminally through the reticulo-omasal orifice (Stevens 1970; Sutherland 1976; France and Siddons 1993). The dilution effect of water intake, and any fluid exchange between the gut epithelial tissue and the rumen digesta, as influenced by changes in pH and osmotic potential both in the rumen and also in any other compartment along the gastro-intestinal tract also affects VFA concentration.

The total VFA production in the rumen is determined by the quantity of OM (substrate) fermented and the rate at which it is fermented (Leng 1970a; France and Siddons 1993), with the total quantity of the fermentable substrate being made up of the roughage and the supplement(s). Besides adding to the total quantity of fermentable substrates in the rumen, the supplement may also have a stimulatory effect on microbial fermentation and therefore contribute to higher total VFA production. In the present study, *in sacco* degradation results showed that sucrose supplement neither enhanced nor impaired the degradation of barley straw in the rumen. Therefore, it can be presumed that the sucrose supplement only played the role of boosting the quantity of fermentable substrates in the rumen over and above those already provided by the roughage (Obara *et al.* 1991). As a result animals with higher dietary and basal roughage intakes were expected to have higher rates of total VFA production, and therefore higher VFA concentration. This expectation was supported by the total VFA concentration in the rumen of

animals on dietary treatments $\mathbf{E_0}$, $\mathbf{E_R}$ and $\mathbf{E_{RA}}$ that were proportional to the level of intake. However, the same argument does not explain why animals supplemented with sucrose entirely through the abomasum ($\mathbf{E_A}$) had the lowest total VFA concentration (65.4 mmol/L), in spite of their intake that was similar to that of animals on dietary treatment $\mathbf{E_R}$. Furthermore, animals on $\mathbf{E_0}$ and $\mathbf{E_A}$ had similar feed intakes and did not have any sucrose supplement in the rumen, yet their total VFA concentrations differed significantly (84.3 vs 65.4 mmol/L). This indicates that factors other than intake may have influenced the total VFA concentration in the rumen of animals supplemented with sucrose wholly through the abomasum.

In this study, there was evidence of extensive hind gut microbial fermentation activity in animals receiving the sucrose supplement entirely through the abomasum and to a lesser extent those receiving the supplement through both ruminal and abomasal routes. The faeces produced by these animals were very moist and of low DM content, which indicated significant loss of water from the body through the faeces. The mean daily urine output in the animals on the four dietary treatments during the four periods (822 ±50 ml/d (SD)) was not significantly different. A substantial proportion of water is lost from the body through urine, in addition to other avenues such as faeces, sweating and the respiratory surfaces. Although individual animal water intakes were not monitored, animals on these two dietary treatments ($\mathbf{E_A}$ & $\mathbf{E_{RA}}$) may have offset this high water loss by drinking more water, and also absorbing more fluid from the rumen. If this was indeed the case, then the net effect of higher water intake (dilution), and fluid absorption (including VFAs) from the rumen would be to depress total VFA concentration in the rumen. This may explain the unexpectedly low total VFA concentration in the rumen of the animals supplemented with sucrose through the abomasum ($\mathbf{E_A}$), and to a lesser extent the slightly lower mean total VFA concentration for animals on dietary treatment $\mathbf{E_{RA}}$.

As much as this proposition may appear plausible, it is not clearly corroborated by the estimates of rumen liquid kinetics in these animals. A higher water intake is expected to increase the dilution in the rumen, and since the maximum volume of the rumen does not change much in the short term for a given body weight, the extra fluid entering the rumen would have to be removed by absorption across the rumen epithelium and outflow to the lower gut. If the latter avenue was more dominant, then it follows that the rumen outflow rate would be expected to increase and the rumen emptying and filling time (T½) shortened considerably. However, the results on rumen liquid kinetics did not show any significant difference in rumen fluid volume, dilution rate, daily

outflow rate, and rumen T½ between animals on the four dietary treatments. Furthermore, if microbial growth in the rumen is not limiting, any increase in dilution and outflow rate may have a beneficial effect in increasing the flow of microbial biomass from the rumen to the small intestines, thereby supplying more microbial protein to the host animal (Kennedy and Milligan 1978a; Zin and Owens 1983; Robinson *et al.* 1985; Owens and Goetsch 1986). However, this was not supported by the results for microbial protein synthesis, though the mean daily allantoin excretion and microbial N production (g/d) values for the abomasally supplemented animals tended to be higher than those on other treatments. Nevertheless, if the dilution rate was the main cause of low total VFA concentration in the abomasally supplemented animals, then presumably the extra fluid in the rumen was removed predominantly by absorption across the rumen epithelium rather than through outflow. This would be consistent with the hypothesis that these animals drank more water primarily to re-hydrate their body tissues following sustained loss of water through faeces (and possibly urine), an objective that can only be realized through higher water absorption from the gut.

The percentage of acetate in the total VFA was highest in animals on the treatments that did not include any sucrose supplementation in the rumen, i.e. 70 ($\mathbf{E_0}$) and 71% ($\mathbf{E_A}$), and lowest (i.e. 68.8 & 63.5%) in those that received the sucrose supplement wholly or partly through the rumen ($\mathbf{E_R}$ & $\mathbf{E_{RA}}$). These results also showed that, while the presence of sucrose supplement in the rumen was quite effective in increasing total VFA concentration, it also favoured a higher percentage of propionate and butyrate. This was evident from the results of molar proportions of various VFA which showed acetate: propionate: butyrate ratio of 69:21:8 ($\mathbf{E_0}$), 61:29:9 ($\mathbf{E_R}$), 71:20:6.5 ($\mathbf{E_A}$) and 64:27:7.4 ($\mathbf{E_{RA}}$). These results are in agreement with those of other workers (Syrjala 1972; Chamberlain *et al.* 1985; (Rook *et al.* 1987). The propionate/acetate and G/E ratio also appeared to be increased by sucrose supplementation wholly or partly through the rumen. Dietary composition is a major factor influencing both total VFA concentration, and also the ratio of various VFAs in the rumen (Wolin 1981; Russell and Strobel 1991; Brockman 1993). Most roughage is slowly fermented in the rumen producing a VFA pattern characterized by a high proportion of acetate (France and Siddons 1993). This study showed a high molar proportion of acetate, especially in the unsupplemented animals or those supplemented with sucrose abomasally. However, other workers have found that when the basal roughage is supplemented with a readily fermentable carbohydrate energy source such as soluble sugars or starch, the fermentation pattern in the rumen tends to change in favour of higher propionate/acetate ratio

(France and Siddons 1993). When sheep fed fresh lucerne were infused with sucrose (190 g/d) intraruminally total VFA concentration was not higher than in the controls (infused with H_2O) but the molar concentrations and the ratio of acetate, propionate and butyrate are similar to those reported in this study (Obara *et al.* 1991).

Propionate is considered to be highly glucogenic and when its proportion in total VFA is high it can be expected to increase gluconeogenesis in the liver (Wolin 1981; Ørskov 1982c; Preston and Leng 1987). Acetogenic substrates, especially acetate, on the other hand are mainly used to meet the immediate energy needs of the animal and any surplus is conserved as fat in the adipose tissue (Cronje *et al.* 1991). However, an efficient utilisation of acetate for oxidative metabolism to generate ATP is dependent on adequate amounts of oxaloacetate to prime the TCA cycle, and the synthesis of fat is dependent on the supply of NADPH which is mainly obtained by the metabolism of glucose via the pentose phosphate pathway. Both oxaloacetate and NADPH are mainly sourced from glucose metabolism (Crabtree *et al.* 1987; Preston and Leng 1987; Cronje *et al.* 1991). Some workers have suggested that the acetate not utilised for ATP production or conserved as fat may be wastefully oxidized to heat through substrate cycle leading to the high heat increment normally associated with intake of roughage in ruminants (Blaxter 1962; 1967; MacRae and Lobley 1982; MacRae *et al.* 1985; Cronje *et al.* 1991). Inadequate glucose supply may therefore reduce the capacity of body tissues to synthesise fat from acetate, with the heat from futile cycles becoming a major constraint to voluntary intake, especially in ruminants in hot tropical environments (Preston and Leng 1987). A high availability of glucose is expected to lead to a higher efficiency in the utilisation of fermentation products, especially acetogenic substrates, and thus allow a higher voluntary intake (Wolin 1981). The molar percentage of propionate to total VFA in the rumen is considered to be one of the most important criteria for ranking the nutritive value of forages as feeds for ruminants (Leng 1982c; Norton and Poppi 1995).

In this study, animals that received a sucrose supplement wholly or partly through the rumen ($\mathbf{E_R}$ & $\mathbf{E_{RA}}$) generally had a higher molar percentage of propionate and therefore higher predicted glucogenic index (G/E) than the control or abomasally supplemented animals. Animals supplemented with sucrose abomasally though having a fermentation pattern characterized by high acetate and therefore much lower propionate/acetate and G/E ratio, might have benefited from intestinal digestion of sucrose and absorption of glucose. However, these suggestions were

not supported by the acetate clearance tests which showed no significant differences in the acetate clearance characteristics. This was rather surprising, especially when the rumen fermentation results indicated a higher glucogenic potential index (**G/E**) for intraruminally supplemented animals (i.e. $\mathbf{E_R}$ & $\mathbf{E_{RA}}$), while the abomasally supplemented animals ($\mathbf{E_A}$) were expected to have even higher tissue glucose from the alimentary (intestinal) absorption. However, this presumes that all the sucrose is hydrolysed in the small intestines, but there are indications that sucrase (invertase) enzyme activity in ruminants is rather low (Walker 1959b; Siddons 1968; Ørskov *et al.* 1972).

It seems that the higher glucogenic potential predicated on the basis of rumen fermentation parameters and/or intestinal absorption of glucose, did not necessarily translate to a more rapid acetate clearance in the body tissues of supplemented versus the unsupplemented animals. There was however, a general trend that pointed to a better performance of the abomsally supplemented animals. There was, however, a general trend that pointed to a better performance of the abomasally supplemented animals. These animals had the highest acetate clearance rate (0.16 mol/h) and the shortest clearance half-life (0.94 h). It is also notable that the abomasally supplemented animals and those supplemented with sucrose entirely through the rumen had the highest dietary DM intake and total apparent DM digestibility. It is probable that sucrose supplementation intraruminally or abomasally may have enhanced glucogenic potential in the body tissues and increased their capacity to metabolise acetate, but the differences were masked by the large variation in the data obtained in this study. Large variation in acetate clearance parameters is not unique to this study. Large variation in acetate clearance parameters was also noted by Fonseca *et al.* (2001) when reporting on higher acetate clearance rate with increasing proportion of maize (100-300 g/kgDM) supplement in lambs fed urea-treated straw basal diet. The higher acetate clearance rate when maize was used at higher proportion was attributed to higher intestinal digestion of starch that escaped ruminal fermentation and absorbed as alimentary glucose (Fonseca *et al.* 2001). Similarly, Cronje *et al.* (1991) reported on higher acetate clearance rates when propionate or propionate and acetate were added to the basal roughage diet of sheep, or sheep supplemented with protein. Given that sucrose was not effectively digested in the small intestines of the sheep mainly due to the low sucrase activity in ruminants (Ørskov *et al.* 1972), it is presumed that the abomasally supplemented animals obtained most of the glucose from absorbed propionate following microbial fermentation of sucrose in the hindgut.

The changes following ingestion in the molar percentage of butyrate showed that it was also influenced by supplementation of sucrose, with the animals receiving the supplement entirely through the rumen generally having the highest percentage. A relatively high percentage of butyrate in the rumen of animals supplemented with sucrose or in diets high in molasses has been reported before (e.g. Syrjala 1972; Chamberlain *et al.* 1985; Habib 1988). The proportion of butyrate in the rumen also appeared to vary inversely with that of other VFAs, thus suggesting that it was produced mainly at the expense of these other VFAs (i.e. isobutyrate, valeric and isovaleric). The proportion of other VFAs in the rumen of animals on all the four dietary treatments was generally very low, and this may be attributed to the low content of preformed protein in the basal diet as a high proportion of N was in form of NPN. These other VFAs that comprise of branched-chain VFA arise mainly from microbial degradation (hydrolysis and deamination) of protein, peptides and amino acids of dietary and microbial origin (Nolan and Leng 1972; Kennedy and Milligan 1980b; Mackie and White 1990).

4.4.4. Liquid kinetics in the rumen

Animals supplemented with sucrose wholly through the rumen had the largest rumen fluid volume, highest dilution rate (0.007/h) and outflow rate (14.7 L/d), and the shortest T½ (9.9 h). The performance of animals supplemented with sucrose through both rumen and abomasum ($\mathbf{E_{RA}}$) was lower but close to those on treatment $\mathbf{E_R}$. However, the differences between the treatments were not significant probably due to the large variations in the estimates of rumen turnover among the animals. A high rumen fluid turnover may arise from a high saliva output associated with intense rumination (Djouvinov and Todorov 1994) and/or influx of fluid from the body tissues into the rumen due to changes in osmolality in the rumen digesta as influenced by dietary intake (Russell and Chow 1993). In the present study all the animals were on the same type of basal diet, and therefore any difference in rumen turnover rates due to osmolality could only be attributed to the presence of sucrose supplement in the rumen as was the case in animals on dietary treatments $\mathbf{E_R}$ and $\mathbf{E_{RA}}$. As discussed earlier (**4.4.3**), it is also possible that supplementation of sucrose entirely through the abomasum may have resulted in higher water intake and therefore more fluid in the rumen. However, it is highly unlikely that the extra fluid in the rumen was removed by outflow rather than absorption as this would have increased outflow which is not supported by these results.

4.4.5. Protozoa count in the rumen

The high total protozoa numbers per ml in the rumen of animals supplemented with sucrose entirely intraruminally is consistent with reports from other workers giving supplements of soluble sugars, including molasses (Bird and Leng 1984; Habib 1988). A high protozoa count in the rumen is also associated with high proportion of butyrate (Luther *et al.* 1966; Habib 1988), a trend that was also noted in the present study. Although no attempt was made to quantify the protozoa on the basis of their species, the highest proportion of protozoa consisted mainly of *Entodinium spp.* There was also evidence of large ciliates (*Holotricha*), especially in animals supplemented with sucrose entirely through the rumen. A large population of protozoa, especially the entodiniomorphs can be expected in the rumen of animals supplemented with readily fermentable carbohydrates such as sugars and starch (Bird *et al.* 1979; Newbold *et al.* 1986b). Within the rumen, protozoa have been known to take up carbohydrate quite rapidly, therefore making it unavailable to amylolytic bacteria that would otherwise have fermented it very rapidly to VFA leading to a depression of rumen fluid pH (Williams and Dinusson 1973; Mackie *et al.* 1978; Kariya *et al.* 1989). A low rumen pH can reduce fibre digestion in the rumen and is also likely to impair growth of protozoa as well (Newbold *et al.* 1986b).

The rapid decrease in protozoal numbers noted in the intraruminally supplemented animals within the first 4 h after feeding at 09.00 and again at 16.00 h may have been as a result of the marked fall in rumen pH. The reduction in numbers coincided with the times when the 1st and 2nd doses of sucrose were administered to the supplemented groups. As expected, the variation was more pronounced in the dietary treatments that included at least some sucrose supplementation through the rumen (i.e. $\mathbf{E_R}$ & $\mathbf{E_{RA}}$), than in the control or abomasally supplemented animals. It is noteworthy that these are also the animals that experienced the largest variation in rumen fluid pH. The total protozoa numbers and proportions of various species in the rumen are highly influenced by rumen pH, which in turn depends on the type of diet available to the animal (Newbold *et al.* 1986a, b). The distribution of protozoa in the various ecological niches in the rumen that includes; rumen fluid and sequestration within the rumen epithelium and fibre particles is influenced by the time post-feeding (Dehority 2003). Therefore, the conventional method of sampling the rumen fluid alone is not a very reliable method for estimating the total protozoa population (or biomass) in the rumen (Leng 1989b; Bergen 2004; Sylvester *et al.* 2004). However, in situations where this method is to be relied upon to estimate total protozoa count

based on only a few samplings per day, it has been proposed that the most ideal time for sampling would be about 3 h post-feeding, when most of the protozoa are present in the rumen fluid ingesting the available dietary substrates such as soluble sugars and starch (Dehority and Tirabasso 1989). This is confirmed by the results of present study that showed fairly stable protozoa numbers in the rumen fluid 3 h after feeding, and the numbers remained stable for the rest of the period until it was once again disrupted by the 2nd dose of sucrose in animals receiving whole or part of the sucrose supplement intraruminally.

In spite of a possible beneficial role of protozoa in ameliorating rumen fluid pH, a high protozoa population in the rumen is undesirable because of their negative effect on microbial protein supply to the host animal (Bird *et al.* 1979; Bird and Leng 1984). Protozoa are known to engulf bacteria and therefore reducing their numbers in the rumen. This predatory behaviour is also responsible for the increased microbial N turnover in the rumen, and they also resist washout from the rumen to the small intestines (Hungate *et al.* 1971; Weller and Pilgrim 1974; Nolan 1975; Ulyatt *et al.* 1975b). Although the microbial N production did not differ significantly between the dietary treatments, it was notable that the lowest mean value (18.1 g N/kg OMADR) was for animals on treatment $\mathbf{E_R}$ that received sucrose intraruminally; these sheep had the highest total protozoa count. The reduction of microbial protein flow to the small intestines due to a high total protozoa population in the rumen is likely to reduce the amount of total protein available to the animal (Bird *et al.* 1979). This in turn reduces the protein: energy ratio in the fermentation products absorbed from the gut (Leng 1982a; Bird and Leng 1984), and contributes to low animal productivity (Bird and Leng 1979; Leng *et al.* 1984; Nolan 1989c). The negative effect of protozoa on productivity is likely to be even more pronounced in animals subsisting on low quality roughage, where microbial protein is the main source of amino acids absorbed from the small intestines (Bird and Leng 1979, 1984; Preston and Leng 1987).

4.5. Summary and conclusions

When the animals fed urea-treated basal roughage were supplemented with sucrose wholly or partly through the rumen, cellulolytic activity was not adversely affected. However, there was also no evidence of sucrose supplementation having the *catalytic effect* (suggested by Preston and Leng 1987) on microbial fermentation, apart from the effect of boosting the total quantity of fermentable substrates in the rumen. Although the supplementation with sucrose wholly or partly

through the rumen generally resulted in a higher total VFA concentration and a higher propionate to acetate ratio, and therefore higher predicted glucogenic potential, this did not translate to an enhanced capacity to metabolise acetate at the tissue metabolism level. Similarly, this study did not unequivocally demonstrate that abomasally supplemented sucrose enhanced glucogenic potential in the body tissues. Rather, there was evidence that abomasally supplemented animals were unable to cope with large amounts of sucrose administered via the abomasum, this lead to extensive hindgut fermentation and poor faecal consistency. The effect on fermentation in the large intestine is attributed to low activity of sucrase enzyme in the small intestines of ruminants (Walker 1959b; Siddons 1968), and sucrose escapes to the hindgut where it is fermented by microbes (Ørskov *et al.* 1972). Although intestinal digestion of sugars (and starch) is considered to be more "energetically efficient" than the fermentative digestion in the fore- and hindgut (Leng 1982c; Dixon and Stockdale 1999; Harmon and McLeod 2001), this only applies where the host animal's endogenous digestive enzymes in the small intestines are capable of effectively digesting the carbohydrate.

It is concluded that supplementation of low quality basal roughage with readily digestible carbohydrate sources such as sucrose, is likely to increase total VFA production in the rumen, and also to change the rumen fermentation pattern in favour of higher propionate/acetate ratio. This increases the absorption of DE from the rumen and the energy is also more balanced in glucogenic to acetogenic substrates. Moreover, this is achieved without adversely affecting rumen microbial degradation and therefore, intake of the basal roughage, provided the proportion of the supplement (sucrose) in the total diet is not high. However, the beneficial effect of the sucrose supplement in enhancing microbial protein supply depends on the fermentability of the basal roughage. By-passing rumen fermentation in favour of the more "efficient" intestinal digestion may enhance glucose supply in the body tissues further, but the proportion of sucrose that can be efficiently hydrolysed in the small intestines needs to be determined more precisely. This is mainly due to the limitations associated with postruminal digestion of readily digested carbohydrates in ruminants.

5. CHAPTER FIVE: GENERAL DISCUSSION, CONCLUSIONS AND FURTHER STUDIES

5.1. General discussion and conclusions

High-fibre, low-protein forages also referred to as low quality roughages (mainly crop residues and over-mature native pastures) are an important feed resource for supporting ruminant livestock production, especially in the tropical areas. However, these abundantly available feed resources are largely under-utilised by ruminants, mainly due to low digestibility in the rumen, leading to low rates of digesta clearance from the rumen and therefore low voluntary intake. Furthermore, when these roughages are fermented in the rumen the absorbable end-products are imbalanced in terms of their P:E ratio and also their glucogenic: acetogenic ratio for meeting the needs of host animal tissues. These adverse ratios lead to an inefficient tissue metabolism of digestion products and generate a high heat increment that can lead to heat stress and low feed intake of ruminants, especially under tropical conditions (Preston and Leng 1987, Leng 1990). Because of low voluntary intake the animal may not obtain adequate ME and nutrients for maintenance and so they lose body condition, and ultimately liveweight. This results in very low animal productivity in the tropics, even as the animal protein requirements in these areas continue to increase due to the rapidly growing human population.

In an attempt to address this low digestibility impediment to improved intake of low quality roughage, most attention has focused on two main areas: the high cell wall constituents (CWC), and the low content of rumen degradable nutrients (N and S), as the two main factors contributing to low digestibility and voluntary intake (Preston and Leng 1987; Leng 1989a, 1990). Various forms of physical and chemical treatments have been reported as being effective in increasing digestibility and intake of basal roughage in ruminants. This is mainly due to their effect in reducing particle sizes of the refractory material and/or increasing the susceptibility of the roughage to microbial degradation in the rumen (Sundstol 1978; Lam *et al.* 1992; Lowry and Kennedy 1996). However, these treatments on their own are generally inadequate to achieve high digestibility and intake, mainly due to the basal roughage being deficient in key nutrients that are required by the ruminal cellulolytic microbes for optimal growth (Mathison and Milligan 1971; Leng 1990).

It has for a long time been presumed that once this low digestibility hurdle was removed voluntary intake would improve, leading to delivery of more ME and nutrients to body tissues. However, in recent times attention has been directed to nutrient imbalance at the tissue metabolism level as a possible constraint to increased intake in ruminants. In particular, attention has been given the inadequacy of protein relative to energy or P: E ratio in the body tissues (Illius and Jessop 1996), and the proportion of glucogenic to acetogenic substrates in the digestion or fermentation products absorbed from the gut (Leng 1982c, Leng 1990; Cronje *et al.* 1991). Early work by Egan (1965a, 1977) and others showed that, when the amino acid supply in the body tissues was improved through abomasal infusion of casein, voluntary intake of low quality basal roughage was significantly increased. It was therefore, hypothesised that nutrient imbalance in the body tissues rather than low digestibility *per se* constrained voluntary intake of basal roughage in ruminants. Egan (1977) argued that, provided that tissues are well supplied with balanced nutrients, especially glucose or glucogenic substrates such as propionate and amino acids, ruminants could alleviate the low digestibility hurdle by allowing additional gut distention to accommodate a higher digesta load, and a longer MRT to increase the extent of digestion.

There are therefore opportunities for manipulating rumen fermentation and intestinal digestion through supplementation to stimulate higher absorption of glucogenic substrates (glucose and amino acids) in order to deliver more balanced ratios of nutrients for tissue metabolism. The results should be a more efficient utilization of digestion products, especially the conservation of surplus acetogenic substrates as fat for later use rather than their wasteful oxidation through futile cycles with unwanted heat generation (MacRae and Lobley 1982; MacRae *et al.* 1985; Crabtree *et al.* 1987, 1990). Efficient nutrient capture leads to lower heat increment in the animal and may even stimulate higher basal intake and therefore improved productivity. The interaction between rumen fermentation, intestinal digestion, and voluntary intake is summarized in **Figure 5.1**. The author's studies were therefore undertaken to establish the effect of various supplements (NPN, protein and energy) in improving rumen fermentation and voluntary intake.

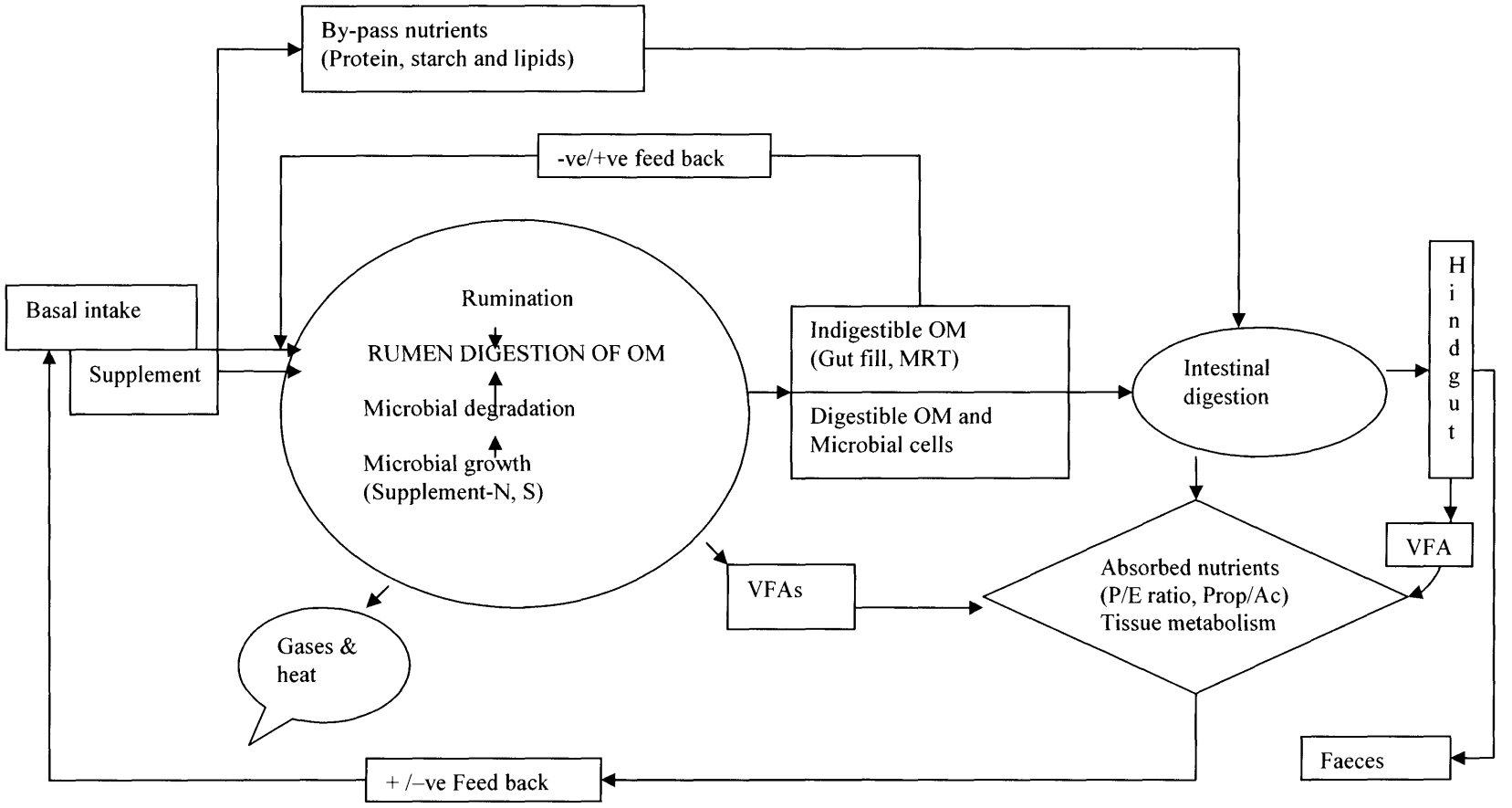


Figure 5.1. The interaction between rumen digestion (fermentation), intestinal digestion and voluntary intake in ruminants.

5.1.1. Preliminary study

The preliminary study (**Exp 1**) was initiated with a hypothesis that correcting nutrient deficiency associated with low quality basal roughage (N, S, etc) would stimulate higher digestibility and therefore intake. It was further hypothesized that the presence of supplemental energy could stimulate even higher microbial growth and fermentation activity after the N deficiency was removed through supplementation with an NPN (urea) or pre-formed protein source leading to higher intake. The preliminary study therefore investigated the role of urea or protein (CSM) with or without energy supplementation in enhancing voluntary intake of oaten chaff basal roughage in sheep. The study showed that basal intake was not increased by urea or protein supplementation irrespective of whether an energy supplement was included or not. However, total dietary intake was increased by protein or protein and energy supplementation but not by urea. It was evident that supplementation with protein and energy resulted in a higher dietary intake without stimulating any increase in basal intake. Therefore, the higher dietary intake was as a result of (increased) intake of the supplement rather than basal roughage, which would be expected considering that both the protein and energy supplements, but not urea contain digestible OM that can yield energy. Like many other trials conducted over a relatively short period of time, this study did not establish any superiority of preformed protein over urea in stimulating basal intake (Kropp *et al.* 1977; Redman *et al.* 1980; Peterson *et al.* 1985; Iwanyanwu *et al.* 1990).

The lack of response in basal roughage intake to urea or protein supplementation was attributed to the moderate N content in the basal roughage (11.8 gN/kgDM or 7% CP), leading to the relatively high intake of the basal diet observed in this study of 785 g/d (73.2 g/kg$^{0.75}$) which was equivalent to about 3.2%BWT. Though low quality roughage are categorized as those forages containing low protein content (5-8% CP) (Leng 1990), response to urea supplementation has only been observed in those that are quite low in N (Körster *et al.* 1996) or relatively high in potentially digestible OM. Very low OM digestibility can limit the intake response to urea supplementation, mainly because intake of such roughage by ruminants is constrained by long MRT, as it normally takes longer for the highly refractory plant material to be comminuted to particle sizes that are small and dense enough to enable them to be cleared from the rumen through the reticulo-omasal orifice (Poppi *et al.* 1981a, b; Lechner-Doll *et al.* 1991; Murphy and Kennedy 1993). For example, there was no response in intake when cows grazing on poor

quality native pastures (6.8% CP, 485 g/kgDM IVOMD) were supplemented with iso-nitrogenous quantities of urea, dried poultry waste (DPW) + urea, soybean meal (SBM), DPW + SBM, or DPW as N sources. Also animals grazing on poor quality winter pastures (4.5-6.0% CP) did not show any basal intake response to undegradable dietary protein supplementation (Hollingworth-Jenkins *et al.* 1996; Lam *et al.* 1997). Available literature shows that responses in intake to urea supplementation of low quality roughage are quite variable, and are mainly influenced by N content (Körster *et al.* 1996), and the proportion of digestible OM relative to crude protein or DOM: CP (Hogan 1996; Jordan *et al.* 2002).

It is concluded from this study that when the N content of the basal roughage is not too low (<5% CP) and/or when the ratio of potentially digestible OM relative to the available CP less that 7:1, it is unlikely for any response in basal roughage intake to be realized with urea (or any other NPN) supplementation. Furthermore, as this study has shown, supplementation of the basal roughage with a protein (with or without energy) is likely to stimulate higher dietary intake while that of the basal roughage is either depressed (substitution effect) or unchanged.

5.1.2. Effect of sucrose supplementation intraruminally or abomasally on basal intake

A comprehensive investigation was undertaken (**Exp 2**) to test the hypothesis that increasing production and absorption of glucogenic substrates from the gut (mainly rumen and small intestines) would enhance glucogenic potential in the body tissues, and therefore stimulate higher voluntary intake. This study was also undertaken to compare the relative efficiencies of rumen fermentation and postruminal digestion of iso-energetic quantity of sucrose (energy) in enhancing the tissue clearance of an intravenous load of acetate. It was therefore, further hypothesized that a the supplementation regime that increased the glucose supply to the body tissues would have the greatest effect in enhancing tissue metabolism of acetate and acetate clearance rate, would also stimulate intake of the basal roughage. The study therefore involved supplementation of basal roughage diet with iso-energetic quantities of sucrose; intraruminally ($\mathbf{E_R}$), abomasally ($\mathbf{E_A}$) or through both routes in equal proportions (50:50) ($\mathbf{E_{RA}}$).

The study showed that when animals were supplemented with sucrose intraruminally, there was a higher ruminal fermentation activity that elevated total VFA concentration in the rumen, and

consequently lowered the rumen fluid pH. The lowest mean daily pH for the four treatments was in the animals receiving sucrose input entirely through the rumen ($\mathbf{E_R}$). However, only a moderate decrease in pH was noted in the rumen of animals supplemented with sucrose through both intra-ruminal and abomasal routes ($\mathbf{E_{RA}}$), while that of unsupplemented (control) animals or those supplemented with sucrose entirely through the abomasum was relatively higher (6.32-6.44). Although supplementation of basal roughage with readily fermentable carbohydrates may reduce rumen fluid pH and therefore depress cellulolytic activity (Mould *et al.* 1983; Mould and Ørskov 1983), this only becomes pronounced when the pH falls below 6.0, the minimum value in the range (6.0-7.0) considered suitable for optimal growth and activity of cellulolytic microbes, and remains below this level for a prolonged period (Miller and Muntifering1985; Hoover 1986). In this study intraruminal supplementation with sucrose did not adversely affect rumen microbial degradation of barley straw *in sacco;* degradation parameters such as rate of degradation, lag time, and potential degradability showed no significant difference between the unsupplemented and supplemented animals. The effective buffering of copious saliva flow stimulated by the high fibre content in the basal diet was probably the reason for pH remaining above 6.0, and, as there was adequate ammonia to supply the N needs of fibre digesting bacteria, digestibility was unaffected by ruminally adminisrtered sucrose.

The study showed that the total VFA concentration in the rumen of animals on the four dietary treatments varied widely over the 24-h feeding period. Though the VFA concentration in the rumen at any time is determined primarily by production (fermentation) and removal (absorption and outflow) other factors such as dilution from water intake and salivary outflow, and the trans-epithelial water movement may be important. This study also showed that as well as stimulating higher total VFA production, intra-ruminal sucrose supplementation also markedly changed the fermentation pattern in the rumen, mainly in favour of higher propionate: acetate ratio, and to some extent also elevated the proportion of butyrate. In contrast, when the same quantity of sucrose was administered entirely through the abomasum, the VFA fermentation pattern in the rumen was characterized by a relatively high proportion of acetate and was very similar to that of the unsupplemented animals. Because propionate is a glucogenic substrate, it can be expected that a fermentation pattern that is high in propionate relative to acetate is likely to result in higher gluconeogenesis in the liver. This will boost the glucose supply for tissue metabolism (Leng 1982c; Cronje *et al.* 1991). Similarly, abomasal supplementation of sucrose was expected to result in higher alimentary glucose absorption and to increase glucose supply to the body tissues,

provided that sucrase activity in the intestines was high enough to hydrolyse all the sucrose to glucose and fructose. Given the relatively "higher efficiency" of intestinal digestion compared to the "inefficiencies" associated with rumen microbial fermentation, and the energy cost of gluconeogenesis in the liver, it was expected that the abomasally supplemented animals would end up with a higher quantity of circulating glucose than animals in the other dietary treatments. However, this was not supported by the acetate clearance rates results for which the unsupplenmented animals and those supplemented abomasally with sucrose were not significantly different. However, the clearance curves had considerable variability and the mean rates tended to point to a higher acetate clearance by abomsally supplemented animals. Therefore, there is a possibility that differences in acetate clearance rates between dietary treatments were masked by the large variation in the estimates of acetate clearance constant.

The relatively higher dietary and basal DM intake in animals supplemented with sucrose entirely through the rumen or abomasum may be attributed to a number of factors acting either individually or together. Firstly, high digestibility in both dietary treatments($\mathbf{E_R}$ and $\mathbf{E_A}$) may have resulted in a higher intake, though caution is needed because *in sacco* results showed that microbial degradation in the rumen was not significantly different between the four dietary treatments. This suggests that differences in total apparent DM digestibility between these two dietary treatments and the others were mainly due to post-ruminal (hindgut) digestion. However, when this is taken within the context of the fact that the highest proportion of DM in basal roughage is normally digested in the foregut, with up to 25-30% being digested in the hindgut (Ulyatt *et al.* 1975a), then it is highly unlikely that higher digestibility was the only factor responsible for the high intake in the intraruminally and abomasally supplemented animals. Secondly, as mentioned above there is a possibility that the predicted higher glucogenic potential following sucrose supplementation wholly intraruminally or abomasally may have indeed resulted in a higher acetate metabolism rate. A higher acetate clearance rate may assist the animal to metabolise VFA energy more quickly and presumably more efficiently through conversion to LCFA and therefore stimulate basal intake (Leng 1990; Cronje *et al.* 1991).

5.1.3. Effect of sucrose supplementation on N digestion and metabolism

The results of this study also showed that supplying of sucrose intraruminally or abomasally can bring about major differences in N digestion and metabolism. Nitrogen intake was highest in

intraruminally or abomasally supplemented animals due to the higher total and basal DM intake, given that the basal diet was quite high in N (22.2 g/kgDM). However, since most of the N in the basal roughage was mainly in a form that is highly degradable in the rumen (see **4.4.1**), most of it was probably rapidly absorbed across the rumen epithelium as ammonia and transported to the liver for conversion to urea and excreted in urine (McDonald 1948). This is supported by the high urinary N excretion in all the four dietary treatments that accounted for nearly 60% of the total N intake.

The faecal N excretion was generally high in all animals supplemented with sucrose, and more so in those in which all or part of the sucrose supplement was delivered through the abomasum. The high faecal N excretion in the abomasally supplemented animals was attributed mainly to the extensive microbial fermentation in the hindgut of sucrose that was probably not effectively digested and absorbed in the small intestines. Sucrase Iinvertase) activity in the small intestines of ruminants is generally low (Ørskov *et al.* 1972), making it difficult for ruminants to effectively digest and/or absorb large quantities of readily fermentable carbohydrates in the small intestines (Ørskov *et al.* 1971; DeGregorio *et al.* 1982; Siciliano-Jones and Murphy 1989a, 1989b). Any undigested and/or unabsorbed carbohydrate will end up in the hindgut where it is extensively fermented by microbes to VFA, and unlike the VFA or ammonia that are readily absorbed into the blood, most of the microbial biomass from hindgut fermentation is voided in faeces (Øskov *et al.* 1971b, 1972). When readily digestible carbohydrate reaches the hindgut and is fermented to VFA and gases, the microbial biomass is also added to the fecal N, thereby contributing to the depressed N digestibility, even without necessarily affecting the amino acid N absorbed into the body tissues (Kay 1969). Moreover, extensive microbial fermentation in the hindgut, combined with the poor buffering capacity in the hindgut compared to the rumen, may predispose the animal to lactic acidosis (Rowe 1994; Rowe 1997).

Microbial N synthesis in the rumen is mainly influenced by the supply of fermentable OM, ammonia, and minerals such as S (Tamminga 1982a; Leng 1982c; Kelly *et al.* 1993). However, for maximum flow of the microbial biomass to the small intestine, synthesis has to be complemented by an appropriate rumen turnover rate for both the fluids fraction (dilution) and the solids in the digesta. Considering that, in this study, rumen ammonia concentrations were not limiting in any of the four dietary treatments, and that, on average, extensive rumination must have ensured reasonable dilution rates in the rumen, it was expected that microbial protein

synthesis in the rumen and supply to the intestines would be enhanced by intraruminal supplementation with sucrose. However, this was not the case because sucrose supplementation, irrespective of the avenue used, did not significantly increase microbial protein supply beyond the level achieved with the unsupplemented basal roughage diet (control). This suggests that fermentable OM and ammonia were not limiting microbial synthesis in the rumen and flow to the intestines. It is unclear what other factor(s) may have limited microbial growth efficiency in the rumen given that the microbial N production determined in this study of 18-19 gMN/kg OMADR was on average only 40% of the upper limit of the normal range of efficiencies (14-49) suggested by ARC (1984). However, given that the microbial fermentation of most low quality roughage is dominated by the slow-growing celluloytic microbes (bacteria and anaerobic fungi), it is possible that the dilution rates obtaining in the rumen (0.04-0.07/h) were the most appropriate for that growth rate. Moreover, a large proportion of cellulolytic microbial biomass in low quality basal roughage is associated with particles, and therefore any increase in dilution rates is unlikely to stimulate higher microbial growth efficiency. Furthermore, changes in composition of microbial groups in the rumen occasioned by intraruminal supplementation of sucrose, (e.g. increase in protozoa numbers) whose net benefit is not always positive, may have reduced microbial growth efficiency.

5.1.4. Conclusion

It is concluded from this study (**Exp 2**) that ammoniation of low quality roughage with urea increases the N content and the degradability of the roughage in the rumen. This results in a higher production of fermentation products (VFA and microbial cells) as shown by the high total VFA concentration. A further increase in the production of fermentation products was achieved through supplementation with readily fermentable carbohydrate (sucrose). This also had the added benefit of changing the fermentation pattern in the rumen mainly in favour of higher propionate: acetate ratio which enhanced the glucogenic potential in the body tissues and may have promoted more efficient metabolism of acetogenic energy substrates (acetate and butyrate). Although the supplementation with sucrose intraruminally had the potential to reduce ruminal pH and therefore depress cellulolytic activity, this did not happen mainly because the basal diet was adequately supplied with N leading to high ammonia concentration in the rumen and the fibre content in the basal diet stimulated sustained rumination which ensured that salivary secretions were able to buffer the rumen contents and maintain pH above 6.0.

It is possible to increase further the glucogenic potential in the body tissues of the ruminants by having dietary carbohydrate by-pass rumen fermentation and undergo the more "efficient" intestinal digestion. However, as was shown by this study, when sucrose is used the proportion of by-passed carbohydrate that can be efficiently digested and absorbed postruminally is probably low. The intestinal digestibility of sucrose is uncertain and requires further study. Taking into consideration the apparent limitation of sucrose digestion postruminally, it is concluded that supplementation via the rumen may be the most viable option if sucrose is to be used as a supplement for ruminants subsisting on low quality basal roughage.

5.2. Suggestions for further studies/ research

This study has shown that there are opportunities for utilising readily fermentable carbohydrates such as sucrose as supplements to boost the level of fermentable substrates in the rumen and absorption of fermentation products (VFA and microbial cells). The enhanced rumen fermentation is also characterized by high propionate: acetate ratio and therefore higher supply of glucogenic to acetogenic substrates to host tissues. Further studies are needed to establish why the extra glucogenic potential that was predicted to occur on the basis of rumen fermentation parameters did not translate unequivocally to a more efficient metabolism of acetate.

Compared to monogastric, ruminants generally tend to have a low sucrase (invertase) activity in the small intestines (Walker 1959b; Siddons 1968), and this is the major cause of the extensive hindgut fermentation when large quantities of sugar are digested postruminally (Ørskov *et al.* 1972). The problem of hindgut fermentation was mild in those animals supplemented 50:50 with sucrose via the rumen and abomasum ($\mathbf{E_{RA}}$), and even the animals that received all sucrose abomasally ($\mathbf{E_A}$) seemed to adapt to the sucrose supplementation over time (one month). A longer trial is required to establish whether the animals can adapt to handling larger amounts of sucrose by inducing higher sucrase secretion in a way similar to what has been observed with starch digesting enzymes (Clary *et al.* 1967; Beever and Armstrong 1969). It also needs to be established whether the adjustment can be accelerated by starting with small amounts of sucrose and gradually increasing the daily ration. Moreover, it also need to be established whether the gut tissues in various parts of the GIT can adapt to facilitate transport of larger quantities of dietary sucrose or its digestion products (glucose & fructose), given that such adaptation has been

speculated on the basis of *in vitro* studies (Zhao *et al.* 1998). In this study, it was apparent that the optimal proportion of sucrose that can be by-passed to the abomasum without causing extensive hindgut fermentation is between 0 and 50% of dietary sucrose supplement. Further studies need to be conducted to determine more precisely the exact proportion of dietary sucrose that can be by-passed to the abomasum to optimise alimentary glucose absorption and minimize wasteful fermentation of sucrose in the hindgut. In addition, there is need for investigations to establish the most viable and practical way of protecting sugars such as sucrose from rumen fermentation so that they can be intestinally digested, and for the method to have practical application under field conditions.

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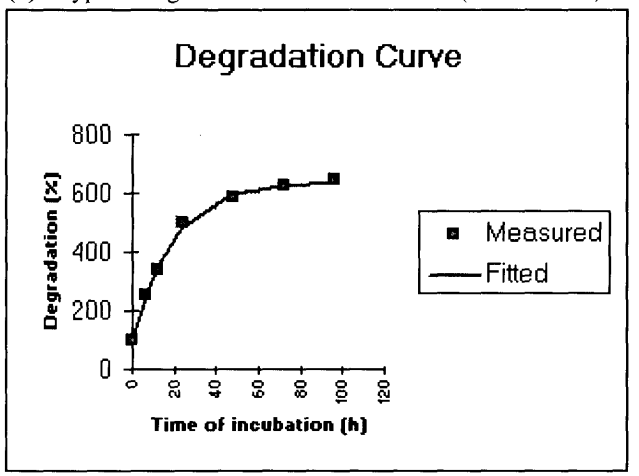
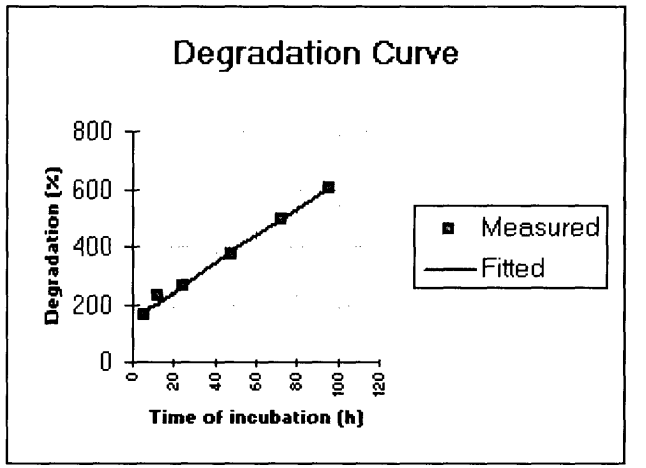
APPENDICES

APPENDIX I. The *in sacco* data for all the four animals in four periods (NB asymptotic curve could not be fitted for this data of animal **No 916**, P2, T1)

(a). Typical degradation curve for the data (15 out of 16).



(b) Plot for T1 period 2 (Animal No 916) only



Treatment T1(E0)

	P1T1 (3492)	P2T1 (916)	P3T1 (3498)	P4T1(3500)
a	100.9	142.7	82.8	89.3
b	541.1	2357.5	564.9	510.6
c	0.052	0.002	0.038	0.047
Sum of sq.	480.60	833.86	2213.87	1578.23
No. of obs.	7	6	7	7
RSD	10.96	**16.67**	**23.53**	19.86

Treatment T3 (EA)

	P1T3 (915)	P2T3(3498)	P3T3(3492)	P4T3 (916)
a	107.0	89.6	87.6	84.6
b	550.0	563.6	434.1	544.0
c	0.051	0.040	0.019	0.028
Sum of sq.	374.07	1708.09	1378.52	3108.94
No. of obs.	7	7	6	7
RSD	9.67	20.66	21.44	27.88

P1T2 (916)

a	103.1
b	494.3
c	0.044
Sum of sq.	1666.79
No. of obs.	7
RSD	20.41

P2T2 (3492)

a	93.5
b	523.1
c	0.028
Sum of sq.	1370.83
No. of obs.	7
RSD	**18.51**

P3T2(915)

a	73.8
b	573.0
c	0.037
Sum of sq.	2859.53
No. of obs.	7
RSD	26.74

Treatment T2 (ER)

P4T2 (3498)

a	80.1
b	541.7
c	0.042
Sum of sq.	3864.13
No. of obs.	7
RSD	31.08

Treatment T4 (ERA)

	P1T4 (3498)	P2T4(915)	P3T4(916)	P4T4(816)
a	106.4	81.7	93.2	109.1
b	507.6	558.2	587.3	539.1
c	0.053	0.042	0.024	0.014
Sum of sq.	24.21	3394.22	900.95	1720.09
No. of obs.	7	7	7	7
RSD	2.46	29.13	15.01	20.74

Appendix II. Summarised rumen liquid kinetics (Cr-EDTA) and acetate clearance rate data for the four periods.

Cr-EDTA data	Animal	Regression equation	COD R2	Rate const. k	y' at t=0 ln[Cr]	y= [Cr]0 [Cr] at t=0	Rumen vol. (litres)	outflow rate (L/d)	T1/2 (h)
Period 1	T1 3492	y1 = -0.0724x + 2.1496	R2 = 0.8718	0.0724	2.1496	8.581	6.52	11.32	9.57
	T2 916	y2 = -0.0755x + 1.8343	R2 = 0.9712	0.0755	1.8343	6.261	8.93	16.19	9.18
	T3 915	y3 = -0.0716x + 1.7872	R2 = 0.9104	0.0716	1.7872	5.973	9.36	16.09	9.68
	T4 3498	y4 = -0.0729x + 1.985	R2 = 0.985	0.0729	1.9850	7.279	7.68	13.44	9.51
Period 2	T1 916	y1 = -0.0477x + 1.6095	R2 = 0.6845	0.0477	1.6095	5.000	11.18	12.80	14.53
	T2 3492	y2 = -0.0676x + 2.1313	R2 = 0.9824	0.0676	2.1313	8.426	6.64	10.77	10.25
	T3 3498	y3 = -0.0398x + 2.1754	R2 = 0.5916	0.0398	2.1754	8.806	6.35	6.07	17.41
	T4 915	y4 = -0.0705x + 1.7316	R2 = 0.9042	0.0705	1.7316	5.650	9.90	16.75	9.83
Period 3	T1 3498	y1 = -0.0586x + 1.8435	R2 = 0.9449	0.0586	1.8435	6.319	8.85	12.45	11.83
	T2 915	y2 = -0.0674x + 1.6083	R2 = 0.9777	0.0674	1.6083	4.994	11.20	18.11	10.28
	T3 (3492*)	y3 = -0.010x + 2.2444	R2 = 0.6407	0.0100	2.2444	9.435	5.93	1.42	69.30
	T4 916	y4 = -0.0461x + 1.6224	R2 = 0.7307	0.0461	1.6224	5.065	11.04	12.22	15.03
Period 4	T13500(915*)	y1 = -0.0417x + 1.8473	R2 = 0.9513	0.0417	1.8473	6.343	8.82	8.82	16.62
	T2 3498	y2 = -0.0689x + 1.9029	R2 = 0.9723	0.0689	1.9029	6.705	8.34	13.79	10.06
	T3 916	y3 = -0.0444x + 1.5899	R2 = 0.8555	0.0444	1.5899	4.903	11.41	12.15	15.61
	T4 3492*	y4 = -0.0138x + 1.8048	R2 = 0.7081	0.0138	1.8048	6.079	9.20	3.05	50.22

Acetate data	Animal	Regression equation	COD R2	Rate const. k (/min)	y' at t=0 ln[Ac]	y= [Ac]0 [Ac] at t=0	Blood Ac. compart.(L)	Acetate clearance rate (mol/hr)	T1/2 (h)
Period 1	T1 3492	y1 = -0.0091x + 1.2415	R2 = 0.2621	0.009	1.24	3.461	52.01	0.10	1.27
	T2 916	y2 = -0.0025x + 0.4246	R2 = 0.0545	0.003	0.42	1.529	117.73	0.03	4.62
	T3 915	y3 = -0.0071x + 0.5581	R2 = 0.7178	0.007	0.56	1.747	103.01	0.08	1.63
	T4 3498	y4 = 0.0018x - 1.023	R2 = 0.0504	0.002	1.02	2.782	64.71	0.02	6.42
Period 2	T1 916	y1 = -0.0114x + 1.3688	R2 = 0.7417	0.011	1.37	3.931	45.79	0.12	1.01
	T2 3492	y2 = -0.0045x + 0.422	R2 = 0.2351	0.005	0.42	1.525	118.03	0.05	2.57
	T3 3498	y3 = -0.0222x + 0.9891	R2 = 0.5408	0.022	0.99	2.689	66.94	0.24	0.52
	T4 915	y4 = 0.0077x - 1.9147	R2 = 0.051	0.008	1.91	6.785	26.53	0.08	1.50
Period 3	T1 3498	y1 = -0.0079x + 1.1437	R2 = 0.4901	0.008	1.14	3.138	57.35	0.09	1.46
	T2 915 *	*	*		*	*	*	*	*
	T3 3492*	y3 = -0.0119x + 1.3772	R2 = 0.3103	0.012	1.38	3.964	45.41	0.13	0.97
	T4 916	y4 = -0.0034x + 0.2746	R2 = 0.0284	0.003	0.27	1.316	136.78	0.04	3.40
Period 4	T1 3500(915*)	y1 = -0.0079x + 1.1558	R2 = 0.2018	0.008	1.16	3.177	56.67	0.09	1.46
	T2 3498	y2 = -0.0179x + 1.6261	R2 = 0.9777	0.018	1.63	5.084	35.41	0.19	0.65
	T3 916	y3 = -0.018x + 1.1823	R2 = 0.7789	0.018	1.18	3.262	55.18	0.19	0.64
	T4 3492*	y4 = -0.0129x + 1.5789	R2 = 0.6233	0.013	1.58	4.850	37.12	0.14	0.90

Appendix III. Primary data on the spiking of rumen fluid samples to determine Cr recovery and validatation of the method used.

Sample Serial No	Animal /Sample No	Sample Treatment	Schott bottle wt.	Sample wt.	Dilution to 25g	Actual wt	Actual H2O addded	Dilution Factor	Cr conc. µg/ml	After correct. for dil.	% Recovery
P2/57	Unspiked (209)	T4S5	88.8308	2.5001	113.8308	113.8834	25.0526	10.0206	0.386	3.8680	
P1/32	Unspiked (210)	T4S6	89.3468	2.5137	114.3468	114.3663	25.0195	9.9533	0.430	4.2799	
P3/122	Unspiked (213)	T4S5	93.9685	2.5211	118.9685	119.0037	25.0352	9.9303	0.378	3.7536	
P4/185	Unspiked (214)	T2S3	91.3495	2.5162	116.3495	116.3966	25.0471	9.9543	0.696	6.9282	
209*	P2/T4S5/74-915	Random-11	94.7510	2.5479	119.7510	119.7888	25.0378	9.8268	2.990	29.3822	
210*	P1/T4S6/76-3498	Random-12	89.9397	2.5445	114.9397	114.9439	25.0042	9.8268	3.020	29.6768	
211	Cr-only	25µL	89.2555	0.0250	114.2555	114.2797	25.0242	1000.9680	2.740	2742.6523	
212	Cr-only	50µL	93.9743	0.0472	118.9743	118.9836	25.0093	529.8581	5.370	2845.3377	
213*	P3/T4S5/75-916	Random-21	93.8427	2.5516	118.8427	118.8690	25.0263	9.8081	2.950	28.9338	
214*	P4/T2S3/76-3498	Random-22	93.4905	2.5385	118.4905	118.5039	25.0134	9.8536	3.300	32.5169	
215	Cr-only	25µL	89.7462	0.0253	114.7462	114.7654	25.0192	988.9012	2.800	2768.9233	
216	Cr-only	50µL	95.2728	0.0497	120.2728	120.2794	25.0066	503.1509	5.370	2701.9204	
217	CR-EDTA (Stock)	0.101g Cr	44.7750	0.1010	94.7750	96.5970	51.8220	513.0891	5.450	2796.3356	
	Mean Cr conc.				2742.652	2845.338	2768.923	2701.9204	2796.335644	2771.033876	% Recovery
209	P2/T4S5/74-915	Random-11	94.7510	2.5479	119.7510	119.7888	25.0378	9.8268	2.990	29.3822	96.99
210	P1/T4S6/76-3498	Random-12	89.9397	2.5445	114.9397	114.9439	25.0042	9.8268	3.020	29.6768	93.97
213	P3/T4S5/75-916	Random-21	93.8427	2.5516	118.8427	118.8690	25.0263	9.8081	2.950	28.9338	93.73
214	P4/T2S3/76-3498	Random-22	93.4905	2.5385	118.4905	118.5039	25.0134	9.8536	3.300	32.5169	94.86
								2742.65232	2768.92332	2755.78782	

*spiked samples

Mean recovery

94.89 **(95%)**