Effects of Excess Nitrogen Intake on Nutrient Utilisation and Partitioning in Lactating Ewes

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of M.Appl.Sc.

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by B. Malik

The effects of different forms of excess nitrogen (amino acids vs ammonia) on the partitioning of nutrients between milk production and body weight change in lactating ewes suckling twin lambs were investigated. Effects on nutrient intake, ewe and lamb live weight changes, milk yield and composition, and nitrogen (N) balance were assessed.

Nine abomasally cannulated lactating 4 year-old Coopworth ewes with mean initial weight of 66.41 ± 5.90 kg each suckling twin lambs with initial litter weight of 5.72 ± 1.22 kg were used. All ewes were fed the same basal diets in 12 equals meals at 2-hour interval. Treatments consisted of abomasal infusions of water (control), casein (150 g/d), and ammonia (85 g/d) in two periods with a carry-over design. Lambs were fed with milk from their own mothers throughout the experimental periods and also given the same amount of 200 g/d of creep feed in the second period.

Ewe dry matter intakes and live weight gain (LWG) were not different between treatments. Lamb LWG was lowest in the ammonia group ($P<0.05$). Casein infusion tended to increase ($P<0.10$) mean milk yield by 33% while ammonia infusion tended ($P<0.10$) to reduce it by 15% of that of control. Milk fat yield was lower ($P<0.05$) in the casein group while no differences were found in milk protein, lactose, total solids, and solid non fat (SNF) yields. Ewes in the ammonia group had the lowest milk protein ($P<0.05$), highest fat ($P<0.01$), and lowest milk SNF concentrations. Milk lactose and total solids concentrations were not affected by treatments.

Dry matter and organic matter digestibilities, digestible dry matter and digestible organic matter intakes were not different between treatments. Casein and ammonia treatments increased N digestibilities ($P<0.05$) and slightly increased N intake ($P<0.10$).

Nitrogen retention per kg metabolic weight was highest in the ammonia group ($P<0.05$). The efficiencies of absorbed N utilisation for milk protein synthesis were higher in the control and casein groups ($P<0.05$) while that for body protein synthesis was higher in the ammonia group ($P<0.05$). Compared to ewes in the control group, those in the ammonia group showed a higher preference for partitioning the absorbed N into body protein synthesis by about 17% ($P<0.05$) and a lower preference for partitioning the absorbed N into milk protein synthesis by about 20% ($P<0.05$). No differences in the partitioning of absorbed N between body and milk protein syntheses were found between the control and casein groups.

It was concluded that the form of excess N (ammonia vs amino acids) differentially affects the partitioning of nutrients between milk production and body weight change in lactating ruminants.

Keywords : excess nitrogen, casein, ammonia, abomasal infusion, twin lambs, lactating ewes.

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CHAPTER 1

INTRODUCTION

1.1. Background to the study

Metabolisable protein (MP) supply to ruminant animals comes from two sources: microbial crude protein (MCP; 60-80%) and undegraded but digested dietary protein (UDP; 20-40%). MCP is synthesised by rumen microbes from rumen degradable protein (RDP), subject to the availability of fermentable metabolisable energy (FME) (Oldham, 1996). Optimum MCP synthesis is achieved where there is a well-balanced supply of FME and RDP. When the availability of FME is limiting relative to RDP (or RDP is in excess relative to FME), the capacity of rumen microbes to capture nitrogen (N) from RDP is limited, which leads to an increase in ammonia (NH_3) concentration in the rumen. The accumulating NH_3 is then absorbed into blood, transported to the liver, converted to urea, and excreted in urine (Beever, 1996).

MP provides the animal with amino acids for body tissue or milk protein synthesis. If the amount of amino acids supplied is in excess of the capacity of the animal to secrete them in milk or store them in body tissue, they will be deaminated with the carbon skeleton used as an energy substrate in various metabolic pathways. The N in the amino groups (NH_2) is converted to, and excreted as urea in urine (Martin and Blaxter, 1965).

NH_3 resulting from excess RDP and/or from deaminated excess amino acids can be considered as two separate sources of excess N excreted by ruminants. New Zealand pastures are high in crude protein (CP) content and provide excess RDP in particular. For instance, the CP content of spring pastures in the Waikato district has been reported to vary from 200-300 g/kg dry matter (DM) (Moller *et al.*, 1993), while Rusdi (1996) reported a CP content of ryegrass in the Lincoln area up to 274 g/kg DM. The high content of CP in pasture is thought to be disadvantageous for several reasons. Firstly, losses of protein in the net transfer of ingested protein to MP will occur when CP content is higher than approximately 210 g per kg of digestible organic matter (DOM), corresponding to 130 g per kg DM at a digestibility of 70% and 150 g per kg DM at a digestibility of 80% (Poppi and McLennan, 1995). Clearly, NZ pasture

exceeds this 'limit' by a considerable margin and extensive losses of pasture protein in the form of urinary N are observed (Cruickshank *et al.*, 1985).

Secondly, urea formation from excess protein and NH_3 requires energy which constitutes a loss of energy for production, therefore decreasing the efficiency of metabolisable energy (ME) utilisation. As a result, the energy loss associated with excreted urinary N decreases the proportion of ME in digestible energy (NRC, 1989). For example, when dietary CP content increased from 190 to more than 230 g/kg DM, the fat corrected milk (FCM) yield decreased from 24.5 to 23.1 kg/day, equivalent to 4.5 MJ NE/day (Danfaer *et al.*, 1980, cited in Oldham, 1984 and NRC, 1989). Data from a number of authors show that estimates of the energy cost of urea synthesis range widely from 15 to 50 kJ per g of N (Martin and Blaxter, 1965; Blaxter, 1967; Tyrrell *et al.*, 1970; Twigge and Van Gils, 1984).

Thirdly, excessive protein intake has also been found to be deleterious to animal reproduction. Sonderegger and Schürch (1977) observed a lengthened interval of parturition to first service by 4-5 days in cows for each 100 g of excess digestible protein provided per day. Jordan and Swanson (1979) concluded that feeding cows with a diet of 19.3% CP resulted in a longer period of 'days open' (106 days), compared to cows receiving diets with 12.7 % CP (69 days) or 16.3% CP (96 days). Fewer service per conception (1.67) were required in groups receiving 12.7% and 16.3% CP compared to that in the 19.3% group (2.47). Reasons for these changes are unclear.

Finally, the conversion of ammonia into urea in mammals occurs in the liver through a cyclic mechanism: the urea cycle (Lehninger, 1982). To form one molecule of urea, one NH_2 group from amino acids combines with one NH_2 group derived from NH_3 (Beever, 1996). Even though theoretically there are a number of N containing metabolites which may contribute NH_2 groups to urea synthesis, amino acids are probably the only N sources available in sufficient quantities, in particular where large amounts of NH_3 are presented to the liver (Lobley *et al.*, 1995), e.g. from excessive intake of RDP as can be expected with NZ pastures.

New Zealand dairy cows consume protein well in excess of their requirements, both RDP and MP (Rusdi and van Houtert, 1997). Negative correlations between blood urea or milk urea levels and milk fat or milk protein

have been observed in a study of nine commercial dairy herds over two springs in 1990 and 1991 in New Zealand where cows grazed pasture alone. There were also positive correlations between urea levels and pasture protein contents (Moller *et al.*, 1993).

While overseas studies (Sonderegger and Schürch, 1977; Jordan and Swanson, 1979a) emphasise the detrimental effects of excessive protein intake on reproductive performance, New Zealand dairy cows do not appear to exhibit poor reproductive performance relative to their overseas counterparts (see Macmillan *et al.*, 1996). In addition, New Zealand dairy cows loose relatively little live weight in early lactation. This phenomenon, perhaps can be explained by the observation that sheep have been shown to give priority to excretion of excess NH_3 over excess AA (Lobley and Milano, 1997). This could occur based on the fact that even though each of the N atoms of urea are believed to come from balanced inputs of mitochondrial NH_3 (via carbamoyl phosphate axis) and cytoplasmic NH_3 (via cytosolic aspartate), it has been demonstrated that NH_3 can provide N for both carbamoyl phosphate and aspartate (Luo *et al.*, 1995). Furthermore, the ratio of NH_3:AA in diet has been shown to affect the ratio of NH_3-N:AA-N in urine (Lobley *et al.*, 1996). Cows with excess MP are faced with a higher concentration of AA relative to NH_3 and may have to mobilise body protein to provide N from NH_2 to be combined with N from NH_3 derived from deamination of AA in urea synthesis. However, the deamination of AA may provide these animals with some energy from carbon skeletons that might be used to drive the urea cycle and produce more milk. Cows with excess RDP are faced with high amount of NH_3 and may not need to catabolise as much body AA to provide NH_2 for urea synthesis. However, these cows still need some energy for urea synthesis that may be obtained by reducing the availability of ME for lactation or mobilising body fat. On the other hand, cows which receive mixed diets (i.e. balanced diets) are not faced with an excess protein relative to ME and may be able to mobilise body tissue to support lactation more effectively than in the case in cows receiving unbalanced diets with a large excess of protein. It can then be hypothesised that the source of excess N (NH_3 *vs* amino acid) plays a role in nutrient partitioning in lactating ruminants. It is intended, therefore, to assess the effect of excess N intake and the effects of different sources of excess N (NH_3 *vs* amino acid) on partitioning of nutrients

between milk production and body weight change in lactating ruminants, using the ewe as a model.

1.2. Hypothesis

The hypothesis to be tested in this project is that the form of excess N (NH_3 *vs* amino acid) differentially affects the partitioning of nutrients between milk production and body weight change in lactating ruminants.

1.3. Objectives

The objective of this project is to assess the effects of casein and ammonia infusions on nutrient intake, body weight gain, milk yield and composition, and nitrogen balance in lactating ewes suckling twin lambs.

CHAPTER 2

REVIEW OF LITERATURE

In this chapter, literature on nitrogen metabolism in ruminants is reviewed. The importance of supply of protein and energy in optimising the utilisation of degraded dietary nitrogen by rumen microbes to synthesis microbial crude protein and the metabolism of absorbed AA by the host animal is discussed. Nitrogen metabolism in the rumen and liver with emphasise on urea synthesis and its consequences to the host animal is covered.

2.1. The supplies of protein and energy to ruminants

It has been well accepted that protein supply to ruminant animals comes from two different sources, namely rumen microbes and by-pass protein. Rumen microbes synthesise dietary protein degraded in the rumen into MCP. By-pass protein or UDP is dietary protein that escapes degradation in the rumen (Webster, 1992; AFRC, 1993; Meehan, *et al.*, 1996). These two different protein sources provide the host ruminants with metabolisable protein (MP). MP describes the quantity of feed and microbial protein digested and absorbed as a mixture of amino acids (AA) which is available to host ruminants for their metabolism (Burroughs *et al.*, 1975; AFRC, 1993; Oldham, 1996). The contribution of MCP to the total AA entering the small intestines is substantial. About 75% of MCP is true protein and about 85% of it are digestible and absorbable from small intestines providing the host animal with a main supply of AA including the ten essential AA (AFRC, 1993; Nolan, 1993). In their review, Clark *et al.*(1992) estimated the proportion of microbial nitrogen (N) that passes to the small intestines of dairy cows to be 59% on average with a range between 34 to 89%. In dairy cows, the requirement of AA for milk protein synthesis increases as milk yield increases. Therefore, it is important to maximise the supplies of AA from microbial protein synthesis in the rumen and to make sure that dietary AA that escape rumen degradation are complementary to microbial protein (Clark *et al.*, 1992; Stern *et al.*, 1994).

In ruminant animals the importance of energy supply is recognised for both rumen microbes and host animal. Dietary constituents in the rumen

undergo fermentation by rumen microbes. This fermentation results in the production of volatile fatty acids (VFA), mainly acetate, propionate and butyrate, carbon dioxide (CO_2) and methane (CH_4) (Merchen and Bourquin, 1994). During the fermentation process, some energy, in the form of fermentable carbohydrates, is utilised by rumen microbes for the synthesis of MCP (France and Siddons, 1993). The VFAs are absorbed through the rumen wall and used as energy source for various metabolic functions in ruminal tissues (Forbes and France, 1993). For rumen microbes, VFAs are the end products of metabolism but for the host animal they are the major source of absorbed energy (Cotta and Hespell, 1986; Corbett, 1987; France and Siddons, 1993) as they provide 50-80% of metabolisable energy (ME) supply (Merchen and Bourquin, 1994).

Carbohydrates including cellulose, hemicellulose, pectin, starch and soluble sugars are the principal substrates for fermentation (Sutton, 1979; France and Siddons, 1993). Dietary proteins also make some contribution that can be significant when the diet contains high rumen degradable protein (RDP). The contribution from lipids is very small (France and Siddons, 1993; Beever and Cottrill, 1994; Meehan *et al.*, 1996).

The synthesis of MCP is believed to depend mainly on the availability of energy resulting from fermentation and the availability of N as ammonia and some AA from degradation of dietary protein (Thomas and Clapperton, 1972). The supply of energy for the use of rumen microbes in MCP synthesis is now commonly expressed as fermentable metabolisable energy (FME). FME is defined (Beever and Cottrill, 1994) as the ME concentration of a feedstuff minus the ME contributed by dietary fats and fermentation products in feeds that have been ensiled or undergone fermentation (Beever and Cottrill, 1994; Meehan *et al.*, 1996).

2.1.1. The relationship between protein and energy supply

The synthesis of MCP in the rumen and the passage of AA into small intestine may be decreased with a deficiency of any nutrients. However, the two nutritional factors that are most likely to be limiting are protein and energy (Clark and Davis, 1983; Hoover and Stokes, 1991; Clark *et al.*, 1992). In high producing dairy cows, the amount of milk produced and the efficiency of feed

utilisation are influenced by the type of carbohydrate given and the ratio of protein to energy in the diet (Clark and Davis, 1980). Using diets that supply 80 and 120% of estimated protein and energy requirements (2 x 2 factorial) in dairy cows in the 6th-10th week of lactation, Gordon and Forbes (1970) reported the associative effects of level of protein and energy intake. They found that the effect of protein and energy on milk yield and composition was related to the level of the other in the diet. It was also shown that partitioning of ME into millk energy output was greater (63%) than into bodyweight change (50%) with the high, than with the low, protein level. Paquay *et al.* (1973) found that the optimal ratio of digestible N to ME was influenced by the stage of lactation. The values (g N intake/MJ ME) were noted to range from 1.55 in the first 3 months to 1.1 beyond 10 months. These workers also concluded that fermentable carbohydrate in the rumen stimulated the utilisation of digestible N by rumen microbes, provided more ME to the animal and increased milk production.

In terms of microbial protein synthesis, the important relationship between protein and energy supplies to the synthesis of MCP has been well recognised. Rapid synthesis of MCP is stimulated by the large and simultaneous supplies of both fermentable N and ME (Huber and Herrera-Saldana, 1994). When the rate of RDP degradation is faster than the rate of energy production (in the form of adenosine triphosphate, ATP), the efficiency of MCP synthesis will reduce which leads to an excess of rumen ammonia. In addition, amino acids will be fermented as an energy source which will also contribute to an increase in ammonia accumulation (Nocek and Russel, 1988). This accumulating ammonia is transfered via portal blood to the liver and excreted as urea in the urine (Huber and Herrera-Saldana, 1994). Conversely, when the amount of fermentable energy exceeds the amount of fermentable N, the synthesis of MCP will be limited to the amount of fermentable N (Nocek and Russel, 1988; Huber and Herrera-Saldana, 1994). The ATP resulting from carbohydrate fermentation will instead be used by rumen bacteria to synthesise storage polysaccharide (Stewart *et al.*, 1981).

The metabolism of absorbed AA by the host animals is also subject to the availability of energy. The incorporation of AA into cell protein will occur only when energy is available (Asplund, 1994). Protein synthesis is an energy-

consuming process. An estimate of 3-5 molecules ATP are required for each peptide bond synthesised (Summers *et al.* 1988) suggesting that energy is the major controlling factor in protein synhesis (Asplund, 1994). When there is a deficiency of dietary energy as VFA (from cabohydrate), ruminants have an ability to utilise absorbed AA and body fat to supply energy for body or milk protein synthesis (Asplund, 1994). Glucose plays an important role in energy metabolism in mammals but ruminants absorb no or little glucose directly from the gut and must rely almost entirely on gluconeogenesis to meet their requirement (Armstrong and Smithard, 1979; MacRae and Lobley, 1986). Gluconeogenic AAs can act as energy sources (MacRae and Lobley, 1986) and when energy supply is lacking and glucose is limiting, gluconeogenic AAs will be metabolised for energy even if AA supply is limiting. This can result in an impaired supply of AA for protein synthesis (Asplund, 1994).

2.1.2. Protein and carbohydrate fermentability

Matching the supply of N and energy in the rumen is important to optimise the synthesis of MCP by rumen microbes and to increase the efficiency of nutrient utilisation (Sinclair *et al.*, 1993; Sinclair *et al.*,1995; Witt *et al.*, 1997). The characteristics of carbohydrates and proteins in feedstuffs determine the availability of these nutrients through fermentation by rumen microbes. Carbohydrates are considered of particular importance because they are the main sources of energy for rumen microbes (Firkins, 1996).

Carbohydrates in feedstuffs can be differentiated into structural and nonstructural components. Structural carbohydrates are insoluble in neutral detergent solution and less degradable (Nocek and Russel, 1988). They include pectin, cellulose, hemicellulose, and lignin (Chase, 1995). Nonstructural carbohydrates (NSC) are not included in the cell wall matrix and not recovered in neutral detergent fibre (NDF). Soluble NSC degrade rapidly and are almost completely fermented (90-100%) in the rumen. NSC include sugars, starches, fructans, galactans, pectins, β-glucans (van Soest *et al.*, 1991; Chase, 1995).

With respect to their susceptibility to ruminal degradation, dietary proteins are differentiated into three distinct fractions (Ørskov and McDonald, 1979). Quickly degradable protein (QDP) is considered as a fraction that is

quickly degraded in the rumen. Another fraction is slowly degraded (slowly degradable protein, SDP). QDP and SDP contribute to rumen degradable protein (RDP). The last fraction escapes rumen degradation (undegraded dietary protein, UDP). These classes of dietary protein are derived from the rates of degradation of feed proteins held in nylon bags within the rumen for various periods of time (Ørskov and Mehrez, 1977), commonly up to 48 hours for concentrates and up to 72 hours for forages (AFRC, 1993).

The notion that fermentability of NSC and RDP has a large effect on ruminal digestion and MCP production has been studied by many workers (e.g. McCarthy *et al.*, 1989; Herrera-Saldana and Huber, 1989; Herrera-Saldana *et al.*, 1990; Stokes *et al.*, 1991a,b). Stoke *et al.* (1991a) studied a continuous culture in which three different diets containing 25, 37, and 54% of dry matter (DM) as NSC were given. Within each NSC level, five diets with different amounts of RDP were prepared. The ratio of NSC to RDP in these diets ranged from 1.9-8.9:1. The level of NSC up to 37% DM optimised carbohydrate availability and at all levels of NSC, bacterial efficiency increased with increasing level of RDP. It was concluded that maximum fermentation in a continuous culture of rumen contents was achieved when diets contained 37% or more NSC and when the ratio of NSC to RDP was <3. Using three diets with different ratios of NSC to RDP, Stoke *et al.* (1991b) found that greater MCP was supported by diets containing 31 or 39% NSC and 11.8 or 13.7% RDP in DM, respectively. In reviewing data of prior studies, Hoover and Stokes (1991) noted a high correlation ($r = 0.94$) between synthesis of MCP per unit of carbohydrate digested and intake of RDP as intake of RDP increased from 5 to about 20% DM. Results from some other studies were in agreement with the above findings while others were not. In cows, rapidly degradable carbohydrate and protein (barley plus cotton seed meal) gave higher milk production (Herrera-Saldana and Huber, 1989) and MCP synthesis (Herrera-Saldana *et al.*, 1990) compared to less ruminally degradable diets of milo and brewer grains. However, more soluble and degradable carbohydrate (i.e. barley) did not increase the passage of AA to the small intestine (McCarthy *et al.*, 1989) nor the milk production when given synchronously with urea (Casper and Schingoethe, 1989) and a synchronous release of energy and N supply did not enhance MCP synthesis in the rumen (Henning *et al.*, 1993). In reviewing the studies in which

improved synchrony of energy and N release in the rumen has been claimed to increase MCP synthesis, Chamberlain and Choung (1995) noted that the effect of synchronisation was confounded by the effect of characteristics of the feeds, as changes in synchronisation had often been achieved by manipulation of different dietary ingredients.

2.2. Nitrogen metabolism in ruminants

Before entering the intestine, feedstuffs consumed by ruminants are all initially exposed to fermentative activity in the rumen. Fermentation of feedstuffs in the rumen results in the production of short-chain VFA (principally acetate, propionate and butyrate), CO_2 and CH_4. The VFA are absorbed through rumen wall and used by host animal as energy sources. Ammonia, free amino acids and other simple N compounds are also produced from breakdown of N-containing feedstuffs. During the fermentation process, energy (ATP) is yielded and utilised to support microbial growth and the synthesis of microbial protein from N compounds. The microbial protein and part of the dietary protein that escapes rumen fermentation pass to the abomasum and small intestine and supply amino acids to ruminant animals (Czerkawski, 1986; Mackie and White, 1990; Aharoni and Tagari, 1991; Broderick *et al.*, 1991; France and Siddons, 1993; Chamberlain and Wilkinson, 1996).

2.2.1. Mechanism of dietary protein degradation in the rumen

The nitrogen transactions in the rumen are described diagramatically in Figure 2.1. In the rumen, RDP is mostly broken down to ammonia (NH_3) and VFA. This process involves a number of microbial activities such as protein hydrolysis, peptide degradation, amino acid deamination and fermentation of resultant carbon skeletons (Cotta and Hespell, 1986).

Ruminal peptide breakdown is a two-step process. Initially, the main amino-peptidase activity found in mixed ruminal bacteria breaks di- and tri-peptides rather than single amino acids from peptide chains. The enzymes involved in this process are dipeptidyl peptidase (DPP). The di- and tripeptides resulted from the activity of DPP are then hydrolised into free amino acids by separate di- and tripeptidase activities (Tamminga, 1979; Wallace *et al.*, 1990; Wallace, 1996).

The mechanism of protein degradation varies somewhat between bacteria and protozoa. With bacteria, the protein chain is broken down into smaller parts by hydrolisis of some or all of its peptide bonds. This process occurs outside the bacterial cell. The peptides and amino acids resulting from this process are then transported inside the bacterial cells and the peptides undergo further hydrolysis to amino acids. Thereafter, the amino acids are either combined into bacterial protein or broken down to VFA, NH_3, CO_2, CH_4, and some fermentation heat. End products of this degradation are voided back into the surrounding medium (Tamminga, 1979). With protozoa, proteolysis of dietary protein is initiated by an engulfment of protozoa to small feed particles. The proteolysis occurs inside the protozoal cell. Some of the resulting amino acids are incorporated into protozoal protein while the remainings are often voided into surrounding medium rather than being degraded further (Coleman, 1975; Tamminga, 1979).

2.2.2. Sources of nitrogen for rumen microbes

Ammonia, amino acids, peptides, urea, nucleic acids, and other N-containing compounds including nitrate and choline are the sources of N for microbial protein synthesis in the rumen with ammonia as the primary source

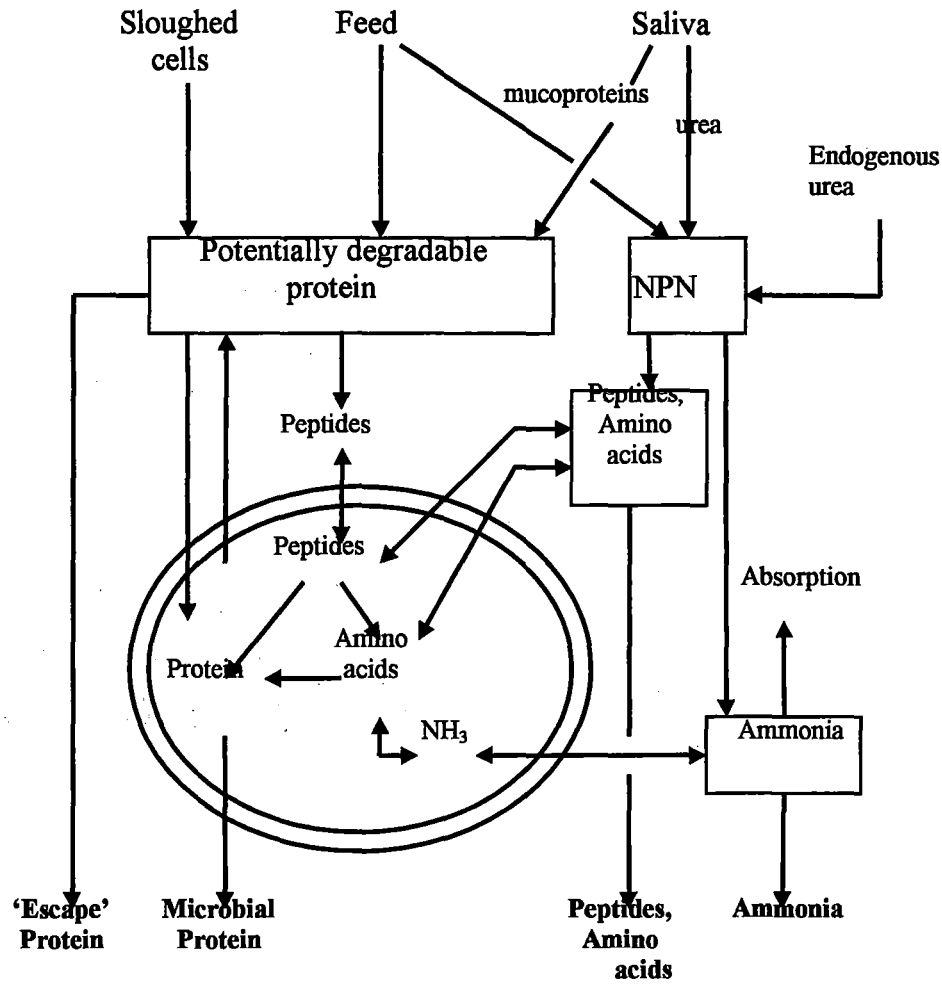

Figure 2.1. A model of nitrogen transactions in the rumen. The ovals represent the microbial cell wall (after Nolan, 1993)

(Blackburn, 1965; Allison, 1970; Al-Rabbat *et al.*, 1971; Wallace *et al.*, 1997). Nolan and Leng (1972) calculated ammonia and amino acid contributions to total microbial N as 80% and 20%, respectively. A study by Pilgrim *et al.* (1970) showed that in sheep fed a high-N diet (lucerne hay), an average of 63% of bacterial N and 38% of protozoal N were derived from ammonia. In sheep fed a low-N diet (wheaten hay), the values were 77% and 54%, respectively.

The major fate of peptides and amino acids arising from proteolysis is to be taken up by the microorganism and then to be incorporated into protein or broken down into ammonia. Non-ammonia N sources, predominantly peptides and amino acids, contribute about 10-50% of microbial N (Nolan, 1975; Leng and Nolan, 1984). Rumen microbes seem to have no specific requirement for amino acids. This was shown in an early study of Loosli *et al.* (1949) where large amounts of ten essential amino acids were synthesised in the rumen of ruminants fed urea as the only source of N. Virtanen (1966) found that in cows fed a protein-free diet, the rumen microbes were still be able to synthesise all amino acids that make up milk protein. However, more recent studies (Argyle and Baldwin, 1988; Cruz Soto *et al.*, 1994) demonstrated that rumen microbial growth was greatly stimulated by peptides and amino acids when rapidly fermentable energy sources are available. This phenomenon, however, is not likely to occur if available energy sources are slowly degradable. Chikunya *et al.* (1996) found that ruminal fermentation was not stimulated by soluble protein when fibre was slowly degraded. The potential stimulatory effect of peptides and amino acids on the growth of rumen microbes is, therefore, dependent on the nature of energy sources (Cruz Soto *et al.*, 1994; Wallace, 1996).

Urea is broken down very rapidly in the rumen resulting in ammonia that can be utilised by rumen microbes to synthesis microbial protein. The urea entering the rumen comes either from feed or from recycled urea by endogenous salivary excretion or diffusion across the rumen wall (Virtanen, 1966; Roffler and Satter, 1975; Nolan *et al.*, 1976; Kennedy and Milligan, 1980). This urea recycling is of great importance in allowing ruminant animals to survive under conditions of limited N supply (Blackburn, 1965).

The rate of urea hydrolysis in the rumen is very rapid and usually faster than the rate of ammonia assimilation. This phenomenon can lead to ammonia overflow, rapid absorption of ammonia into portal circulation, and inefficient N

retention (Chalupa *et al.*, 1970; Wallace *et al.*, 1997) or even ammonia toxicity. The addition of urea, therefore, will be of benefit only to animals given low protein, high energy diets with ammonia concentration in the rumen not greater than 50 mg/l l rumen fluid (Roffler and Satter, 1975). The feeding of readily available carbohydrates is important when urea is added to a diet in order to synchronise ammonia production and ammonia utilisation (Allison, 1970).

2.2.3. Nitrogen metabolism in the liver

Metabolic activity in the liver tissues is intense which is indicated by its oxygen consumption. In cattle, the uptake of oxygen in the liver contributes about 18-26% of whole-body oxygen consumption (Huntington and Reynolds, 1987; Eisemann and Nienaber, 1990; McBride and Kelly, 1990; Reynolds *et al.*, 1991a). In ruminants, amino acid metabolism in the liver is important not only for protein synthesis but also for gluconeogenesis and ureagenesis (Bergman and Heitmann, 1978). Protein synthesis is a major fate of amino acids utilisation in the liver of ruminant. Protein synthesis in liver tissues accounts for 9-13% of whole-body protein synthesis in sheep and cattle (Davis *et al.*, 1981; Eisemann *et al.*, 1989).

In ruminants, the absorption of glucose from the gastrointestinal tract is negligible. They must rely almost entirely on gluconeogenesis from various substrates as the major source of blood glucose (Wolff and Bergman, 1972; Armstrong and Smithard, 1979; Armstrong and Weekes, 1983; MacRae and Lobley, 1986). Glucose synthesis occurs mainly in the liver (about 85%) and also in the kidneys (about 15%) (Bergman *et al.*, 1974) with carbon skeletons provided by propionate, lactate, and AA (Bergman and Heitmann, 1978; Huntington, 1990; Demigné *et al.*, 1991). The quantity of AA contributing to glucose carbon is variable. In sheep, it was indicated that 11-32% of the glucose turnover is derived from AA (Wolff and Bergman, 1972; Bergman and Heitmann, 1978; Lindsay, 1982). In cattle, AA contribution to hepatic glucose production is 12-16% (Huntington, 1990). Glycine, alanine, glutamine, glutamate, and serine are highly glucogenic AA that have been observed (Bergman and Heitmann, 1978). Alanine and glutamine make up to 60% of AA

contribution to glucose synthesis (Bergman and Heirmann, 1978; Lindsay, 1980).

Microbial fermentation and metabolism of AA in the gastrointestinal tract and peripheral tissues of ruminants result in ammonia as an end-product which is to be converted into non-toxic urea for excretion. Urea is synthesised in the liver by a cyclical series of reactions known as ureagenesis (Newsholme and Leech, 1983; Meijer *et al.*, 1990; Lobley and Milano, 1997). Urea resulting from this process is predominantly excreted in the urine and some is excreted into the lumen of the digestive tract via saliva or direct transfer from blood (Satter and Roffler, 1975; Huntington, 1989). Alanine, glycine, and glutamine continuously released by gastrointestinal tract and peripheral tissues are taken up by liver. This mechanism provides a way for transporting ammonia to the liver for detoxification through ureagenesis (Bergman and Heitmann, 1978; Reynolds, 1992).

Gluconeogenesis and ureagenesis are interrelated based on the notion that both syntheses depend on, and compete for, ATP (Krebs *et al.*, 1976). In rat isolated hepatocytes, however, no inhibition of urea synthesis was observed because of the competition for ATP by gluconeogenesis (Wojtczak *et al.*, 1978). Another reason is that both gluconeogenesis and ureagenesis share intermediate stages (Krebs *et al.*, 1976). These syntheses share the enzymes involved in the metabolic pathways including aspartate and malate dehydrogenase and mitochondrial metabolite transport systems such as glutamate, aspartate, and 2-oxoglutarate (Martin-Requero *et al.*, 1992). Interference of one process by the other may be expected especially when rates are high (Krebs *et al.*, 1976). However, results of studies as whether the rate of either pathway affects the other are variable. Studies on relationship between gluconeogenesis and ureagenesis were often approached by examining the effect of propionate on ureagenesis. This is based on the notion that in ruminants, in most dietary conditions, the gastrointestinal tract supplies very low glucose and relative high ammonia (Orzechowski and Motyl, 1989) and that propionate is believed to be the main precursor of glucose synthesis (Demigné *et al.*, 1991). Whether propionate influences the rate of ureagenesis is, therefore, worthy of investigation. In sheep infused with ammonia and/or propionate into the

mesenteric vein, no effect of propionate on urea synthesis was observed (Orzechowski *et al.*, 1988). In the presence of 18.2 mM propionate, the rate of ureagenesis by slices of sheep liver was reduced to 40% of that observed in the absence of propionate (Rattenbury *et al.*, 1983). *In vivo* study in non-lactating dairy cows receiving intra-ruminal infusion of urea (Choung and Chamberlain, 1995) showed a reduced rate of ureagenesis when propionate was infused intra-ruminally. In contrast, in isolated sheep hepatocytes, propionate stimulated ureagenesis (Garwacki *et al.*, 1990; Demigné *et al.*, 1991). Glucose synthesis from propionate was reduced by 43% with high concentration of ammonia (4 mM) (Demigné *et al.*, 1991) and by 57.5% with 2 mM inclusion of ammonium chloride (Weekes *et al.*, 1979). Glucose synthesis from alanine and glutamate was inhibited by ammonia (Mustvangwa *et al.*, 1996). Urea (Leonard *et al.*, 1977; Emmanuel and Edjtehadi, 1981), NH_4Cl (Fernandez *et al.*, 1990) treatments and abomasal infusion of casein (Guerino *et al.*, 1991) have also been found to increase hepatic ammonia concentration and reduce glucose production. Changes in citrulline synthesis, which might be due to depressed activity of carbamoyl-phosphate synthetase (CPS I), are likely to be the explanation for the opposite effects of propionate and ammonia (Orzechowski and Motyl, 1989). In urea cycle (Figure 2.2.) in liver mitochondria, CPS I is an enzyme which catalyses the first reaction between ammonia and CO_2 and ATP to produce carbamoyl phosphate. The carbamoyl phosphate then reacts with ornithine to form citrulline catalysed by ornithine transcarbamoylase (Meijer *et al.*, 1978; Newsholme and Leech, 1983). The synthesis of mitochondrial carbamoyl phosphate by CPS I is often considered as the rate-limiting step in ureagenesis (Meijer *et al.*, 1990). However, considering that citrulline synthesis is dependent upon the mitochondrial concentration of both ornithine and carbamoyl phosphate, it is suggested that rather than carbamoyl phosphate, ornithine is likely to be more regulatory for ureagenesis in the liver (Meijer *et al.*, 1978). In contrast to

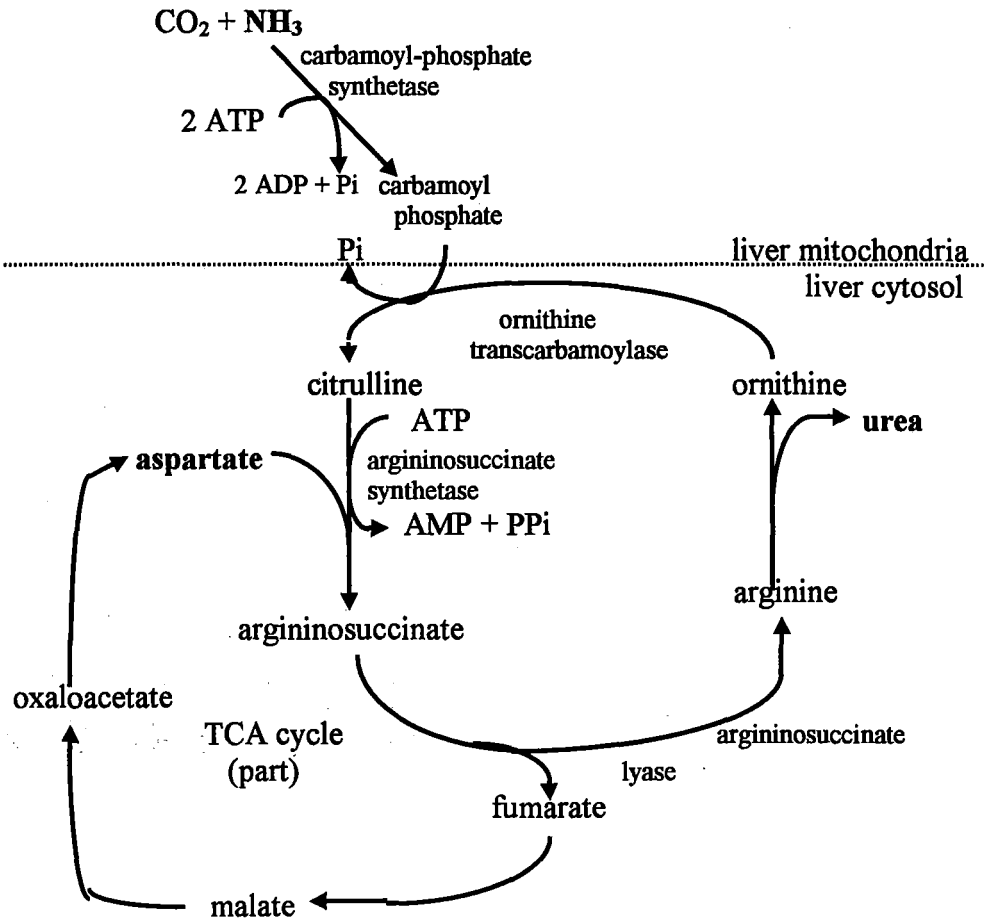

Figure. 2.2. Urea cycle (after Newsholme and Leech, 1983)

propionate, ammonia has been shown to enhance citrullinogenesis (Orzechowski and Motyl, 1989).

With regard to various effects (no effect, negative, or positive) of propionate on ureagenesis, Lobley and Milano (1997), in their review, noted that the pre-incubation in some of these studies was different. In order to allow ureagenesis to proceed, minimum quantities of carbon sources (propionate, lactate, AA, etc.) are required. Graded addition of propionate (0 to 1.6 mM) to ovine hepatocytes incubated in the presence of physiological concentrations of NH_3 and/or AA clearly demonstrated this phenomenon. Initial stimulation of ureagenesis was shown with low propionate up to 0.4 mM above which the inhibition of ureagenesis was observed.

Under normal physiological and nutritional conditions, the synthesis of urea from absorbed NH_3 by the liver is efficient. In a variety of different diets, 70-95% portal NH_3 is extractable by the liver (Parker *et al.*, 1995). NH_3 is very toxic and an acute ammonia toxicity can lead to inappetance, infertility, tetany, coma, and death (Soar *et al.*, 1973; Lobley and Milano, 1997). Severe signs of intoxication are expected to occur in cattle when the concentration of NH_3 exceed 0.8 mmol/l in the arterial plasma (Symonds *et al.*, 1981a,b) or 1.05 in the carotid blood (Davidovich *et al.*, 1977) or 0.57-0.68 mmol/l in the jugular blood (Bartley *et al.*, 1977; Davidovich *et al.*, 1977). The capacity of sheep and cattle liver to metabolise NH_3 is about 1.18-2.13 μmol/min per g liver weight (Linzell *et al.*, 1971; Symonds *et al.*, 1981a,b; Orzechowski *et al.*, 1987).

Inputs of N atoms in ureagenesis come from NH_3 through mitochondrial carbamoyl phosphate synthesis and AA through cytoplasmic aspartate synthesis (Newsholme and Leech, 1983). Within a range of diets, NH_3-N could account for 27-110% to hepatic urea-N formation (Parker *et al.*, 1995) while NH_2-N could contribute 16-30% (Huntington, 1989; Reynolds *et al.*, 1991b). As ruminant liver has a limited capacity to metabolise NH_3 and *in vitro* study (Luo *et al.*, 1995) has shown a possibility of transfer of N from NH_3 to aspartate, then a question arose as whether the liver has a priority to excrete NH_3 or AA if one of these compounds is in excess over the other. In their recent review, Lobley and Milano (1997) addressed this matter by studying two scenarios: enhanced NH_3 or AA supply into sheep fed to 1.5 maintenance. The first scenario was tested by infusion of NH_4HCO_3 at 1.1 mmol/min for 20 min.

This resulted in a maximal ureagenesis at 1.4 mmol urea-N/min. Peripheral hyper-ammonaemia occurred and the removal of NH_3-N to urea-N synthesis increased to 0.89 from 0.46 under basal conditions while the input of N from AA was reduced. When graded amounts, to 2 mmol N/min, of an AA mixture were infused, a maximal rate of ureagenesis, similar to that with NH_4HCO_3 infusion, was observed. Inspite of a lowered apparent NH_3 removal, the absolute hepatic extraction of NH_3 remained constant even though the fractional contribution of NH_3 to ureagenesis decreased. Based on these findings, it could be said that ruminants would give a priority to excretion of excess NH_3 over excess AA. This notion was supported by the results of Luo *et al.* (1995) and Mustvangwa *et al.* (1997) that showed a preferential utilisation of NH_3 by isolated hepatocytes for urea synthesis through both mitochondrial carbamoyl phosphate and cytoplasmic aspartate synthesis.

2.3. Effects of excess nitrogen intake in ruminants

2.3.1. Efficiency of metabolisable protein and metabolisable energy utilisation

A decrease in the efficiency of N utilisation in ruminants may occur with a high nitrogen intake, as a significant proportion of the ingested N may fail to reach the small intestines (Pisulewski *et al.*, 1981; Ulyatt *et al.*, 1988). The loss of N during digestion in the rumen is accelerated when pasture crude protein (CP) is high and rapidly degraded (Wohlt *et al.*, 1978; Corbett, 1987). Losses of protein in net transfer of ingested protein to MP will occur when CP content of pasture is higher than approximately 210 g per kg digestible organic matter. This corresponds to 130 g CP per kg DM at a digestibility of 70% and to 150 g CP per kg DM at a digestibility of 80% (Poppi and McLennan, 1995). These losses of N are largely in the form of ammonia which is absorbed from the rumen and excreted as urinary urea (MacRae and Ulyatt, 1974; Wanjaiya *et al.*, 1993).

Ureagenesis in the liver of ruminant is an energy-demanding process (McBride and Kelly, 1990). Energy required for urea formation from excess AA and NH_3 constitutes a loss of energy for production, therefore decreasing the gross efficiency of ME utilisation. As a result, the energy loss associated with

excreted urinary N decreases the proportion of ME in digestible energy (Schneider *et al.*, 1980; NRC, 1989). For example, when dietary CP content increased from 190 to more than 230 g/kg DM, the fat corrected milk (FCM) decreased from 24.5 to 23.1 kg/day, equivalent to 4.5 MJ net energy (NE)/day (Danfær *et al.*, 1980 as cited in Oldham, 1984 and NRC, 1989). Data from a number of authors show that estimates of the energy cost of urea synthesis range widely from 15 to 50 kJ per gram of N (Martin and Blaxter, 1965; Blaxter, 1967; Tyrrell *et al.*, 1970; Twigge and van Gils, 1984).

Various estimates for energy cost of urea synthesis have been based on the assumption that stoichiometrically 4 ATP are required to synthesis 1 mole urea (Martin and Blaxter, 1965). It, therefore, can be expected that increasing ammonia concentrations will enhance oxygen consumption by the liver as a result of an increased rate of ureagenesis (Reynolds *et al.*, 1992). However, this is not always the case as diets designed to promote NH_3N supply from the gut in sheep (Mustvangwa *et al.*, 1996) and cattle (Reynolds *et al.*, 1991a,b) and infusion of ammonium chloride in the mesenteric vein of lambs (Lobley *et al.*, 1995) have not given any significant increased in liver oxygen consumption. This phenomenon perhaps could be explained by the notion of Newsholme and Leech (1983) that in the synthesis of one molecule of urea only one ATP is required in the urea cycle because NADH is generated to produce three ATP through the conversion of malate to oxaloacetate in the tricarboxylic acid (TCA) cycle (see Figure 2.2.).

2.3.2. Catabolism of amino acids

The conversion of ammonia into urea in mammals occurs in the liver through a cyclic mechanism named the urea cycle (Lehninger, 1982; Newsholme and Leech, 1983). To form one molecule of urea, two amino (NH_2) groups are required (Beever, 1996). One NH_2 group is derived from NH_3 removed from the portal vein by the liver. Through its condensation with mitochondrial CO_2 to form carbamoyl phosphate, this NH_3 enters urea cycle (Newsholme and Leech, 1983; Meijer *et al.*, 1990). Another NH_2 group comes from cytoplasmic aspartate to which glutamate is possibly the immediate N-donor as it is involved in various transamination reactions (Newsholme and

Leech, 1983; Lobley *et al.*, 1995). Theoretically there are a number of N containing metabolites which may contribute NH_2 group to urea synthesis. However, AA are probably the only N sources available in sufficient quantities to furnish the extra N (Lobley *et al.*, 1995).

From this phenomenon, a concept has been developed that increased NH_3 load on the liver can induce a penalty in terms of AA catabolism (Reynolds, 1992; Parker *et al.*, 1995). Several observations where the increase in hepatic urea-N production was greater than that in NH_3-N removed by the liver are believed to support this concept. Reviewing the work of several authors, Parker *et al.* (1995) noted that the potential contribution of hepatic NH_3 uptake to urea-N release ranges from 0.27 to 1.10 or in other words, the magnitude of hepatic urea-N production could be up to 3 times greater than the amount of hepatic NH_3 uptake. In lambs given a 4-day infusion of ammonium chloride into mesenteric vein, NH_3, at the highest, contributed 66% and 54% of urea-N for low and high infusion, respectively, indicating a requirement of major inputs from other N sources. Indeed, the oxidation of leucine was observed to be increased with high NH_3 infusion (Lobley *et al.*, 1995). Compared to cattle fed cereal diets, in cattle fed forage, the uptake of NH_3 by the liver was doubled (Fitch *et al.*, 1989; Huntington, 1989) and the removal of NH_2-N was twofold resulting in a decrease of splanchnic supply of NH_2 to peripheral tissues (Huntington, 1989). An increase in AA oxidation has also been noted by Liu *et al.* (1995) in sheep with a long-term excess of dietary protein intake. In a recent study, Mustvangwa *et al.* (1997) incubated isolated hepatocytes from sheep fed basal diet plus urea with NH_4Cl in the presence of L-[^{15}N]alanine or L-alanine and L-[1-^{14}C]alanine. Even though there was no changes in [1-^{14}C]alanine oxidation, an increase in the production of $^{14}N^{15}N$-urea and $^{15}N^{15}N$-urea was observed lending support to the suggestion that ureagenesis during NH_3 surplus may enhance AA deamination.

Other studies, however, have failed to give any supports to the proposal that ammonia detoxification can induce AA catabolism. A chronic (4 days) intra-mesenteric vein infusion of NH_4HCO_3 in sheep resulted in urea-N equivalent to only 1.2 of the amount of hepatic NH_3 uptake without any alteration in leucine oxidation (Lobley *et al.*, 1996). In sheep hepatocytes incubated with varying levels of $^{15}NH_4Cl$ in the absence or presence of a

physiological mixture of amino acids, Luo *et al.* (1995) observed that instead of $^{14}N^{15}N$, the $^{15}N^{15}N$ was the predominant form of urea-N. The same indication was observed by Mustvangwa *et al.* (1996). With regard to these conflicting results, it may be wise to say, as pointed out by Lobley *et al.* (1996) that any minimised contribution of non-NH_3 compounds to urea synthesis would substantially save such nitrogenous metabolites for anabolic processes.

2.3.3. Reproductive performances

Excessive nitrogen intake has been found to be deleterious to ruminant reproduction. The negative effects on reproduction have been noted in dairy cows fed excess CP regardless of protein sources and degradability (Elrod *et al.*, 1993). Reproductive traits that have been observed to be influenced by excess N intake include interval of parturition to first service (e.g. Sonderegger and Schürch, 1977), period of days open (e.g. Jordan and Swanson, 1979a), service per conception (S/C) (e.g. Edwards *et al.*, 1980), conception rate (e.g. Folman *et al.*, 1981), and pregnancy rate (e.g. Butler *et al.*, 1996).

Several mechanisms by which high N intake effect fertility have been proposed, namely direct effects on sperm, ova, or the uterine environment; alterations in the functions of the hypophyseal-pituitary-ovarian axis which then alter gonadotrophin or progesterone secretion; and imbalances in protein-energy supply (Ferguson and Chalupa, 1989; Canfield *et al.*, 1990). High intake of N leads to increased ammonia and urea N concentrations in blood, reproductive tract, and uterine and vaginal secretions (Jordan *et al.*, 1983; Carroll *et al.*, 1987; Carroll *et al.*, 1988). Urea has been found to have spermicidal effect *in vitro* (Dasgupta *et al.*, 1971; Umezaki and Fordney-Settlage, 1975; Visek, 1982) and detrimental effect to the early development and survival of sheep (Bishonga *et al.*, 1994) and rat (Saitoh and Takahashi, 1977) embryos *in vivo*. A change in uterine pH has also been noted in cattle fed excess either RDP or UDP (Elrod and Butler, 1993; Elrod *et al.*, 1993).

Feeding high CP diets did not give any significant effect on the production of luteinizing hormone (LH) and progesterone in non-lactating dairy cows (Blauwiekel *et al.*, 1986). An *in vivo* study in dairy cattle (Erb *et al.*, 1976a) showed that dietary urea was consistently associated with decreased

plasma LH and increased plasma progesterone. Conversely, a high CP intake induced an increase in serum LH and a decrease in progesterone in cows between the first and second week postpartum (Jordan and Swanson, 1979b). The binding process of LH to ovarian receptors has been shown to be inhibited by a high concentration of urea *in vitro* (Haour and Saxena, 1974) that could result in a decrease in serum progesterone (Jordan *et al.*, 1983). A low concentration of progesterone resulting from a high concentration of urea could lead to an increased embryonic loss as has been shown in rats *in vitro* (Saitoh and Takahashi, 1977). However, decreases in LH concentration were not always accompanied by decreases in progesterone secretion as shown by Howard *et al.* (1987) and Carroll *et al.* (1988).

With regard to reproductive traits, some workers (Treacher, 1976; Sonderegger and Schürch, 1977; Jordan and Swanson 1979a) observed a lengthened interval from parturition to first service in cows fed high CP diets while others (Kaim *et al.*, 1983; Carroll *et al.*, 1988; Canfield *et al.*, 1990) did not. A 4-5 day longer interval of parturition to first service in cows for each 100 g of excess digestible protein provided per day was observed (Sonderegger and Schürch, 1977).

Longer period of 'days open' have been shown in cows fed high CP (Jordan and Swanson, 1979a; Edwards *et al.*, 1980; Folman *et al.*, 1981) while it was not shown in another study (Howard *et al.*, 1987). Jordan and Swanson (1979a) concluded that feeding cows with a diet of 19.3% CP resulted in a longer period of 'days open' (106 days) compared to cows receiving diets with 12.7% CP (69 days) or 16.3% CP (96 days).

Fewer S/C were required in groups fed lower CP (Jordan and Swanson, 1979a; Edwards *et al.*, 1980). However, in some studies, S/C were not different in either lower or higher CP groups (Erb *et al.*, 1976b; Howard *et al.*, 1987). Groups receiving 12.7% and 16.3% CP required only 1.67 S/C compared to 2.47 S/C of 19.3% CP groups (Jordan and Swanson, 1979a). Conception (Folman *et* al., 1981; Ferguson, 1988; Canfield *et al.*, 1990) and pregnancy (Ferguson, 1988; Butler *et al.*, 1996) rates have been noted to be lower in higher CP groups while these traits were shown not different in either groups in another study (Howard *et al.*, 1987).

2.4. Conclusions

A well-balanced supply of protein and energy is required to optimise the efficiency of utilisation of ruminally degraded N by rumen microbes to synthesis MCP. An appropriate supply of energy is also required for metabolism of absorbed AA by the host animals. An excess of NH_3 resulting from microbial fermentation of RDP and/or metabolism of AA in the gastrointestinal tract and peripheral tissues of ruminants has to be excreted as urea through the urine. The synthesis of urea brings some consequences to the host ruminant including reduced efficiency of MP and ME utilisation, possibility of AA catabolism, and possibly impaired reproductive performance. The preference of ruminants to excretion of excess NH_3 over excess AA has been shown. It, therefore, is worthwhile to asses whether different sources of excess N will give different consequences to ruminants and lactating ruminants in particular.

CHAPTER 3

MATERIALS AND METHODS

3.1. Time and site of experiment

The experiment was conducted from July to October 1997 at the Johnstone Memorial Laboratory of Lincoln University, Canterbury, New Zealand.

3.2. Animals

Nine lactating 4 year-old Coopworth ewes with mean initial weight of 66.4 ± 5.9 kg at lambing were randomly allocated into 3 groups of 3 animals each. All ewes were cannulated intra-abomasally. Surgery was done 8 weeks before lambing. Once they lambed, all the ewes were allocated to individual metabolic crates. Each ewe was suckling twin lambs. Lambs of each ewe were allocated into individual crates next to that of their mothers. Each crate occupied by the lambs had an access to that of the ewes. Total lambs used were 18 with mean initial weight of 5.7 ± 1.2 kg at the start of the experiment.

3.3. Feeds

All ewes were provided with the same basal diets consisting of 29.5% barley, 20% lucerne, 0.5% $NaHCO_3$, and 50% commercial standard concentrate on a dry matter basis. The commercial standard concentrate was supplied by Weston Milling (Rangiora, NZ) and consists of brollard, wheat, barley, coconut (copra) meal, limestone, molasses, salt, and vitamin premix (Weston Calf Premix). Diets were designed to provide 80% of metabolisable energy (ME) for lactating ewes and adequate supplies of rumen degradable protein (RDP; 294 g/d) and metabolisable protein (MP; 280 g/d) (AFRC, 1993). Diets were given *ad libitum* and rations were provided as twelve similar portions daily by means of automated feeders. Two weeks were given as an adaptation period. The composition and nutritive value of the diet is given in Table 3.1.

All lambs suckled milk from their own mothers. Access for suckling was given three times a day: in the morning, midday, and afternoon for 5-10 minutes each. In the second period, in addition to milk, the lambs were provided with diets consisting of 59% barley, 40% lucerne, 1% $NaHCO_3$ at a rate of 200 g/lamb/day.

Table 3.1. Ingredients and nutritive values of experimental diets

Ingredient/nutrient	g/kg of dry matter
Barley	295
Lucerne	200
$NaHCO_3$	5
Commercial Standard Concentrate*	500
Organic Matter	930
Crude Protein	145
Metabolisable Energy (MJ/kg DM)**	11.2

* consists of brollard, wheat, barley, coconut (copra) meal, limestone, molasses, salt, and vitamin premix (Weston Calf Premix)

** estimated from the equation of Alderman (1985)

3.4. Infusates

Casein, ammonia, and water infusions were given intra-abomasally to the ewes. The infusates were prepared to supply each ewe with 150 g casein (20 g N) per day in the form of sodium caseinate and 85 g (1.08 mol) ammonia (15 g N) per day in the form of ammonium bicarbonate (NH_4HCO_3) (150 g/d of sodium caseinate or 85 g/d of ammonium bicarbonate solubilised in 2 l of water). The infusates were prepared daily and the infusion lines were maintained regularly. Infusates were continuously infused by a peristaltic pump CPP30 (Chemlab, England) at the rate of 2 l/24 h. Water was infused as control.

3.5. Experimental design

The experiment was conducted in two periods with a carry-over design. In each period each ewe received either ammonia, casein, or water infusion per abomasum

. In period 1, two, three, and four ewes received ammonia, casein, and water infusions, respectively. In period 2, four ewes received ammonia infusion

while three and two other ewes received casein and water infusions, respectively. The infusions covered five weeks in each period. The actual amount of infusates infused was recorded daily. At the start of each period the infusates were given at 33% strength by the first day, 67% strength by the second day, and full strength by the third day thereafter. Treatments' allocation in each period is given in Table 3.2.

Table 3.2. Treatments allocation

Ewe	Period 1	Period 2
1	casein	casein
2	casein	control
3	casein	ammonia
4	control	ammonia
5	control	control
6	ammonia	casein
7	control	ammonia
8	control	casein
9	ammonia	ammonia

3.6. Feed intake

Feed intake was recorded daily from the difference between the amount of feed given and the amount of feed refusal. Feed offered and refused were sub-sampled for chemical analysis.

3.7. Live weight gain

All ewes and lambs were weighed weekly. Live weight gain per day of both ewes and lambs was calculated by regression of live weight on time, using all weight recordings.

3.8. Milk yield and composition

The amount of milk produced by each ewe was determined by a weekly hand-milking for 5 consecutive weeks in each period. On the day of milking, at 0830 h the ewes were given intramuscular injection of 2 ml oxytocin each and their lambs were immediately given access for suckling. After 5-10 minutes the

lambs were taken from the ewes and the udders of the ewes were cleared out by hand-milking and time was recorded. About six hours later, another intramuscular injection of 2 ml oxytocin was given to each ewe before hand-milking started. At the end of milking, time was recorded and the amount of milk produced by each ewe was corrected into g/ewe/d by multiplying it by 4. Samples of 60 g milk from individual ewe were taken and stored at -20 °C for later chemical analysis. Milk composition (protein, fat, lactose, total solids, and solids non fat) was analysed on Foss Milko-scan 133B (Foss Electric, Denmark) with calibration based on Rossbiot-bub and Kjeldhal for fat and protein, respectively at Meadow Fresh Foods (Canterbury) Limited Christchurch. Milk gross energy content was estimated using the equation recommended by Šebek and Everts (1993).

3.9. Nitrogen balance and feed digestibility study

Nitrogen balance and digestibility studies were performed in the third week of both periods. Collections of faeces and urine were made over 5 days. The animals were held in metabolism crates equipped with separator sieves and chutes. Two 10 litre polyethylene buckets were placed under each crate to keep urine and faeces produced by the ewes. The urine was delivered to the bucket which contained 400-500 ml glacial acetic acid (99.85%) as preservatives. Such amount of acetic acid used was based on the observation that to reduce pH of 1 litre urine down to about 3, a total of 400 ml acetic acid (99.85%) was needed. Faeces and urine from the buckets were weighed and 10% of those was taken as sub-samples daily. Bulked samples of faeces and urine for over 5 days were stored at -20 °C for later chemical analysis.

During the 5-day collection period, lambs were given access to their mothers for suckling. To avoid contamination of urine and faeces from lambs to those of the ewes, the lambs were fitted out with baby nappies while they were suckling.

Faecal samples were freeze dried. Feeds and faeces were analysed for dry matter (DM) by oven drying at 95°C for 48 h and for organic matter (OM) by ashing at 550 °C for 8 h. The analysis of N for feeds, faeces, and urine was performed by Kjeldahl method on Tecator Kjeltec Auto 1035

Analyser (Sweden). The analysis of fat for feeds and faeces was performed by adapted Soxlet method on Tecator Soxtec System HT 1043 Extraction Unit (Sweden). The procedures were as described by the manufacturer. Acid detergent fiber (ADF) content of feeds was analysed using methods described by van Soest (1973). Metabolisable energy (ME) content of feeds was estimated by an equation recommended by Alderman (1985).

3.10. Nitrogen kinetics

An isotope study was performed to investigate whole-body protein turnover in the fifth week of both periods. The aim was to estimate amino acid flux using a tracer of L-[4,5-^{3}H(n)]-leucine and ^3H_2O. The methods used in this study were adopted from those of Cronjé *et al.* (1992). Ewes were inserted with catheters in both jugular veins on the day preceding to tracer infusions. A solution containing 250 μCi L-[4,5-^{3}H(n)]-leucine in sterile physiological saline (9 g/l NaCl) was continuously infused intravenously to each ewe over 12 h at a rate of about 0.25 ml/min. Four days after this infusion, a single injection of 200 μCi ^3H_2O in saline solution was administered intravenously to determine the irreversible loss rate (ILR) of body water. Data resulted from these procedures was to be used to calculate the oxidation rate of [^{3}H]-leucine.

3.10.1. Sampling and analysis

Blood samples for analysis of leucine specific radioactivity (SR) were collected into heparinized tubes at intervals of 0, 0.5, 1, 2.5, 5, 6, 8, 9.5, 11, and 12 h after commencement of the infusion. Samples were centrifuged and plasma was stored at -20 °C for later analysis.

Blood plasma samples were deproteinised with acetonitrile (CH_3CN) (3 ml/ml plasma) (Jones and Gilligan, 1983). Deproteinised plasma was centrifuged for 5 minutes at 9500 rpm. Plasma leucine was derivatised with orthopthalaldehyde (OPA) reagent (1 μl OPA/10 μl deproteinised plasma sample) (Jones and Gilligan, 1983). Plasma leucine concentration was determined by high-performance liquid chromatography (HPLC) (LC Module 1 Waters Associates, USA). Chromatography was performed by a reverse phase

column (C8 Water Radial-Pak Cartridge Type 8NVC84μ) at room temperature. Leucine was detected by fluorescence with wavelengths of 365 nm for excitation and 470 nm for emission (Shimadzu 12F-535). Solvents used in the chromatography were methanol (A) and 0.1 M ammonium dihydrogen orthophosphate ($NH_4H_2PO_4$) and pH was adjusted to 6.4 with 4M NaOH (B). The methods used were 0-10 min 50% A + 50% B at the flow rate of 2 ml/min, 10-20 min gradiant up to 1.0A at 2 ml/min, and 20-25 min 1.0A at 2 ml/min. Fractions from HPLC were collected within 0.5 and 1 minute intervals and 1 ml of these was mixed with 10-12 ml scintillation liquid (Optiphase 'HiSafe' 3, SC/9205/21). Beta emission of the fractions was counted on Wallac 1409 Liquid Scintillation Counter (Wallac, Finland).

The estimate of fractional contribution of [^{3}H]-leucine to the ILR of body water was to be obtained from the SR of urinary ^3H_2O. For this purpose, urine excreted during the infusion of L-[4,5-^{3}H(n)]-leucine was collected quantitatively into 30-75 ml glacial acetic acid (99%) at the intervals of 0, 0.5, 1, 2.5, 5, 6, 8, 9.5, 11, and 12 h after commencement of the infusion. Over the following four days, urine was collected in daily basis into 400-500 ml glacial acetic acid (99%). Samples were stored at -20 °C for later analysis. ^3H_2O of the urine was isolated by vacuum sublimation and counted in the same scintillation liquid as used for leucine isolates.

A single injection of 200 μCi ^3H_2O in saline solution was administered at four days after L-[4,5-^{3}H(n)]-leucine infusion to determine the ILR of body water. Samples of urine excreted at 1, 5, 10, 15, 21, and 24 h after ^3H_2O injections were collected into 30-500 ml glacial acetic acid (99%). Samples were stored at -20 °C for later analysis. ^3H_2O of the urine was isolated by vacuum sublimation and counted in the same scintillation liquid as used for leucine isolates. SR of urine was used to estimate the ILR of ^3H_2O.

3.10.2. Calculation

The intention was that the leucine flux rate would be calculated as follows:

Infusion rate (DPM/min)

$$\text{Flux } (\mu\text{mol/min}) = \frac{}{\text{SR at plateau (DPM/}\mu\text{mol)}}$$

Leucine oxidation rate was to be calculated from the proportion of tritium infused as L-[4,5-^{3}H(n)]-leucine which appeared in the body ^3H_2O pool during the leucine infusion. Water pool size and ILR were calculated as follows:

$$\text{SR at time t} = A.e^{-kt}$$

$$\text{Pool size (g)} = \frac{\text{Dose } ^3H_2O \text{ injected (DPM)}}{\text{A (DPM/g)}}$$

$$\text{ILR (g/h)} = \text{Pool size (g)} \times \text{k (/h)}$$

3.11. Statistical analysis

Results were average corrected means across periods I and II. Data were subjected to analysis of variance by REML procedures of Genstat 5 Release 4.1 (Lawes Agricultural Trust, 1997). The effects of pre-treatment and treatment were used in the fixed model. The value of 0 was used as pre-treatment for all sheep in period I. The values of 1,2,3 (representing control, casein and ammonia, respectively) as treatments in period I were used as pre-treatments in period II.

CHAPTER 4

RESULTS

4.1. Infusions

The intra-abomasal infusions of casein and ammonia were well maintained throughout the experimental periods. The mean actual amount of casein infused was 145.62 ± 4.40 g/ewe/d (20.25 g N/d) and of ammonia was 84.99 ± 1.77 g ammonia/ewe/d (12.59 g N/d).

4.2. Feed intake

Mean dry matter intake (DMI) from feed differed significantly between treatments ($P<0.05$). DMI were 2.16, 2.22, and 1.92 kg/ewe/d for ewes in control, casein, and ammonia treatments, respectively. When expressed as per kilogram metabolic body weight, however, the mean DMI were not different ($P>0.25$). DMI per kilogram metabolic body weight were 103.1, 104.3, and 95.6 g/kg $W^{0.75}$/d for ewes in control, casein, and ammonia treatments, respectively. Mean DMI for each treatment is presented in Table 4.1.

Table 4.1. Mean dry matter intakes (DMI), metabolisable energy (MEI) and metabolisable protein intake (MPI) in lactating ewes given abomasal infusions of casein, ammonia or water (control)

Parameter	Control	Casein	Ammonia	sed*
Feed DMI (kg/ewe/d)	2.16[a]	2.22[a]	1.92[b]	0.11
Infusate DMI (kg/ewe/d)	0	0.14	0	-
Feed DMI (g/kg $W^{0.75}$/d)	103.1	104.3	95.6	5.67
MEI (MJ/ewe/d)**	24.2	24.9	21.5	-
MPI (g/ewe/d)***	210	242	192	-

[a,b] : means in the same row with different superscripts are different ($P<0.05$)
* : standard error of differences
** : estimated the equation of Alderman (1985)
*** : estimated from AFRC (1993)

4.3. Live weight gain

Mean ewe live weight (LW) over the whole period was significantly different ($P<0.05$) between treatments: 57.5, 59.3, and 56.5 kg/ewe for control, casein, and ammonia treatments, respectively. However, mean ewe live weight gain (LWG) was not different: 77.6, 103.8, 97.5 g/ewe/d for control, casein, and ammonia treatments, respectively.

In contrast, both mean LW and LWG of the lambs were found to be significantly different between treatments. Mean lamb LW was 15.6, 16.6, and 14.9 kg ($P<0.01$) for control, casein, and ammonia treatments, respectively. Mean lamb LWG was significantly ($P<0.05$) lower (65.9 g/d) in ammonia group compared to those in other groups (132.1 and 165.5 g/d for control and casein groups, respectively). One lamb of the ewe in cage 10 (casein) died during the experiment due to pneumonia. However, since the effect of this loss on mean lamb LW of its sibling might not be more than 10%, the sibling was not excluded from the experiment. LWG of the ewes and lambs are presented in Table 4.2. Effect of treatments on lamb LWG is illustrated in Figure 4.1.

Table 4.2. Mean live weight (LW) and live weight gain (LWG) of lactating ewes given abomasal infusions of casein, ammonia or water (control) and of their lambs

Parameter	Control	Casein	Ammonia	sed*
Ewe LW (kg/ewe)	57.5^{b}	59.3^{a}	56.5^{b}	0.91
Ewe LWG (g/d)	77.6	103.8	97.5	46.57
Lamb LW (kg/lamb)	15.6^{B}	16.7^{A}	15.0^{B}	0.46
Lamb LWG (g/d)	132.1^{a}	165.5^{a}	65.9^{b}	21.18

^{A,B} : means in the same row with different superscripts are different ($P<0.01$)
^{a,b} : means in the same row with different superscripts are different ($P<0.05$)
* : standard error of differences

4.4. Milk yield and composition

The effects of treatments on milk yield and composition are summarised in Table 4.3. Compared to control, casein infusion tended ($P<0.10$) to increase

milk yield by 33% while ammonia infusion tended to decrease it by 15%. The amount of milk yielded was 1383, 1841, and 1174 g/d for ewes in control, casein

Table 4.3. Mean milk yield and composition in lactating ewes given abomasal infusions of casein, ammonia or water (control)

Parameter	Control	Casein	Ammonia	sed*
Yield (/d)**				
Milk (g)	1383	1841	1174	309.3
Milk Protein (g)	87.6^{ab}	110.4^{a}	65.3^{b}	17.9
Milk Fat (g)	97.5	115.2	80.0	21.3
Milk Lactose (g)	65.7	89.4	54.9	15.3
Milk Total Solids (g)	260.0	328.6	208.8	54.7
Milk SNF (g)	162.6^{ab}	213.4^{a}	128.8^{b}	35.2
GE (MJ)	6.9	8.5	5.4	1.4
Composition (g/kg)				
Milk Protein (P)	62.7^{a}	61.0^{a}	56.3^{b}	2.1
Milk Fat (F)	65.7^{AB}	59.3^{B}	75.1^{A}	5.3
Milk Lactose	47.8	47.9	46.6	0.9
Milk Total Solids	183.6	175.5	185.8	5.1
Milk SNF	118.0^{A}	116.2^{A}	110.6^{B}	2.0
GE (MJ/kg)***	4.8	4.5	4.7	0.2
Milk F/P Ratio (g/g)**	1.0^{ab}	0.9^{b}	1.4^{a}	0.1

^{A,B} : means in the same row with different superscripts are different ($P<0.01$)
^{a,b} : means in the same row with different superscripts are different ($P<0.05$)
* : standard error of differences
** : means over 5 weeks
*** : estimated from the equation of Šebek and Everts (1993)

and ammonia groups, respectively. Milk protein and solids non fat (SNF) yields were significantly ($P<0.05$) increased by casein infusion (110.4 and 213.4 g/d for milk protein and SNF yields, respectively) while they were significantly decreased by ammonia infusion (65.3 and 128.8 g/d for milk protein and SNF yields, respectively). Trends ($P<0.10$) to increase yields by casein infusion and decrease yields by ammonia infusion were found in milk total solids, milk lactose as well as milk gross energy (GE). No significant effect of infusions was found on milk fat yield.

While milk protein concentration was depressed (56.3 g/kg; $P<0.05$), milk fat concentration was increased (75.1 g/kg; $P<0.01$) in ammonia infusion compared to those in casein infusion (61.1 and 59.4 g/kg for milk protein and

milk fat concentrations, respectively). Significant increases ($P<0.01$) in milk SNF concentration (110.6 g/kg) and milk fat to protein (F/P) ratio (1.36) were also shown in ammonia group. No significant effects were found on either milk lactose, milk total solids or milk GE concentrations. The significant effects of treatments on milk yield and composition are illustrated in Figures 4.2 and 4.3.

4.5. Nitrogen balance and feed digestibility

Data of nitrogen (N) balance and feed digestibility are summarised in Table 4.4. Ewes given casein infusion had significantly ($P<0.05$) higher organic matter (OM) digestibility by 10 and 9% compared to those in control and ammonia infusion, respectively. Compared to that in control (53.3 g/d), total N intake increased ($P<0.05$) significantly by casein infusion (65.4 g/d) and slightly by ammonia infusion (57.5 g/d). When expressed as g/kg $W^{0.75}$, N intake tended ($P<0.10$) to be higher in casein and ammonia groups (3.04 and 2.89 g/kg $W^{0.75}$, respectively) than did it in control group (2.50 g/kg $W^{0.75}$). Compared to that of control, ammonia infusion significantly increased ($P<0.05$) N digestibility by 21% while casein infusion increased it by 13%. No difference was found in dry matter (DM) digestibility and digestible DM and digestible OM intakes.

Faecal N excretion was lower ($P<0.05$) in ammonia treated ewes (13.9 g/d) than that of control (19.7 g/d) and casein treated (18.5 g/d) ewes but they were not significantly different when expressed as g/kg $W^{0.75}$. Both casein and ammonia infusions led to a significantly higher amount of N excreted in the urine. The amount of N excreted in the urine in the control group was 17.7g/d compared to 27.2 and 24.9 g/d in the casein and ammonia groups, respectively. When expressed as g/kg $W^{0.75}$, the differences were also significant ($P<0.01$) and the values were 0.8 in the control group compared to 1.3, and 1.2 in the casein and ammonia groups, respectively. Nitrogen loss in the milk was increased by casein infusion (19.2 g/d) and was decreased by ammonia infusion (9.6 g/d) ($P<0.05$) and when expressed as g/kg $W^{0.75}$, the values were 0.93 and 0.48 ($P<0.10$) for casein and ammonia infusions, respectively. Nitrogen retention was significantly ($P<0.05$) higher in the ammonia group (8.8 g/d) compared to that in the other groups (2.1 and 0.4 g/d in control and casein groups, respectively).

Expressed as g/kg $W^{0.75}$, the values for N retention were 0.09, and 0.00 for ewes in control and casein groups, respectively compared to 0.44 for those in ammonia group.

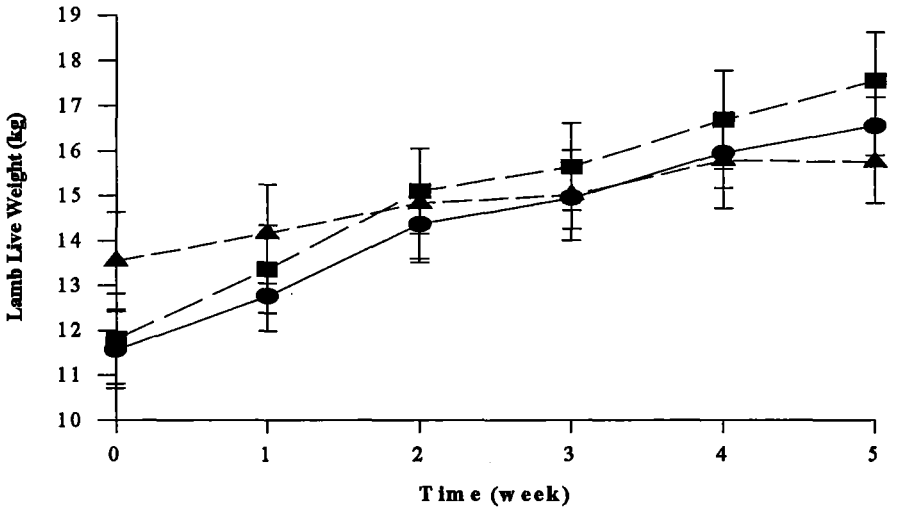

Figure 4.1. Mean lamb live weights in ewes given abomasal infusions of water as control (●), casein (■), and ammonia (▲). Vertical lines denote standard error of mean.

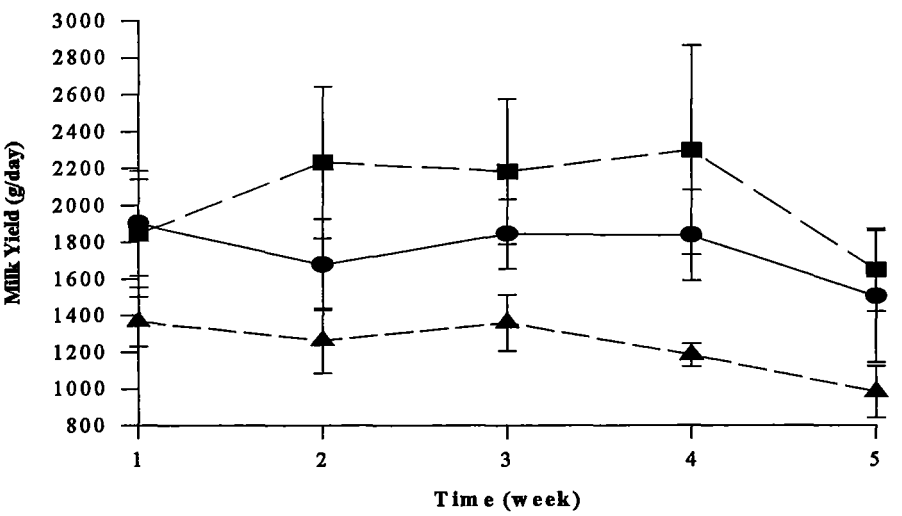

Figure 4.2. Mean milk yields in ewes given abomasal infusions of water as control (●), casein (■), and ammonia (▲). Vertical lines denote standard error of mean.

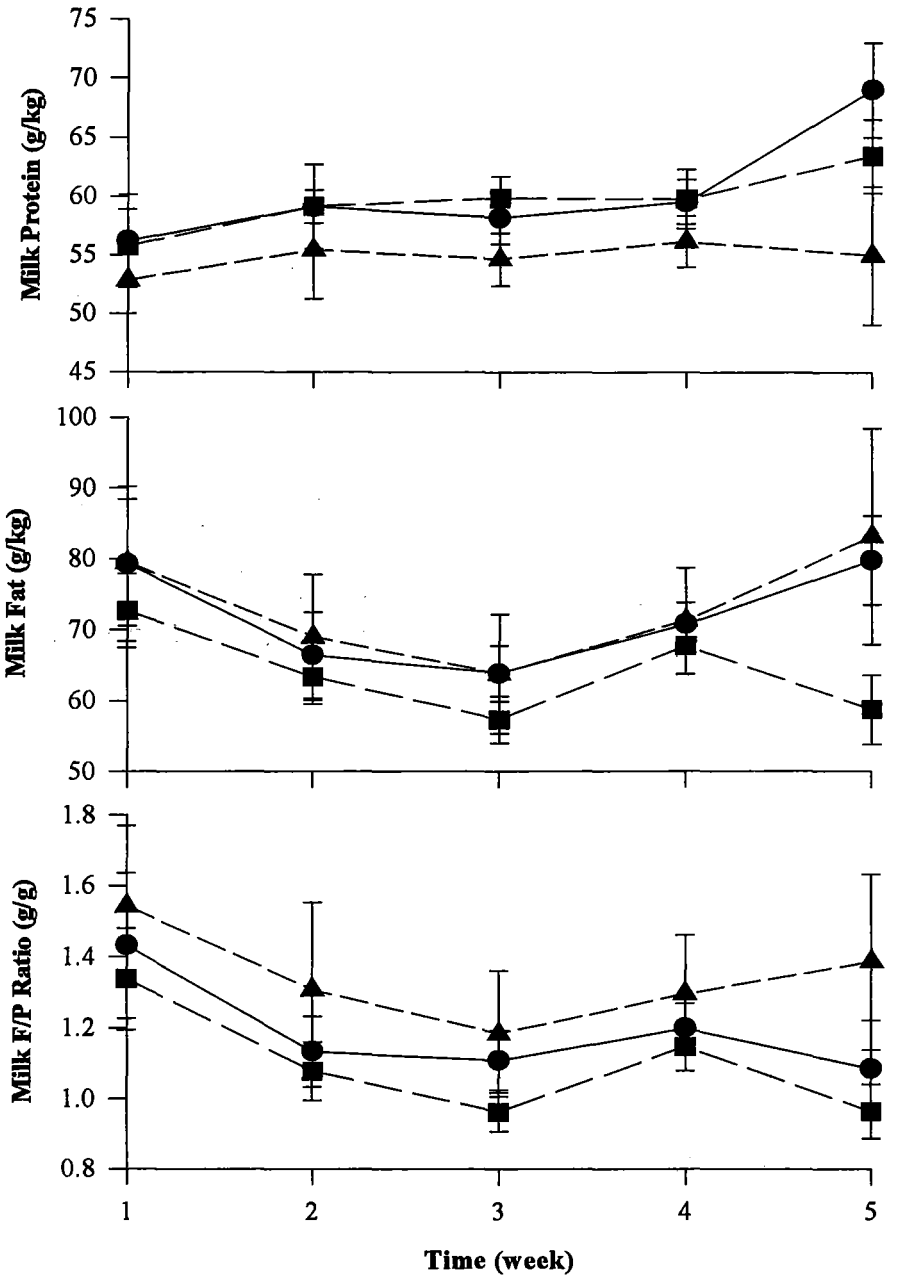

Figure 4.3. Mean milk protein (top panel), fat (middle panel) composition, and fat to protein (F/P) ratio (bottom panel) in ewes given abomasal infusions of water as control (●), casein (■), and ammonia (▲). Vertical lines denote standard error of mean.

Table 4.4. Feed apparent digestibility and nitrogen balance in lactating ewes given abomasal infusions of casein, ammonia or water (control)

Parameter	Control	Casein	Ammonia	sed*
Apparent digestibility (g/kg)				
Dry Matter (DM)	732.5	808.9	746.2	19.0
Organic Matter (OM)	756.6^{b}	835.1^{a}	769.4^{b}	19.1
Total Nitrogen	620.6^{b}	701.8^{ab}	752.1^{a}	32.0
Intake (/d)**				
Digestible DM (kg/ewe)	1.6	1.6	1.4	0.1
Digestible DM (g/kg $W^{0.75}$)	77.5	73.0	71.9	7.6
Digestible OM (kg/ewe)	1.6	1.5	1.4	0.1
Digestible OM (g/kg $W^{0.75}$)	74.6	70.2	69.2	7.3
N Balance				
N Intake (/d)				
g/ewe				
diet	53.3	45.2	44.9	4.2
infusion	0	20.2	12.6	-
total	53.3^{b}	65.4^{a}	57.4^{ab}	4.1
g/kg $W^{0.75}$				
diet	2.5	2.1	2.3	0.2
infusion	0	0.9	0.6	-
total	2.5	3.0	2.9	0.2
N Loss (g/d)				
Faecal (/ewe)	19.7^{a}	18.5^{ab}	13.9^{b}	2.1
(/kg $W^{0.75}$)	0.9	0.9	0.7	0.1
Urine (/ewe)	17.7^{b}	27.2^{a}	24.9^{a}	2.6
(/kg $W^{0.75}$)	0.8^{B}	1.2^{A}	1.2^{A}	0.1
Milk (/ewe)	12.6^{ab}	19.2^{a}	9.6^{b}	4.0
(/kg $W^{0.75}$)	0.6	0.9	0.5	0.2
N Retention (g/d)				
/ewe	2.1^{b}	0.4^{b}	8.8^{a}	2.6
/kg $W^{0.75}$	0.1^{b}	0.0^{b}	0.4^{a}	0.1
Absorbed N (g/kg $W^{0.75}$)***	1.6^{b}	2.2^{a}	2.2^{a}	0.2
N Utilisation (% absorbed N)				
Urine N	54.8	67.7	59.0	5.6
Utilised N	45.2	32.3	41.0	5.6
Milk N	42.5^{a}	40.2^{ab}	22.6^{b}	8.4
Body N	0.7^{b}	-6.1^{b}	17.7^{a}	6.9

^{A,B} : means in the same row with different superscripts are different (P<0.01)

^{a,b} : means in the same row with different superscripts are different (P<0.05)

* : standard error of differences

** : includes infusate

*** : calculated as (total intake N - faecal N)

The amount of N absorbed (calculated as total N intake minus faecal N) was significantly higher ($P<0.05$) in treated ewes (2.2 g/kg $W^{0.75}$ in both casein and ammonia) than that in control (1.6 g/kg $W^{0.75}$). The proportion of the absorbed N excreted in the urine tended ($P<0.10$) to be higher in treated ewes (54.8, 67.7, and 58.9% for control, casein, and ammonia, respectively). In contrast, the proportion of the absorbed N that was utilised for body and milk protein synthesis tended ($P<0.10$) to be lower in treated ewes (32.3 and 41.0% in casein and ammonia) compared to that of control (45.2%). Compared to that of control, ewes infused with ammonia utilised absorbed N for milk protein synthesis by 20 percentage units less and they utilised absorbed N for body protein synthesis by about 17 percentage units more ($P<0.05$). No significant difference was found in the proportion of the utilisation of absorbed N for milk and body protein syntheses in control and casein treated ewes.

4.6. Nitrogen kinetics

The study of nitrogen kinetics could not be established due to some difficulties with radioactivity measurement. Some steps of the methods were well performed but some were not. The deproteinisation of blood plasma samples was initially done with sulfosalicylic acid (0.5 g/ml plasma) as suggested by Cronjé *et al.*, 1992. However, the supernatants obtained were not clear suggesting that some precipitation might have occurred. Another deproteinisation was done with acetonitrile (CH_3CN) (3 ml/ml plasma) (Jones and Gilligan, 1983). The method worked well and resulted in clear supernatant.

The determination of the concentration of plasma leucine by high-performance liquid chromatography (HPLC) on both infusate and plasma samples was well performed . About 65% of counts was found in the leucine peak. Other authors (Jones and Gilligan, 1983; Cronjé *et al.*, 1992) have not given any comments on the rate of recovery of plasma leucine.

Beta emission of the fractions collected from the HPLC was counted on a liquid scintillation counter. However, the radioactivity counted was too low even when the number of fractions collected from the HPLC was increased by up to 5 times (bulked up from 5 collections). The counting of beta emission was

then done directly on the deproteinised plasma samples (not the fractions collected from HPLC). Compared to that of the collected fractions from the HPLC, the direct count on the deproteinised plasma samples resulted in higher counts of radioactivity. The values of these counts are illustrated in Figure 4.4. It can be seen from this figure that compared to those of the expected total plasma ^{3}H radioactivity, the counts of plasma ^{3}H radioactivity measured were still very low (about 1/1000). This might suggest that the actual amount of L-[4,5-$^{3}H(n)$]-leucine infused was considerably lower than planned (250 μCi) that possibly be due to a miscalculation in the preparation of the isotope infusion. The expected total plasma ^{3}H radioactivity was the total accumulating radioactivity in the blood plasma with an assumption that there was no metabolism of amino acid (i.e. leucine). The values for this were calculated based on the assumptions that blood volume in a sheep is about 58 ml/kg body weight and blood plasma volume is about 63% of blood volume (Ruskebusch *et al.*, 1991). It was noted also that while Cronjé *et al.* (1992) found that the radioactivity reached the plateau within 6 hours after infusion, there seemed to be no plateau shown in the present study.

The estimate of fractional contribution of [^{3}H]-leucine to the irreversible loss rates (ILR) of body water was not obtained. This was due to a difficulty in isolation of $^{3}H_2O$ of the urine by vacuum sublimation. The problem rose from the fact that the amount of glacial acetic acid added to the urine was very high (30-500 ml) compared to that used by Cronjé *et al.* (1992) which was only 1-2 ml. Such amount of acetic acid used was based on the fact that at the N balance study, it was found that 400 ml acetic acid glacial was needed to reduce pH of 1 litre urine down to about 3. However, this high amount of acetic acid affected the isolation of $^{3}H_2O$ of the urine by vacuum sublimation as the boiling point of acetic acid (115 °C) is close to that of water (100 °C).

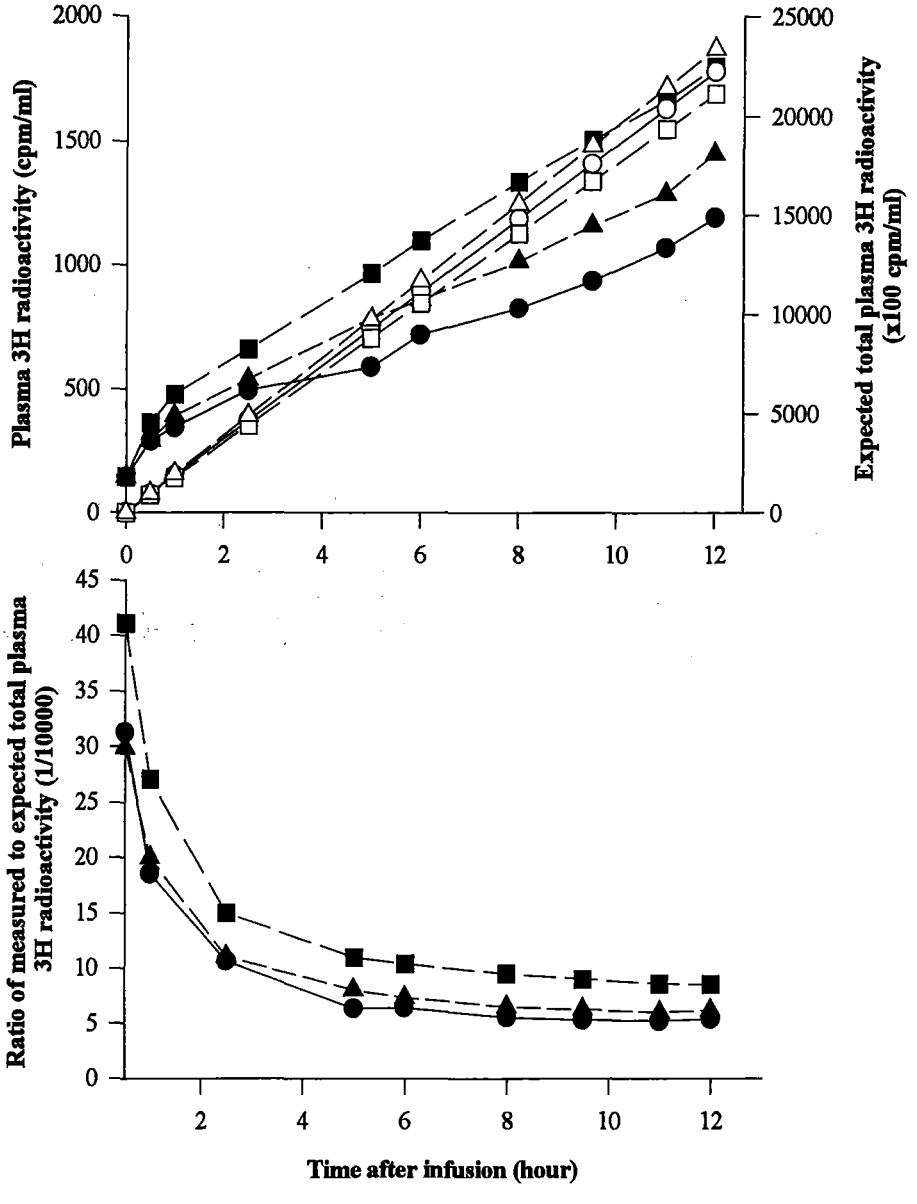

Figure 4.4. Measured plasma ^{3}H (●: control, ■: casein, ▲: ammonia) and expected total plasma ^{3}H radioactivities (O: control, □: casein, Δ: ammonia) (top panel) and their ratio (●: control, ■: casein, ▲: ammonia) (bottom panel) over 12 hours after infusion in ewes abomasally infused with casein, ammonia, and water as control.

CHAPTER 5

DISCUSSION

5.1. Feed intake

Ewes in casein infusion had a DMI (g/d) 3% higher than did the ewes in the control group (Table 4.1). Ammonia infusion gave a greater effect by reducing DMI up to 11% of that of control. However, these effects were likely to be related to the animal body sizes. When they were expressed in g/kg metabolic body weight, the figures were not different: 103.1, 104.3, and 95.6 g/kg $W^{0.75}$/d for ewes in control, casein, and ammonia, respectively. At the beginning of the experiment, the animals were well randomly allocated to each treatment based on their live weights. At this time the mean live weights of the ewes were 66.4, 66.1, and 66.9 for control, casein, and ammonia, respectively. However, during the adaptation period, DMI of the ammonia treated animals dropped considerably resulting in these animals having a lower body weight at the start of the experiment.

The effects of casein infusion on DMI are variable. Decreases in DMI have been noted in lactating cows (Broderick *et al.*, 1970) and steers (Houseknecht *et al.*, 1992; Bruckental *et al.*, 1997) abomasally infused with casein. On the other hand, Whitelaw *et al.* (1986) found that DMI increased gradually from 9.08 to 9.73 kg/d in lactating cows given gradual abomasal casein infusion from 0 to 600 g/d. Increases in DMI were also noted by Rogers *et al.* (1979) in dairy cows abomasally infused with casein and Egan (1965) and Egan and Moir (1965) in sheep duodenally infused with casein. The result of present study is, however, in accordance with those of most workers who found that abomasal or duodenal infusions of casein showed no effect on DMI in lactating dairy cows (Hale *et al.*, 1972; Clark *et al.*, 1973; Spires *et al.*, 1973; Derrig *et al.*, 1974; Vik-Mo *et al.*, 1974; Rogers *et al.*, 1984; Cant *et al.*, 1991; Choung and Chamberlain, 1993; Hurtaud *et al.*, 1993; Guinard *et al.*, 1994), lactating ewes (Barry, 1980), lactating goats (Ranawana and Kellaway, 1977a,b) and growing lambs (Beermann *et al.*, 1991).

No effect of ammonia infusion on DMI in the present study supports the result of Moorby and Theobold (1997) who infused ammonium acetate to lactating cows. Feeding urea has not resulted in differences in DMI in lactating cows (Susmel *et al.*, 1995; Lines and Weiss 1996) and in lambs (Ørskov *et al.*, 1973). In contrast, abomasal infusions of urea have been shown to increase DMI in sheep (Egan, 1965; Egan and Moir, 1965).

Cant *et al.* (1991) noted that variation in DMI responses are the results of many interrelated factors including ration palatability, energy density of the basal diet, nutrient digestibility, milk energy output, stage of lactation, and duration of study. In the present study, the effects of increasing nitrogen supply by-passing the rumen on feed intake were likely to be related to an improved protein status of the animal as noted by Clark (1975). Basal diets used in the present study and others (ie. Ørskov *et al.*,1973; Derrig *et al.*, 1974; Vik-Mo *et al.*, 1974; Beermann *et al.*, 1991; Choung and Chamberlain, 1993; Guinard *et al.*, 1994) in which post-ruminal administrations of protein or non protein nitrogen did not affect DMI were relatively high-protein diets. The positive effects of casein infusions on DMI were noted in animals fed with low-protein roughage diets (Egan 1965; Egan and Moir, 1965), silage which provided unbalanced amino acids (Rogers *et al.*, 1979), and N-limited diets (Whitelaw *et al.*, 1986).

5.2. Live weight gain

Ewe live weight gain (LWG) was not different between treatments. These results were consistent with those of Barry (1980) in which no significant differences of live weight gain were found in pasture-fed lactating ewes abomasally infused with casein. Supplementation of undegradable dietary protein (UDP, i.e. fishmeal) to ewes suckling twin lambs did not give any effects on ewe live weight change (Cowan *et al.*, 1981; Sheehan and Hanrahan, 1989). Results from other studies, however, showed that abomasal casein infusion increased the rate of live weight loss (Ørskov *et al.*, 1977) and ammonium bicarbonate inclusion tended to reduce body weight (Shukla and Talpada, 1974) in lactating cows. Positive ewe live weight gain in this study might be related to level of milk production. Frey *et al.* (1991) found that with average milk yield during week 4-

12 of lactation of 1055 ml/d, supplementation of UDP to grazing ewes suckling twin lambs resulted in the ewes in positive live weight gain. In the present study, mean milk yields during the experimental periods (week 4 to 14 of lactation) were 1174, 1383, and 1841 g/d for ammonia, control, and casein groups, respectively. In studies when body weight declined as lactation progressed, ewes yielded about 1500 to 2100 g/d of milk during the 11 weeks of lactation in the low and high herbage allowances, respectively (Gibb *et al.*, 1981).

Mother's milk and grazed herbage are two main sources of nutrients for lambs (Treacher, 1983) in most sheep production systems. In the present study, lambs were suckling their mothers throughout the experimental periods. Creep feed, i.e. pelleted barley and lucerne, was given only in period 2 with the amount limited to 200 g/d. Therefore, milk was the major source of nutrients for lambs in the present study. Milk production and composition are two aspects that are generally considered as major determinants affecting lamb LWG. Milk is a source of highly digestible energy and protein that are utilised very efficiently (Treacher, 1983). Results of Mayes and Colgrove (1983) showed that during 3.5 months of lactation, apparent digestibilities of milk fat and protein in lambs were 0.99 and 0.97 on average, respectively. Apart from lactose which must be hydrolysed before it can be absorbed, pre-ruminant animals depend mainly on milk fat as energy source (Walker, 1979). Results from the study of Susin *et al.* (1995) lend support to this notion. They found that lambs receiving different amount of milk but similar amount of milk fat showed a similar LWG.

In the present study, the higher LWG in lambs of casein group might be explained by the finding that although milk fat yield was not statistically different between treatments, lambs in the casein group received highest milk fat (115.2 g/d) while those in ammonia group only received 80.0 g/d. In addition, lambs in casein group received higher milk protein (110.4 g/d) than those in ammonia group (65.3 g/d). Furthermore, compared to control, casein infusion tended ($P<0.10$) to increase milk yield by 33% while ammonia infusion tended to reduce it by 15%. The importance of the quantity of milk received by lambs in relation to their LWG has been noted by many other workers (e.g. Wallace, 1948; Slen *et al.*, 1963; Folman *et al.*, 1966; Butterworth *et al.*, 1968; Acharya and Bawa, 1971; Torres-Hernandez and Hohenboken, 1980; Treacher, 1983). Comparing

the effect of litter size on the correlation of milk yield and lamb growth rate, Slen *et al.* (1963) and Snowder and Glimp (1991) concluded that in twin lambs, milk drawn by the lambs was more a limiting factor on lamb growth rate than it was in single lambs. In the present study, although there were no significant differences in milk fat nor milk gross energy (GE) yields between treatments, the correlation analyses (Figure 5.1. and 5.2.) showed that throughout the experimental periods there were high relationships between milk yield (MY), milk protein (MP), milk fat (MF), and milk GE yields with lamb LWG. Coefficient of correlation (r) of LWG with MY was 0.55 (P=0.02) which was similar to that of Torres-Hernandez and Hohenboken (1980) in ewes suckling twin lambs for the entire 15 weeks of lactation. These authors found that LWG of twin lambs increased 0.07 kg for each additional litre of milk produced or in other words, 14.3 liter additional milk is needed by the lambs to gain each additional kilogram body weight. The figures in the present study were very close in which 12.5 kg of milk was needed for the lambs to increase LWG by 1 kg (LWG = 0.08 + 0.08 MY).

Coefficients of correlation (r) between LWG and MF, MPr, and GE yields were 0.51 (P=0.03), 0.45 (P =0.06), and 0.53 (P =0.02), respectively. From Figures 5.1. and 5.2., it can be seen that indeed in each relationship most lambs in ammonia group had LWG values under the regression lines that might suggest that lambs of ammonia group had an inferior LWG than did their counterparts in control and casein groups.

5.3. Milk yield and composition

Abomasal casein infusion tended (P<0.10) to increase milk yield by 33% while abomasal ammonia infusion tended (P<0.10) to reduce it by 15% (Table 4.3.). Most results of other studies (Table 5.1.) showed that post-ruminal infusions of casein increased milk yields in lactating cows (Spires *et al.*, 1973; Derrig *et al.*, 1974; Vik-Mo *et al.*, 1974; Rogers *et al.*, 1979; Rogers *et al.*, 1984; Whitelaw *et al.*, 1986; Choung and Chamberlain, 1993; Hurtaud *et al.*, 1993; Guinard *et al.*, 1994; Griinari *et al.*, 1997) by up to 32% and lactating goats (Ranawana and Kellaway, 1977a,b) by 22% (Table 5.1.). A trend in

increased milk yield (P>0.05) was reported in lactating ewes (Barry, 1980) abomasally infused with casein plus methionine. No increases in milk production have also been noted in lactating cows (Broderick *et al.*, 1970; Cant *et al.*, 1991; Hurtaud *et al.*, 1993). Inclusions of ammonia or urea in the diets have been reported to reduce milk production in lactating cows (Shukla and Talpada, 1974) and lactating ewes (Tanev, 1971, 1973; Farid *et al.*, 1984). However, no differences in milk yield have been noted in lactating cows as the results of ammonia and urea supplementations (Lines and Weiss, 1996) or duodenal infusion of ammonia (Moorby and Theobold, 1997).

Table 5.1. Effect of postruminal infusions of casein on milk yield and composition relative to control

Amount infused (g/d)	**Spe cies**	**Changes in**					**Authors**
		Milk yield (%)	**Fat (% unit)**	**Protein (% unit)**	**Fat yield (%)**	**Protein yield (%)**	
145 Nac	**ewe**	**+ 33.0**	**ns (-0.6)**	**ns (-0.2)**	**ns (+18.2)**	**ns (+26.0)**	**present study**
800 Nacm	cow	ns	ns	+ 6.2	ns	+ 11.6	Broderick *et al.*, 1970
400 Nac	cow	+ 7.5	- 0.3	ns	na	+ 8.9	Vik-Mo *et al.*, 1974
400 Nac	cow	+ 5.6	ns	+ 0.2	na	na	Derrig *et al.*, 1974
300 Nac	cow	+ 21.6	na	na	na	na	Rogers *et al.*, 1979
462 Nac	cow	+ 6.9	ns	ns	ns	+ 9.9	Rogers *et al.*, 1984
600 lac	cow	+ 32.3	ns	+ 0.3	+20.0	+ 40.8	Whitelaw *et al.*, 1986
330 cas	cow	+ 25.2	- 0.4	+ 0.3	+15.0	+ 36.5	Choung and Chamberlain, 1993
500 Nac	cow	ns	- 0.2	ns	ns	ns	Hurtaud *et al.*, 1993
762 Cac	cow	+ 10.8	+ 0.1	+ 0.2	+13.8	+ 19.1	Guinard *et al.*, 1994
45 cas	goat	+ 22.0	ns	ns	+17.8	+ 23.4	Ranawana and Kellaway, 1977a

Nacm: sodium caseinate + methionine; Nac: sodium caseinate; lac: lactic caseinate; Cac: calcium caseinate; cas: casein
na : not available
ns : not significant

It can be seen from Figure 4.2. that throughout the experimental periods the mean milk yield of the ewes in the casein group was higher than that of their counterparts in the control and ammonia groups. Unfortunately, because of a precaution not to put too much stress to the animals in the adaptation period,

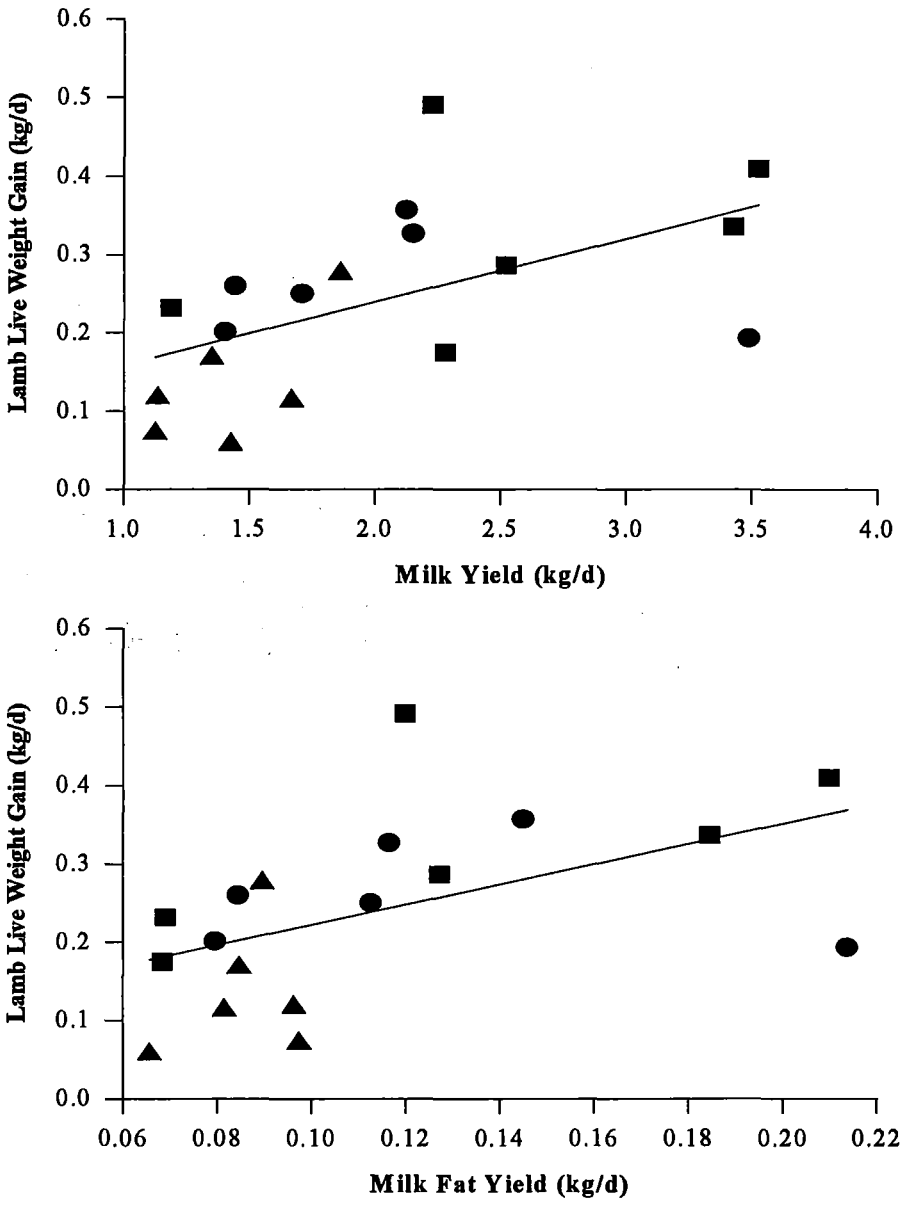

Figure 5.1. Relationships between lamb live weight gain and milk yield (MY) (top panel, Y = 0.08 + 0.08 MY, r = 0.55, P = 0.02) and milk fat yield (MF) (bottom panel, Y = 0.09 + 1.29 MF, r = 0.51, P = 0.03) for individual ewes (6 ewes in each group) given abomasal infusions of water as control (●), casein (■), and ammonia (▲).

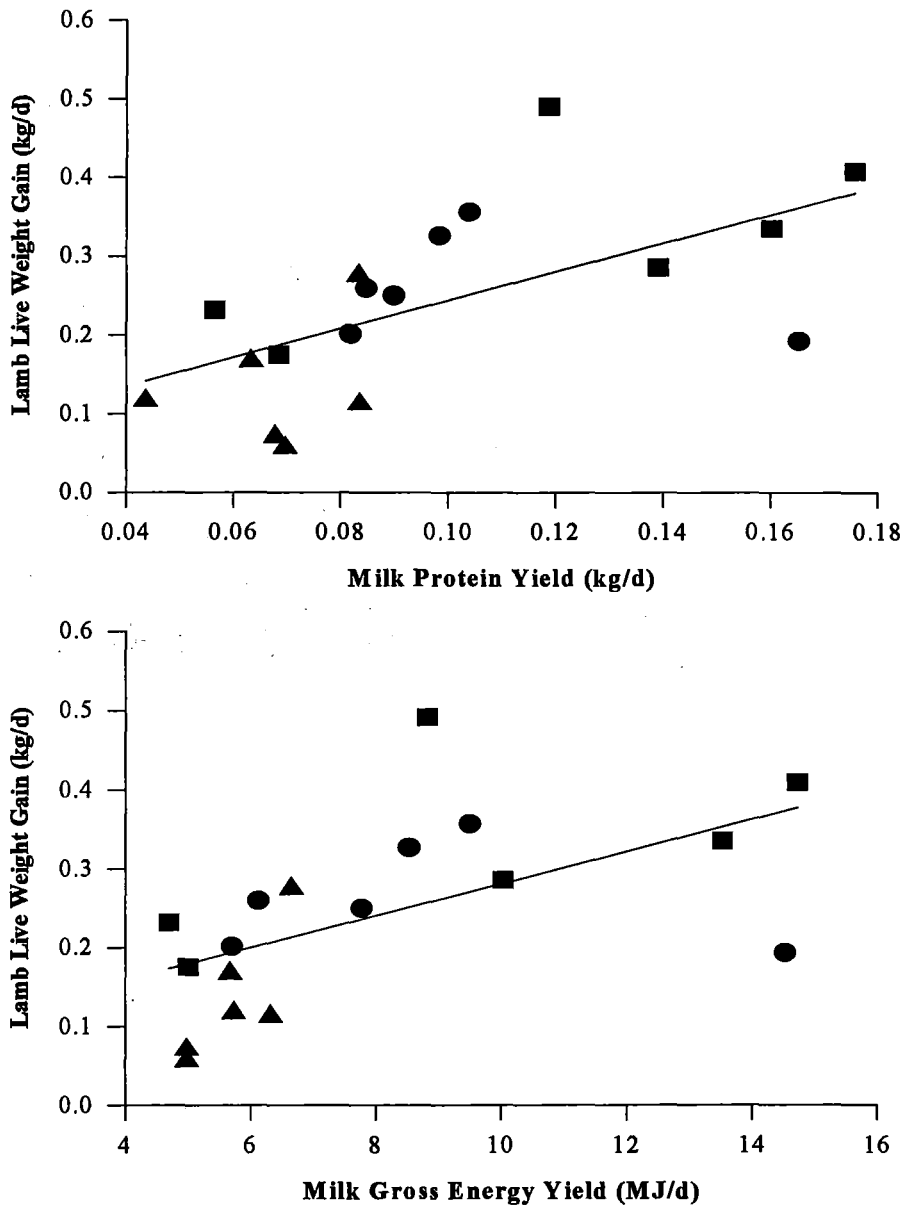

Figure 5.2. Relationships between lamb live weight gain and milk protein yield (MPr) (top panel, Y = -0.13 + 2.18 MPr, r = 0.45, P = 0.06) and milk gross energy yield (GE) (bottom panel, Y = -0.16 + 0.03 GE, r = 0.53, P = 0.02) for individual ewes (6 ewes in each group) given abomasal infusions of water as control (●), casein (■), and ammonia (▲).

no milk yield was measured at the time just before the treatments started (0 week). It would be very noteworthy if there was data of milk yield at 0 week time to determine whether the differences in milk yield were due to treatment effects or simply due to animal factors.

Compared to ewes in the control group, ewes in the casein group received an additional 146 g/d of casein which equal to 19 g/d of N in the form of amino acids supplied to the abomasum. With regard to the relationship of intestinal supply of non-ammonia nitrogen (NAN) and milk production in ewes, Gonzales *et al.* (1982) found that for every g of NAN reaching the abomasum, milk yield increased by 0.08 g ($y = 0.08X - 0.41$). In the present study, ewes in the casein group yielded 458 g more milk than did those in the control group. With an additional supply of 19 g/d of NAN reaching the abomasum, this figure was far too small compared to what is expected (1.52 kg) if the regression equation of Gonzales *et al.* (1982) was used. However, this small response of milk yield to casein infusion in the present study might be due to the low ability of the ewes to produce milk or limited ME supply. The estimate ME intakes of all groups in this study, ie. 24.2, 24.9 and 21.5 MJ/ewe/d (Table 4.1.) were lower than the ewes' requirement of ME (AFRC, 1993), ie. 35.9, 36.9 and 32.7 MJ/ewe/d for control, casein and ammonia groups, respectively.

Armstrong (1982) proposed mechanisms by which protein supply affects milk yield. Increasing DMI and overall ration digestibilities and therefore enhancing the supply of energy available for milk production is an 'indirect' effect to which the response of milk yield to variation in protein supply might be attributable. This does not seem to be the case in the present study as no differences were found in DMI, DM nor OM digestibilities (Table 4.1 and 4.4). Instead, the response of milk yield to casein infusion might be attributable to the 'direct' effects by supplying the animal either with essential AA for milk synthesis, additional AA as carbon sources for glucoce production, or AA which can induce changes in hormonal status (growth hormone, GH, in particular) (Clark, 1975; Armstrong, 1982).

Rogers *et al.* (1980) compared the effects of protected and unprotected casein supplementations to lactating cows grazing pasture. An increase of 12% in milk yield resulted from protected casein compared to an insignificant increase

of 3% from unprotected casein. This increase in milk yield was not accompanied with increases in DMI implying a direct effect of casein supplementation on milk production. The higher response of milk yield to protected casein might also support the notion that providing the animal with AA in the intestines enhances milk synthesis. Furthermore, post-ruminal infusion of casein in lactating cows has been observed to increase the concentrations of leucine, isoleucine, phenylalanine, valine, and total essential AA in the blood plasma (Broderick *et al.*, 1970; Derrig *et al.*, 1974; Spires *et al.*, 1973; Spires *et al.*, 1975; Choung and Chamberlain, 1993; Guinard *et al.*, 1994).

With regard to the fate of casein in supplying AA as a carbon source for glucose synthesis, it has been recognised that glucose is the major precursor for the synthesis of lactose by mammary glands (Armstrong, 1982). In ruminants, the absorption of glucose from the gastrointestinal tract is negligible. They must rely almost entirely on gluconeogenesis from various substrates as the major source of blood glucose (Wolff and Bergman, 1972; Armstrong and Smithard, 1979; Armstrong and Weekes, 1983; MacRae and Lobley, 1986). Glucose synthesis occurs mostly in the liver with propionate, lactate, and glucogenic AA as the sources for carbon skeletons (Bergman *et al.*, 1974; Bergman and Heitmann, 1978; Huntington, 1990; Demigné *et al.*, 1991). The importance of mammary uptake of glucose for milk secretion has also been shown in lactating goats (Hardwick *et al.*,1961; Linzell, 1967). Cows on the onset of lactation have shown to enhance the utilisation of glucose for milk synthesis (Bennink *et al.*,1972) and 60% of glucose taken up by mammary gland is utilised for lactose synthesis (Thomas, 1983). To determine whether the response of milk yield to casein administration is through the way of supplying glucogenic AA to increase the availability of glucose to the mammary glands, Ranawana and Kellaway (1977b) compared the responses of lactating goats to the infusions of glucose and casein. They found that although with both casein and glucose infusions, the arterial concentrations of glucose were high, so that the availabilities of glucose to the mammary glands were enhanced, no increase in milk yield was found with glucose treatment. It has also been reported that compared to isocaloric and isonitrogenous infusion of glucose, monosodium glutamate, and urea, infusion of casein showed its superior effect on increasing milk yield in lactating cows

(Spires *et al.*, 1975). These facts indicated that the increases in milk yield following the infusions of casein were not attributable to gluconeogenesis from casein. This notion was in agreement with those in other studies (Vik-Mo *et al.*, 1974; Ørskov *et al.*, 1977; Rogers *et al.*, 1979) in which the post-ruminal infusion of casein, not glucose, was found to increase milk production in lactating cows.

In term of changes in hormonal status induced by casein, it has been found that the concentration of GH was increased in lactating goats abomasally infused with casein (Oldham *et al.*, 1978) and in dairy cows fed treated-casein (Oldham *et al.*, 1982). It has been observed that plasma GH concentration in dairy-type cattle which produce higher milk yield was higher than that in the beef-type cattle (Bines and Hart, 1978). Moreover, injection of GH increased milk production and feed utilisation in dairy cows (Machlin, 1973) and greater milk yields in ewes have been observed to be associated with higher concentrations of GH in the plasma (Barry, 1980). These observations lend support to the suggestion that responses in milk yield from abomasal infusion of casein might be exerted through hormone action.

Neither concentrations of AA, glucose, nor GH in the blood plasma was measured in the present study. However, the trend of higher milk yield in ewes in the casein group might be attributable to one or more factors, individually or collectively, in the 'direct' effect referred to above.

As about 80% of the extra N was found in urine, high rate of urea synthesis in the liver might be expected in casein treated ewes as a result of high supply of AA and in ammonia treated ewes as a result of high supply ammonia. A recent study by Lobley and Milano (1997) suggested that when the animals are faced with a high concentration of ammonia derived from deamination of excess AA, they might have to catabolise body protein to provide the "second" N atom needed in ureagenesis. In contrast, as both Ns used in urea synthesis can be derived from ammonia, ewes with excess ammonia would not need to mobilise body protein. However, while ewes in casein group might be able to gain some energy from carbon skeletons resulting from catabolism of AA to drive the urea cycle and to increase milk production, those in ammonia group, in order to drive the urea cycle, might have to mobilise body fat or reduce the availability of

metabolisable energy (ME) for lactation. The different effects of casein and ammonia infusions on milk yield in the present study support this hypothesis. Unfortunately, the nitrogen kinetic study failed to establish whether the treatments affected the metabolism of AA.

The non-significant effect of casein on milk protein concentration was not in accordance with results from other studies (Table 5.1.) which showed that post-ruminal casein infusions or treated-casein supplementation increased milk protein concentration in lactating cows (Broderick *et al.*, 1970; Derrig *et al.*, 1974; Spires *et al.*, 1975; Rogers *et al.*, 1980; Choung and Chamberlain, 1993; Guinard *et al.*, 1994). Even though not statistically significant, casein infusion increased milk protein yield by 26% which was in accordance with results from other studies (Table 5.1.) in lactating cows (Broderick *et al.*, 1970; Spires *et al.*, 1973; Spires *et al.*, 1975; Rogers *et al.*, 1984; Whitelaw *et al.*, 1986; Choung and Chamberlain, 1993; Guinard *et al.*, 1994), lactating goats (Ranawana and Kellaway, 1977a), and lactating ewes (Barry, 1980). The inclusions of urea or ammonia in the diets have been reported to reduce milk protein concentration (Tanev, 1971) and milk protein yield (Tanev, 1973; Farid *et al.*, 1984) in lactating ewes as shown by ammonia-infused ewes in the present study.

The lower milk fat concentration in casein group compared to that in ammonia group in the present study was in accordance with results of most other studies. Casein infusion decreased milk fat concentration in cows (Vik-Mo *et al.*, 1974; Choung and Chamberlain, 1993; Hurtaud *et al.*, 1993). Lactating cows fed urea had higher milk fat concentration than those in a control group (Hale *et al.*, 1972; Lines and Weiss, 1996). Inclusion of ammonium sulfate increased milk fat concentration in ewes (Tanev, 1971). A recent study of Moorby and Theobold (1997) showed that duodenal infusion of ammonia increased milk fat concentration in dairy cows.

Neither milk lactose concentration nor milk lactose yield was affected by treatments. This was not unexpected as the concentration of lactose in milk is considered to remain relatively constant. Changes in lactose concentration as a result of dietary manipulation seems to be very small and unlikely (Sutton, 1989). Lactose is osmotically active and functions as a regulator of water content of milk since its synthesis brings about a flow of water which dilutes other milk

constituents, such as protein and fat (Davies *et al.*, 1983; Sutton, 1989). As the concentration of milk lactose was not different between treatments, thus, the differences in milk fat and protein concentrations were not related to dilution effects.

In the present study, it was shown that providing the animals with by-pass N in different forms, amino acids (casein) or ammonia, gave different effects on milk composition. Higher milk protein concentration resulted from casein infusion while higher milk fat concentration resulted from ammonia infusion. The milk protein response might be explained as follows. Schingoethe (1996) pointed out that improved protein nutrition in lactating ruminants will be achieved if the by-pass protein supplied was as good a quality as the rumen microbial protein or complemented the deficiencies in the microbial protein. In other words, the supply of ruminally undegraded protein has to increase the supply of amino acids that are limiting production in absorbable form. This notion has been emphasised by the results of Chen *et al.* (1993) in which they found that with equal ruminal protein degradabilities, high quality protein (a blend of blood, fish, and soyabean meal) resulted in greater milk protein concentration than did low quality protein (corn gluten meal). As mentioned before, in many studies (e.g. Choung and Chamberlain, 1993; Guinard *et al.*, 1994) casein infusions have been found to enhance the concentrations of some essential AA in blood plasma, including methionine and lysine that are considered as the most limiting AA for milk protein synthesis under many dietary situations (Schwab *et al.*, 1992; Schingoethe, 1996). As the effect of post-ruminal infusion of casein on milk protein concentration is exerted through its ability to correct certain AA deficits (DePeters and Cant, 1992), it, therefore, is not unexpected that in the present study, ewes in the casein group had a greater milk protein concentration than did those in ammonia group.

With regards to milk fat concentration, it is still uncertain how casein and ammonia infusions showed the contrasting effects. None of the workers (Tanev, 1971; Moorby and Theobold, 1997) whose studies showed the positive effects of ammonia administration on milk fat concentration has given a clear explanation. However, there is a theory of milk fat depression in high protein-fed ruminants based on the concept that there is competition for circulating

precursors for fat synthesis between mammary gland and adipose tissue that is regulated through changes in insulin concentration (McGuire *et al.*, 1995; Griinari *et al.*, 1997). Insulin has been known to have a stimulatory effect on lipogenesis and inhibitory effect on lipolysis in adipose tissue (Bauman and Elliot, 1983; Sutton *et al.*, 1986). According to this theory, an increase in insulin release will preferentially channel free fatty acids to adipose tissue resulting in a decreased supply of milk fat precursors to mammary gland and thus, depress milk fat production (Emery, 1973; Jenny *et al.*, 1974; Hart, 1983; Brockman and Laarveld, 1986; Sutton *et al.*, 1986). Casein infusions have been reported to increase insulin concentration in the plasma (McAtee and Trenkle, 1971; Barry, 1980; Istasse *et al.*, 1987), although others have failed to find this (Oldham *et al.*, 1978; Oldham *et al.*, 1982; Guinard *et al.*, 1994). Whether the lower milk fat concentration of ewes in the casein group in the present study can be explained by this theory is uncertain.

5.4. Nitrogen balance and feed digestibility

Dry matter (DM) digestibility was not affected by treatments. Compared to control, post-ruminal casein infusion has also been found not to increase DM digestibility in lactating cows (Rogers *et al.*, 1984). Organic matter (OM) digestibility is closely related to energetic feeding value of the diets (van der Koelen and van Es, 1973). However, as the significant increase in OM digestibility in casein group did not lead to a higher digestible OM intake compared to those in the other groups, it can be said that all the animals received equal energy supply as planned. Abomasal infusions of casein and ammonia led to a higher apparent digestibility of N. This is in accordance with the results of Wright *et al.* (1998) in which N digestibility was increased with increasing supply of UDP. The increased N digestibility in the present study was a result of high post-ruminal digestibility of casein (about 88%) and ammonia (about 100%).

Total nitrogen intakes, when expressed in g/kg metabolic weight (for the remaining N balance discussion, the parameters discussed will be expressed in per kg metabolic weight when applicable), tended to be higher ($P<0.10$) in casein and ammonia group. Since the contribution of N from diet was not different, the

higher N intakes in the treatment groups were, as intended, brought about by N from the infusions. Insignificant differences in faecal N between treatments might be related to DMI which were not different either. Faecal N loss was found to be highly correlated with DMI rather than with N intake (Stallcup *et al.*, 1975; Kwan *et al.*, 1984).

Infusions of casein and ammonia into the abomasum significantly increased the loss of N through urine. The total intakes of N of the casein and ammonia treated ewes were about 1.2 times higher than that of the control ewes. However, the amounts of N excreted in the urine of casein and ammonia treated ewes were about 1.5 times higher than that of control ewes. When it was corrected for the amount of N from the diet, approximately 80% or more of additional N intake in casein and ammonia treated ewes was excreted through the urine. The higher loss of N in the urine in treated ewes was not unexpected. Urinary N has been found to be the main route of N excretion when N supply is in excess (Susmel *et al.*, 1995; Wright *et al.*, 1998). Urinary N losses are derived from some sources including rumen loss, the replacement of metabolic losses in the gut, the incorporation of dietary protein N into microbial nucleic acids, loss in maintenance, and losses that are due to an inefficient conversion of absorbed amino acids into milk and body protein (Tamminga, 1992). Of these, the latter is more likely to be the case in the present study.

Ewes in the casein group tended ($P<0.10$) to have an ability to utilise more N for milk N production rather than for body protein. This can be seen from the fact that milk N tended to be higher in casein treated ewes and lower in ammonia treated ones while a contrasting phenomenon was shown in N retention in which casein treated ewes had the lowest and the ammonia treated ewes had the highest N retention ($P<0.05$). A negative N retention has been shown in cows in early lactation as a result of body protein mobilisation for milk protein secretion (Paquay *et al.*, 1972). Due to difference in experimental design used, no such relationship could be shown in the present study.

The highest N retention in ammonia treated ewes was not, however, accompanied with high ewe LWG as no difference was found in ewe LWG (Table 4.2). This occurrence has also been observed by Lobley *et al.* (1985). Regarding this concern, it has been noted that this is a common feature when N

retention measured by total collection method, as used in the present study, is compared with LWG (MacRae *et al.*, 1993; Spanghero and Kowalski, 1997). If the mean N retention values in the present study are converted by adopting the coefficients of 6.25 (assuming a body protein content of 16%) and 4 (assuming body protein is associated with water in an average ratio of 1:3; Blaxter, 1989), then the values of 53, 11, and 220 g/d of ewe LWG are expected compared to measured values of 77, 104, and 98 g/d in control, casein, and ammonia groups, respectively. It can be seen that while N retention was apparently overestimated in the ammonia group, it was underestimated in the control and casein groups. The overestimation of N retention can result from overestimation of N intake and incomplete recovery of excreta, scurf, and digestive tract gases (Spanghero and Kowalski, 1997). Comparative studies by MacRae *et al.*, (1993) showed that compared with slaughter measurement, the N balance technique overestimated net protein accretion by 0.24 in growing lambs.

If this was not the case in the present study, then the observation of Komaragiri and Erdman (1997) perhaps can give another explanation. By using the D_2O dilution technique, these workers measured body tissue mobilisation in lactating cows at -2, 5, and 12 week postpartum. They found that over the wide range of total body energy observed (2000-16000 MJ), the proportions of fat and protein energy change consistently. This suggested that mobilisation and probably repletion of body tissue reserves during lactation result in relatively constant proportions of fat and protein being lost or gained. The lower N retention in casein treated ewes in the present study might have been compensated by body fat gain and the higher N retention in ammonia treated ewes might have been accompanied by a loss of body fat resulting in no differences in LWG in both groups. As discussed earlier, it has also been suggested that due to hormonal changes, a channelling of free fatty acids to adipose tissue might have occurred in casein treated ewes. This phenomenon is supported by the approximate energy balance calculation shown in Table 5.2. If the expected values of LWG converted from the mean N retention values were

Table 5.2. Approximate energy balance (MJ/d unless otherwise specified)

Parameter	Control	Casein	Ammonia
Method 1			
Gross energy intake[1]	39.74	43.39	35.33
Digestible energy intake[2]	29.83	28.69	26.22
Urine energy[3]	0.41	0.63	0.57
Methane energy[4]	3.58	3.12	3.15
Metabolisable energy intake[5]	25.84	24.62	22.50
Metabolisable energy for maintenance[6]	10.44	10.69	10.31
Metabolisable energy for lactation[7]	10.59	13.71	8.40
Body protein energy[8]	0.31	0.06	1.30
Metabolisable energy for body protein synthesis[9]	1.04	0.21	4.32
Metabolisable energy for body fat synthesis[10]	3.77	0.29	-0.53
Net Energy for fat synthesis[11]	2.64	0.01	-0.76
Expected body fat synthesis (g/d)[12]	67	0.36	-19
Method 2			
Lean tissue deposited (g/d)[13]	53	11	220
Measured LWG (g/d)	77	104	98
Expected body fat synthesis (g/d)[14]	24	93	-122

[1] : GEI = 18.4 MJ/kg (feed DMI + infusate DMI) (SCARS, 1990)
[2] : DEI = 19 MJ/kg DOMI (McDonald *et al.*, 1995)
[3] : urine E = 23 kJ/g urine N (McDonald *et al.*, 1995)
[4] : methane E = 0.12 DEI (McDonald *et al.*, 1995)
[5] : MEI = DEI - (urine E + methane E)
[6] : MEm = 0.5 MJ/kg $W^{0.75}$ (Sykes and Nicol, 1983)
[7] : MEl = GEmilk/kl; kl = 0.35 MEI/GEI + 0.42 (SCARS, 1990)
[8] : body protein E = 23.6 MJ/kg body protein (McDonald *et al.*, 1995)
[9] : MEp = GE in body protein/kp; kp = 0.3 (Sykes and Nicol, 1983)
[10]: MEf = MEI - (MEm + MEl + MEp)
[11]: NEf = MEf * kf or MEf/kf (if MEf is negative); kf = 0.7 (Sykes and Nicol, 1983)
[12]: body fat E = 39.3 MJ/kg (McDonald *et al.*, 1995)
[13]: calculated as (N retained x 6.25 x 4)
[14]: calculated as (body protein synthesis - measured LWG)

compared with the measured ones (method 2, Table 5.2), then the values of 24, 93, and -122 g/d were obtained that might represent the expected values for body fat synthesis in control, casein, and ammonia groups, respectively. It can be seen from the energy balance calculation (method 1, Table 5.2) that while those in control and casein groups had positive availability of ME for body fat synthesis (3.77 and 0.02 MJ/d, respectively), ewes in ammonia group mobilised body fat equivalent to 0.53 MJ/d of ME. It was likely that the effect of high rate of body

protein synthesis on LWG in ammonia treated ewes was compensated by the high mobilisation of body fat.

From Table 5.2., it can also be seen that even though the figures are quite different, calculations used either method 1 or 2 showed the same tendency that ewes in casein group were able to synthesis body fat (0.36 or 93 g/d) while those in ammonia group had to mobilise it (-19.23 or -122 g/d). This observation supports the theory based on the work of Lobley and Milano (1997) that ewes that receive excess AA, as was the case in casein group, would have to mobilise body protein to provide N from amino group (NH_2) to be combined with N from ammonia derived from deamination of AA in urea synthesis. However, the deamination of AA may provide the animals with some energy from carbon skeletons that might be used to drive the urea cycle and for milk production. In contrast, as both Ns used in urea synthesis can be derived from ammonia, ewes with excess ammonia would not need to mobilise as much body protein. However, in order to drive the urea cycle, these animals still need some energy that might be obtained by either reducing ME for lactation or mobilising body fat. Further investigation in this matter is, however, needed.

Referring to the work of Kwan *et al.* (1977), the efficiency of N utilisation is defined as the percentage of N absorbed which is used for milk plus body tissue deposited (Table 4.4). Compared to those of control ewes, the amount of N absorbed was significantly higher by 37 percentage units ($P<0.05$) in both groups of treated ewes while the efficiencies of N utilisation tended ($P<0.10$) to be lower by 4-13 percentage units in treated ewes. These inverse relationships between N intake and N utilisation were supported by the results of previous studies (Kwan *et al.*, 1977; Lynch *et al.*, 1991). In lactating cows, although theoretically, the efficiency of the conversion of absorbed N to milk and body tissue protein could be of 43%, under practical conditions, the figures were only 16-25% (van Vuuren and Meijs, 1987). The lower efficiencies of N utilisation might be caused by the relatively higher N excretions through urine in treated ewes. Calculated as a mean difference from control (Table 5.3.), the additional N intakes in treated ewes originated from infusions were 0.54 and 0.39 g/kg $W^{0.75}$ for casein and ammonia group, respectively. Of these, 0.48 and 0.64 were utilised for both milk and body protein syntheses in casein and ammonia

treated ewes, respectively. These high efficiencies of additional N utilisation may suggest that the supply of N from basal diets was not adequate.

Table 5.3. Efficiency of additional nitrogen utilisation in lactating ewes given abomasal infusions of casein and ammonia relative to control

Parameter	Casein	Ammonia
Additional N intake and utilisation (g/kg $W^{0.75}$/d)		
Intake	0.54	0.39
Total utilised	0.26	0.25
Milk	0.35	-0.10
Body	-0.09	0.35
Efficiency of additional N utilisation		
Total utilisation	0.48	0.64
Milk	0.65	-0.26
Body	-0.17	0.90

The partitioning of absorbed N to milk and body N was different ($P<0.05$) between treatments. Ewes in casein group did not significantly utilise absorbed N for milk and body protein synthesis with different proportions to those in control group. About 40% of absorbed N was utilised to produce milk N with no more than 0.75% absorbed N was deposited as body protein. Contrastingly, ewes infused with ammonia utilised the absorbed N for body protein and milk protein syntheses with different proportions. Compared to those of control, the percentage of absorbed N utilised for milk protein synthesis decreased by about 20 percentage units while that for body protein synthesis increased by about 17 percentage units. Further calculations as shown in Table 5.3. showed that 65% of additional intake from casein infusion was used to synthesise milk protein without any proportion of it was used for body protein synthesis. Additional N intake from ammonia infusion, on the contrary, was used for body protein synthesis by 90% without any proportion of it was used for milk protein synthesis.

In a recent study, Carruthers and Neil (1997) studied the effects of feeding pastures with different N contents (17.6 *vs* 13.2% CP) in lactating cows. Cows fed high N pasture had an additional N intake of 105 g/d but compared to

that of cows fed low N pastures, the difference in milk N output accounted for only 4% of extra N eaten, with most of the N consumed (83%) was excreted in the urine. The protein contained in these pastures were in the forms of RDP that provided the animals with excess of ammonia as was the case in ammonia treated ewes in the present study. The negative effect of high RDP intake on the proportion of apparently absorbed N retained in milk has also been noted in other studies (van Vuuren and Meijs, 1987; Mackle *et al.*, 1996).

Results of the present study suggest marked changes in nutrient partitioning between milk production or body weight change as the source of excess N is casein or ammonia. If these results are confirmed then they have big implications to dairy cows. Feeding excessive nitrogen to dairy cows not only is costly expensive but also leads to excessive loss of urinary nitrogen to the environment. In addition, it might mean that in early lactation there is some benefit to ammonia overload as it leads to less body weight (N) loss and good reproductive performance. Meanwhile, increasing AA supply can be done in later lactation to increase milk yield and milk protein yield.

However, there are a number of points for consideration in interpreting the results of this study. Protein metabolism was studied using N balance which is often considered to overestimate N retention and underestimate N excretion and exaggerate LWG. Nitrogen retention in body is calculated as a net of other losses from N intake and is also very dependent on the measurement of milk N. The responses of milk yield to the treatment effects depend principally on the base levels of milk production that were not measured. Whether the response on milk yield is really due to treatments or simply due to animal effects need to be clarified. An inclusion of glucose in ammonia infusion to equal energy supply in casein infusion is another notion that deserves further investigation.

5.5. Conclusions

Results of the present study demonstrated that abomasal infusions of casein and ammonia had differential effects on milk production, milk composition, and efficiency of N utilisation in lactating ewes. Abomasal casein infusion slightly increased milk production and reduced milk fat concentration but did not significantly affect milk protein concentration. Abomasal ammonia infusion slightly reduced milk production, significantly reduced milk protein concentration, and increased milk fat concentration. Excessive supply of N, in the form of both AA and ammonia, led to a slightly decreased efficiency of absorbed N utilisation for productive purpose. Ewes in casein treatment had a preference to utilise absorbed N for milk protein synthesis while ewes in ammonia treatment showed a preference for utilising absorbed N for body protein synthesis rather for milk protein synthesis. These findings confirmed the hypothesis that the form of excess N (amino acid *vs* ammonia) differentially affects the partitioning of nutrients between milk production and body weight change in lactating ruminants. Implications of excess N intake in the form of AA and ammonia on N metabolism in lactating ruminants need to be investigated further.

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