I. General Introduction

There has been heated discussion in the literature on what aspect - breeding or feeding - should be given most attention in improving animal production in a harsh environment such as the tropics It has been claimed that improving animal genotype through breeding programs is both more feasible and can provide better long-term benefits than attempts to change the environment by improving animal management and nutrition, even though the latter seems to offer immediate benefits (Barker, 1994). Thus Cunningham (1991) demonstrated that a crossbreeding program which combined the high milk yield of temperate (*Bos taurus*) dairy cattle and the heat tolerance of tropical (*Bos indicus*) cattle increased milk production by 100% compared to that of local cattle.

On the other hand, Webster (1991) maintained that selected breeds are unlikely to absolutely 'outperform' indigenous breeds as they will experience some reduction in their production capacity under tropical environments. Preston and Leng (1987) demonstrated that animals of improved genotype are superior to the local breeds only when environmental conditions are favourable, while under stressful environments, improved breeds offer no advantage.

Leng (1990) further suggested that differences in productivity between breeds could disappear when animals are fed correctly supplemented diets. When offered a low quality unsupplemented hay, for example, Brahman cattle have been shown to be more efficient in utilising feed than Brahman x Hereford or Hereford cattle. When the protein to energy ratio (P/E ratio: the relative proportion of absorbed protein to VFA energy) of the diet was increased by supplementation with by-pass protein meal, however, the superiority of Brahman cattle in feed efficiency was eliminated. The responses to bypass protein supplementation, however, have been reported to vary with the genotype of animals, the nature of the basal diet used and the environmental conditions (Leng *et al.*, 1977). Hogan (1996) also emphasised the point that the current productivity of most ruminants which rely on forages is considerably less than their genetic potential. The gap between potential and the actual will be further widened with any increase in genetic potential without a corresponding improvement in feeding management.

Thus the interactions between environment and genotype have significant impact on productivity of tropical livestock (Preston and Leng, 1987), and in order to obtain a more meaningful practical outcome, research should be aimed towards providing a clear understanding of the interaction between genotype and nutrition in ruminant production (Oldham, 1995). While the importance of other factors affecting production is well recognised, the present study was undertaken to explore the possibilities of manipulating the nutritional status of ruminants - with particular reference to bypass protein supplementation - in order to improve their ability to produce under tropical environments. Increasing ruminant productivity by improving feed utilisation is most relevant to tropical regions since most small-holders have a preference for improving the productivity of indigenous breeds within existing systems based on available feed resources.

Low ruminant productivity in the tropics has been identified as a direct effect of high ambient temperature and/or humidity and is also due to the low quality, compared to that in the temperate regions, of feedstuffs available and the interaction between these climate factors and nutrition (Church, 1970; Devendra and Burns, 1983; Leng, 1990). It has been hypothesised (Leng, 1990) that under tropical conditions, increasing the P/E ratio of the absorbed nutrients by providing additional protein which escapes rumen fermentation can minimise total heat production and heat stress in animals and thus increase feed intake and ruminant productivity. This theory is, however, based on a compilation of results from a number of experiments in which environmental conditions may have varied considerably and, as a consequence, it requires examination under more controlled conditions. A series of experiments has therefore been carried out to establish the effects of bypass protein supplementation on performance of ruminants exposed to controlled high ambient temperatures.

II. Review of Literature

Chapter 1. Digestion and metabolism in ruminants

The ruminant digestion system is characterised by a symbiotic relationship between rumen microorganisms, which are capable of synthetising fibre digesting enzymes, and the host that enables ruminants to survive on high-fibre plant materials (Brockman, 1993; Theodorou and France, 1993). With the ability to synthesise high quality animal products such as meat and milk from low quality forages, ruminants have significant potential to contribute to improving the welfare of the world human population (Beever, 1993).

Ruminants differ from monogastric animals due to the presence of a complex stomach which consists of four compartments; reticulum, rumen, omasum and abomasum. The first two compartments, which are often referred to collectively as the reticulo-rumen, are only separated by the reticulo-rumen fold and as a result, feed particles can easily flow from one to the other. The reticulo-rumen is the place where most bacterial activity occurs. The function of the omasum is to screen large feed particles and to absorb water, acids and other substances. The function of the abomasum, on the other hand, resembles that of the stomach of monogastric animals (Hungate, 1966; Church, 1970)

The rumen provides an anaerobic environment suitable for microbial growth. The temperature in the rumen ranges from 38 to 42°C and the pH ranges from 5.5 to 7.0. This relatively constant pH is maintained by the buffering action of bicarbonate and the phosphate salts of saliva, which is secreted in copious amounts (Church, 1970; Theodorou and France. 1993). The rumen is one of the most dense and complex natural microbial ecosystems, inhabited by three principal groups of microorganisms namely bacteria, protozoa and fungi, of which, over 200 species of rumen bacteria and at least 20 species of protozoa have been identified (Czerkawski, 1986). The concentrations of bacteria, protozoa and fungi in the rumen range from 10^9-10^{10}, 10^5-10^6 and 10^3-10^5 per ml of rumen fluid respectively (Theodorou and France, 1993). These numbers, however, are affected by the amount and composition of dietary nutrients entering the rumen as well as by other factors such as the

competition between protozoa and bacteria for substrates (Hungate, 1966; Church, 1970; Preston and Leng, 1987).

1.1. Rumen fermentation

The soluble components of ingested nutrients such as carbohydrate and proteins are fermented by rumen microbes. On the other hand, undegraded components such as bypass protein escape rumen fermentation and pass to the small intestines where they are subject to digestion by the host's enzymes, and become available for absorption (Leng, 1986). The end-products of rumen fermentation are volatile fatty acids (VFA; e.g. acetate, propionate and butyrate), ammonia, methane and sometimes lactic acid, and microbial cells (Preston and Leng, 1987; Brockman, 1993; Russell and Strobel, 1993).

As far as the host animal is concerned, the products of fermentation that are nutritionally valuable are the VFAs and the microbial cells (Nolan, 1993). Although the VFAs are microbial waste products, VFAs are the main energy source for the host, accounting for about 70-80% of energy disappearing from the rumen and representing 50 to 70% of digestible energy intake (Brockman, 1993; France and Siddons, 1993). Rumen microorganisms utilise the intermediates of carbohydrate fermentation (triose phosphate, pyruvate and malate) as sources of carbon skeletons for synthesis of polymers for their own cells. They also utilise ATP generated during the fermentation process for both their maintenance and growth (Nolan, 1993; Russel and Strobel, 1993). The microbial cells, after being digested in the small intestines, become a principal source of amino acids for the host (Beever, 1993; Nolan, 1993).

1.1.1. Production, absorption and metabolism of volatile fatty acids

Carbohydrates, principally polysaccharides (hemicelluloses, cellulose, pectins, fructans and starches), some disaccharides such as sucrose, and monosaccharides such as glucose, are the major sources of energy both for the rumen microbes and for the host (Church, 1970). The soluble components of carbohydrates are initially degraded in the rumen to hexoses and pentoses and are then fermented via pyruvate to produce VFAs. Hexoses are metabolised to pyruvate through the Embden-Meyerhof glycolytic pathway, as illustrated in Figure 1.1. Acetate and butyrate are both formed from acetyl CoA, while propionate is formed from pyruvate mainly through the succinate pathway and alternatively through the acrylate pathway.

Excesses of reducing power during the formation of acetate and butyrate from hexose are mainly used during methane formation and also for propionate formation (France and Siddons, 1993).

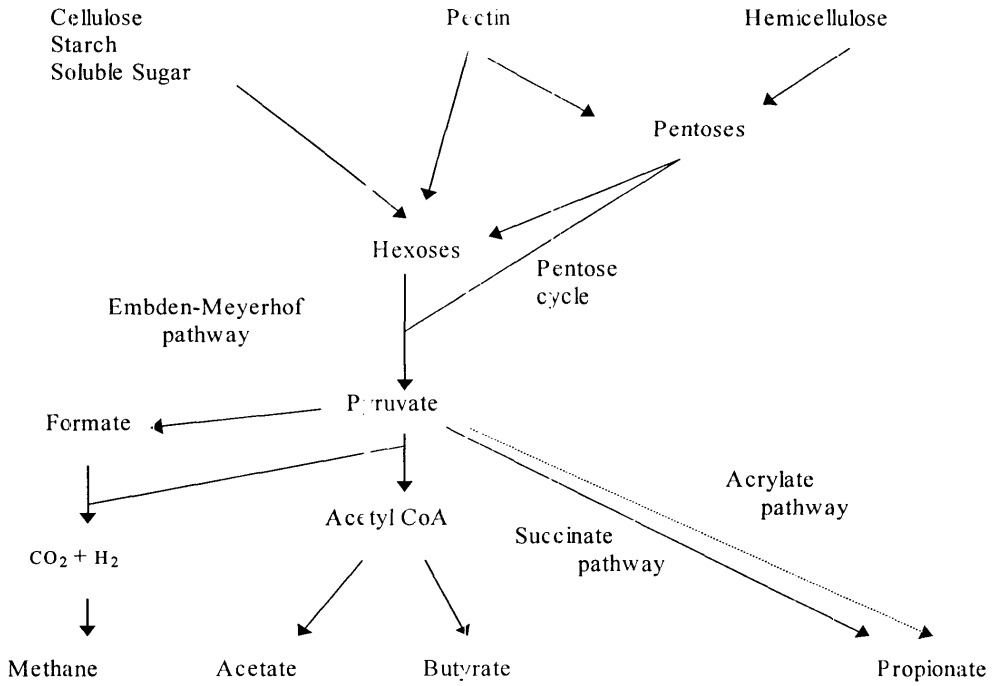

Figure 1.1. A schematic representation of the major pathway of carbohydrate metabolism in the rumen (from France and Siddons, 1993).

The whole reactions of hexose fermentation can be summarised as follows:

hexose $\rightarrow$ 2 pyruvate + 4H

pyruvate $\rightarrow$ acetate $+CO_2$ +2H

2 pyruvate $\rightarrow$ butyrate $+ 2CO_2$

pyruvate + 4H $\rightarrow$ propionate + H_2O

CO_2 + 8H $\rightarrow$ methane $+2H_2O$

It has generally been observed that roughage diets produce the highest molar proportion of acetate; concentrate diets generate higher proportions of propionate, even though the proportion of acetate remains the highest on all diets (Orskov and MacLeod, 1990; Beever, 1993; France and Siddons, 1993; Dijkstra, 1994). Beever (1993) summarised the reactions involved in VFA formation, highlighting the

changes in individual VFAs produced from carbohydrate fermentation in high forage, high cereal and high molasses diets as follows:

high forage diet :

1 mol carbohydrate → *1.34 mol acetate* + 0.45 mol propionate + 0.11 mol butyrate + 0.61 mol methane + 4.62 mol ATP

high cereal diet:

1 mol carbohydrate → 0.90 mol acetate + *0.70 mol propionate* + 0.20 mol butyrate + 0.38 mol methane + 4.38 mol ATP

high molasses diet:

1 mol carbohydrate → 0.94 mol acetate + 0.40 mol propionate + *0.33 mol butyrate* + 0.54 mol methane + 4.5 mol ATP

However, this classification, which is based on the type of available substrates, has been considered an oversimplification, because other factors such as interactions between available substrates and between species of rumen microbes also affect the VFA produced (Dijkstra, 1994). The amount of soluble carbohydrate, for example, may reduce the pH of rumen fluid and this can affect the rate of fermentation of structural carbohydrate (Sutton, 1985; Dijkstra, 1994; Orskov, 1994). Similarly, whether carbohydrate is fermented or directly incorporated into microbial cells depends on the availability of protein (Russel and Hespell, 1981; Dijkstra, 1994). Moreover, fermentation of starch and sugars by bacteria will theoretically yield more propionic acid, and less acetic and butyric acid, than would be if the same substrates were fermented by protozoa (Dijkstra, 1994).

The normal concentration of VFA in the rumen ranges between 70 and 130 mM, but it can vary from 30 to 200 mM (France and Siddons, 1993). Apparently, VFAs are absorbed by simple diffusion through the rumen wall (Preston and Leng, 1987). Since the pK of VFA is low (about 4.8) and the pH of rumen fluid is normally between 6-7, the VFAs in rumen fluid exist mainly in dissociated form, but on absorption these acids become undissociated (Dijkstra, 1994; Orskov, 1994). Based on a model proposed by Gäbel and Martens (1991), Dijkstra (1994) described the mechanism of VFA absorption through the rumen epithelium as illustrated in Figure 1.2. The model suggests that only undissociated VFAs can diffuse through the membrane due to the lipophilic characteristics of the epithelium, and after entry they become dissociated due to the higher pH of the cell (approximately 7.0) compared to

the low pK of the VFA. Theoretically, because almost all VFAs in the rumen are dissociated, only a very small proportion of the VFA produced is absorbed by diffusion. However, as illustrated in Figure 1.2, continuous supply of H^+ ions in the rumen fluid by Na^+/H^+ exchange, the rate of which is increased when pH falls, ensures a high rate of VFA absorption (Dijkstra, 1994).

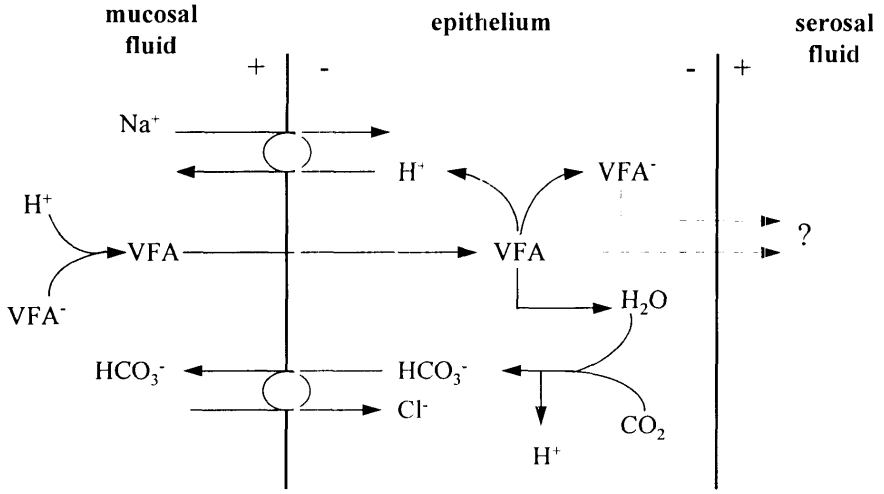

Figure 1.2. A proposed model for the transport mechanism of VFA from the rumen (from Gäbel and Martens, 1991; adapted by Dijkstra, 1994)

MacLeod and Orskov (1984) and Orskov (1994) also confirmed that the rate of VFA absorption is dependent on the pH of the rumen fluid, and propionic and butyric acids are absorbed more rapidly than acetic acid when rumen pH is low.

Acetate, propionate and butyrate can all be oxidised to generate energy, but only propionate can be used as a precursor for net glucose synthesis. Given the fact that, in most dietary conditions, ruminants absorb very little or no glucose from dietary sources, the role of propionate as a glucose precursor is very important (MacRae and Lobley, 1982; Cridland, 1983; Preston and Leng, 1987; Brockman, 1993; France and Siddons, 1993; Dijkstra, 1994).

Acetate is also used for fat synthesis with an obligatory requirement for NADPH. The ratio of acetate and butyrate to propionate can determine the efficiency of VFA utilisation for production. A low proportion of propionate (e.g. in roughage based diets) can limit the availability of NADPH and suppresses the process of fatty acid synthesis from acetate. Higher proportions of propionate and an ample supply of amino acids in concentrate diets, on the other hand, increase the production of

NADPH and therefore improve the efficiency of utilisation of acetate (MacRae and Lobley, 1982; Preston and Leng 1987).

1.1.2. Microbial protein pr)duction

The efficiency of microb al cell synthesis and therefore the amount of cells available for digestion in the small intestine can vary considerably (Russell and Strobel, 1993). Low efficiency of microbial protein synthesis is often observed when ruminants are fed low quality roughages (Oosting *et al.*, 1995). Factors affecting this efficiency include the level of nitrogen intake (Hume *et al.*, 1970), amino acids, peptides (Hume, 1970b; Leng and Nolan, 1984; Oosting, *et al.*, 1995), branched-chain fatty acids (VFA) (Hume, 1970a; Oosting, *et al.*, 1995) and minerals, especially S, K, P and Co (Hume and B rd, 1970; Preston and Leng, 1987; Oosting, *et al.*, 1995). The efficiency is also affected by the maintenance ATP requirement of microbes (energy required for motility, replacement of cells, synthesis of extracellular protein and polysaccharides and for active transport of nutrients), the retention time of microbes in the rumen (Leng and Nolan, 1984; Preston and Leng, 1987; Oosting *et al.*, 1995) and the predation of bacteria by protozoa (Preston and Leng, 1987).

Nitrogen metabolism in the rumen can be illustrated as in Figure 1.3 (Nolan, 1993). Soluble dietary protein is fermented by rumen microbes to peptides and amino acids. The amino acids may be directly incorporated into microbial protein, deaminated to produce branched chain fatty acids (VFA) or converted to ammonia. That ammonia may be utilised by the rumen microorganisms for protein resynthesis by incorporating it into their own cells. Excess ammonia is absorbed across the rumen wall and, after conversion to urea in the liver, is excreted in the urine or recycled to the gut via saliva or across the gut wall.

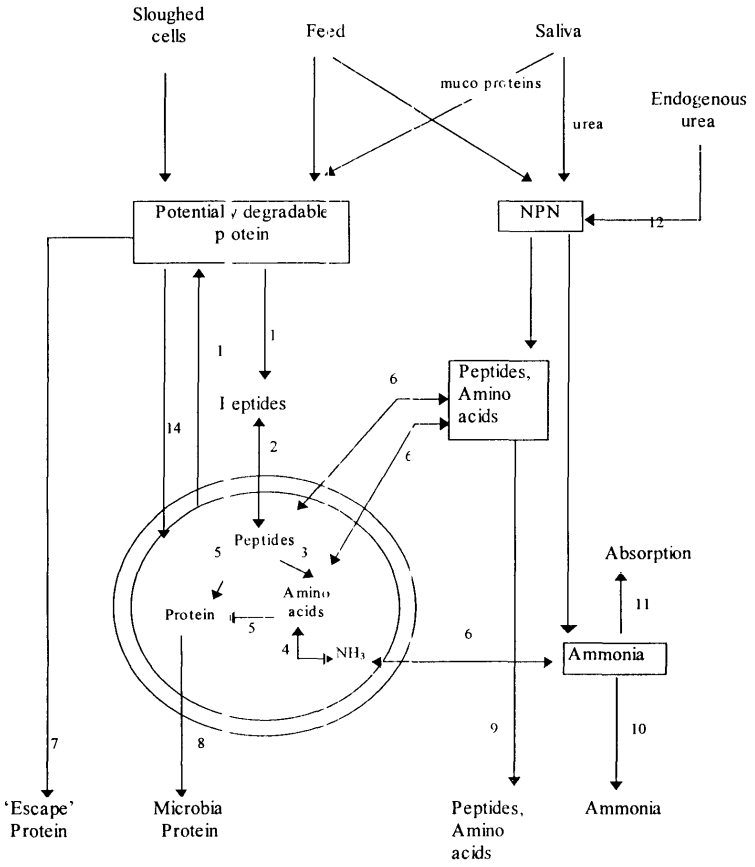

Figure 1.3. Schematic representation of nitrogen transactions in the rumen (Nolan, 1993).
(The ovals = microbial cell wall; 1=proteolitic by bacterial, protozoal and fungal proteases; 2=carrier-mediated peptide uptake across microbial cell walls; 3=peptidolysis; 4= amination/deamination; 5= protein synthesis; 6=microbial assimilation/excretion or equilibration of amino acids and ammonia; 7=protein not hydrolised before efflux from rumen (UDP); 8= microbial protein efflux; 9=efflux of extra-cellular peptides and amino acids; 10= efflux of extra-cellular ammonia; 11= absorption of ammonia through the rumen wall; 12=movement of endogenous urea through the rumen wal ; 13= N compounds excreted by living cells and debris of lysed cells; 14=engulfment of proteinaceous particles by protozoa).

It is apparent that rumen ammonia has an overriding role in nitrogen metabolism and microbial protein synthesis (Leng, 1986). The optimum concentration of rumen ammonia at which microbial growth is maximum varies with diet. Based on an *in vitro* study, Satter and Slyter (1974) suggested that the optimum concentration of rumen ammonia for maximum bacterial growth was 50-80 mg NH_3-N/L of rumen fluid. In an *in vivo* study, on the other hand, Slyter *et al.* (1979) observed that a concentration of rumen ammonia of 22 mg NH_3-N/L was sufficient for maximum microbial synthesis. Preston and Leng (1987), however, suggested that on straw based diets much higher levels of rumen ammonia are required. The digestibility of straw in cattle still increased with increases in ammonia level up to 80 mg NH_3-N/L, and dry matter intake increased until the ammonia level reached 200 mg NH_3-N/L. In addition, Mehrez *et al.* (1977) reported that the optimum

concentration of ammonia for maximum dry matter disappearance from barley grains in the rumen was 200-270 mg NH_3-N/L.

According to Orskov and Grubb (1978), the capacity of rumen microbes to synthesise protein is to a large extent determined by the amount of substrate fermented, and when the fermentability of the diet increases the nitrogen requirement of the microbes is also increased. The requirement for nitrogen in a diet based on NaOH-treated barley straw was thus higher than on untreated straw because NaOH treatment increases the potential organic matter digestibility of the straw.

At low ammonia concentrations, bacteria fix ammonia by a two-step ATP-consuming reaction, i.e. conversion of glutamine to glutamate, and then to 2-oxoglutarate as illustrated in Figure 1.4. When the ammonia concentration is high, on the other hand, ammonia is assimilated by glutamate dehydrogenase, which is probably similar to the process found in soil bacteria. This mechanism does not require ATP (Leng and Nolan, 1984; Nolan, 1993).

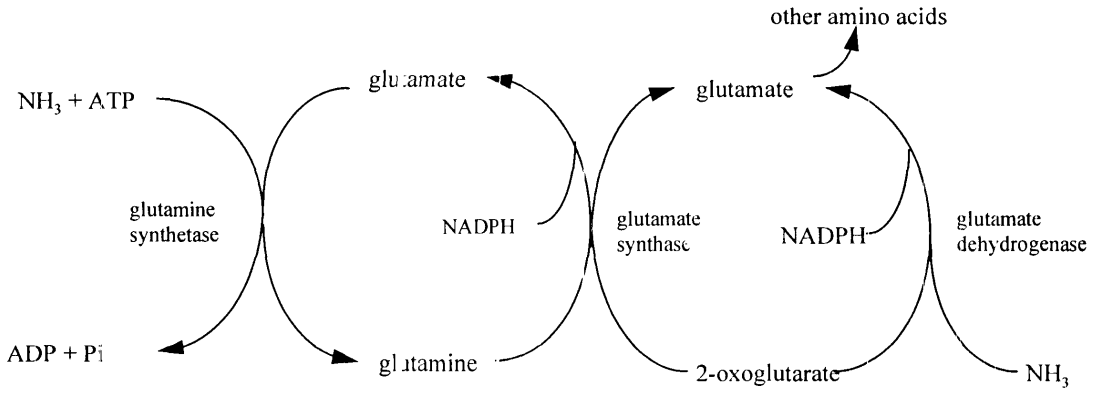

Figure 1.4. Two-step ammonia assimilation by bacteria (Brown *et al.*, 1974).

The consequence of ammonia fixation via glutamine synthetase, when the concentration of ammonia in the rumen fluid is low, is that the high ATP cost reduces the efficiency of microbial growth (Schaefer *et al.*, 1980).

1.2. Efficiency of microbial growth and protein requirements

The efficiency of ATP utilisation affects the partitioning of fermented organic matter (FOM) between the microbial cells, VFA and methane production. Based on stoichiometry of rumen fermentation, Nolan (1989) simulated the relationship between the proportion of glucose converted either to VFA or incorporated into microbial cells at increasing efficiency of ATP utilisation (Figure 1.5). Figure 1.5 indicates that there is competition for ATP utilisation between VFA production and microbial cell synthesis. Increased efficiency of microbial cell production (Y-ATP, g dry cells/mole ATP available) leads to an increase in microbial cell synthesis but a reduction in the production of VFA.

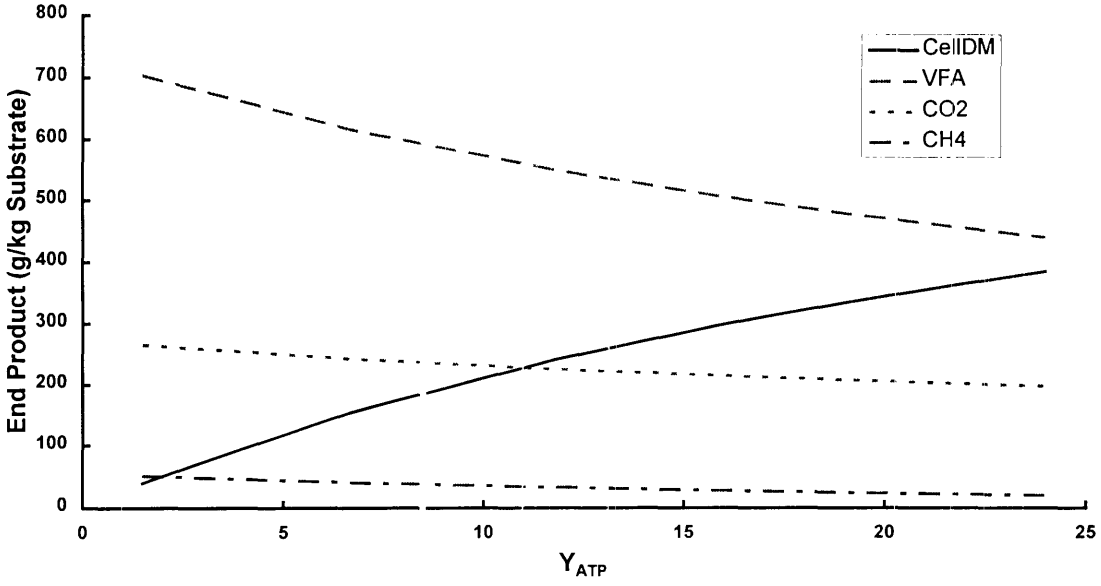

Figure 1.5. Prediction of microbial cells and VFA production from 1 kg glucose at increasing levels of microbial cell growth efficiency ($Y\text{-}_{ATP}$). The prediction is based on Czerkawski (1986) and is for an animal given a good quality diet (Nolan, 1989).

Orskov (1994) suggested that the ratio of VFA to microbial cells can be manipulated in two ways.

1. By altering the lysis and turnover of protein in the rumen:

   Microbial biomass produced per unit of carbohydrate fermented tends to be reduced by protein turnover within the rumen. The engulfment of bacteria by protozoa will reduce the microbial biomass, and thus defaunation can increase the excretion of allantoin, an indication of microbial cell production.

2. By adjustment of the particulate dilution rate:

   The rate of outflow of microbial cells is always less than the rate of synthesis because the rumen bacteria attach to fibrous particles in colonies. Thus increasing the particulate dilution rate may increase the microbial yield per unit of carbohydrate fermented.

Microbial protein can supply the majority of the protein required by the host. However, during early growth, late pregnancy and early lactation the amount of nitrogen obtained from microbial protein is below requirements (Figure 1.6; Orskov, 1970). Under these conditions additional protein escaping rumen fermentation is required to meet the high requirements (Orskov, 1970; Loerch *et al.*, 1983; Leng, 1986; Hussein and Jordan, 199).

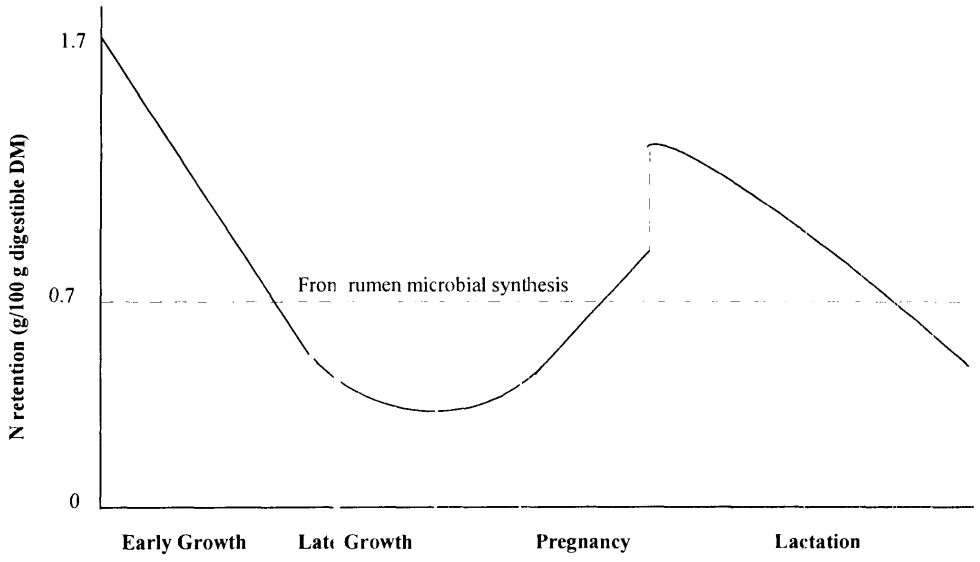

Figure 1.6. Protein requirements relative to microbial protein production for different stages of growth, pregnancy and lactation (Orskov, 1970).

In general, about 60 - 80% of the total amino acid intake of ruminants fed roughage-based diets is provided by microbial protein (Merchen and Titgemeyer, 1992). Kaufmann and Lupping (1982) suggested that, at best, 60 - 70 per cent of the protein requirements of high-yielding dairy cows can be supplied by rumen bacteria. To maintain milk production over 20 l/day, however, they indicated that cows should be supplemented with bypass protein.

1.3. Measurements of microbial N supply

There have been various methods developed to estimate microbial N supply in ruminants. These include the methods based on the use of a protein-free purified diet, amino acid profile (AAP), the use of microbial markers such as diaminopimelic acid (DAPA), ribonucleic acid (RNA), and those based on ^{35}S, ^{15}N, and ^{32}P isotopes (Orskov, 1982). In the study of Siddons *et al.* (1982), for example, five commonly used methods, i.e. the RNA, DAPA, ^{35}S, ^{15}N, and AAP were compared to estimate duodenal microbial protein in sheep. They found that the two isotope methods, ^{35}S and ^{15}N, gave the most reliable results.

However, all these methods are technically impractical as they require an accurate measurement of digesta flow and post-ruminally cannulated animals to obtain samples from the abomasum or duodenum (Chen and Gomez, 1992; Puchala and Kulasek, 1991; Balcells *et al.*, 1993). Thus, a more recently developed approach, the urinary purine derivatives method, is a simple, non-invasive technique that enables estimation of microbial supply (Topps and Elliott, 1965; Rys *et al.*, 1975; Chen *et al.*, 1990 a,b,c; Chen *et al.*, 1991; Chen and Gomez, 1992; Balcells *et al.*, 1993). For these reasons, the purine derivative method is used and especially reviewed in this study.

The use of the purine derivative method is based on the assumption (McAllan and Smith, 1973) that practically all nucleic acids flowing through the small intestines of ruminants are of microbial origin. This is because the purine contents of most ruminant feeds are very low and dietary purines are extensively degraded in the rumen (Chen and Gomez, 1992 .

In the small intestines, microbial nucleic acids flowing from the rumen are degraded extensively. The purine nucleotides are hydrolised to nucleosides and free bases and both are absorbed from the small intestine. The absorbed purines are then

degraded to their derivatives i.e. hypoxanthine, xanthine, uric acid and allantoin (Figure 1.7) and are excreted in urine. Thus, the amount of these purine derivates excreted in urine should relate to the amount of microbial protein absorbed (Chen *et al.*, 1990a,b,c; Chen and Gomez, 1992).

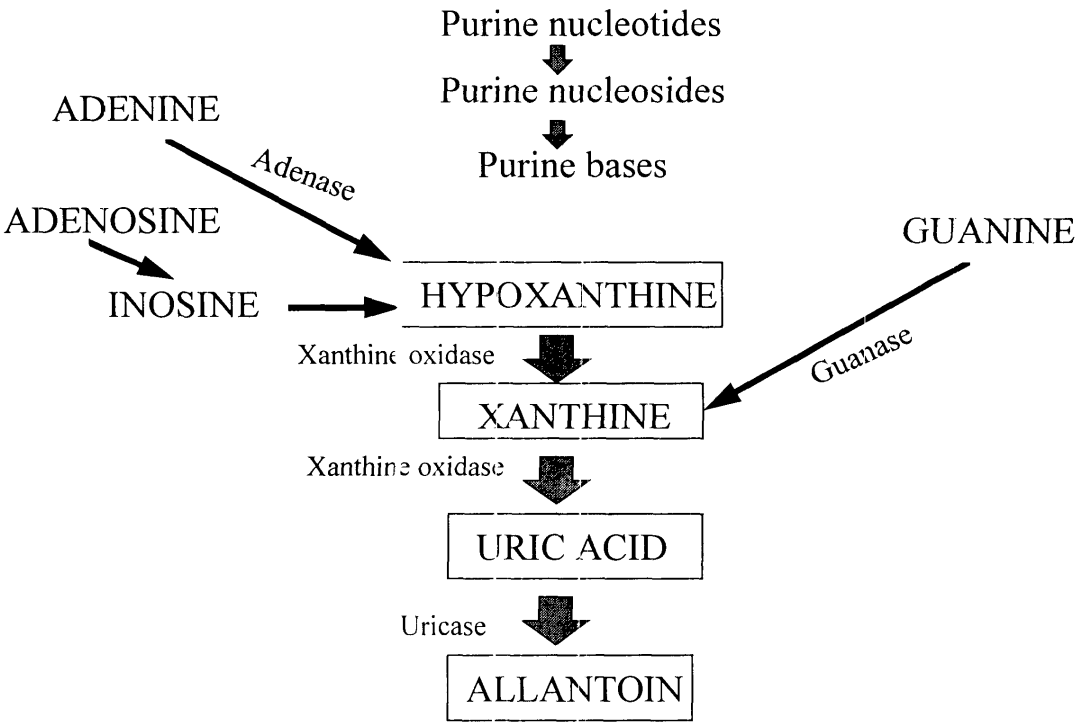

Figure 1.7. Degradation of purine nucleotides and formation of purine derivatives (Chen and Gomez 1992)

The metabolism of absorbed purine derivatives differs in sheep and cattle due to the higher activity of intestinal mucosal xanthine oxidase in cattle than in sheep. Thus in cattle, almost all absorbed purines are converted into uric acid, leaving a very small amount to be incorporated into tissue nucleic acids. In sheep, on the other hand, the purines can either enter the liver unchanged, or be incorporated into tissue nucleic acid, and only the proportions that are not incorporated into tissue nucleic acid will be completely degraded into the end products, hypoxanthine, xanthine, uric acid and allantoin. Thus, there is always a net endogenous contribution to daily excretion of PD in cattle, but this is negligible in sheep (Chen *et al.*, 1990a; Chen and Gomez, 1992).

The above differences in purine metabolism lead to the need to use different equations for estimating purine absorption (X, mmol/d) from PD excretion in urine

(Y, mmol/d) in cattle and in sheep as follows ($W^{0.75}$ = kg metabolic liveweight of the animal):

Cattle: $Y = 0.85X + (0.385\ W^{0.75})$

$X = (Y\text{-}0.385 \times W^{0.75}) / 0.85$

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

$X = Y - (0.150W^{0.75}\ e^{-0.25X}) / 0.85$

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

where: $f(X_n) = 0.84X + (0.150W^{0.75}\ e^{-0.25X}) - Y$

$f'(X_n) = 0.84 - (0.038W^{0.75}\ e^{-0.25X})$

A knowledge of the digestibility, N content and ratio of purine : total N of microbial purines enables the calculation of microbial N supply in ruminants. Chen and Gomez (1992), proposed the following equation for both sheep and cattle, in which the values 0.83, 70 and 11.6 : 100, respectively, are used. The use of this equation is also based on the assumption that the purine to protein ratio in mixed rumen microbes is not affected by diet or other factors.

Microbial N supply (g/d) = $X \times 70 / 0.83 \times 0.116 \times 1000$ (for sheep and cattle)

$= X \times 0.727$

Throughout the current study, the calculation of microbial N supply was based on the daily excretion of urinary allantoin only. To estimate the total daily excretion of purine derivatives, the relationship proposed by Balcells *et al.* (1991) was used. Balcells *et al.* (1991) suggested that the relationship between urinary excretion of allantoin (Y, μmol/kg$^{0.75}$ BW) and purines infused duodenally (X, μmol/kg$^{0.75}$ BW) can be described by the following equation:

$Y = 0.8015X - 43.7$ and thus $X = (Y+43.7) / 0.8015$

Chapter 2: Ruminant production in tropical environments

Despite the fact that about 53% of the world cattle population, 32% of the sheep, 75% of the goats and 92% of the buffalo inhabit the tropics, animal protein production from ruminants is lower in the tropics than in the temperate regions (only 34% and 21%, respectively of total world beef and milk production). The priority is thus to improve the productivity of individual animals instead of increasing the numbers of tropical ruminants (Stobbs and Minson, 1980).

Productivity of farm animals in the tropics is affected mainly by three environmental factors i.e. disease, high ambient temperature and inadequate feed supply (Berbigier, 1991). For the purpose of this thesis, the effects of the last two factors and their interactions will be discussed.

2.1. Climate

The tropics lies between 23° North and South latitudes. The region from 5 to 7° latitude north and south of the equator, especially in the Congo basin, part of the Guinea coast of Africa, the Indian subcontinent, the Malaysian peninsula, Indonesia, New Guinea, the southern Philippnes and the Amazon basin, is known to have an "equatorial climate" which is characterised by high temperature and humidity (Johnson, 1987), even though in some parts of the region such as eastern Indonesia, a hot-dry climate is also found (Macfarlane, 1968). The minimum and maximum ambient temperatures of a typical location in the humid tropics ranges from 19 to 26°C and 26-33°C respectively, with relative humidity of 75-80% (Roman-Ponce, 1987).

The negative effect of climate on animal production is most severe in this region due to a combination of the effects of high temperature and high humidity. The various indigenous breeds of livestock of the region are well adapted, have good fertility and resistance to disease, but have evolved to be of small size with slow growth rate and low milk production (Johnson, 1987).

Ambient temperature is the principal element of climate which affects the physiological functions of an animal (McDowell, 1980). High ambient temperature depresses an animal's feed intake by affecting the appetite and satiety centres of the

brain which have an important role in the regulation of feed intake (NRC, 1981b; Young and Degen, 1981; Leng *et al.*, 1993), and increases each of water intake, respiration rate and rectal temperature (Appleman and Delouche, 1958; Beaver *et al.*, 1989).

A low feed intake reduces the rate of passage of digesta and allows extended time for rumen digestion (Warren *et al.*, 1974; Koes and Pfander, 1975). As a result, digestibility of dry matter (Young and Degen, 1981; see Figure 2.1), cellulose, acid detergent fibre (ADF) and neutral detergent fibre (NDF) are elevated (Christopherson, 1985) when feed intake is depressed. Erasmus *et al* (1965) reported that for each 10°C increase in ambient temperature there was an increase of one per cent in the digestibility of energy. Erasmus *et al* (1965) also found that Shorthorn steers had a higher digestion coefficient of NFE at high (31 to 34°C) ambient temperature than at lower (16 to 18°C) temperature. No significant differences were found, however, in the digestibility of dry matter, crude protein or crude fibre. In addition, ambient temperature had no effect on Afrikaner steers in terms of their digestibility of these nutrients.

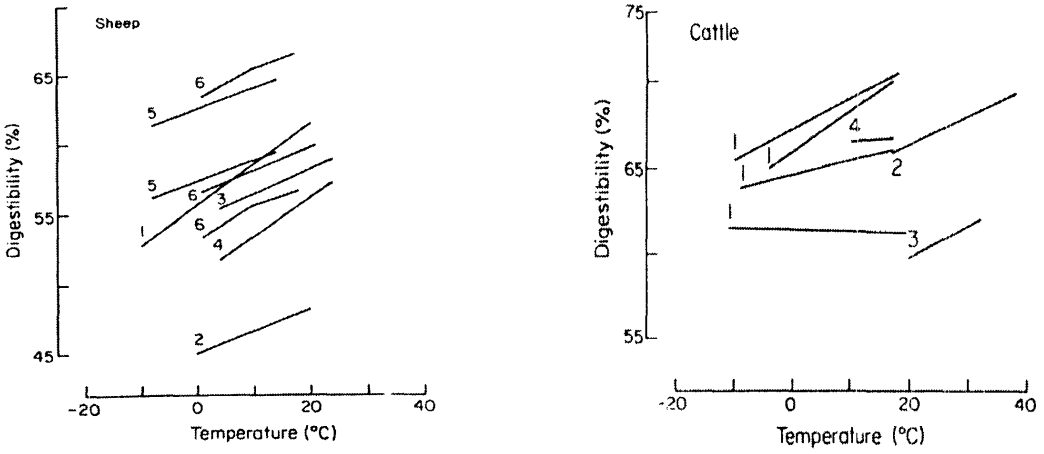

Figure 2.1. Effect of ambient temperature on feed digestibility in sheep and cattle (**Sheep**: 1. Christopherson, 1976; 2. Kennedy *et al.*, 1976; 3. Kennedy *et al*, 1977; 4. Kennedy and Milligan, 1978; 5. Nicholson, *et al.*, 1980; 6. Westra and Christopherson, 1976. **Cattle**: 1. Christopherson, 1976; 2. Colditz and Kellaway, 1972; 3. McDowell et al., 1969; 4. Wohlbier and Schneider, 1965) see Young and Degen (1981).

However, Figure 2.1. also indicates that few studies have been conducted at temperatures above 30°C.

The effect of ambient temperature has been found to be non-significant on the digestibility of concentrate rations. Thyroid hormones are suggested to have a

significant role in controlling digestive function and metabolism in animals under high ambient temperature (Young and Degen, 1981).

High ambient temperature can however, also negatively affect total digestibility due to increased water intake and reduced feed intake. High water intake can dilute the bacterial population, increase the rate of passage (reduce digestion time in the rumen) and limit the contact between bacterial enzymes and substrate, and as a result, reduce digestibility (Koes and Pfander, 1975; Owens and Isaacson, 1977).

It thus appears that the effects of high ambient temperature on the digestibility of feeds are variable (Erasmus *et al*, 1965). The tendency for animals in the tropics, generally, to suffer from low productivity (Copland, 1985; Leng, 1990) is probably explained by the fact that depressed dry matter intake (Koes and Pfander, 1975) overwhelms any advantages that might arise from increased digestibility.

Voluntary dry matter intake is thus an important parameter in animal nutrition because it is a basic factor determining nutrient supply, feed utilisation and the level of production (Devendra and Burns, 1983; Leng *et al.*, 1993; Sauvant *et al.*, 1991). Reduced dry matter intake has negative effects on the nutritional status of ruminants in a hot environment, a fact demonstrated for example by Hafez (1968) who reported a remarkable fall in milk production in Holstein, Brown Swiss and Brahman cows when ambient temperatures were increased from 21°C to 27°C (Figure 2.2). The reduction in milk production at high ambient temperature illustrated in Figure 2.2 was the result of a reduction in feed intake. McDowell (1980) also reported that reduced feed intake, associated with increases in body temperature and respiration rate as ambient temperatures increased, resulted in a 25 to 50% fall in milk production depending on the level of ambient temperature and length of exposure.

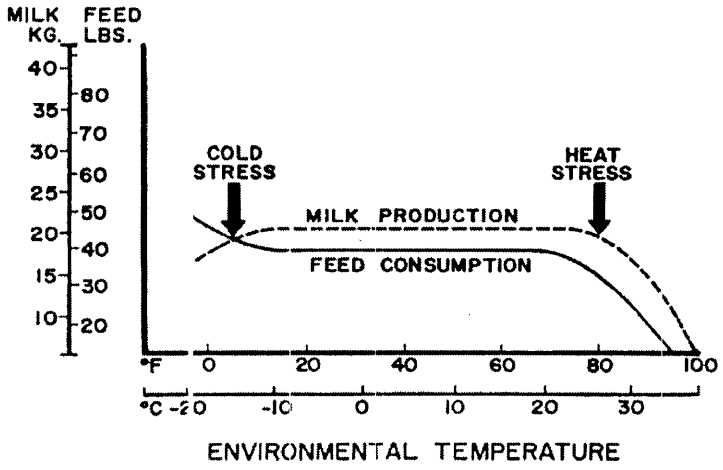

Figure 2.2. Feed intake and milk production in cattle at various ambient temperatures (from Hafez, 1968)

High ambient temperature also reduces liveweight gain and feed efficiency. Ames and Brink (1977) reported that liveweight gain and feed efficiency of crossbred wether lambs fed a pelleted ration and exposed to temperatures that increased 5°C every 12 days from -5°C to 35°C were significantly lower when temperatures were below or above a suggested thermoneutral zone for maximum gain of 10-15°C (Figure 2.3).

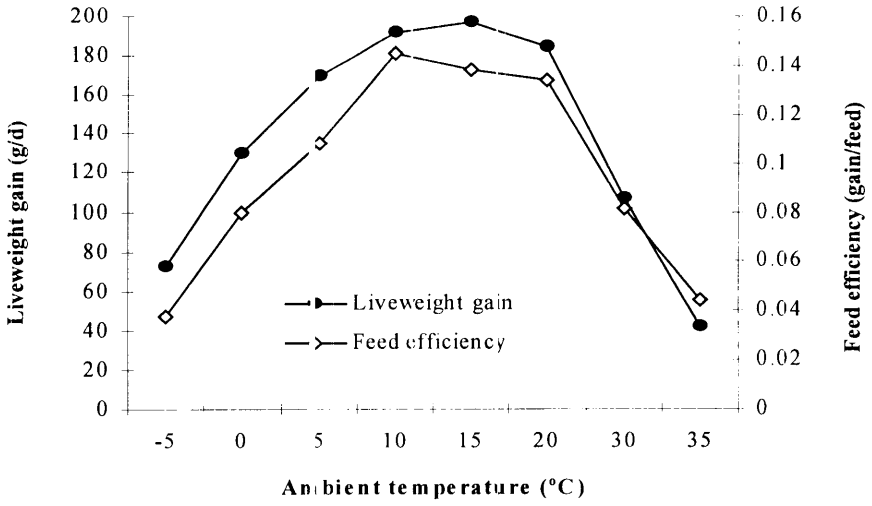

Figure 2.3. Effect of ambient temperature on liveweight gain and feed efficiency in Crossbred wethers (from data of Ames and Brink, 1977).

The reductions in liveweight gain outside the thermoneutral zone demonstrated in Figure 2.3 were considered to be a result of a higher maintenance energy requirement during both cold and heat stress. Ames and Brink (1977) argued that feed intake declined during heat stress and increased during cold stress, but the latter increased at a slower rate than the increase in requirement of energy for maintenance.

2.2. Feed availability and utilisation

Crop production for human consumption is the main objective of farming in most tropical countries but farmers maintain animals as part of the crop production system (McDowell, 1980). One of the major consequences of this system to ruminant production is that only less fertile land is available for growing forage and animals depend greatly on agricultural residues (Leng and Devendra, 1995). The latter are well known to be poor in essential nutrients, particularly protein, and are low in digestibility (Carangal and Calub, 1987; Dixon, 1987).

High temperature is the major factor that stimulates tropical forages to mature rapidly (McDowell, 1980), and it also increases the rate of lignification of cell walls. It also stimulates rapid metabolic activity which reduces the pool of metabolites in the contents of the plant cell (Van Soest, 1982, 1994). Consequently, the quality of tropical forages is generally lower than that of temperate forages (McDowell, 1980; Stobbs and Minson, 1980), mainly due to lower nitrogen and soluble carbohydrate contents, but there is also a higher proportion of structural cell wall components, and consequently lower DM digestibility (Stobbs and Minson, 1980; Van Soest, 1994). The DM digestibility of tropical forages is generally 15 units lower than that of temperate forages (Van Soest, 1994).

On the other hand, the quantity (kg of DM) of forages produced per unit time is greater in the tropics due to the rapid growth of tropical plants. In regions such as Asia (Carangal and Calub, 1987; Leng and Devendra, 1995), for example, an enormous amount of biomass from agriculture and industrial byproducts (e.g. rice straw) is available and has become a major source of feed for ruminants. However, by including a significant amount of agro-industrial byproducts, which are well known to be of poor quality (Carangal and Calub, 1987; Dixon, 1987) in the diets, ruminants are more likely to suffer from low feed intake and low productivity than if they were fed better quality pasture.

Low quality roughage is not only responsible for low ruminant productivity but also contributes to high methane production which contributes to the greenhouse gases that cause global warming. As one of the end-products of rumen fermentation, methane is produced at a higher rate on high forage diets than on high cereal ones (Beever, 1993). About 18% of the total greenhouse gases consists of methane, and it is estimated that 15-25% of world methane production is generated by ruminants, which mostly reside in developing, tropical countries (Leng, 1991; Chantalakhana, 1994).

2.3. Acetate metabolism and utilisation of low quality roughages

It has long been recognised that ruminants produce better, in terms of higher energy retention, on concentrate or mixed diets than on forage diets (MacRae and Lobley, 1982). This is in part due to a higher heat production from forage diets which results in a lower efficiency of utilisation of metabolisable energy in high-fibre diets compared with concentrate diets (MacRae and Lobley, 1982; Orskov and MacLeod, 1990). The exact explanation for these differences between diets is still a matter subject to controversy.

The efficiency of utilisation of feed energy by an animal depends on the level of metabolic heat production (Webster, 1981). Owing to extensive fermentation in the rumen, 50-80% of the digestible energy intake in ruminants is absorbed as VFAs (Ballard *et al*, 1969; Bergman, *et al.*, 1965), of which more than half is from acetate (Tyrrell *et al.*, 1979, Pethick *et al.*, 1981; Brockman, 1993).

Considering the significant role of acetate in energy supply, its possible roles in the process of heat production and thus its impact on the efficiency of utilisation of metabolisable energy in ruminants will be discussed in the following section.

2.3.1. Wasteful oxidation of acetate vs high energetic cost of digestion

Acetate is the most abundant VFA in ruminants, accounting for 70-75% of the total VFA produced in the rumen (Hungate, 1966). When acetate concentration is elevated, about 67-75% of the acetate produced is absorbed through the rumen wall and the rest flows out of the rumen in the liquid phase (Peters *et al.*, 1992).

Acetate is non-glycogenic and thus cannot be temporarily stored as glycogen (McClymont, 1952). Consequently, it must be either converted to fatty acids through lipogenesis or oxidised. When acetate absorption is extensive, a significant proportion of it would be oxidised for non-productive purposes. Thus, McClymont (1952) suggested that the high heat increment of feeding (HIF), which may count for up to 60% of dietary ME, can be associated with a high 'specific dynamic action' of acetate. McClymont (1952) thus hypothesised that the low efficiency of ME utilisation in ruminants can be associated with wasteful oxidation of acetate. The studies of Amstrong and Blaxter (1957a,b) and Amstrong, *et al.* (1957, 1961), which generally showed that acetate cannot be utilised by ruminants as efficiently as propionate or butyrate, suppor the hypothesis of McClymont (1952). Some later studies (e.g. Orskov and Allen, 1966 a,b,c), however, tended to contradict the 'acetate theory' put forward by McClymont (1952). Orskov *et al.* (1979) maintained sheep by intra gastric infusion of VFAs, casein and electrolytes and changed the ratios of acetate : propionate : butyrate from 85: 5: 10 to 45 : 45 : 10 and found that the efficiency of utilisation of ME (K_f) remained constant at about 0.60 (Table 2.1) Thus, the theory that high acetate production increases heat production was apparently discredited.

Table 2.1. Effect of altering the molar proportions and energy proportions of acetic, propionic and butyric acids on the efficiency of utilisation of ME above maintenance (K_f) as determined by intragastric studies in lambs (Orskov *et al.*, 1979).

	Molar proportions (mmol/mol)				
Acetic acid	850	750	650	550	450
Propionic	50	150	250	350	450
Butyric acid	100	100	100	100	100
	Energy proportions (J/kJ)				
Acetic acid	720	590	480	390	300
Propionic	70	210	330	430	530
Butyric acid	210	200	190	180	170
Determined K_f values	0.59	0.61	0.61	0.57	0.64

MacRae and Lobley (1982) countered Orskov *et al.* (1979) and argued that the lower heat production from concentrate diets is due to a more efficient use of acetate in body tissues. Glucose or its precursors (propionate or amino acids) are not limiting with concentrate diets and this will ensure the adequacy of NADPH which facilitates

the conversion of acetate into fatty acids. Thus, MacRae and Lobley (1982) and later MacRae *et al.* (1985) argued that the amount of casein infused into the abomasum in the experiment of Orskov *et al.* (1979) was too high (23% of ME intake). The excess amino acid from casein could have generated sufficient NADPH, thus avoiding an insufficiency of NADPH which may impair the utilisation of acetate in ruminants on forage-based diets. With forage diets, acetate absorption is often relatively high but propionate and amino acid absorptions are not sufficient to generate NADPH and the anabolism of acetate to fatty acids may be impaired (MacRae and Lobley, 1982; MacRae *et al.*, 1985).

Orskov and MacLeod (1990) further suggested that there was no significant difference in heat production during the metabolism of acetate and propionate. In their later study, Orskov and MacLeod (1993) found that increasing the proportions of acetate from 0.36-0.91 but reducing the proportions of propionate from 0.56-0.01 at a constant proportion of butyrate of 0.08, did not increase heat production. At a concentration of acetate of above 0.75, the plasma and urinary concentrations of β-hydroxybutyrate increased. When acetate concentration was over 0.80, acetate was excreted in the urine and heat production declined when the proportion of acetate was above 0.86. The results indicated that metabolic crisis did not occur when the level of acetate was within the levels observed in rumen contents of fed animals. At an extremely high concentration of acetate, heat production even decreased, probably due to loss of acetate in the urine.

Thus, Orskov and MacLeod (1990, 1993) and Orkov (1994) claimed that the difference in heat production from roughage and concentrate diets is due to differences in the 'energetic cost of digestion'. According to this concept, roughage diets require more energy for eating and ruminating, and other activities associated with eating such as standing up, than do concentrate diets. Therefore, reducing the "work" associated with prehension, ingestion and digestion, for example by grinding and/or pelleting of long roughages, could be expected to reduce the time spent eating and ruminating and therefore to increase the efficiency of utilisation of absorbed energy.

Oxidation of acetyl-CoA through the citric acid cycle is dependent on the continuous regeneration of oxaloacetate. An adequate supply of oxaloacetate is required to "prime" the cycle and oxaloacetate can initially be generated from

glucose or its precursors (Orskov and MacLeod, 1990). Thus, an insufficiency of glucose or its precursors (principally propionate or glucogenic amino acids) could have two effects simultaneously, i.e. to limit acetyl Co-A entry into the citric acid cycle causing acetate to build up, and to reduce the availability of NADPH from glucose metabolism to allow the acetate to be used for fatty acid synthesis.

Some workers have emphasised the possible importance of the substrate cycle as a means of eliminating acetate which is not required for fatty acid synthesis, or not able to be used for this purpose because inadequate supplies of NADPH are available. Thus one 'futile cycle' is suggested to be a 'substrate cycle' between acetate and acetyl-CoA, as way of avoiding a metabolic excess of acetate when oxaloacetate is limited (MacRae and Lobley, 1982; MacRae *et al.*, 1985), as illustrated in (Figure 2.4).

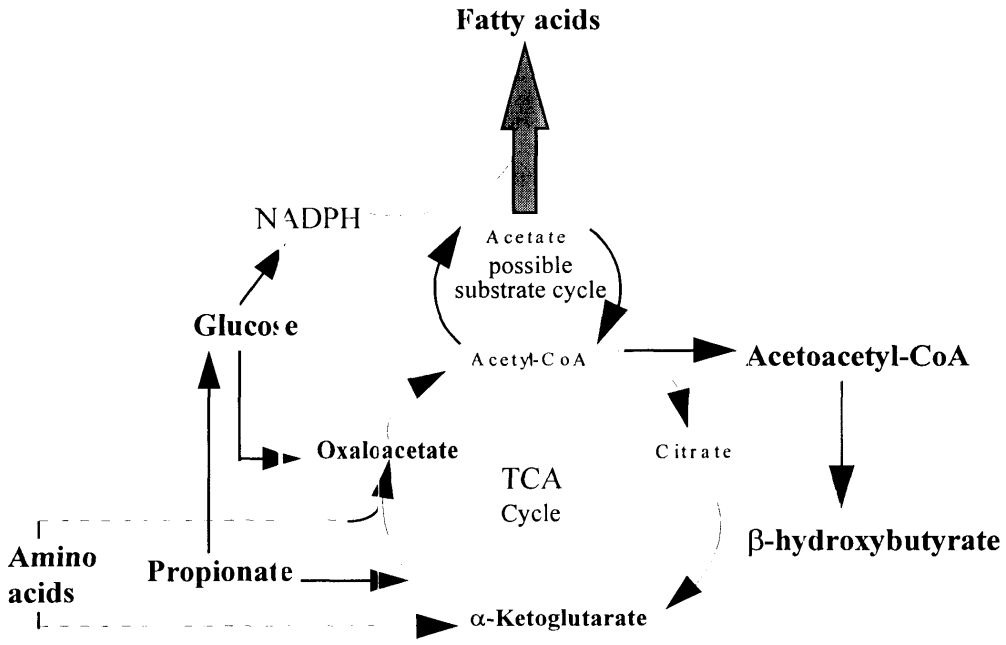

Figure 2.4. Schematic representation describing the relationship between glucose and its precursors and acetate metabolism (based on MacRae and Lobley, 1982; Preston and Leng, 1987).

Scollan *et al.* (1988) reported that the activity of the enzymes in a similar substrate cycle in adipose tissue was greater in Blackface sheep fed sugarbeet pulp (which produced more acetate) than in those fed barley (which produced less acetate). Jessop *et al.* (1990) also found that such a substrate cycle exists in sheep liver cells,

and suggested that this cycle could increase heat production and thus the heat increment of feeding in ruminants producing high amount of acetate. However, Crabtree *et al.* (1987) concluded that substrate cycle in hindlimb muscle of sheep contributed only about 0.5% of the total heat production of an animal. Crabtree *et al.* (1990) also reported that heat production from a similar substrate cycle in the mitochondria and cytoplasm of rat hepatocyte accounted for approximately 1% of total heat production. Thus the studies of Crabtree *et al.* (1987, 1990) indicate that the contribution of substrate cycles to total animal heat production is likely negligible.

Jessop *et al.* (1991) and Jessop and Leng (1993), on the other hand, suggested that imbalance of the absorbed nutrients induced either by a high acetate concentration or by deficiency in glucose precursors increases the rate of sodium pumping across cell membranes (Na^+, K^+ -ATPase activity). Utilisation of ATP in this way could increase the rate of acetate oxidation. As this is a major energy-consuming process (Jessop and Leng, 1993), the energy expenditure of animals on imbalanced diets could be increased and, if this is the case, the efficiency of utilisation of ME in forage diets would be reduced.

When the various theories for the lower efficiency of use of energy in forage based-diets are evaluated, it is not possible to exclude the possibility that the efficiency of metabolism of acetate is reduced when glucogenic precursors are limiting in cells. However, the argument that the low efficiency is related to the "work of digestion" also seems reasonable.

When ambient temperatures are above or below the animal's zone of thermoneutrality, energy retention is reduced (Graham *et al.*, 1959; Blaxter and Wainman, 1961; McDowell *et al.*, 1969; Ames *et al.*, 1971; Brinks and Ames, 1975; Ames and Brinks, 1977) probably because more energy is needed for thermoregulation. In the tropics, where the majority of ruminant feeds are of low digestibility and have high cell wall content and would produce high concentrations of acetogenic substrates, and thus create imbalances in the supply of absorbed nutrients, the efficiency of utilisation of feeds will probably decrease further. However, information concerning the efficiency of utilisation of fibrous feeds of different quality is limiting. In this thesis, two approaches have been taken, i.e. to improve the availability of NADPH via glucose and gluconeogenesis by providing additional amino acids which escape rumen fermentation, and to reduce the "work"

of digestion by grinding of a low quality basal diet. Acetate metabolism was examined at high ambient temperature, to simulate tropical conditions.

2.3.2. Methods of studying acetate metabolism

The metabolism of VFAs has been discussed in Chapter 1, and in this section, the method of tracing acetate metabolism will be briefly discussed.

As discussed earlier, the absorbed acetate can either be used for synthesis of fatty acids or completely oxidised through the TCA cycle. Acetate enters the TCA cycle after being converted to acetyl-CoA, which is then oxidised to CO_2 and H_2O. The energy released by oxidation is conserved in the reduced electron carriers, NADH and FADH, which are themselves oxidised when their high-potential electrons are transferred through the respiratory chain to O_2, which is then reduced to H_2O. The energy release from electron flow is delivered as a proton gradient across the mitochondrial membrane which is then used to phosphorylate ADP to ATP - a reaction catalysed by ATP synthase (Lehninger *et al.*, 1993).

Although oxidative pathways were first delineated in the 1950's (see Kleiber, 1960), and Lehninger et al.,(993) stated that each mole of acetyl-CoA passing through the TCA cycle produces 2 moles of CO_2, Cridland (1983) points out that these 2 moles of CO_2 are not from the acetyl-CoA carbons, but from the oxaloacetate molecule that becomes labelled when the labelled acetyl-CoA enters and undergoes one turn of the cycle (Figure 2.5) and then condenses with acetyl-CoA on the subsequent turn of the cycle (Figure 2.6).

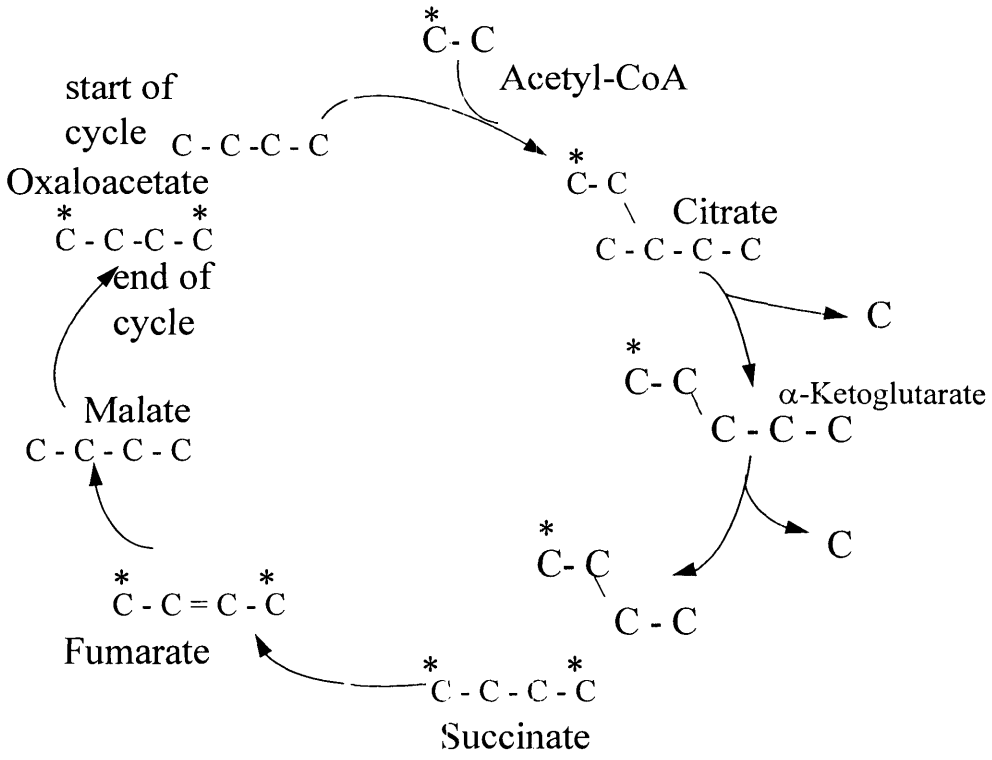

Figure 2.5. The positions of labelled (*) carbon atoms in the TCA cycle intermediates when acetyl-CoA labelled on the carboxyl carbon enters the cycle (after Cridland, 1983)

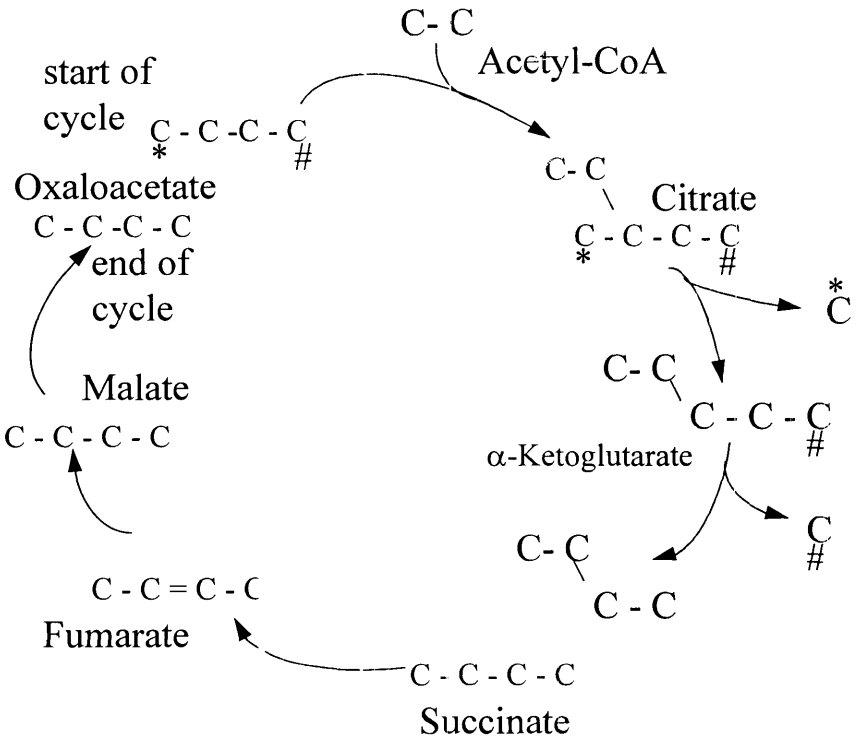

Figure 2.6. The positions of labelled (* and #) carbon atoms in the TCA cycle intermediates when oxaloacetate labelled on the carboxyl carbons continues the cycle (after Cridland, 1983)

Therefore, labelled acetate can be used to trace the flow of carbon atoms through the TCA cycle, and thus the proportion of acetate carbons which are not recovered in the blood as CO_2 is considered to have been used for fatty acid synthesis. This assumes complete recovery and full equilibration. In studies reported in this thesis, ^{14}C-sodium acetate was used.

2.4. Nutrition and climate interactions

As illustrated in Figure 2.7, high ambient temperature and humidity in the tropics directly or indirectly affect animal performance. The high ambient temperatures and humidities which occur in some parts of the tropics, impose heat load to animals resulting in increased respiration rate and rectal temperature, and at a point they cannot dissipate their metabolic heat at a rate sufficient to maintain homeothermy, feed intake, and thus productivity will eventually be depressed (Berbigier, 1991; Leng, 1990; Young, 1993; Webster, 1991).

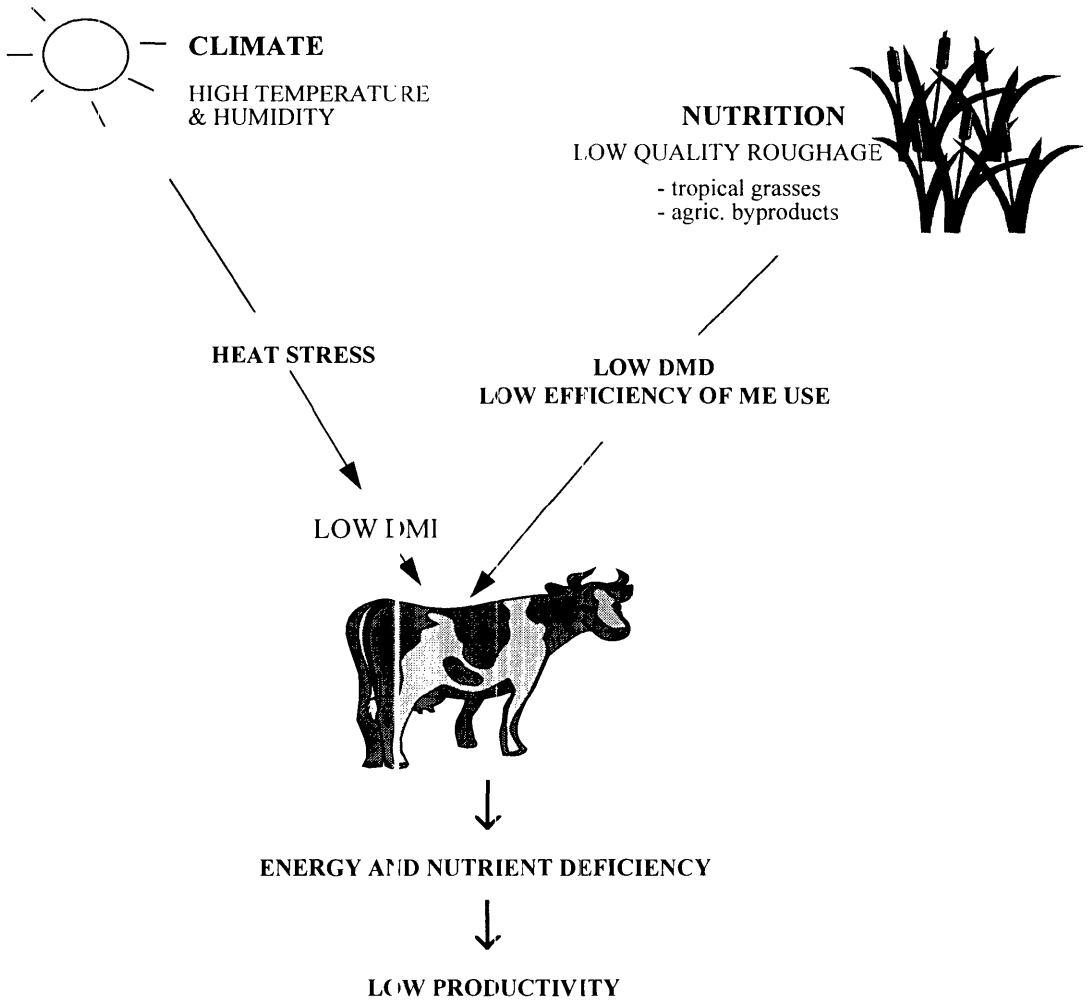

Figure 2.7. A diagram to illustrate the combined effects of climate and nutrition on ruminant productivity in the tropics (based on Blaxter, 1977; Yousef, 1985; Leng, 1990; Orskov and MacLeod, 1990; Young, 1993)

Figure 2.7 also indicates that the depression in productivity of tropical ruminants is exacerbated by the fact that the tropical forages are lower in nitrogen and soluble carbohydrate contents, but are higher in structural cell wall components, and consequently have a lower DM digestibility (McDowell, 1980; Stobbs and Minson, 1980; Van Soest, 1994). High ambient temperature stimulates a rapid growth of tropical forages (McDowell, 1980) which increases the rate of lignification of cell walls (Van Soest, 1982, 1994).

Many workers have concluded that improved productivity due to nutrition in ruminants is a result of an increased ratio of propionate relative to other VFAs produced in the rumen, or an increased ratio of protein to energy in the absorbed nutrients. Lindsay *et al.* (1989) reported that the rumen modifiers; monensin,

lasalocid and avoparcin improved growth rate both in pen-fed and grazing cattle, and that monensin and avoparcin increased propionate production and so improved the efficiency of fermentation of molasses based diets. Similarly, bypass protein supplementation, which theoretically improves the ratio of protein to energy absorbed by a ruminant, appeared to increase the efficiency of utilisation of low quality roughage (see for example, Preston and Leng, 1987; Leng, 1989, 1990; Perdok and Leng, 1990).

Leng (1990) has suggested that, in cold environments, acetate may be utilised efficiently as ruminants need to increase heat production to maintain body heat balance although how the extra heat is generated biochemically is not entirely clear as discussed above. Under tropical conditions, however, where ruminants do not have to utilise acetate for maintaining body heat balance, and where low protein-high fibre roughage is the major component of ruminant diets, acetate may be oxidised wastefully through the so called 'futile cycle'.

However, as discussed in section 2.3.1, it can be concluded that any improvement in the performance of ruminants due to bypass protein supplementation does not appear to be a result of improved efficiency of acetate utilisation. Low productivity of ruminants fed high-fibre-low-protein diets is simply due to insufficient availability of energy resulting from both low DMI and low efficiency of utilisation of ME.

Chapter 3. Nutritional strategies to improve ruminant productivity in the tropics

The low quality of most available feedstuffs (forages and agro-industrial byproducts) has been identified as a major nutritional constraint for ruminants in the tropics. The efficiency of utilisation of these feed materials is lower compared with concentrate/mixed diets, and the theories discussed in Chapter 2 need to be explored further to define feeding strategies which may improve the utilisation of these feed resources. In the following sections some of the most important strategies available to improve the efficiency of utilisation of tropical feedstuffs will be discussed.

3.1. Optimising rumen digestion

The need to extract more nutrients from very mature forages and agro-industrial by-products is increasingly appreciated world-wide. Attention has been focussed on the fate of the fibrous particulate fraction of rumen digesta and on the nutritional implications of controlling rumen function (Kennedy and Murphy, 1988). Fibrous feeds which are low in essential nutrients, especially nitrogen and sulphur, suffer a slow rate of digestion in the rumen with the consequence that DMI is limited due to the time required to remove materials from the rumen (Dixon, 1987; Kennedy and Murphy, 1988). Improving the digestibility and efficiency of utilisation of feed by physical and chemical treatments, and by supplementation of the diets with nutrients essential for microbial growth is necessary if a high level of production is to be achieved. In this review, relevant treatments of low quality forages will be discussed.

3.1.1. Chemical and physical treatments

The maximum capture of energy by ruminants from forages is determined by the balance between the time allowed for fermentation in the rumen and particulate dilution rate. This is of particular importance for mature tropical forages which are characterised by a slow rate of passage accompanied by poor fermentation that together lead to low DMI and productivity (Kennedy and Murphy, 1988). Sufficient time must ideally be allowed to achieve maximum extraction of nutrients from the feed, but the retention time in the rumen is limited to 48-60 h (Orskov, 1994).

Various chemical and physical treatments have been widely applied to improve the utilisation of low quality roughages. The most common chemical treatments applied to fibrous feeds that have been reported in the literature are hydrolytic treatments (mostly with NaOH) and ammoniation (using anhydrous ammonia, NH_4OH or urea).

Based on an extensive review of low quality roughage treatments with NaOH, Males (1987) concluded that treatment with NaOH generally increased both *in vitro* and *in vivo* DM digestibility of wheat, barley and rice straws. Improvements in *in vitro* digestibility were mostly greater (52.9±13.4%; from 8 studies) than those *in vivo* (18.3±2.8%; from 9 studies). NaOH treatment has also been reported to increase *in vivo* OM digestibility of rye straw from 460 to 760 g/kg and of barley straw from 520 to 760 g/kg (Berger *et al.*, 1994). Liveweight gain in cattle was increased by 8, 33 and 207% when NaOH-treated straw was fed at <50% (7 studies), 50 and 60% (4 studies), and above 70% (2 studies) of the diet respectively (Males, 1987).

NaOH treatment by the so-called wet method (straw soaked in diluted NaOH for 3 days before being washed to remove chemical residues) has two disadvantages; the residual NaOH can create an environmental problem (Males, 1987; Berger *et al.*, 1994) and some soluble materials (150-200 g/kg of straw DM) are lost after treatment because the treated materials are washed prior to feeding (Berger *et al.*, 1994).

For these reasons, dry treatments have been developed, but the resultant OM digestibilities have been found to be somewhat lower than those resulting from the wet method. In one study, for example, Wanapat *et al.* (1985) reported that the OM digestibilities of untreated, dry-treated and wet-treated barley straws were 524, 678 and 757 g/kg respectively. For the same reasons, ammoniation has also been widely used to treat low quality roughage. However, the increase in digestibility of roughages due to ammoniation is not as dramatic as that obtained by NaOH treatment (Berger *et al.*, 1994).

The most popular physical treatment to increase the utilisation of high fibre roughage for ruminants is grinding and pelleting (Berger *et al.*, 1994). Based on earlier publications (Minson, 1963; Beardsley, 1964; Moore, 1964; Meyer *et al.* 1965; Osbourne *et al.*, 1976 and Beever *et al.*, 1981), Berger *et al.* (1994) concluded that, while grinding and pelleting reduces particle size and the digestibility of forages,

feed intake, microbial protein p oductic n and feed efficiency may be increased due to an increased particulate dilutic n rate. The net energy content of mature forages is increased by grinding, largely as a result of a reduced heat increment and less energy being required for eating and ru minating (Osbourne *et al.*, 1976; Berger *et al.*, 1994).

3.1.2. Supplementation with limiting nutrients

Digestion of poor qualit' roughages such as straw is limited mainly by the availability of dietary N. Run en NH_3 is the major source of the N required for bacterial growth and is a good indicator for monitoring and predicting the requirements for dietary N supplementation (Balcells *et al.*, 1993). Amino acids, peptides, branched-chain fatty acids and minerals (especially sulphur) are other limiting factors determining the efficiency of utilisation of poor quality roughage by ruminants (Hume, 1970a,b; Hume *et al.*, 1970; Hume and Bird, 1970; Leng and Nolan, 1984; Oosting *et al.*, 1995). Supplementation of low quality roughage with these limiting nutrients genera ly improves the efficiency of utilisation of the diets, but the responses have been variable.

This variability can in pa rt be associated with the type, and thus the nitrogen content, and the potential digestibility, of the basal diets. Physical treatments (e.g. chopping, grinding) that reduce the particle size of the diet, and chemical treatments (e.g. NaOH or ammonia treatments) that to a certain extent increase susceptibility of the fibrous materials to microb al attack, also determine the extent to which the basal diet can be degraded in the rumen. This in turn determines the optimum level of nitrogen supplementation to obtain a maximum response (Orskov and Grubb, 1978). The variability can also be associated with differences in the species, breed or physiological state of the host ruminant, as will be discussed in subsequent sections.

On a basal diet of dried sugarcane (Soetanto, 1987), supplementation with molasses-urea blocks containing 3 or 6 percent of urea increased rumen ammonia concentrations in mature sheep from 60-70 mgN/L to about 80 mg N/L and over 100 mg N/L, respectively, and increased DMI and liveweight gain. The effect of level of urea on the disappearance of dry matter from nylon bags incubated in the rumen was, however, not significant. In th s work. the actual liveweight gains were only 19 g/d and 24 g/d for sheep with acces s to blocks containing 3% and 6% urea, respectively.

Supplementing ewes fed NaOH-treated barley straw with 3 g urea N/d significantly increased DMI and digestibility, but as urea-N intakes were increased to 6, 9 and 12 g/d, no further significant increases in DMI and DMD were observed (Balcells *et al.*, 1993). Oosting *et al.* (1995) observed that supplementation with either casein or potato protein significantly increased voluntary DMI, digestibility and the efficiency of microbial protein synthesis (when measured by the amino acid profile method) in wethers fed ammoniated wheat straw. When the purine derivatives method was applied, however, the increase was non-significant for the potato protein supplement.

Liveweight gain of Etawah-cross does was significantly higher on native grass + molasses-urea block (MUB) *ad libitum* + 625 g gliricidia/d or native grass + 625 g gliricidia + 50:50 mixture of fish meal:MUB than on native grass only or native grass + MUB (Mastika and Wodzicka-Tomaszewska, 1994). Thus supplementing low quality forages with nutrients essential for microbial growth has generally improved performance of ruminants, presumably due to increases in the rate of fibre degradation and microbial protein synthesis.

However, it is clear from Chapter 1 that optimising rumen fermentation is not in itself sufficient to meet the protein requirements of ruminants that have the potential to achieve high levels of production. Microbial protein synthesis can meet protein requirements of the host only when the latter are low, e.g. during late growth or at maintenance. To support high levels of production, protein additional to that which can be supplied by microbial synthesis is required (Orskov, 1970).

3.2. Bypass protein supplementation

The terminology used to describe the dietary protein which is not degradable in the rumen and passes intact to the duodenum has been variable. Kempton *et al.* (1976) and Preston and Leng (1987) used "bypass protein" to indicate that the protein "passes intact from the rumen to the duodenum". Van Soest (1994), on the other hand, suggested "escape protein" to distinguish it from the "bypass" of ingested nutrients through a closed esophageal groove into the abomasum, which was demonstrated by Orskov *et al.* (1970) and Orskov (1982). Other terminologies, "undegraded dietary protein" (UDP), (SCA, 1990; AFRC, 1993) and "protected protein" have also been used. However, Preston and Leng (1987) maintained that "escape" protein may include suckled milk protein, which is readily degradable in the rumen but passes intact to the intestines due to reflex closure of esophageal groove

during suckling. It appears that no terminology has been universally adopted, and which terminology is used depends on the context of discussion and what aspect is to be stressed. Thus, bypass protein is used in this thesis because the present study deals with adult ruminants, and because no attempt is made to close the esophageal groove, this terminology will only describe dietary protein escaping rumen fermentation. Also, key references quoted in this thesis use this terminology.

Bypass protein can be obtained from feedstuffs that contain proteins which escape rumen degradation (these include cottonseed meal, soy bean and fish meal), or can be produced by protecting feed protein through chemical (formaldehyde, tannins, glutaraldehyde) or physical (heating, pelleting) treatments. Chemically-treated bypass protein sources are preferred only when cheaper, naturally-occurring alternatives are not available (Kempton and Leng, 1977). Solubility is a term commonly used to indicate the likely degree of fermentation that will occur in the rumen; the higher the solubility, the lower the bypass protein content of a feedstuff. Table 3.1 describes the solubility of the dietary protein in several bypass protein sources.

Table 3.1. Solubility (in a buffer solution) of the protein (% fermented) in a range of feedstuffs (from Kempton *et al.*, 1976; Kempton and Leng, 1977).

Feed	Solubility	Feed	Solubility
Casein	100	Grains	30-50
Fresh clover	75	Meat meal	30
Silage	70-80	Fish meal	20-80
Rye-grass	65-100	Soybean meal	20-70
Lupin	65	Formaldehyde peanut	20
Peanut	65	Heated cottonseed	20
Lucerne hay	60	Heated linseed	20
Wilted silage	50-70	Heated peanut	20
Dry clover	45	Formaldehyde casein	0-10
Dehydrated Lucerne	40		

However, solubility does not usually reflect the degradability of feed protein. Some soluble proteins, for example serum albumin, ovalbumin, chloroplast protein extract and soluble protein from soybean- and rapeseed meal are relatively resistant to ruminal degradation (Mahadevan *et al.*, 1980; Preston and Leng, 1987). The presence of disulphide bonds and tannins also increase the resistance of protein to protease (Preston and Leng, 1987).

3.2.1. The necessity for bypass protein supplementation in the tropics

Under conditions of high ambient temperature and humidity in the tropics, the low quality of most available forages may further depress total DMI (Leng, 1990). The maximum rate of microbial protein synthesis is most likely to be lower than that which can be achieved in temperate regions. Improving ruminant productivity in the tropics thus cannot be achieved only by maximising microbial protein synthesis.

In a cold environment ruminants increase metabolic heat production to keep the body warm (Blaxter, 1962). Those in the tropics face the difficulty of dissipating excessive body heat due to the combined effects of heat gained from the environment and from the metabolism of low quality roughage. When ambient humidity is high, the problem is exacerbated because dissipation of body heat by means of evaporative cooling becomes less effective. The only other strategy then available to the animal is to reduce feed intake in order to reduce heat generation, and thereby to maintain body heat balance (Devendra and Burns, 1983; McDonald *et al.*, 1988; Leng, 1990). It is difficult for ruminants in the hot-wet tropics, where night temperatures of 28-30°C are common, to dissipate metabolic heat at a rate equivalent to that associated with the feed intake of high yielding animals under temperate conditions (Leng, 1991). Thus, it has been suggested that for highly productive animals, the amount and proportion of dietary protein that escapes rumen fermentation should be increased. This suggestion has been advanced for growing lambs (Bunting *et al.*, 1992), finishing cattle (White *et al.*, 1992) and lactating cows (Higginbotham *et al.*, 1989; Chen *et al.*, 1993; Huber *et al.*, 1994) experiencing heat stress.

Leng (1990) suggested that tropical conditions could in fact be an advantage to ruminants, as animals then do not have to oxidise acetogenic substrates or body fat to maintain body temperature. The relative excess of these energy-yielding substrates then available could theoretically be utilised for production if their energy was balanced with supplementary bypass protein. Leng (1990) thus argued that if adequately supplemented with small amounts of deficient essential nutrients, ruminants in the tropics might produce at even higher levels than their counterparts in the temperate regions fed a similar basal diet. If correct, this theory has immense implications for tropical animal production. The evidence for it is examined below.

3.2.2. Responses of ruminants to bypass protein supplementation

The pioneering work by Egan and Moir(1965) Preston and Willis (1970), Orskov *et al.* (1973) and Ferguson (1975) in the area of bypass protein (see Kempton *et al.*, 1976) have demonstrated that additional amino acids can stimulate VFI, liveweight gain and wool growth in ruminants. The responses to bypass protein supplementation, however, are affected by a number of factors, including the productive capacity and physiological state of the animal, environmental conditions, the quality of the bypass protein source used and the nature of the basal diet (Leng *et al.*, 1977).

There have been various experiments conducted to assess the responses of ruminants to bypass protein supplementation. Hennessy *et al.* (1983) reported that the liveweight gain of Hereford steers fed chaffed pasture hay was significantly improved from -35g/d to 425g/d and 730g/d when the chaff was supplemented with 600g/d (P1) and 1200g/d (P2) of a protein meal (76.4% CSM + 9.5% meat meal + 9.5% fish meal + 2% ammonium lignosulphate + 2.6% minerals), respectively. No significant effect on liveweight gain was observed when the same basal diet was supplemented with high-energy sorghum pellets at levels of 560g/d (S1) or 1120g/d (S2). When the two supplements were combined, however, increases in liveweight gain were 355 (P1S1), 590(P1S2), 585(P2S1) and 555 (P2S2) g/d.

The responses to bypass protein observed by Hennessy *et al.* (1983) were somewhat confounded by differences in the concentration of ammonia in the rumen. The ammonia N concentrations in the rumen fluid of cattle which received the high-energy supplement (at 560 and 1120 g/d respectively) of 10.2 and 11.1 mg N/l were in fact much lower than the suggested minimum level of 50 mg/l (Satter and Slyter, 1974), and these low levels may have contributed to the reductions of 29 and 23% respectively observed in hay intake. It is also interesting to note that when the high-energy supplement was offered in addition to these two levels of protein-rich supplements, liveweight gains were lower than those achieved by the highest protein supplement (P2) alone. The high starch content of the energy supplement could have reduced the population of cellulolytic bacteria, and such a reduction would be consistent with the reductions observed in the intake of the basal diet. However, the total DMIs, which may thus have explained the lower liveweight gains, were not reported by Hennessy *et al.* (1983).

Increasing the level of CSM supplement from 0 to 200, 400, 600, 800 and 1000 g/d increased liveweight gain from 500 to 961 g/d in Hereford steers grazed on oat stubble, and from 128 to 613 g/d on dry pasture. The improvement in gain was a combined effect of increased forage intake and of the higher ME content of the supplement (Smith and Warren, 1986a). On mature annual pasture, on the other hand, Hereford steers lost 214 g/d when no supplement was given. Supplementation with 900 g/d of CSM, lupin, oats plus isobutylidene diurea or oats alone improved the liveweight gains to 321, 119, 47 and 47 g/d respectively (Smith and Warren, 1986b)

Feeding untreated or stack-ammoniated rice straw supplemented with 0, 0.4, 0.8 or 1.2 kg/d of a 42% CP protein meal (60% CSM, 30% soybean meal and 10% meat meal) increased liveweight gain in cattle from -8 g/d on unsupplemented rice straw to 648 g/d on ammoniated straw plus 1.2 kg protein meal/d. The response to protein meal was significantly ($P<0.001$) higher with ammoniated straw (mean = 491g/d) than with untreated straw (mean=224g/d) (Perdok and Leng, 1990).

Ortigues *et al.* (1990) reported that supplementation of chopped barley straw with 0(F0), 76(F1) or 152 (F2) g fish meal/Kg basal diet and fed at both low (L, 1.1 M) and high (H, 1.5 M) levels significantly increased liveweight gain in dairy heifers. On the high plane of feeding, however, the second increment of fish meal supplement (F2) resulted in a smaller response than the first (F1). The mean liveweight gains were 170, 296, 434, 468, 651 and 710 g/d for LFO, LF1, LF2, HFO, HF1 and HF2 respectively.

Hennessy *et al.* (1994), on the other hand, found that supplementation of Hereford (H), Brahman x Hereford (BH) or Brahman (B) cows which were grazed on low or medium quality subtropical pastures for 130 days after calving with 750 or 1500g/d of cottonseed meal had no direct or indirect effect on the growth rate of calves from birth to weaning. The only significant effect of the supplement was to reduce the inter-calving interval in the cows.

A part of the variation in liveweight responses that has been observed with bypass protein supplementation may be due to differences in the quality of the basal diet (Ortigues *et al.*, 1990). Thus the higher digestibility of ammoniated than of untreated straw was associated with higher total DMI and therefore increased liveweight gain in cattle supplemented with protein meal (Perdok and Leng, 1990). In contrast, supplementation of high-concentrate diets (Smith *et al*, 1980) or a high

quality forages (Steen, 1985) with bypass protein have resulted in only small responses.

If some protein from the supplement is degraded in the rumen, as is common (Kempton and Leng, 1977), an elevation in the concentration of rumen ammonia can be expected (Ortigues *et al.*, 1990), and in animals on a low quality diet, this can itself contribute significantly to an increase in digestibility and hence total DMI. The carbohydrate component of bypass protein supplements, which often accounts for more than 50% of their mass, also provides a considerable amount of readily fermentable energy which can be utilised in conjunction with degraded nitrogen, by the microbes, for microbial protein synthesis. This carbohydrate component thus contributes to the total protein available to the host (McLennan *et al.*, 1995) and the positive responses to bypass protein supplements which have been commonly observed may be a combination of the effects of providing one or more of rumen degradable protein, soluble carbohydrate and bypass protein.

An increase in intake of the basal diet, which increases the total DMI, is the major mechanism by which supplementation with bypass protein increases ruminant productivity (McLennan *et al.*, 1995). High intake of the basal diet is only possible if its potential degradability is high enough to enable the available nitrogen from the supplement to be utilised optimally (Orskov and Grubb, 1978). For low quality basal diets, this can be achieved by treatment with alkali (Saxena *et al.*, 1971; Coombe *et al.*, 1979; Males, 1987; Adebowale, *et al*, 1989), cellulotic enzymes (Nakashima and Orskov, 1989) or by grinding and pelleting (Berger, *et al.*, 1994).

In a model proposed by McLennan *et al.* (1995, see Figure 3.1), the maximum level of liveweight gain that can be achieved in cattle through bypass protein supplementation is about 1 kg/d. With grain (energy-rich) supplements, on the other hand, and subject to the adequacy of rumen degraded nitrogen, liveweight gain can be increased further to an ultimate level above that achieved with protein meal alone. The model indicates, however, that up to the maximum response obtained from protein meal (1 kg/d at a DMI of 1.5% liveweight), the amount of protein meal required to achieve a given response is less than the requirement for grain. A gain of 0.6 kg/d, for example, is achieved at supplement levels of 0.4% and 0.8% LW for protein meal and grain respectively.

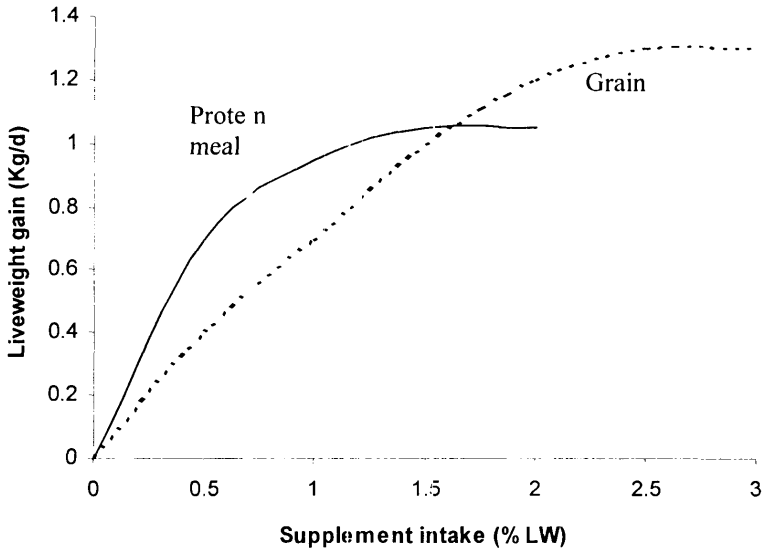

Figure 3.1. Theoretical model of response of liveweight response to protein meal or grain supplementation (McLennan *et al.*, 1995).

Beyond the level that can be utilised to obtain a weight gain of about 1 kg/d, the advantage of protein supplement is probably limited by other factors such as limited availability of energy to utilise the available protein (Ortigues *et al.*, 1990; McLennan *et al.*, 1995). Liveweight gains higher than 1 kg/d can probably be achieved by additional grain supplementation at high levels of protein supplement, but this requires further study (McLennan *et al.*, 1995). Feeding grains alone, despite the greater potential to increase weight gain, is less attractive to farmers in the tropics than is protein supplementation because most grains are expensive and are needed for human consumption.

Despite their limited potential for practical application, grain supplements were used in the current study as a first step in understanding the importance, if any, of balancing high rumen levels of soluble nitrogen with soluble carbohydrates at relatively high levels of urea supplementation. The hypothesis tested was that it is more efficient and economical to use urea/soluble carbohydrate supplements than those containing bypass protein. If that proved to be so, practical feeding systems could be developed for the tropics based on materials such as cassava and rice bran as alternative to grain as energy sources.

In summary, low quality of the available feedstuffs and high environmental temperature (and humidity) are the major factors limiting ruminant production in the tropics. Low efficiency of utilisation of ME from high-fibre, low protein diets has

long been recognised, but the exact explanation as to what causes this low efficiency is still unclear. Even though none of the available theories on the efficiency of ME utilisation in forage and concentrate diets has been universally accepted (see Chapter 2), some evidence suggests that balancing the protein to energy ratio in the absorbed nutrients, as by supplementation with bypass protein, can improve the utilisation of low quality roughage by ruminants. On the other hand, minimising physical work (and thus energy expenditure) related to the processes of eating and digestion has also been shown to improve productivity. Thus, in an attempt to increase the efficiency of utilisation of low quality forages by ruminants in the tropics all these theories need to be explored further. In the current study the responses to bypass protein supplementation were examined in a series of experiments with sheep in a controlled environment with different types of basal diet, and with different availabilities of soluble carbohydrate.

The aims of the study were:

1. To examine a theory (Leng 1990) that bypass protein supplementation can improve productivity of ruminants fed low quality roughages under tropical conditions.
2. To examine that improved responses to bypass protein supplementation reported in the literature were due to improved efficiency of acetate for fatty acid synthesis due to better supply of amino acids (as a precursor of glucose), or simply due to improved intake of digestible organic matter.

The main hypotheses tested were:

1. That bypass protein supplementation will improve the utilisation of low quality roughage in ruminants housed at high ambient temperature (Experiments 3 - 6).
2. That additional soluble carbohydrate in the form of barley grain will improve the response of ruminants housed at high ambient temperature to bypass protein rich supplementation (Experiment 7).
3. That grinding (and pelleting) of low quality basal diets will improve performance of ruminants housed at high ambient temperature at a greater degree than that achieved through bypass protein supplementation (Experiment 8).
4. That bypass protein supplementation will reduce the rate of acetate oxidation and thus increase its utilisation for fatty acid synthesis.

III. Experimental

Chapter 4. Responses of goats to different protein-rich supplements (preliminary experiments)

4.1. Introduction

Protein and energy are the major limiting factors in animal production (Bird, 1996) and, in developing countries, ruminants are fed native grasses and a significant amount of agricultural by-products such as rice straw which are high in fibre but low in protein content and digestibility (Leng, 1990). The low feed intakes observed when ruminants are fed low quality roughage can be associated with an insufficiency of absorbed nutrients and an imbalance between them, with the result that ME cannot be efficiently used for liveweight gain (Perdok and Leng, 1990). Thus, supplementation of low quality roughages with the essential nutrients required by rumen microbes, especially nitrogen and sulphur, is expected to increase the supply of energy and protein available to the host (Preston and Leng, 1987).

Even though the host's need for protein can be met mainly by protein derived from microorganisms in the rumen (Leng, 1986; McDonald *et al.*, 1988), during early life the amount of microbial protein synthesised in the rumen is insufficient to support a high growth rate. Consequently, protein that is less degradable in the rumen and which is thus available for digestion and absorption in the small intestines is needed to meet the high protein requirements of fast growing stock (Orskov, 1970).

Traditional protein sources such as fish-, soybean- and cottonseed- meals are amongst the most expensive feed ingredients. If protein supplementation is to become a reality in the tropics, less expensive materials that are relatively rich in protein will need to be used. In Indonesia, materials such as kapok seed, copra meal and soybean curd/tofu by-product, are all potentially rich in protein, but have not been extensively studied as feed supplements for ruminants. Two preliminary experiments were therefore conducted at Mataram University on Lombok, Eastern Indonesia, to investigate the responses of Kacang goats to supplementation with these locally available protein sources.

In the first experiment, the effects on performance of four protein supplements; soybean curd byproduct, copra meal, kapok seed and fish meal were examined in

goats fed a basal diet of native grass. In the second, mixtures of copra meal and kapok seed, containing 100% copra meal, 90% copra meal + 10% kapok seed, 80% copra meal + 20% kapok seed, 70% copra meal + 30% kapok seed or 60% copra meal + 40% kapok seed on a weight basis, were given *ad libitum* in a separate feeder in addition to a basal diet of rice straw or native grass to assess their effects on the performance of goats. The hypotheses tested were: (1) that the protein supplements would improve the balance of nutrients consumed by the goats and hence improve feed digestibility, total DMI and liveweight gain, and (2) that kapok seed (an inexpensive ingredient) can replace copra meal as a protein supplements for goats.

4.2. Materials and Methods

4.2.1. Experiment 1

In Experiment 1, 20 male Kacang goats aged 5 to 6 months and weighing 12±0.41 kg were randomly divided in declining order of liveweight into 5 groups; A, B, C, D and E. All goats were housed in individual metabolism cages within open-sided barns and were fed a basal diet of native grass (chopped to 5-8 cm). Goats in group A received only the basal diet *ad libitum*, while those in groups B, C, D and E received the same basal diet supplemented with soybean curd by-product (SC), copra meal (CM), cracked kapok seed (KS), and fish meal (FM) respectively. Nutrient compositions of feedstuffs used in this experiment were not measured, and were taken from published results as described in Table 4.1a.

Table 4.1. Nutrient compositions (% DM) of feedstuffs used in Experiments 1 and 2.

	DM	CP	NFE	EE	CF	Ash	Reference
Native grasses	23.7	8.4	?	0.9	16.6	8.4	a
Kapok seed	86	31.7	26.7	9.7	24.0	9.7	a
Soybean curd byproduct	16	26.3	30.4	6.3	16.3	4.2	b
Copra meal	86	21.6	49.7	10.2	12.1	6.4	c
Fish meal	86	72.9	3.8	9.7	1.8	11.8	c
Rice straw	86	3.7	37.4	1.7	35.9	21.2	c

a : Sewet (1994); b: Lubis (1963); c: Hartadi *et al.* (1980).

The amount of supplements provided was estimated (based on their CP content as described in Table 4.1a) to meet the crude protein requirement of 43g CP/d (29 g/d for maintenance and 14 g/d for growth at 50 g/d; NRC, 1981a). It was expected that the goats would obtain 22g CP/d from the basal diet and thus the amounts of the

different supplements offered were adjusted to allow for an intake in each case of 21g CP (3.36 gN)/d. This is equivalent in crude protein content to 50 g/d cottonseed meal (42%CP) which is recommended as the amount to be supplemented to weaner sheep grazing dry pasture (Leng, 1986).

In order to ensure that the goats ate all their supplement allowance the supplements were offered at 09.00 h each day, before the grass was offered at 10.00 h. Data collection was carried out for 8 weeks (from 28 April to 16 June 1994) during the rainy season. Feed and water intakes were measured daily, total collections of faeces and urine were conducted for 7 consecutive days during week 7. Rumen fluids for ammonia concentration were collected before feeding and at 3 and 6 h after feeding during week 8. All goats were weighed weekly (before feeding) to measure the rate of liveweight gain.

4.2.2. Experiment 2

Experiment 2 was started immediately after Experiment 1 finished, using all goats used in Experiment 1. Owing to scabies infection that might have occurred since Experiment 1, all animals were treated with 0.2 mg Ivomec/kg liveweight subcutaneously 3 times, with 7-day intervals between treatments (Manurung *et al.*, 1990) before data collections were conducted. During this experiment, the 20 goats were rerandomised according to liveweight and arranged into 5 new treatment groups. Animals in each group were offered *ad libitum* (at a level of at least 15% in excess of previous day's intake) fresh, as harvested, rice straw (chopped to 5-8 cm long) and (also *ad libitum*) either 100%CM, 90%CM+10%KS, 80%CM+20%KS, 70%CM+30%KS or 60%CM+40%KS, respectively. In this experiment, kapok seed was selected because it was the cheapest of the supplements used in Experiment 1. Copra meal was among the most preferred supplements in Experiment 1 and was used in the supplement mixture in Experiment 2 to investigate its ability to stimulate the intake of kapok seed.

Data collection was carried out for 8 weeks (19 July to 13 September 1994). Initially, rice straw was the basal diet, but from week 6 this was changed to fresh native grass because some animals had almost stopped eating the rice straw and two of them died due to inappetence. All protein supplements were fed *ad libitum.*

Variables measured were feed and water intakes (daily), dry matter digestibility (during week 5 and again during week 8), rumen ammonia concentrations (at 6 h after feeding in week 5 and in week 8) and liveweight gain (weekly, before feeding). Data from both experiments were analysed by one-way or two-way analysis of variance (ANOVA) on the Minitab 8.2 program (Ryan *et al.*, 1985).

4.3. Results

4.3.1. Experiment 1

DMIs of goats fed different types of supplement are presented in Table 4.2.

Table 4.2. Dry matter intake (DMI, g/d), water intake (WI, ml/d), dry matter digestibility (DMD, %) and liveweight gain (LWG, g/d) in goats fed fresh, chopped native grass and different protein supplements in Experiment 1.

	Supplement					P
	None	SC	CM	KS	FM	value
DMI:						
Grass	324±16	334±15	341±19	300±16	330±18	0.50
Grass+Suppl.	324±16^{a}	400±15^{ab}	431±20^{b}	350±16^{a}	360±19^{a}	<0.01
WI	294±28	332±34	375±88	273±9	331±25	0.58
DMD	32.6±8	56.4±3	48.9±5	45.7±5	43.1±9	0.18
LWG	-13±3^{a}	13±6^{b}	3±4^{ab}	-11±5^{a}	11±4^{b}	0.02

SC = soybean curd by-product; CM = copra meal; KS = cracked kapok seed; FM = fish meal
Means within rows with different superscripts differ (P<0.05)

Grass intake was not significantly (P>0.05) affected by the type of protein supplement, and the overall range of group mean intakes was only 300-341 g/d. The intake of grass was lowest in goats offered cracked kapok seed. Despite the non-significant differences in grass intake, type of supplement did significantly affect the overall DMI (Table 4.1), with the highest value of 431 g/d being recorded on the copra meal treatment. The intakes of all supplements were less than the amounts offered.

Except for the group fed soybean curd, mean dry matter digestibilities were all less than 50%. DM digestibility was lowest on the control diet (32.6%), but although values were higher on all treatments (up to 56.4% on copra meal) the differences were non significant (P>0.05).

Water intake did not differ significantly between treatments and the intake was less than 500 ml/d in all cases. Individual WI records varied from a low of 254 to 550 ml/d.

As shown in Figure 4.1, rumen ammonia concentrations were all below 30 mg N/L before feeding and increased significantly (P<0.05) to 36 - 60 mg/l at 3 and 6 hours after feeding.

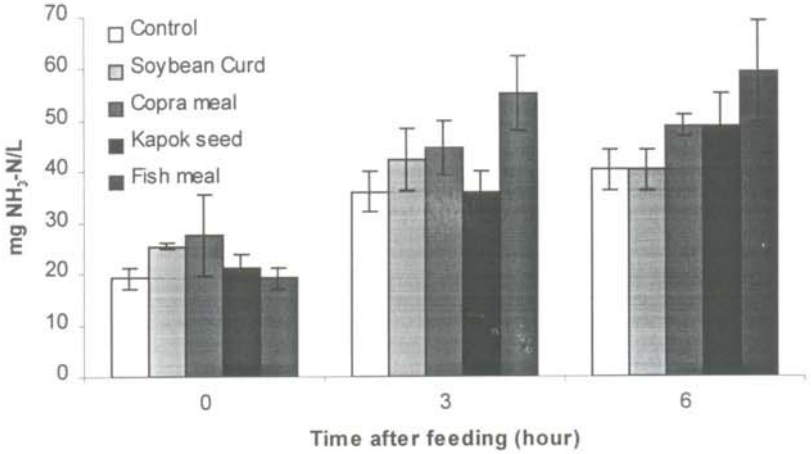

Figure 4.1. Effect of protein supplements on rumen ammonia concentration at different times after feeding in goats in Experiment 1.

Liveweight gains differed significantly (P=0.02) between treatments, but the values recorded were very small and some groups (Table 4.1.) lost weight. Those which were supplemented with soybean curd had the highest mean liveweight gain (13 g/d; none of the individual goats lost weight) followed by those fed fish meal (11 g/d); goats on copra meal could only maintain their liveweight (3 g/d). Goats receiving no supplement and those offered cracked kapok seed lost weight gradually throughout the experiment (13 and 11 g/d, respectively).

4.3.2. Experiment 2

The DMIs of goats in the second experiment are presented in Table 4.2. In all treatments the values were not significantly different.

The intakes of both basal diets (rice straw and native grass) were not significantly affected by the level of kapok seed in the concentrate. The intake of concentrate tended to decrease (P = 0.14) with an increase in the proportion of KS in the concentrate mixture, and straw intake (during weeks 1 - 5) tended to increase (P = 0.17) following the reduction in concentrate intake. The intake of grass during weeks 6 - 8 was similar on all treatments.

Table 4.3. DMIs of goats in Experiment 2 (both basal diets and concentrates were fed *ad libitum*).

	%KS : %CM in concentrate					P
	0:100	10:90	20:80	30:70	40:60	value
Weeks 1-5[1]:						
Straw	195±31	164±37	207±64	236±26	237±55	0.17
Concentrate	116±29	99±26	96±10	84±12	81±4	0.14
Total	311±41	263±53	303±55	320±31	318±54	0.45
Weeks 6-8[2]:						
Grass	208±21	202±22	218±14	221±9	226±10	0.28
Concentrate	163±21	133±38	137±20	125±14	100±25	0.07
Total	371±33	335±51	355±26	346±17	326±59	0.24

1: basal diet = rice straw; 2: basal diet = fresh native grass

As in Experiment 1, water intake did not differ significantly between dietary treatments. However, mean water intakes were significantly higher during weeks 6-8 (425-676 ml/d) than during weeks 1-5 (355-513 ml/d).

Apparent dry matter digestibilities for the second experiment are shown in Table 4.4, from which it is evident that values were not significantly affected by supplement.

Table 4.4. Effect of proportion of kapok seed relative to copra meal in the concentrate mixture on dry matter digestibility (%) in Experiment 2.

Week	%Kapok seed : %Copra meal in supplement					P
	0:100	10:90	20:80	30:70	40:60	value
1-5	37.4±7.7	28.0±10.8	31.8±9.5	42.4±8.3	43.2±15.3	0.44
6-8	61.5±2.8	59.6±6.6	53.8±9.9	59.2±5.2	54.6±5.2	0.43

Rumen ammonia concentration was not affected by level of kapok seed in the concentrate, but was significantly ($P<0.01$) higher when the grass basal diet was offered than during the period when rice straw basal diet was used (Figure 4.2).

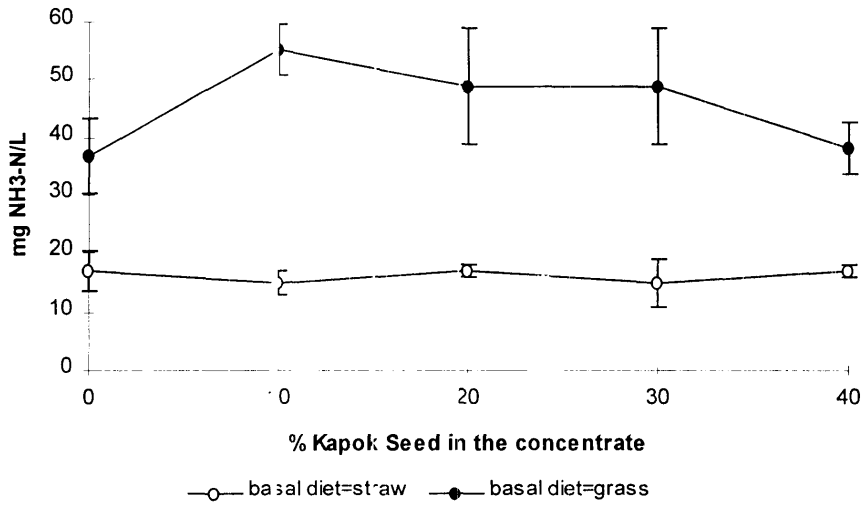

Figure 4.2. Rumen ammonia concentration (mg NH_3-N/L) at 6 h after feeding of goats fed rice straw or grass basal diets

The overall weight gains during the second experiment differed significantly between treatments (Table 4.5).

Table 4.5. Effect of proportion of kapok seed relative to copra meal in the concentrate mixture on liveweight gain (g/d) of goats in Experiment 2.

Week	%Kapok seed : %Copra meal in supplement					P
	0:100	10:90	20:80	30:70	40:60	value
1-5	14	-5	-14	-10	-22	0.20
6-8	112^{c}	81^{abc}	30^{a}	59^{abc}	81^{abc}	0.03
Overall	63^{c}	38^{abc}	8^{a}	24^{abc}	31^{abc}	0.05

Liveweight gains were mean values of weekly gain
Means within rows with different superscripts differ (P<0.05)

When rice straw was fed as the basal diet in weeks 1-5, all goats, except those supplemented with 100% copra meal, lost weight and the liveweight loss in general increased as the proportion of kapok seed in the concentrate mixture increased. The differences in weight gain, however, were not significant during that period.
Significant treatment differences in liveweight gain did, however, occur when the basal diet was changed to fresh grass (starting from week 5), as indicated in Table 4.5 and Figure 4.3.

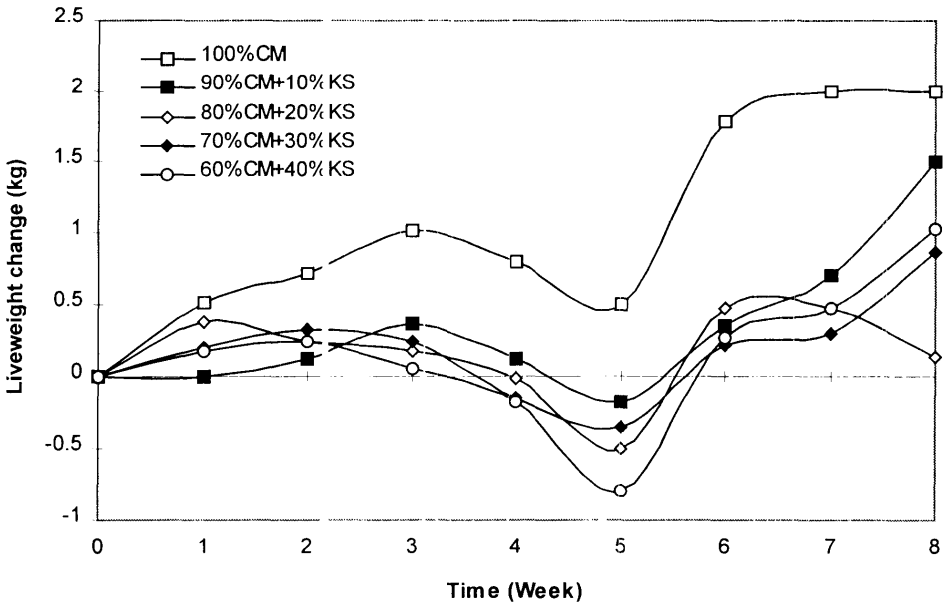

Figure 4. 3. Effect of increasing proportion of kapok seed (KS) relative to copra meal (CM) in the concentrate mixture on liveweight change during Experiment 2 (the basal diet was changed from rice straw to fresh grass starting from week 5).

4.4. Discussion

In Experiment 1, when native grass was supplemented with cracked kapok seed, soybean curd byproduct, copra meal and fish meal at levels of N equivalent to 50 g cottonseed meal, the total digestible DMI was barely adequate to meet the requirement for maintenance of liveweight in young Kacang goats which initially weighed 12±0.41 kg. This lack of response to supplementation was probably due to insufficient intake of essential nutrients, especially nitrogen and sulphur, for microbial growth. The intakes of all supplements (and hence of nitrogen) were less than that offered. Consistently, low ammonia concentrations were observed in the rumen fluid up to 6 hours after feeding and these values were less than the minimum 50 mg NH_3-N /L of rumen fluid suggested as being necessary for optimum microbial growth by Satter and Slyter (1974). This inadequate rumen N status may have contributed to the low DMIs recorded.

The low DMIs of goats in this experiment can also be associated with the feeding management. Devendra and Burns (1983) suggested that combined feeds of grasses, shrubs and leaves are preferred by goats, as such mixtures allow the animals

to select according to their needs. The amounts of feed offered in the current experiments were, however, only 10 to 15% in excess of the previous day's intakes, and the opportunity for selection by the goats was thus limited.

It seems peculiar that the concentration of rumen ammonia did not increase significantly when protein-rich supplements were fed. It was expected that at least part of the protein in the supplement would have been susceptible to microbial degradation to ammonia which would thus have increased the concentrations of ammonia in the rumen fluid. However, a transitory rise in rumen ammonia may have possibly been missed because the samples of rumen fluid were taken before feeding and then at 3 and 6 h after feeding. It is possible that the ammonia concentration increased markedly during the first 2 hours after feeding, because the supplements were mostly eaten during the first hour, and declined before the 3-hour sampling. A source of readily fermentable nitrogen such as urea sprayed onto the basal diet would have ensured an adequate concentration of ammonia in the rumen fluid for most of the day.

The animals used in this study were brought from a mountainous area where they had previously browsed on trees and shrubs, and, based on day-to-day observations, they appeared to be slow to adapt to the pen-feeding system and to new feeds, despite being allowed a 4-week adaptation period before data collection began. Unfortunately, feed intake during the adaptation period was not recorded. The basal diets offered were of single roughages (either rice straw or native grass) chopped into 5-8 cm pieces and this could also have reduced the acceptability of the feeds to the goats, and thus contributed to the low intakes recorded. Putra *et al.* (1994) reported that hanging gliricidia branches up-side down (A) increased the intake by Kacang goats compared to similar feed cut into 10 cm and put in feed troughs (B), cut into 50 cm pieces and put in feed troughs (C), or D placed in a raised rack (the mean intakes were 135, 113, 125 and 133 g DM/$W^{0.75}$ for A, B, C and D, respectively). Putra *et al.* (1994) suggested that the significantly higher feed intake of goats in treatment A was most likely to be due to the browsing habit of the goat.

Among the supplements, soybean curd by-product and copra meal were most readily eaten by the goats. For practical purposes, copra meal can thus be considered to be the best of the supplements tested, both due to its widespread availability, low price and acceptability to goats. Soybean curd by-product is also relatively cheap and is acceptable to goats, but its availability is limited by its high moisture content (16%

dry matter). Raw kapok seed was considered best of all on the basis of price and protein content, but it was less acceptable to the goats compared with other supplements. Further study of the reason for its low intake by goats is required.

When copra meal and raw kapok seed were offered *ad libitum* during the second experiment, the intake of concentrate increased to an average of 95 g/d when rice straw was the basal diet, and the mean increased further to 132 g/d after the basal diet was changed to fresh grass (Table 4.3). The intake of concentrates on both basal diets declined as the level of kapok seed in the concentrate mixture increased. This again suggests that copra meal is more readily accepted by goats than raw kapok seed, and indicates that the goats were well able to detect the presence of raw kapok seed in the mixture. Cyclopropenoic acid (Shin, 1994) and gossypol (Cheeke and Shult, 1985), which are toxic substances in kapok seed, could have affected the intake of this supplement.

The mean water intake of goats in Experiment 1 was less than 400 ml/d, and was slightly higher in Experiment 2 (417 ml/d) when the basal diet offered was rice straw. When the basal diet was changed to fresh grass, however, water intake increased significantly to an average of 543 ml/d. The significant increase in water intake when the basal diet was changed from rice straw to fresh grass in Experiment 2 was probably due to an increase in DMI (from 303 to 346 g/d).

The change in the basal diet from rice straw to grass increased rumen ammonia levels from less than 20 to about 40-50 mg NH_3-N/L of rumen fluid. The latter value is very close to the lower end of the range of ammonia concentrations suggested by Satter and Slyter (1974) to be sufficient for efficient microbial growth in the rumen. The increases in concentrate intake, feed digestibility and rumen ammonia concentration experienced when the basal diet was changed from rice straw to fresh grass were associated with a significant increase in liveweight gain (Figure 2). However, this improvement in liveweight gain could also be attributed, in part at least, to gut fill or a form of compensatory growth since almost all goats lost weight during Experiment 1 and the first 5 weeks of Experiment 2.

The supplement/concentrate intake in Experiment 2 was much higher than, for example, that recommended by Leng (1986) for growing lambs, but supplementation still did not increase either the DMI or the liveweight of goats given a basal diet of rice straw. Mineral supplementation was not incorporated in this study and it is possible that intakes of some essential minerals were insufficient to fully meet the

need for the rumen microbes and that this may have limited the rate of rumen fermentation (Preston and Leng, 1987).

Lack of response in liveweight gain when rice straw was fed as the basal diet was also related to the low digestibility of that straw (less than 50%) and as a result, insufficient total digestible organic matter was ingested by the goats. A similar response was also reported by Salayog (1991). In that work, Border Leicester x Merino wethers lost weight at 73.0, 47.2, 47.1 and 26.0 g/d when fed rice straw *ad libitum* and supplemented with urea (20 g/kg air dry rice straw), urea + molasses (100 g/d), cottonseed meal (100 g/d) and urea + cottonseed meal + molasses, respectively.

When the ME intakes were estimated using the SCA (1990) equation: M/D=0.17 DMD%-2.0 (Table 4.6) and compared with the corresponding recommended values (NRC, 1981) for 12 kg goats, the observed liveweight losses in Experiment 1, and during weeks 1-5 in Experiment 2, were as expected.

Table 4.6. Calculated metabolisable energy concentration of feeds (M/D, MJ/kg DM) and estimated ME intakes of goats (MEI, MJ/d) in Experiments 1 and 2.

Experiment 1:

	Grass	Grass+SC	Grass+CM	Grass+KS	Grass+FM
M/D	3.54	7.59	6.31	5.77	5.33
MEI	1.15	3.04	2.72	2.02	1.92

Experiment 2:

	%KS : %CM in concentrate supplement				
	0:100	10:90	20:80	30:70	40:60
M/D					
weeks 1-5	4.36	2.76	3.41	5.21	5.34
weeks 6-8	8.45	8.13	7.15	8.06	7.28
MEI					
weeks 1-5	1.35	0.72	1.03	1.67	1.70
weeks 6-8	3.13	2.72	2.54	2.79	2.37

However, the above calculated ME intakes based on the DMD values appear to be underestimates. Even though the ME intakes in both experiments were all below the predicted maintenance requirements, the extent of weight losses in Experiment 1 and in weeks 1-5 of Experiment 2 were not as severe as those predicted from the ME intakes. In fact, all goats gained weight after the basal diet was changed

to fresh grass in Experiment 2. These discrepancies between predicted and actual values could be related to technical errors that might have occurred during the digestibility trials. The feeds (basal diets) used were collected from various places and even though grab samples were taken from each bag of feed, the DM content could have varied to an unknown extent, resulting in possible miscalculation of DMD.

Lack of responses to protein-rich supplements observed in these two preliminary experiments, however, cannot be taken as a true reflection of the quality of these supplements. Some nutrients essential for the growth of rumen microbes such as N were not provided at all times, and their absence may thus have limited the ability of rumen microbes to digest the basal diet. Urea and minerals should thus have been included with the basal diet in conjunction with the protein supplements. The goats used in these experiments were collected from various sources and were unfamiliar with feeding on chopped forage in troughs. The lack of responses to supplements may thus have been at least partly due to failure of behavioural adaptation to the feeding system used.

In the subsequent experiments, to overcome these limitations, basal diets of uniform quality were used, and were supplemented with limiting nutrients, especially N and S. Animals of uniform genotype and age were used to minimise possible genetic variations in growth parameters. Also, all subsequent experiments were conducted in temperature-controlled rooms to avoid variations in the effects of environments on the animals.

Chapter 5: Effects of high ambient temperature and CSM supplementation on performance of crossbred lambs

5.1. Introduction

It has been suggested that fermentation of low quality forages produces high ratios of acetate to total volatile fatty acids in the rumen (Orskov and MacLeod, 1990; Beever, 1993: France and Siddons, 1993). Under tropical environments, specifically of low crude protein intakes of roughage diets, a combined effect of the products of fermentation of low quality roughage such as acetate together with the direct effects of high ambient temperature and humidity, may increase the total heat load on an animal. This may force an animal to reduce its feed intake in order to avoid additional heat stress (Appleman and Delouche, 1958; Devendra and Burns, 1983; McDonald *et al.*, 1988: Leng, 1990).

A theory proposed by Leng (1990) predicts that supplementation of such an animal with bypass protein may improve the protein to energy ratio of the absorbed nutrients resulting in a more efficient use of acetogenic substrates. This may reduce the total heat load, and if so the animal's feed intake might then be maintained, or even increased.

The two experiments reported in this chapter were thus designed to: i) study the effect dietary supplementation with a source of bypass protein (cottonseed meal, CSM) on the performance at high ambient temperature of growing crossbred wethers fed low digestibility roughage (Experiment 3), and ii) quantify the effects of declining night-time temperature on dry matter intake (Experiment 4). As it was not possible to obtain typical tropical feeds (tropical grasses or rice straw) at Armidale when the experiment was begun (a period of severe local drought), chaffed wheaten hay which contained no grain was used instead.

The hypotheses tested were:

1. That CSM may improve feed intake and liveweight gain; and
2. 2. That the improvement in feed intake due to CSM supplementation will be greater when night-time temperature is reduced below day-time temperature.

5.2. Materials and Methods

5.2.1. Experiment 3

The use and treatments of animals in this experiment and in subsequent experiments reported in this thesis were approved by the Animal Ethics Committee of University of

New England. Sixteen unshorn Border Leicester x Merino lambs aged 6 months and weighing 24.9±0.37 kg were allocated into a 2x2 factorial design (2 diets and 2 ambient temperatures). Each group was fed either wheaten chaff + 2% urea *ad libitum* or wheaten chaff + 1% urea *ad libitum* + 100g/d cottonseed meal. The CSM supplement was fed once daily, togeher with morning feeds. Animals from each diet treatment were randomly divided into two groups: the first group was housed in a climate chamber at 17±3°C and the other group in a second climate chamber at 35±2°C. The relative humidity in these two climate chambers was maintained at 65%. Both rooms were illuminated daily from 06.00 to 18.00 h. Animals were penned individually. Feed was provided once daily (at 08.30 hours) at a level of about 15% in excess of previous day's intake, and drinking water was freely available at all times.

The experiment lasted for 8 weeks after a 3-week adaptation period. Feed intake (FI - to measure dry matter intake, DMI) and water intake (WI) were recorded daily. Measurements of rectal temperature (RT) and respiration rate (RR) were taken every fourth day (at 14.00-15.00 h). Faecal collection for determination of organic matter digestibility (OMD) was carried out for 7 consecutive days in week 6. Liveweight was recorded weekly before feeding to estimate daily liveweight gain (LWG). During week 8, samples of blood (by jugular venipuncture), to measure blood plasma urea level, and of rumen fluid (by stomach tube, at 3 h after feeding), to measure rumen ammonia nitrogen (NH_3-N) concentration, were collected.

5.2.2. Experiment 4

Experiment 4 was conducted immediately after Experiment 3 using only the 8 lambs previously housed at high temperature (35°C). These animals were maintained for another 4-week trial to study the effect on performance of a day temperature (from 08.00 to 16.00 hours) of 35°C in conjunction with a lower night temperature. The night temperature (from 18.00 to 08.00 hours) was progressively reduced by 2°C every 3 days over a period of 30 days.

Diet, water and light treatments were the same as in Experiment 3 (basal diets and CSM supplement) were offered once daily (at 07.00 - 08.00 h. The basal diets was provided in a square bucket, while the CSM was provided in a small bucket (ice cream container placed inside the square bucket. Refusals were measured twice daily, at 07.00 - 08.00 h and at 17.00 - 18.00. Measurements of RR and RT were made every third day (the last day

of each temperature period), also between 07.00 and 08.00 h (before ambient temperature was increased to the "day" setting) and 17.00 and 18.00 h (before ambient temperature was reduced to the "night" setting) Liveweight was recorded before feeding at the beginning and at the end of the 30-day trial to enable an estimate of daily LWG to be made.

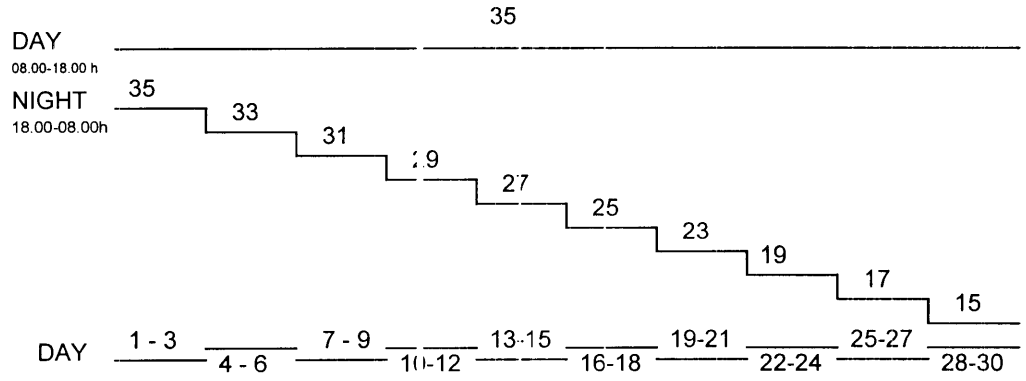

Figure 5.1. Ambient temperature settings in the climate chamber during Experiment 4.

Statistical analysis

Data from Experiment 3 were subjected to analyses of variance (ANOVA) for a factorial design, while those from Experiment 4 were analysed by a one way ANOVA for repeated measures. All data analyses were performed on the Minitab 8.2 computer program (Ryan *et al.*, 1985).

5.3. Results

5.3.1. Experiment 3

Thermoregulatory responses

Thermoregulatory respc nses of lambs to temperature and diet were measured in terms of RR and RT. RF. was significantly affected by ambient temperature (P<0.01), but not by diet (Tabl(5.1 and Figure 5.2).

Table 5.1. DMI (g/d), WI (L/d), RR (resp/min), RT (°C), and LWG (g/d) of crossbred lambs fed w heaten chaff+2% urea (control) or wheaten chaff+1% urea+100 g CSM/d (CSM+) at low and high ambient temperature (mean± SE, N=4).

	17±3 °C		35±2 °C		P value		
	Control	CSM+	Control	CSM+	T	D	TxD
RR	92±8	9(±3	202±6	180±12	.00	.21	.31
RT	39.4±.03	39.(±.08	40.8±.07	40.9±.05	.00	.07	.55
DMI							
Chaff	783±11	83' ±35	590±47	525±43	.00	.89	.13
Chaff +CSM	783±11	92(±35	590±47	614±43	.00	.04	.13
WI	1.8±.05	2.2 ±.14	2.9±.33	3.8±.50	.00	.06	.45
LWG	45±6	8: ±8	-9±5	-27±11	.00	.18	.00

T = temperature effect; D = diet effec t; TxD = Interaction

The interaction between diet and ambient temperature was non-significant on RR. RT was also significantly (P<0.01) higher at higher ambient temperature. RT of lambs on the CSM+ diet tended to be higher than that of those on the control diet, but the difference only approached significance (P=0.07). The interaction between diet and ambient temperature was a so non-significant.

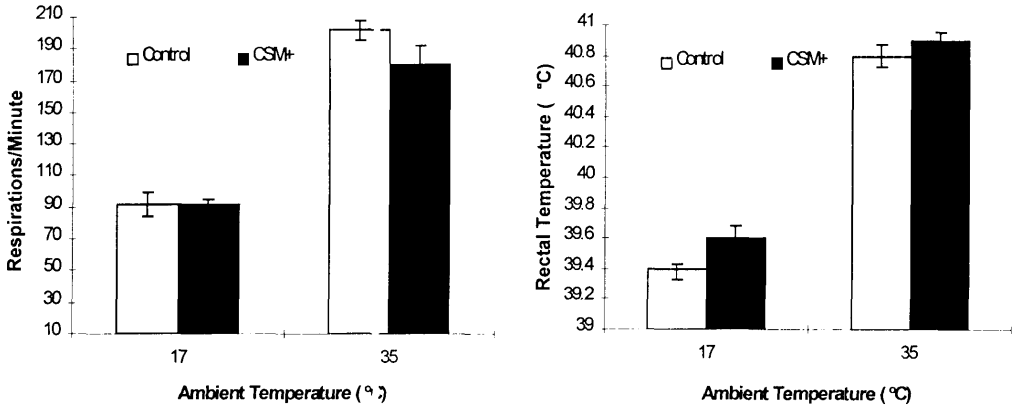

Figure 5.2. Effect of ambient temperature and diet on RR and RT of crossbred lambs (mean±SE, N=4).

Feed and water intake

DMI of chaff was significantly higher (P<0.01) at low than at high temperature but the difference between diets and the interaction between diet and temperature were not significant. Total DMI (chaff+CSM), on the other hand, was significantly higher (P<0.01) at lower temperature than at high temperature, and significantly higher (P=0.04) on the + CSM diet than on the control diet. The interaction between diet and temperature was again non-significant.

The overall WI tended (P=0.06) to be higher on the CSM+ diet than on the control diet. WI at high temperature was significantly (P<0.01) greater than at low temperature. However, the interaction between diet and temperature was non-significant on WI.

The trends in total DMI and WI are illustrated in Figure 5.3.

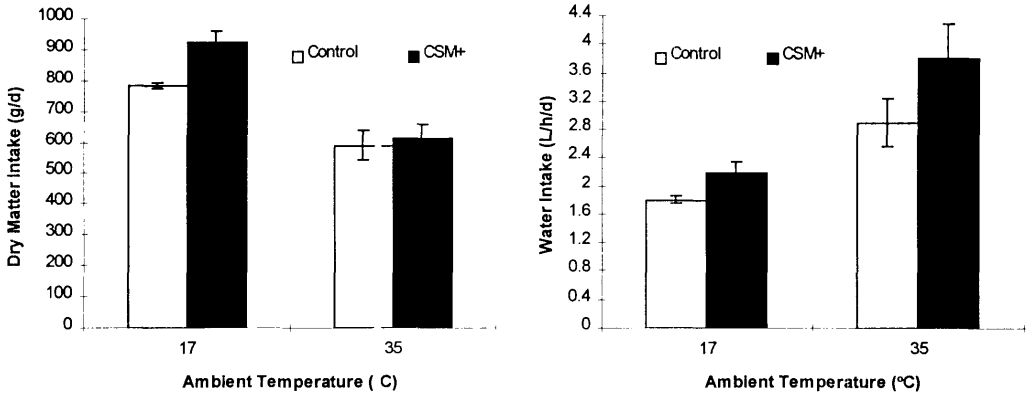

Figure 5.3. Effects of ambient temperature and diet on DMI and WI of crossbred lambs (mean±SE, N=4).

Liveweight gain

The rate of LWG was significantly ($P<0.01$) reduced by high ambient temperature and the interaction between diet and ambient temperature was significant ($P<0.01$). At low temperature, ambs on the CSM+ diet grew significantly faster than those on the control diet. At h gh ambient temperature, on the other hand, those on the CSM+ diet did not perfor n better than those on the control diet, as shown in Figure 5.4, which illustrates the rates of LWG of the groups of lambs and their corresponding estimated ME intakes.

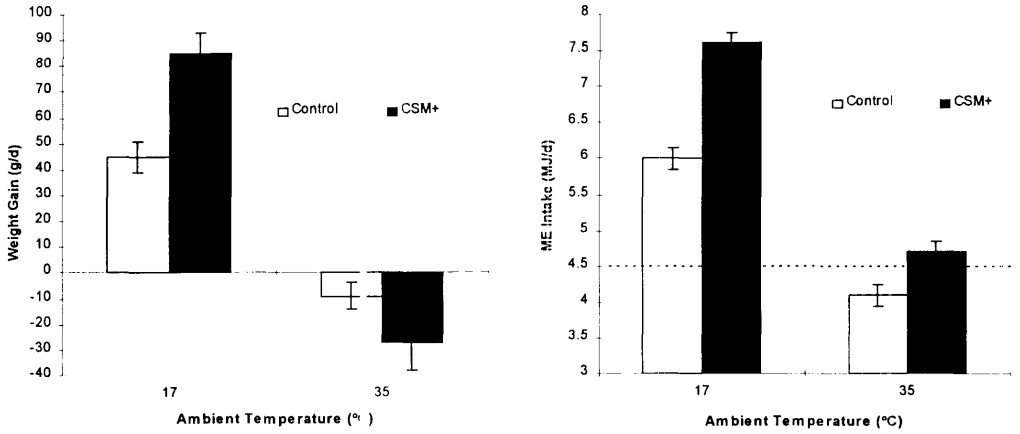

Figure 5.4. Effect of ambient temperature and diet on LWG and estimated ME intake of crossbred lambs in Experiment 3 (mean±SE, N=4; ----- = the calculated maintenance requirement).

Rumen ammonia concentration

The concentration of NH_3-N in the rumen fluid (Table 5.2) did not differ significantly either between diets or ambient temperatures. The mean values from 3 measurements made on weeks 2, 6 and 8 ranged from 310±17 mg N/L in sheep given the CSM supplemented diet at high temperature to 413±20 mg N/L for the control diet at low temperature.

Table 5.2. Rumen NH_3-N (3 h after feeding; mg N/L), plasma urea N (mg N/100ml) and organic matter digestibility (OMD, %) of crossbred lambs fed wheaten chaff+2% urea (control) or wheaten chaff+1% urea+100 g CSM/d (CSM+) at low and high ambient temperature (mean±SE, N=4).

	17±3 °C		35±2 °C		P value		
	Control	CSM+	Control	CSM+	T	D	TxD
NH_3-N							
week 2	387±24	373±21	355±21	342±21	.49	.75	.99
week 6	428±25	345±22	394±22	302±22	.42	.08	.93
week 8	425±24	401±21	399±21	286±21	.13	.14	.32
mean	413±20	373±17	383±17	310±17	.20	.13	.60
Plasma urea N	31.3±.74	28.6±.53	19.1±2.10	12.3±1.49	.00	.01	.20
OMD	62.4±1.13	66.1±.63	59.0±3.25	63.0±2.75	.21	.14	.94

T = temperature effect; D = diet effect; TxD = Interaction

Plasma urea nitrogen

Blood plasma urea nitrogen concentration was significantly higher ($P<0.01$) at low ambient temperature than at high ambient temperature (Table 5.2). The concentration was also higher ($P=0.01$) in the control group than in the CSM+ group. The interaction between temperature and diet, however, was not significant on plasma urea concentration. Figure 5.5 illustrates the relationship between blood plasma urea nitrogen and rumen ammonia nitrogen in the lambs in Experiment 3.

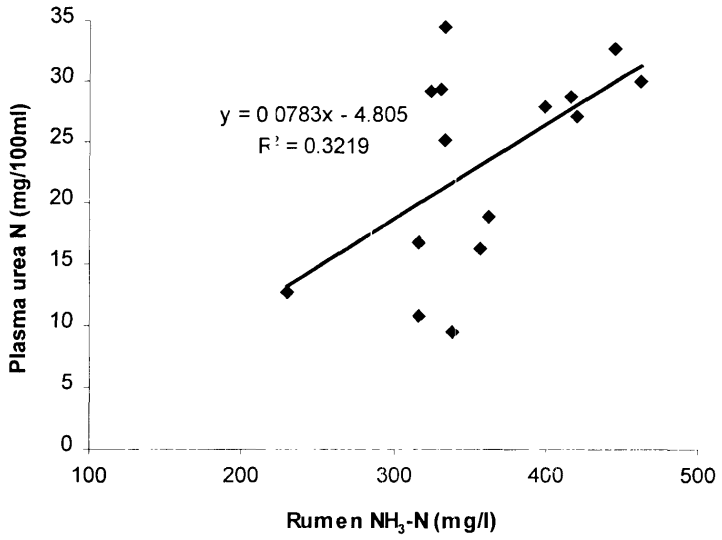

Figure 5.5. Relationship between rumen ammonia N and blood plasma urea N in crossbred lambs in Experiment 3 (data were pooled from both temperatures and diets).

Feed digestibility

As shown in Table 5.2, the digestibility of organic matter was consistently depressed by ambient temperature and the values on the CSM+ diet were generally higher than on the control diet at both ambient temperatures. However these differences were not significant.

5.3.2. Experiment 4

Thermoregulatory responses

As illustrated in Figure 5.6, RR during the day time was generally steady (P=0.37), while at night it significantly (P<0.01) declined over the 30 days as ambient temperature declined. The effect of diet and the interaction between diet and temperature were non-significant on RR.

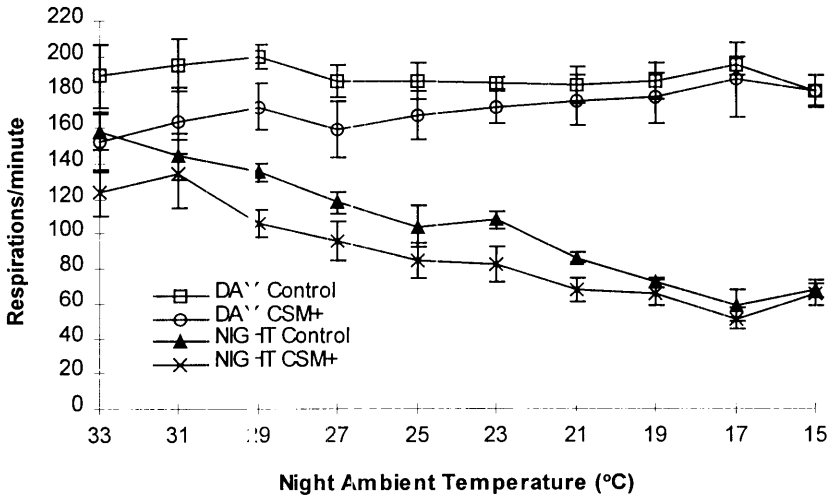

Figure 5.6. Effect of constant day temperature but declining night temperature (2°C per 3 days) on RR of crossbred lambs fed two different diets (mean±SE, N=4).

RT by both day and night (Figure 5.7) declined significantly ($P<0.01$) with declining night ambient temperature. The effects of diet and of the interaction between diet and temperature were non-significant on RT.

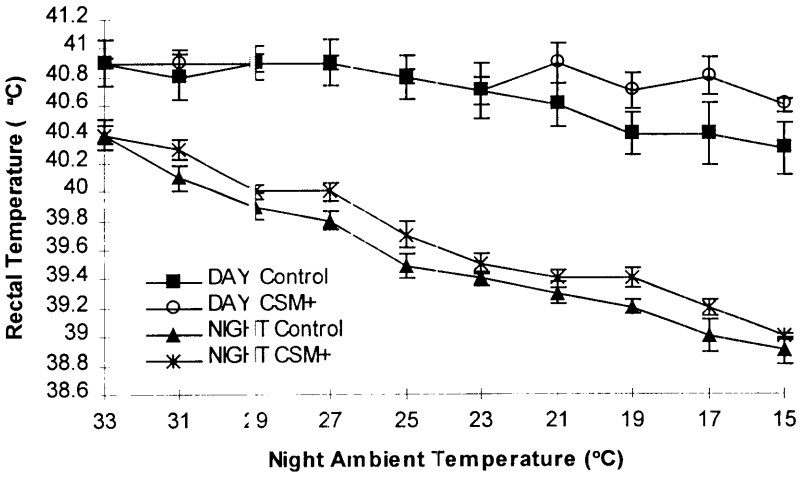

Figure 5.7. Effect of constant day temperature but declining night temperature on RT of crossbred lambs fed two different diets (mean±SE, N=4).

Feed and water intake

There was a significant interaction (P=0.01) between night temperature and diet observed on total DMI (Figure 5.8). As night temperature declined there was a significant increase (P<0.01) in total DMI, but the effect of diet was non significant.

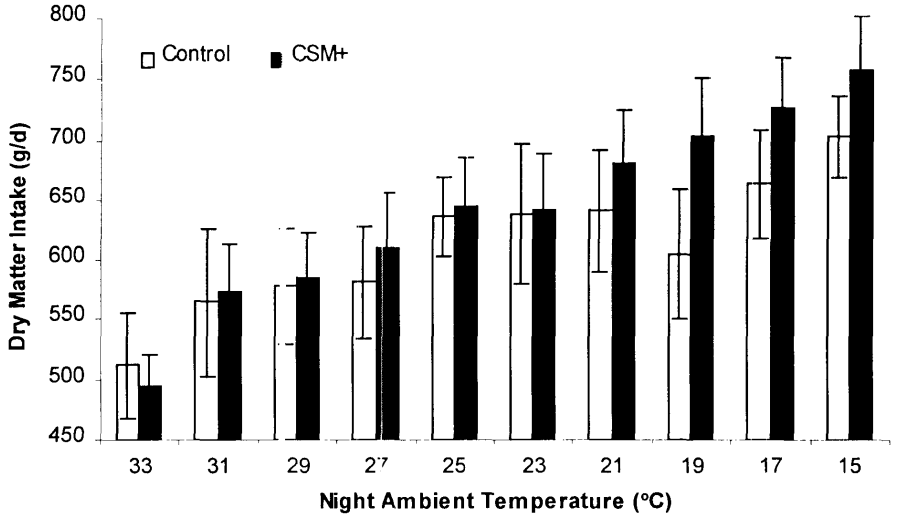

Figure 5.8. Effect of constant day temperature but declining night temperature on total DMI of crossbred lambs fed two different diets (mean±SE, N=4).

As shown in Figure 5.9, chaff intake during the day was significantly (P=0.04) higher in sheep that received the control diet than in those on the CSM+ diet. The interaction between diet and ambient temperature was non significant (P=0.24) on day time chaff intake. Chaff intake at night, on the other hand, was significantly affected by night ambient temperature (P<0.01) but the effect of diet only approached significance (P=0.08), and the interaction between diet and temperature was also non-significant (P=1.00).

The interaction between diet and night ambient temperature was significant (P=0.01) on total chaff intake (Figure 5.9). Total chaff intake (day+night intakes) increased progressively and significantly (P<0.01) as night temperature declined. The total intake was generally higher on the control diet from the beginning of the experiment until night temperature had declined to 19°C. The difference between diets, however, was non-significant (P=0.37).

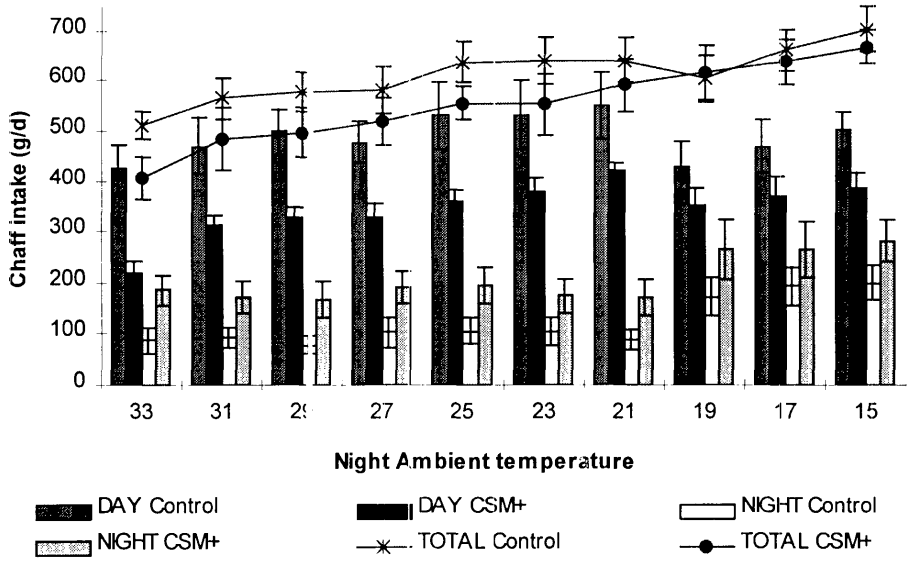

Figure 5.9. Effect of constant day temperature but declining night temperature on chaff intake of crossbred lambs fed two different diets (mean±SE, N=4).

As night ambient temperature declined, there was a significant reduction in day time water intake (P=0.03), night water intake (P<0.01) and total water intake (P<0.01). The effects of diet and the interaction between diet and temperature were non-significant on water intake (Figure 5.10).

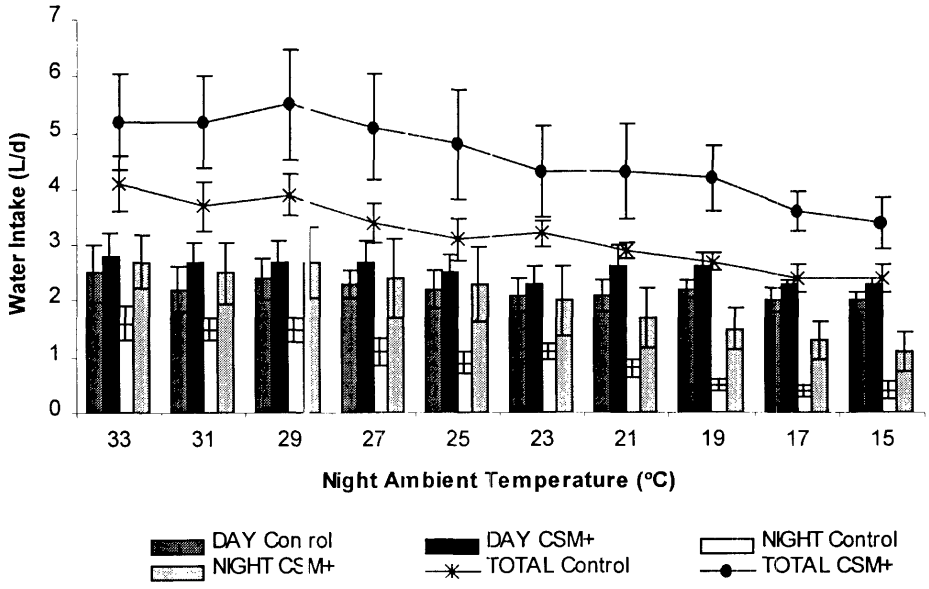

Figure 5.10. Effect of constant day temperature but declining night temperature on water intake of crossbred lambs fed two different diets (mean±SE, N=4).

Liveweight gain and FCR

LWG over the 30-day trial period was significantly higher on the CSM supplemented diet than on the control diet, as shown in Table 5.2.

Table 5.3. Effect of constant day temperature but declining night temperature on liveweight gain (LWG) and feed concersion ratio (FCR) of crossbred lambs fed two different diets (mean±SE, N=4).

	Control	CSM+	P
Initial weight, kg	23.4±1.2	23.4±0.7	1.0
Final weight, kg	24.9±1.3	25.9±0.6	0.5
Gain, g/d	48±7	81±7	0.01
FCR, kg feed/kg gain	13.2±1.5	8.1±0.7	0.02

5.4. Discussion

RR and RT during Experiment 3 significantly increased with ambient temperature regardless of the type of diet. These changes were part of the strategy of the animals to dissipate body heat (Yeates *et al.*, 1975). The relatively high ambient temperature and humidity set in this experiment limited the ability of the animals to reduce body heat through conduction, convection and radiation since the ambient temperature approximated RT. Evaporative cooling by means of increasing RR is an effective strategy for heat-stressed sheep (Bianca, 1968; McDowell, 1980, Johnson, 1987), limited to some extent by the RH experienced. During experiment 4, on the other hand, RR and RT remained relatively high during the day time but, as expected, both declined significantly with reduced night temperature. Irrespective of the details of the changes in RR and RT, the actual values recorded in Experiment 3 indicate that the level of heat stress experienced by the sheep at 35°C was comparable to that observed in the field in the tropics. It is concluded, therefore, that the ambient temperature / humidity regime adopted was successful in mimicking tropical conditions.

In general, DMI declined but WI increased with increased ambient temperature. Significantly higher DMI on the CSM+ diet at low ambient temperature

was probably a result of increased P/E ratio in the absorbed products of rumen digestion in the lambs supplemented with cottonseed meal (Preston and Leng, 1987).

Reduced DMI in both treatments at high ambient temperature was presumably an important strategy adopted by the sheep to minimise their heat production and thus control their body temperature. Acetic acid, which provides energy for most animal tissues (and heat as a product of metabolism, especially in animals given low protein diets), is preferentially produced in low digestibility diets. Heat generated from the fermentation of fibre in low quality forages which contain inadequate levels of protein would be expected to create an additional heat load as a result of the so called 'futile cycles' of acetate metabolism in animals at high ambient temperature (Leng, 1990). Bhattacharya and Hussain (1974) also reported that reductions in DMI and in the utilisation of energy by sheep at high ambient temperature became more severe as the proportion of roughage was increased. While it is acknowledged that there is no simple relationship between RR, RT and DMI and the rate of heat production, the non-significantly different RR, RT and DMI between diets observed at high ambient temperature in the present experiment strongly sugges that under the controlled condition used heat production was not reduced by CSM supplementation. Additional protein from the CSM supplement apparently could not outweigh the negative effects of high temperature. The reduced DMI at high ambient temperature can also be attributed to reductions in thyroid activity due to long-term exposure to heat (Newcomer, 1971; Yousef and Johnson, 1985).

Increased voluntary WI at elevated ambient temperature was probably associated with increased needs for thermoregulation (Bianca *et al.*, 1965; Kare, 1955), for maintaining body water balance in the face of an increased water loss through both respiration (Appleman and Delouche, 1958) and other evaporative cooling mechanisms such as sweating. The slightly elevated WI on the CSM+ diet was probably related to the protein and/or salt content of this supplement, both of which would require more water for excretion of metabolic wastes.

Rumen NH_3-N concentrations were higher than the suggested optimum level of 250 mg NH_3-N (Preston and Leng, 1987) at all sampling times. These high concentrations could be expected to be non-limiting for microbial fermentation in all treatments and thus to have minimised any possible confounding effects of different ammonia concentrations with the effect of level of CSM provided in the diet.

Blood plasma urea nitrogen concentration was significantly higher in sheep on the control diets than in those on the CSM diet, and at low than high temperature. The concentration of NH_3-N in the rumen may, to some extent, have contributed to the observed differences in blood urea N concentration (Figure 5.5). Similarly, the reduction in plasma urea N can be attributed to reduced feed intake (McDowell *et al.*, 1969). From an extensive review Habeeb *et al.* (1992) concluded that blood plasma urea-N in ruminants decreases during heat stress; possibly due to increased recycling of urea-N to the rumen to compensate for any reduced rumen ammonia N level as a result of depression in feed intake. This explanation does not appear to apply in the current work, however, for rumen ammonia N levels in all groups were high (Table 5.2). The plasma urea concentration in the present experiment apparently related to low DMI and thus N intake when ambient temperature was high. Habeeb *et al.* (1992) also suggested that the reduction in blood urea-N at high ambient temperature may be due to increased excretion of urinary nitrogen. Unfortunately, urinary nitrogen excretion was not measured in the present experiment.

Feed digestibility was not affected by either ambient temperature or CSM supplementation in the current experiment. It is likely that the more than adequate soluble nitrogen provided in the form of urea in both diets (as indicated by the ruminal NH_3-N concentration) ensured optimal fermentation in the rumen on all treatments. Leng *et al* (1993) suggested that a concentration of ammonia in the rumen of above 80 mg NH_3-N/l rumen fluid will have little further effect on feed digestibility, and the levels recorded in the present experiment were considerably above that suggested threshold.

The level of DMI is, however, commonly recognised as a major factor affecting the rate of passage and mean retention time of digesta and hence digestibility of a feed (Warren *et al.*, 1974; Christopherson, 1985), and digestibility could thus be expected to increase with reductions in DMI. However, Beede and Collier (1986) suggested that the advantage due to increased digestibility is generally offset by depressed feed intake, resulting in lower availability of nutrients. In the present study, however, a significant reduction in DMI at high ambient temperature did not increase digestibility. It appears likely that the significantly higher WI of sheep at high ambient temperature could have increased the rate of rumen wash out and hence reduced the mean retention time, and reduced digestibility. The factors

may thus have countered the opposing influence of reduced DMI in this case. Concurrent information on fluid and particulate flow rates from the rumen would be needed to verify this suggestion.

It has been suggested that any positive response to bypass protein supplementation (which will vary with the RDP, UDP and soluble CHO levels in the basal diet) may be greater when ambient temperature increases (Leng, 1989). Bypass protein from CSM would be expected to provide additional amino acids for productive functions such as tissue growth. While the rate of liveweight gain in Experiment 3 was almost twice as fast on CSM+ diet as on the control diet at low ambient temperature, differences were non-significant at high temperature.

Apparently, any beneficial effects of CSM supplementation could not outweigh the negative effects on the sheep of the high ambient temperature and humidity in the current experiment. Regardless of diet, feed intake was significantly depressed at high ambient temperature. As a result, less digestible organic matter and less ME was ingested at high ambient temperature than under thermoneutral conditions. In fact, the ME intakes of lambs on the control and CSM+ diets, estimated according to the energy density of feed [(0.16%OMD)- 1.8; SCA, 1990], were 5.97 and 7.56 MJ/d at the low, and 4.13 and 4.69 MJ/d at the high ambient temperature, respectively (Figure 5.4). The calculated ME requirement for maintenance at thermoneutrality for lambs of this weight was 4.54 MJ/d (SCA, 1990) and thus the corresponding predicted liveweight changes are 39 and 82 g/d at low temperature and -11 and 4 g/d at high temperature. These first 3 predicted values were very close to the observed values of 45 and 85, and -9, respectively, but the last value (for sheep on the CSM at 35°C) was higher than that observed. It has been reported that energy retention is reduced when animals are heat stressed (Graham *et al.*, 1959; Blaxter and Wainman, 1961; McDowell *et al.*, 1969; Ames *et al.*, 1971; Brinks and Ames, 1975; Ames and Brinks, 1977) indicating that maintenance energy requirement is increased during heat stress. In the present experiment, a higher ME requirement might have contributed to the loss of weight on both diets at high ambient temperature.

When night time ambient temperature was reduced during Experiment 4, lambs on both treatments gained weight at a rate almost the same as that recorded at low ambient temperature in Experiment 3. This is consistent with the suggestion that low night temperatures facilitated cooling and the reduced heat stress enabled the lambs to increase their DMI. The intermittent high-low (i.e. day-night) temperature during

Experiment 4, which is closer to the real diurnal situation in the tropics, could have allowed the animals to express their capacity to utilise dietary nutrients more efficiently than under a constant high ambient temperature as imposed in Experiment 3. Under the conditions of Experiment 4, lambs fed the more balanced (CSM+) diet may have minimised their dietary-induced thermogenesis and utilised dietary nutrients more efficiently, and thus have grown faster, than those on the control diet. It could also be argued, however, that improved weight gain during Experiment 4 was actually a form of 'compensatory growth' which occurred after a period of weight loss during the preceding 8 weeks (during Experiment 3).

Feed conversion ratios (kg feed/kg gain) during Experiment 4 were lower than they had previously been in the same animals at low ambient temperature during Experiment 3 (13.2 vs 18.4 on the control diet; 8.1 vs 11.1 on the CSM+ diet). Caution must be exercised, however, in extrapolating from these improvements in liveweight gain and feed conversion ratio because Experiment 4 was carried out for only 30 days; gut fill could also have contributed to the observed liveweight gain. Nevertheless, as animals in both treatments experienced similar conditions before Experiment 4 was started, results for liveweight gain and feed conversion ratio can be used, at least, as a relative comparison between the two dietary treatments.

The results of Experiments 3 and 4 demonstrate that, consistent with general findings, high ambient temperature increases RR, RT and WI but reduces DMI and LWG. Supplementation with 100 g/d of CSM could not outweigh the depressing effects of constant high ambient temperature and humidity imposed in the Experiment 3. The digestibility of the basal diet used was probably too low, and the sub-maintenance ME intake observed at high ambient temperature was probably the major factor affecting the loss of weight of sheep fed both the control and the CSM+ diets.

The improved DMI, LWG, and FCR observed when night temperature was reduced cannot be interpreted as being a result of improved efficiency of utilisation of dietary nutrients due to bypass protein supplementation since the results were somewhat confounded by a possible carry-over effects of the preceding dietary and environmental treatments. In the next experiment (Chapter 6), the response of the same lambs to CSM supplementation was reassessed after the animals were reconditioned to eliminate any carry over effects, and a better quality basal diet (oaten chaff) was used.

Chapter 6. Effect of CSM supplementation on the performances of crossbred lambs at either constant high or diurnally high-low ambient temperatures.

6.1. Introduction

In discussion of the results of Experiments 3, the lack of significant responses of sheep to bypass protein supplementation was attributed in part to a constant high ambient temperature - a condition that does not occur in the field. The constant high temperature set in Experiment 3 could have put the sheep under a level of heat stress that could not be alleviated by dietary treatments such as bypass protein supplementation. The results of Experiment 4 suggested that reduced night-time temperature may have improved the efficiency of utilisation of nutrients in sheep fed the CSM+ diet, resulting in a faster growth rate. However, the results were confounded by possible carry-over effects of dietary treatments during Experiment 3. The present experiment (Experiment 5) thus aims to compare the responses of sheep to CSM supplementation at a constant high ambient temperature and at high/low (day/night) temperatures. The experiment was conducted in two stages. The sheep were given a 14-day recovery period between the two stages. It was hypothesised that the level of heat stress would be lower under a diurnally variable temperature regimen than under a constant high temperature, and that the addition of CSM to the basal diet might improve the performance of lambs in the variable, but not in the constant high temperature environment.

6.2. Materials and methods

6.2.1 Experiment 5, Stage 1

Sixteen crossbred (Border Leicester x Merino) wethers, aged 1 year and weighing 4±0.78 kg. were used. All sheep were shorn 4 weeks before the experiment started. Eight animals, randomly chosen, were housed individually in metabolism crates within a climate chamber at a "constant" high ambient temperature of 35±1°C and the other 8 were housed in an adjacent climate chamber with ambient temperatures of 35±1°C by day (from 09.00-18.00 h) and 20±2°C by night (from

18.00-09.00 h). In both rooms, relative humidity was maintained at 60% and the fluorescent lighting was automatically switched on and off at 06.00 and 18.00 h respectively. Four randomly selected animals in each climate chamber were fed *ad libitum* oaten chaff + 2% urea (diet 1) and the other four were fed a basal diet of oaten chaff + 1% urea *ad libitum* and supplemented with 100g CSM/d (diet 2).

Feed and water intakes were measured twice daily (at 09.00 and at 18.00 h) for 2 weeks (days 14-28) after an initial 2-week preliminary period (days 1-13; to allow the animals to adapt to the temperature and dietary treatments). Fresh feed was provided at 09.00 h and the feeders were topped-up at 18.00 h to provide a total feed allowance 15% in excess of the previous day's intake. Water was freely available at all times. RR and RT were recorded twice daily every 4 days. Total collections of faeces were performed for 7 consecutive days during week 4 to estimate dry matter digestibility.

Recovery period

During weeks 5 and 6, ambient temperature in both rooms was reduced to 20°C at all times, but the lighting regime remained the same. All animals were fed *ad libitum* a similar basal diet (unsupplemented oaten chaff) to eliminate the effects of the previous dietary treatments.

6.2.2 Experiment 5, Stage 2

During this stage the basal diet was changed from oaten chaff + 2% urea to oaten chaff only, because supplemental urea was thought to have optimised the rumen function of lambs on the control diet, resulting in no significant effects of CSM supplementation on DMI during Stage 1. Animal allocation and all other treatments remained similar to those of the first stage. Measurements of feed and water intakes, dry matter digestibility, nitrogen balance and purine derivatives were then carried out as described in Stage 1 for another 14-day period. Rumen fluid was collected before feeding and at 3 and 6 h after feeding at the end of week 2 of data collection to measure rumen fluid ammonia concentrations. All animals were weighed at the beginning of Stage 1 and at the end of Stage 2.

Statistical analysis

All data from each stage were subjected to ANOVA for a 2 x 2 factorial design (2 ambient temperatures, 2 diets). Data for rumen fluid NH_3-N from Stage 2 were analysed by ANOVA for repeated measures (2 diets, 4 animals within diet and 3 times of collection). Similarly when comparisons were made between stages, data were analysed by ANOVA for repeated measures. All analyses were performed on the Minitab 10.1 software package (Ryan *et al.*, 1985).

6.3. Results

6.3.1. Stage 1

Results from Stage 1 of Experiment 5 are summarised in Table 6.1.

Table 6.1. DMI (g/d), WI (L/d), RR (resp/min), RT (^{O}C) and OMD (%) of sheep during Stage 1 of Experiment 5 (mean±SE, N=4).

	35°C/20°C		35°C/35°C		Probability[a]		
	Control	CSM+	Control	CSM+	T	D	TxD
DMI:							
Chaff, day	567±25	555±18	562±30	566±15	.92	.96	.90
Chaff, night	445±30	431±21	435±28	425±17	.75	.63	.95
Chaff, total	1012±55	986±39	997±58	990±29	.83	.82	.93
Chaff +CSM	1012±55	1085±39	997±58	1079±29	.83	.12	.93
WI:							
- Day	2.45±.16	2.31±.09	2.02±.04	2.37±.10	.15	.38	.07
- Night	.71±.20	.77±.10	2.11±.07	1.89±.06	.00	.56	.35
- Total	3.16±.35	3.08±.12	4.13±.09	4.26±.03	.00	.92	.64
RR:							
- Day[b]	170±13	186±9	160±14	175±13	.38	.24	.99
- Night[c]	35±2	37±5	162±12	185±5	.00	.12	.18
RT:							
- Day[b]	40.0±.10	40.1±.10	40.0±.05	39.9±.05	.07	.88	.46
- Night[c]	38.4±.10	38.6±.10	39.6±.02	39.6±.10	.00	.14	.45
DMD	52±5	56±1	59±3	57±1	.18	.39	.34

a: T = temperature effect; D = diet effect; T x D = temperature x diet interaction
b: taken at 15.00-16.00;
c: taken at 08.00-0900

Feed Intake

During Stage 1, the intakes of oaten chaff during the day, the night and in total were not affected by either ambient temperature or diet, and the interactions between temperature and diet was non significant (Table 6.1). As well, the total DMI (chaff + CSM) did not differ significantly between diets or ambient temperatures.

Water Intake

The intakes of water during the night and in total were significantly higher at the constant high temperature than in the diurnally variable one, but these intakes did not differ significantly between diets. WI during the day, on the other hand, was not affected either by temperature or diet, and the interactions between temperature and diet were also non-significant. Under the variable day/night temperature regimen of $35^{o}/20^{o}C$, the intake of water during the night was only about 30% of that during the day, regardless of diet. Under the constant high temperature regimen ($35^{o}/35^{o}C$), however, the intakes of water during the day and night were almost identical on both diets and as a consequence the total water intake was significantly higher at $35^{o}/35^{o}C$ than at $35^{o}/20^{o}C$ (Table 6.1).

Thermoregulatory responses

The day-time RR and RT of lambs in the $35^{o}/35^{o}C$ environment were generally similar to those under the $35^{o}/20^{o}C$ temperature during day time. On the other hand, the night-time RR and RT were significantly lower in lambs under the $35^{o}/20^{o}C$ temperature. Supplementation with 100 g/d CSM did not significantly affect thermoregulatory responses, and no significant interactions were observed between temperature and diet.

Feed digestibility

Dry matter digestibility was not significantly affected by either ambient temperature or diet, and the interaction between these factors was also non-significant. The group mean values, however, ranged quite widely from 52 to 59% (Table 6.1).

Recovery period

DMI and WI of lambs during the recovery period did not differ significantly between the previous dietary treatments and ambient temperatures (Table 6.2). Other responses were not measured during this period.

Table 6.2. Effect of previous dietary and ambient temperature treatments on DMI and WI of lambs during the recovery period at 20°C.

Item	Previous temperature and diet				Significance level
	35°C/20°C		35°C/35°C		
	Control	CSM+	Control	CSM+	
DMI (g/d)	1042	066	1057	1059	ns
WI (l/d)	2.8	2.7	2.7	2.9	ns

6.3.2. Stage 2

The results of Stage 2 of Experiment 5 are presented in Table 6.3 and Figure 6.1. Except for the total DMI, the trend of results (Table 6.3) were almost identical to those of Stage 1 (see Table 6.1).

In Stage 2, total DMI was generally higher by an average of 8% than in Stage 1, where urea was included in the basal diet. The total DMI was 10-12% higher ($P<0.01$) on the CSM+ diet than on the oaten chaff diet. The interaction between diet and ambient temperature was non-significant.

Table 6.3. DMI (g/d), WI (L/d), RR (resp/min), RT (^{o}C) and OMD (%) of sheep during Stage 2 (mean± SE, N=4).

	35°C/20°C		35°C/35°C		Probability[a]		
	Control	CSM+	Control	CSM+	T	D	TxD
DMI							
Chaff - day	550±15	572±16	552±28	569±9	.97	.31	.92
Chaff - night	507±11	528±19	527±22	528±15	.58	.52	.56
Chaff - total	1057±24	1010±34	1078±50	1097±21	.79	.39	.73
Chaff+CSM	1057±24	1189±35	1078±50	1187±27	.79	.00	.73
WI:							
- Day	2.27±.13	2.30±.02	1.98±.10	2.35±.11	.26	.07	.12
- Night	1.20±.18	1.15±.06	2.19±.12	2.18±.14	.00	.85	.88
- Total	3.47±.29	3.45±.11	4.17±.14	4.53±.15	.00	.34	.32
RR:							
- Day[b]	163±12	181±8	157±4	172±5	.34	.06	.86
- Night[c]	53±8	52±6	154±6	168±7	.00	.35	.27
RT:							
- Day[b]	39.8±.10	39.8±.02	39.7±.04	39.8±.10	.25	.69	.44
- Night[c].	38.7±.05	38.7±.10	39.5±.02	39.4±.04	.00	.43	.69
DMD	57±1.1	54±0.92	55±0.83	51±4.8	.37	.18	.79

a: T = temperature effect; D = diet effect; T x D = temperature x diet interaction
b: taken at 15.00-16.00;
c: taken at 08.00-0900

The concentration of ammonia in the rumen fluid (Figure 6.1) of sheep receiving the CSM+ diet was significantly higher ($P<0.01$) than in those given the control diet at 35°C/20°C day/night ambient temperatures. At 35°C/35°C, on the other hand, the values did not differ significantly between diets. The effect of time of collection (relative to feeding) and the interaction between diet and time of collection were significant ($P<0.05$) under both ambient temperature regimes; the concentration of NH_3-N in rumen fluid was significantly higher at 3 h after feeding than either before or at 6 h after feeding (Figure 6.1).

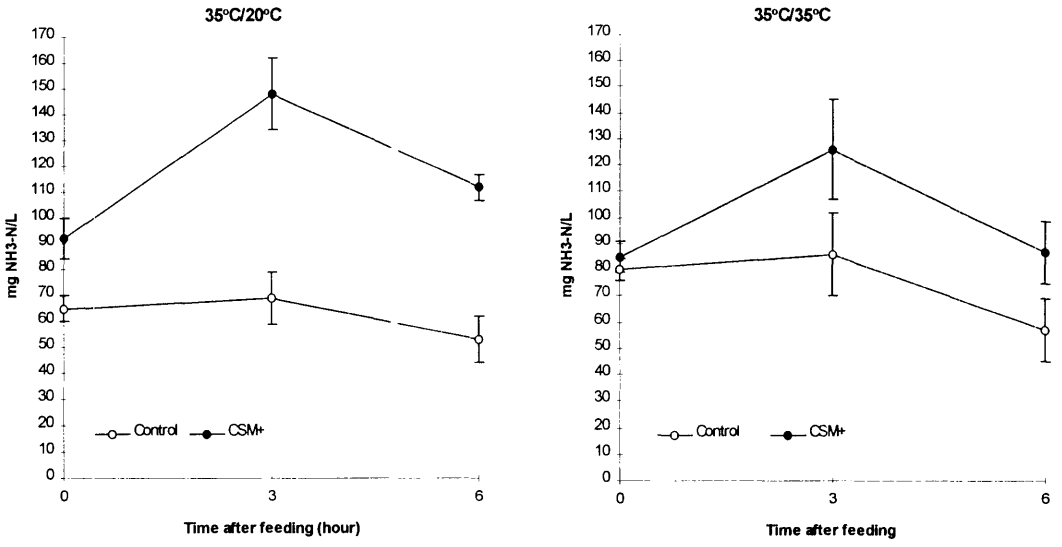

Figure 6.1. Rumen ammonia concentration before feeding and at 3 and 6 hours after feeding in sheep given either the control or the CSM+ diet under 35°C/20°C and 35°C/35°C (day/night) ambient temperatures (mean±SE, N=4).

Liveweight gain

Liveweight gain during the 56-day trial was higher (P=0.02) in lambs fed the CSM supplemented diet (123 g/d at 35/20°C and 118 g/d at 35/35°C) than in lambs on the control diet (90 g/d at 35/20°C and 64 g/d at 35/35°C). These values include the effect of the recovery period because the lambs were not weighed at the end of Stage 1 and at the beginning of Stage 2. The relationship between the digestible DMI and liveweight gain is illustrated in Figure 6.2.

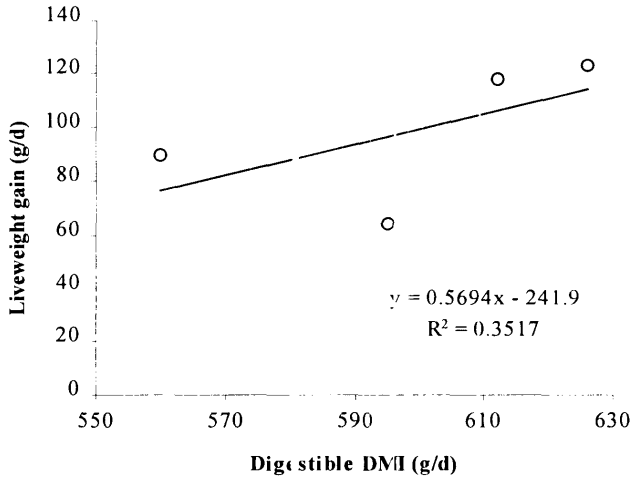

Figure 6.2. The relationship between total digestible DMI and liveweight gain of cross-bred lambs in Experiment 5 (mean of total digestible DMIs in Stages 1 and 2 was used)

6.4. Discussion

During the first stage of this experiment lambs were apparently able to maintain their heat balance at a relatively high level of DMI by increasing their WI, RR and RT. Values for each of these measures were significantly lower when ambient temperature was reduced to 20°C at night in the 35°C/20°C temperature treatment. The relatively high N content of the control diet (9% crude protein) could have provided adequate ammonia N (see Stage 2 for rumen ammonia N concentration) which is the principal source of N for bacterial growth (Hungate, 1966; Leng and Nolan, 1984; Balcells *et al*, 1993). Even though rumen ammonia N can contribute to 40-95% bacterial N (Leng and Nolan, 1984) peptides and free amino acids from oaten chaff might have also increased microbial growth in the rumen (Hume, 1970b). These factors presumably enabled the animals to maintain DMI at the relatively high level of approximately 3% of liveweight. For the same reasons, supplementation with 100 g/d of CSM presumably did not significantly increase DMI. The above characteristics of the basal diet may have limited the response of these particular lambs to bypass protein supplementation (Leng *et al.*, 1977). Rumen distension could also have provided further limitation to DMI. Similarity in DMI was probably the main reason for the similarity in dry matter digestibility in all treatments.

During the 14-day recovery period DMI and WI did not differ significantly from values recorded in the preceding dietary and ambient temperature treatments. Reducing ambient temperature to 20°C during this period did not significantly increased the intake of oaten chaff as compared with that of the basal diet (oaten chaff+1% or 2 % urea) during the first stage when ambient temperatures were much higher. This again demonstrates that these lambs were capable of maintaining a high DMI at these high temperatures.

As expected, with the absence, in Stage 2, of urea in the basal diet, CSM supplementation did stimulate higher DMI ($P<0.01$), probably because N from CSM was available to augment the lower N content of the basal diet. The UDP from the CSM could also have increased the ratio of protein to energy available to the host (Preston and Leng, 1987). However, the actual differences in DMI between sheep on the CSM+ and the control diets ranged only from 109 to 132 g/d, and of that 100 g was CSM. There was thus only a very slight tendency (non-significant) for an effect on intake of the basal diet.

The absence of urea did not depress DMI in Stage 2 relative to that in Stage 1. In fact intake was significantly higher ($P<0.05$) in Stage 2 despite the lower nitrogen content of the basal diet. A time (adaptation) effect may have contributed to the higher DMI in Stage 2.

As explained above, the crude protein content of the oaten chaff used (9% CP) may have been adequate to allow the rumen microbes to maintain an efficient rumen fermentation. This can be explained at least in part by the relatively high rumen ammonia concentration at both temperatures during Stage 2 (see Figure 6.1).

Despite the observed differences in ammonia concentration, digestibility of dry matter did not differ significantly. The ammonia concentrations in all treatments (53 - 148 mg NH_3-N/L rumen fluid, Figure 5.1) were well above the level of 50 mg NH_3-N/L suggested by Satter and Slyter (1974) to be needed for maximum microbial growth. The values recorded here also compare favourably with later reports which suggest the need for higher levels of rumen ammonia. Thus Perdok *et al.* (1988) found that the maximum digestibility of straw by cattle was reached at a rumen ammonia level of 80 mg NH_3-N/L, suggesting that degradation of fibre and starch are optimal at 60-100 mg NH_3-N/L rumen ammonia. However, Kanjanapruthipong (1995) reported that 24 h *in sacco* digestibility of oaten chaff by sheep was not significantly different at rumen ammonia levels of 15, 67, 168 and 213 mg NH_3-N/L.

Although there was a tendency for rate of passage to be increased and thus mean retention time and digestibility to be reduced (Warren *et al.*, 1974; Christopherson, 1985), the relatively high DMI recorded in Stage 2 on both diets and at both temperatures could have contributed to the relatively low digestibilities recorded (55-57%).

As in Stage 1, WI and RR were significantly higher at high ambient temperature. These are well-recognised strategies used by sheep to minimise heat stress and help maintain DMI (Appleman and Delouche, 1958). The effect of CSM supplementation was non-significant on RR and RT, suggesting that heat production was not reduced by additional glucose precursors from the supplement. In fact, day-time RR tended to be higher on the CSM+ diet during Stage 2.

The DMIs in this experiment (Experiment 5) were considerably higher than previously recorded in the same sheep in Experiments 3 and 4; this most likely due to differences in the roughage component of the diet (oaten as opposed to wheaten chaff). In addition, fresh aliquots of chaff were offered twice daily in Experiments 5 and 6, compared to only once daily in Experiments 3 and 4, in order to more closely achieve '*ad libitum*' conditions. The opportunity for selection was thus better in Experiment 5, and the material presented was, on average, fresher.

Liveweight gain was significantly improved by CSM supplementation. Even though the data include the effect of the recovery period, a relative comparison can still be made in liveweight gain because, during the recovery period, all sheep received similar dietary and temperature treatments. These estimates of liveweight gain at least show that, regardless of the nature of the basal diet, CSM supplementation improved liveweight gain. This improvement was possibly contributed to by an increase in total DMI (in Stage 2), and probably by the increased proportion of UDP available for intestinal digestion and absorption due to CSM supplementation (Leng, 1990).