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AMINO ACID SUPPLEMENTATION
AND NITROGEN BALANCE STUDIES FOR
LAMBS CONSUMING FRESH
RYEGRASS/WHITE CLOVER HERBAGE

A
DISSERTATION
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ABSTRACT

The grazing of fresh herbage by ruminants has been demonstrated to result in approximately 30% of the nitrogen derived from the forage subsequently being lost across the rumen. Nitrogen balance studies have demonstrated benefits in terms of increased nitrogen retention as a result of post-ruminal supplementation of nitrogen to lambs receiving either a purified urea diet or consuming fresh herbage.

Eight Dorset Down x Coopworth lambs (38 ± 2.9 kg) fitted with rumen and duodenal cannulae were housed indoors. The lambs were fed freshly harvested ryegrass/white clover pasture at a rate of $0.47 \text{ kg fresh herbage/kgW}^{0.75}/\text{d}$. They each received duodenal infusions of water (control), 3, 6, 9 and 12g casein protein/ $\text{kgW}^{0.75}/\text{d}$ or three levels of a mixture of amino acids (AA, methionine, lysine, histidine and arginine) equivalent to that supplied by the first three casein infusion levels. Treatments were allocated in an 8 x 8 latin square design.

Mean flow of non-ammonia-nitrogen (NAN) to the duodenum was $1.46 \text{ gNAN/kgW}^{0.75}/\text{d}$, with 33% of the forage N intake having being lost across the rumen. The casein infusion of up to $6 \text{ g casein/kgW}^{0.75}/\text{d}$ resulted in significant ($P < 0.01$) linear increases in NR, but with infusion levels above this having resulting in only small NR responses. The NR response to duodenal supplementation of the AA mixture was not significant ($P < 0.5$). This indicates that the NR response from the casein above that obtained with the AA

supplementation was due to the increased duodenal supply of the remaining six limiting essential AA found in the casein.

The coefficient of efficiency of utilisation of absorbed AAN for NR (BVaan) using the first three casein infusion levels was 0.64 ± 0.01 , and with the AA supplementation it was 0.56 ± 0.16 .

Any means whereby a reduction in nitrogen losses across the rumen or an increase in the supply of limiting essential AA could be achieved, would have the potential to significantly increase liveweight gains of lambs consuming fresh ryegrass/white clover.

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Chapter 1.

REVIEW OF LITERATURE

1.0 Introduction

Unlike most monogastric species, grazing animals are able to utilise pasture as a food source with the use of the highly developed reticulo-rumen digestion system. The animal relies on micro-organisms to break down the otherwise indigestible plant constituents into amino acids. This process relies on fermentation, and there is subsequently high levels of ammonia produced, which is utilised by the rumen microbes for protein synthesis. If the rate of ammonia production is greater than the rumen microbes ability to utilise it, then this excess is absorbed from the rumen into the blood stream, and is converted to urea, which is subsequently excreted. Volatile fatty acids, being by-products of rumen fermentation, are absorbed from the rumen and large intestine, and are utilised as an energy source by the host animal. In the grazing ruminant animal, the majority of the protein supply to the small intestine for absorption is rumen microbial protein.

Due to the complexity of the ruminant digestive system, the amino acid requirements of the host animal must be assessed independently of the amino acid requirement of the rumen micro-organisms, and investigations into the influence of protein intake have relied on this relationship. The symbiotic association between the host animal and the rumen micro-organisms is beneficial for both, but there are costs in terms of energy expenditure and protein

destruction involved. One such cost is the availability of amino acids essential for the animals maintenance and/or growth. If the supply of the essential amino acids to the animal is limiting, an increase in their availability will result in an increase in productivity.

Many of the earlier principles of the degradation of protein to amino acids and their eventual utilisation by the ruminant for maintenance and/or growth have been made by analogy to the mono- gastric. The knowledge of amino acid utilisation in the monogastric was more readily available owing to their small size, but more importantly owing to the simplicity of digestive system, i.e the absense of a complex rumen fermentation. However, these principles could not be applied to the ruminant animal. The importance of the role of rumen micro-organisms in dietary protein breakdown and subsequent supply of protein to the host animal was poorly understood, and therefore attainment of knowledge of utilisation of amino acids for production by ruminants was relatively slow.

This literature review looks at investigations into the manipulation of the supply of amino acids for absorption by the ruminant in an attempt to increase the level of production and in doing so possibly increase the efficiency of utilisation of dietary nitrogen.

1.1 Nitrogen requirements of rumen microbes

1.12 Dietary influences on amino acid synthesis in the rumen

The micro-organisms in the rumen of sheep have different nitrogen requirements than those of the host animal. El-Shazly (1951) observed that the power of washed suspensions of rumen micro-organisms to deaminate amino acids depends on the diet and its soluble protein content. Therefore the solubility of the diet is an important consideration in respect to deamination rates. Furthermore, because the rumen micro-organisms synthesise amino acids, the conversion of plant protein to bacterial protein in the rumen would not greatly affect the relative amounts of amino acids in the protein mixture which becomes available for digestion in the abomasum and intestines for a sheep consuming a normal herbage diet (Weller, 1956). Similarly, for a wide range of types of diet, the amino acid composition of the mixed rumen bacterial proteins is almost constant, and the amino acid distribution is similar to that of the bulk protein of plant leaves (Weller 1956). Moreover Storm and Ørskov (1983) found that the amino acid composition of isolated rumen micro-organisms, in particular that of bacteria, was found to be markedly constant between animals.

Bergen (1967) observed through the use of an in vitro enzymatic digestion system and a protein quality index computed from egg protein ratios, that modification of the bacterial population may be an important factor with respect to the nitrogen status of the animal and its response to dietary changes. Further, he found rumen bacteria appeared to be a protein

source of a quality comparable to that of casein. Conditions that favour optimal bacterial growth will consequently lead to increased microbial amino acid production and higher availability of these amino acids for absorption in the small intestine.

1.13 Nitrogen losses in the rumen.

Degradation of plant proteins in the rumen and synthesis of microbial amino acids by micro-organisms, produces volatile fatty acids, carbon dioxide, ammonia and hydrogen as by-products which can be lost during the fermentation process. However there exists a conservation mechanisms in the rumen, such as recycling of nitrogen through the saliva as urea, and the ability to absorb VFA's that are produced. Lewis (1955) found that sheep receiving hay supplemented with casein had the greatest production of ammonia, associated with high rumen acetic acid levels. The proportion of free ammonia, which is rapidly diffused into the portal blood supply through the rumen wall, increases as the pH of the rumen increases (Visek, 1968). Therefore, because ammonia is a major source of nitrogen for rumen bacteria (Smith, 1979), highly digestible dietary protein sources, resulting in elevated rumen pH levels, would increase the dietary protein cost of ruminants.

1.2 Nitrogen requirements of the host animal

1.21 Available Nitrogen for absorption

After microbial fermentation of plant carbohydrates and degradation of plant proteins, the amount of protein and amino acids entering the small intestine for absorption includes 1. dietary rumen by-pass protein, 2. microbial protein and 3. endogenous protein (Jacobson *et al*, 1969).

1.22 Dietary Rumen by-pass protein

Rumen by-pass protein is the plant protein that has escaped rumen degradation, and is absorbed in the small intestine where the lower pH levels and enzymes break up the plant protein into amino acids to be absorbed. The quantity of undegraded dietary protein reaching the small intestine is influenced by the variability in degradability of the various plant protein constituents. Therefore, when the diet is highly degradable in the rumen, there is less undegraded dietary protein, and more opportunity exists for loss of dietary nitrogen from the rumen in the form of ammonia. Any means whereby high quality protein, and perhaps amino acid supplements, can be provided to the host by avoiding rumen breakdown, coupled with microbial growth from non-protein- nitrogen, would offer considerable potential for reducing the dietary protein cost in ruminants (Jacobson 1969).

When the supply of microbial protein does not meet the ruminants amino acid requirement for production, Kempton et al (1978) observed that additions of small amounts of a dietary protein to a low protein cellulose diet, which is not fermented in the rumen but is digested in the small intestine, resulted in an increase in food intake and growth rate. The above observations compliment predictions that a substantial benefit in lamb growth may arise through reducing rumen protein losses of animals grazing high protein content pasture (Poppi et al,1988).

1.23 Absorption of microbial protein

Through the use of paper impregnated with chromic oxides as a marker, MacRae *et al* (1974) found between 65% and 75% of the total amino acids entering the small intestine were apparently digested before the ileum. This suggests that the amounts of total and individual amino acids apparently digested in the small intestine appears to be mainly dependent on the amounts of these constituents reaching the duodenum (MacRae *et al*, 1974). Through estimation of the requirement of tissues for protein in growing lambs, Black *et al* (1972) found that because most diets contain sufficient micro-nutrients, it is the quantity and pattern of absorbed amino acids and the amount of ATP produced which usually determine the capacity of a diet to promote protein synthesis. This was observed by measuring the response in nitrogen retention to changes in protein intake when it was assumed that a). the absorption of nutrients from the digestive tract was accurately assessed, and b). the efficiency of utilization of the absorbed nutrient was taken into account in a feeding trial.

1.24 Endogenous protein

Endogenous protein accounts for only a small fraction of absorbed protein from the small intestine, and is composed of sloughed epithelial cells from the reticulo-rumen and gastro-intestinal tract and various secretions into the abomasum and intestine. Indeed abomasal endogenous nitrogen ranges from only 0.5-2.6gN/d, of which 60-70% is in the form of protein and 10% mucin. Moreover endogenous protein also includes nitrogen

contained in saliva, secreted at perhaps 20L/d in forage-fed sheep, supplying 1-8gN/d, half being present in urea and most of the remainder in protein (Hogan, 1982).

It must be stressed however that endogenous protein is an important consideration when attaining true digestibilities.

1.3 Purified protein diets

1.31 Urea based diets and amino acid supplements

The hypothesis that the amino acid requirement of ruminant animals could be optimally met by rumen microbial synthesis has been studied. Rumen micro-organisms utilise dietary protein as a nutrient source, and so changes in the dietary protein will affect the growth and development of the micro-organisms. Bunn *et al* (1967) found marked changes in the floral population of the rumen for animals fed an amino acid mixture based on the amino acid composition of Alfalfa, compared with animals fed Alfalfa. Therefore there exists the possibility that manipulation of the dietary amino acid content may alter rumen microbial nitrogen arriving at the small intestine.

However, Loosli *et al* (1949) found dietary addition of individual or pairs of amino acids failed to improve animal performance on diets containing urea as the sole source of nitrogen. It could be argued that the small change in rumen microbial nitrogen production resulting from the increased availability of the amino acids would result in only a relatively small increase in rumen microbial nitrogen arriving at the small intestine for absorption and utilisation by the host animal.

1.32 Amino acid supplements

The substitution of either Methionine or Tryptophan for an iso-nitrogenous amount of urea nitrogen in a diet, where urea consisted of 85% of dietary protein, has been shown to increase the retention of absorbed nitrogen above that of lambs fed solely the urea basal ration (Mclaren et al, 1965). A similar response in nitrogen retention resulting from Methionine supplementation to a purified urea diet was found by Loosli et al (1945).

Wright (1971) observed increases of 26% and 21% ($P < 0.05$) in the rate of liveweight gain from supplementation of 0.3% methionine and 4% casein respectively, to an 8% urea protein based diet. Furthermore, Oke et al (1986) demonstrated increased nitrogen retention for lambs fed 0.03% and 0.05% methionine and lysine respectively to a diet of rolled corn and soybran flakes, supplemented with soybean meal. Similarly, Doyle and Bird (1975) found nitrogen retention increased significantly above controls when 3.8g methionine/day was supplemented to a oaten/lucerne chaff based forage diet in sheep. Therefore, given that the amino acid profile of microbial protein is relatively constant (Weller, 1956, Storm and Ørskov, 1983), the responses from supplemental amino acids in urea based diets may be due to the amino acids limiting microbial protein synthesis. However, Doyle and Bird (1975) found that methionine is somewhat naturally resistant to rumen degradation, such that even when fed orally it increases the methionine supply to the animal.

1.4 Diets of a low protein content

A knowledge of the nutrient requirement of the microbial population of the rumen is essential if the best possible use is to be made of poor quality roughage as ruminant feedstuffs (Egan,1964). Indeed, Chalmers et al (1954) observed higher nitrogen retention resulted from less extensive formation of ammonia in the rumen with a herring-meal supplemented urea based diet compared to a casein supplemented urea based diet.

1.41 Low protein diets supplemented with amino acids

Studies involving either corn-soybean meal/wheat straw or isolated soybean as the sole source of dietary protein supplemented with amino acids have not been shown to improve the nitrogen balance in growing lambs, (Scott et al,1972 and Pelaz et al,1979 respectively). This is supported by findings by Weller (1956) in that the amino acid composition of the mixed rumen bacteria proteins does not change for differing dietary protein levels. This observation by Weller (1956) gives support to the hypothesis that the amino acid requirement of ruminant animals could be optimally met by rumen microbial synthesis because all essential amino acids were synthesised in the rumen (Loosli et al,1949). Similarly, Harbers et al (1961) found that lysine supplemented to a high energy diet composed primarily of milo and cottonseed meal did not result in a significant effect on weight gain or efficiency of weight gain in sheep. Similar results were obtained when the nitrogen source was crystalline urea.

All the above studies contradict observations by Loosli *et al* (1945), who found increased nitrogen retention by the addition of methionine to a urea diet. Furthermore, these findings are complicated by Kempton *et al* (1978), who observed that supplements of either urea, casein or formaldehyde-treated casein to a low protein cellulose diet increased feed intake by 27% on average for all three treatments. This suggests that there may be increased digestibility of the low protein cellulose diet through increased availability of an easily obtainable nitrogen source for the rumen micro-organisms, and increased food intake associated with the supplemental protein by-passing the rumen.

1.5 Post-ruminal amino acid supplementation to diets low in available protein

Increased feed intakes like those observed by Kempton *et al* (1978) were observed by Schelling *et al* (1968) for lambs on a purified urea diet, infused with casein into the abomasum. Comparative studies of soybean protein with or without abomasal casein supplementation have shown that low nitrogen retention values were obtained when ammonia nitrogen is absorbed from the rumen more rapidly than the microbes can use it in protein synthesis (Little *et al*, 1967).

Using a mixture of arginine, histidine, lysine, phenylalanine and methionine infused abomasally to lambs fed a purified diet of urea ad-lib, Schelling *et al* (1968) observed an increase in nitrogen retention. The resulting response however was less than that

observed with all ten essential amino acids combined. Nimrick et al (1970) found a positive nitrogen retention response occurred with lysine and methionine infusion, compared to an iso-nitrogenous urea infusion in lambs fed a semi-purified diet containing urea as the only nitrogen source.

1.51 Plasma concentrations of amino acids

Schelling et al (1967) observed decreases in plasma methionine concentrations with increasing dietary protein and amino acid supplementation, but upward trends occurred with most of the other plasma amino acid concentrations, indicating increased utilisation of methionine. Furthermore, he observed that the plasma amino acid concentrations were considerably lower for most of the amino acids for lambs fed a purified diet of urea, but that no significant differences in average daily gain or feed conversion was observed for the various treatments.

Wakeling et al (1970) observed depressions in plasma concentrations of essential amino acids after infusions of methionine and lysine into the duodenum of mature wether lambs receiving a low-protein diet. They hypothesised that if protein synthesis is restricted by the supply of a limiting amino acid, then increasing the amino acid concentration should increase protein synthesis, stimulating demand for the other essential amino acids, leading to a decrease in their plasma concentration. The duodenal infusion of casein and urea nitrogen to sheep fed on a low-protein roughage by Egan (1964) elevated blood urea and ruminal ammonia concentration, demonstrating a return of nitrogen to the rumen and resulting in an increased rate of cellulose digestion in the rumen, with little evidence for loss of nitrogen in the faeces.

1.52 Amino acid infusion as a comparison to a casein supplemented diet.

Schelling *et al* (1968) infused all the essential amino acids to sheep to simulate a casein supplemented diet, with methionine providing the equivalent amount of both the methionine and cystine content of casein. He hypothesised that the resulting increase in nitrogen retention being less than with the casein supplementation may be due to the nonessential amino acids and rate of amino acid absorption eliminating time for protein digestion.

1.53 Rumen protected protein

Protein can be supplied post-ruminally through the diet if it escapes rumen degradation. Formaldehyde treated casein, which protects the casein from rumen degradation, has been demonstrated by Schelling *et al* (1968) to increase nitrogen retention greater than infusion of all the ten essential amino acids via the abomasum. However this was complicated by an associated 15% increase in feed intake, and so the use of a feeding system based on metabolic body size was implemented, which has been adopted by many workers since.

1.6 Supplemental amino acids to a fresh herbage based diet

Ruminants fed ad lib fresh herbages of high digestibility and protein content may nevertheless have a deficiency in the quantity of one or more essential amino acids absorbed from the small intestine (Barry, 1981). Chalmers *et al* (1954) found extensive conversion of casein to ammonia occurred when infused into the rumen. Casein supplemented feed fed to hill ewes on a low plane of nutrition in the later stages of pregnancy was found, through

nitrogen balance studies, to be used very ineffectively, despite the animals need for protein (Chalmers et al, 1954).

Barry (1981) observed that considerable responses were obtained in the performance of young growing ruminants on pasture, by infusing casein plus methionine via the abomasum. He hypothesised that the digestion products from fresh spring pasture fed ad lib were deficient in the supply of essential amino acids relative to that of metabolizable energy. This result, along with results from amino acid supplementation to sheep on low quality diets, points to post-ruminal protein and or amino acid deficiency.

1.61 Efficiency of utilisation of metabolizable energy

The efficiency of utilization of autumn harvested dried grass for fat and protein deposition fed to sheep at maintenance increased from 0.45 to 0.57 with abomasal infusion of 30g casein per day (MacRae et al, 1985). This gives support to the theory that the supply of amino acids can influence the efficiency of utilization of metabolizable energy in sheep given forage rations where the acetate:propionate values are high (MacRae et al, 1985). Furthermore, MacRae et al (1985) observed that as the level of energy intake of spring harvested dried grass was raised from maintenance to 1.5 times maintenance, assuming that the extra amino acids absorbed from the small intestine were not retained as nitrogen but were used as precursors for more NADPH₂ and glycerol phosphate synthesis, then a saving of approximately 100-140 kJ heat/24 hours could account for about 40% of the observed differences in Kpf.

The biological value of absorbed protein can be used to determine the slope of the relationship between nitrogen balance and metabolizable energy intake. Black and Griffiths (1974) used this approach and found that when lambs received insufficient protein, nitrogen balance was independent of metabolizable energy intake, and was linearly related to nitrogen absorption and was influenced by liveweight. When metabolizable energy intakes are above that needed for positive nitrogen balance, a constant proportion of metabolizable energy intake is used in protein synthesis for animals of any given weight (Black and Griffiths, 1974).

Asplund (1986) argues that nitrogen from the deamination of essential amino acids could be used in non-essential amino acid synthesis and that the associated biological value of the diet can be very variable depending on protein content of non-essential compared with essential amino acids.

1.7 Wool growth and amino acid supplementation

Wool growth is a relatively important deposition site for protein received in the diet. Because wool contains 4% sulphur incorporated in cystine molecules, supplementation of sulphur containing amino acids should result in an increased sulphur content of the wool (Reis *et al*, 1962). Increased availability of sulphur amino acids should also increase wool growth. Abomasal infusion of casein, cysteine and methionine were found to increase wool growth and sulphur content of the wool by Reis *et al* (1963).

Wright (1971) similarly increased wool growth by intraperitoneal

injections of 1.5 to 4.5g methionine per day to lambs fed an 8% urea protein based diet.

Further studies by Reis et al (1972) involving abomasal infusion of various amounts of cystine, cysteine and methionine to sheep receiving 800g/day of a mixture of equal parts of chopped wheaten and lucerne hays, significantly increased wool growth rates. He observed an optimal level of methionine to be 1 to 2 g per day. Ten grammes per day was found to reduce the wool growth to the basal level. This may indicate the existence of a level of methionine that is detrimental to wool growth. These results reinforce substantial increases (35-130%) in the wool growth of tattoo patches of sheep obtained by Reis et al (1963) from abomasal infusion of 60 g casein and 2 g of cysteine and methionine per day for 6 weeks.

1.8 Intragastric infusion of volatile fatty acids

Ørskov et al (1979) found no significant difference in nitrogen balance of lambs sustained entirely by intragastric infusion of different molar proportion mixtures of volatile fatty acids, with added abomasal protein. This result suggests that the variable rumen production of volatile fatty acids, which would occur with differing diets, would not have adverse affects on the nitrogen balance.

However, in a study by Tao et al (1974), increasing infusion of methionine to sheep having volatile fatty acids infused intra- ruminally resulted in a curvilinear response of nitrogen balance. The optimal level of methionine was found to be 4.81 to 5 g per

day, thus reinforcing the hypothesis that one or more of the essential amino acids is limiting.

1.9 Diets Low in available protein supplemented with rumen-protected amino acids

1.91 Evidence for limiting amino acids

Armstrong and Annison (1973) reviewed results from workers and found support for the conclusions that either methionine or cyst(e)ine is the first limiting amino acid in the sheep. However, more recent work by Phillips and Walker (1978) where lambs were fed milk replacers, pointed towards lysine being equally limiting to methionine. The observation of lysine being a more limiting amino acid to growth than cystine reinforced observations made by Harper (1958).

1.92 Rumen protected lysine and methionine supplementation

Using a growth experiment for growing-finishing lambs, Mowat et al (1972) observed a weight gain increase and feed efficiency increase of 11% and 9% ($P < 0.05$) respectively through dietary additions of 0.45% encapsulated methionine. The diet consisted of a corn-alfalfa ration supplemented with corn-urea or corn-blood meal-feather meal. Similar results were obtained using a urea basal diet supplemented with 0.4% encapsulated methionine. However 0.6% encapsulated methionine produced a marked reduction in performance, indicating a toxic effect. Substitution of the supplement with soybean, followed by the 0.45% encapsulated methionine, did not result in increased weight gain, probably due to the methionine tending to increase carcass fat (Mowat et al, 1972).

A 33% increase in nitrogen retention was observed by Oke *et al* (1986) by feeding lambs 0.03% and 0.05% rumen protected methionine and lysine respectively, compared with no supplemental methionine and lysine. Abomasal infusion of unprotected methionine and lysine resulted in a similar increase in nitrogen retention, as did abomasal infusion of rumen protected methionine and lysine.

1.93 Plasma methionine and lysine responses

Evidence to support the findings of Phillips *et al* (1978) that lysine may be first equally limiting to methionine was found by Wright *et al* (1988) using increasing addition of rumen protected methionine to the diet and measuring the response in plasma methionine levels. No improvement in lamb nitrogen balance was observed, but there was a detectable inflection point in plasma methionine with increasing dietary levels of rumen protected methionine, indicating that it was being excreted. It follows that this observation could be argued to indicate the existence of a co-limiting amino acid to methionine, possibly being lysine, and would have lead to the need for further studies into the amino acids limiting ruemn microbial protein supply to the small intestine of ruminants.

1.10 Microbial nitrogen supply and limiting amino acids

1.101 Limiting amino acids of microbial protein

Microbial protein usually accounts for the largest proportion of the total amino acid nitrogen entering the small intestine. Therefore, it is meaningful to determine the limiting amino acids of rumen micro-organisms separately because the amino acid composition of the microbial protein is relatively constant (Storm and Ørskov 1984).

Storm and Ørskov (1984) set out to find the order of limiting amino acids in rumen microbial protein. They assessed the efficiency of utilization of absorbed amino acid nitrogen (U), assuming that the difference between U and the maximum efficiency of utilisation of 1.0 is due to one or more amino acids being limiting. Therefore, the supplement required to raise the efficiency of utilisation to the theoretical maximum is $(1-U)/U$. This supplement was then added as a mixture of synthetic amino acids in the same proportions as the absorbed amino acid profile of rumen microbial protein and each amino acid was then removed in turn and the resulting change in nitrogen retention measured. The results showed that methionine was first limiting, followed by lysine, arginine and histidine, as each reduced nitrogen retention when omitted from the amino acid supplement. Therefore, only these amino acids were limiting rumen microbial protein (Storm and Ørskov, 1984).

The finding of histidine being a limiting amino acid in rumen microbial protein is one that has not been demonstrated by many workers, however Bergen *et al* (1968) indicated that histidine could sometimes be limiting, and this could indicate previously unidentified interrelationships.

1.102 Apparent digestibility and net utilization of microbial nitrogen

The coefficient of utilization of rumen microbial nitrogen for protein synthesis was found to be 0.543 by Storm and Ørskov (1983b) through experiments involving lambs entirely sustained by

intragastric infusion of isolated rumen micro-organisms. Furthermore, the true digestibility of rumen microbial nitrogen was found to be 0.813, with gross energy inputs varying from 430 to 860 kJ/kg liveweight^{0.75}. Storm *et al* (1983) also found through intragastric nutrition that the true digestibility value of microbial amino acid nitrogen was 0.85. Since the proportion of amino acid nitrogen accounts for approximately 80% of the nitrogen contained in rumen micro-organisms, manipulation of amino acid nitrogen supply to the small intestine, such as through the use of rumen protected amino acids, should ultimately alter the efficiency of utilisation of AAN for production in the ruminant.

1.11 The apparent need for amino acid supplementation

Through either abomasal or duodenal infusion of essential amino acids, or through the use of the addition of rumen protected essential amino acids to the diets, many workers have shown that certain amino acids are limiting production in ruminants either on a grain or forage based diet. However, under conditions of ad-lib intake on high planes of nutrition, the possibility that energy availability may be limiting for utilisation of the increased supply of essential amino acids must not be overlooked.

1.12 Conclusion

The literature clearly indicates that substantial responses in lamb growth were obtained by increasing the protein supply to the small intestine (Schelling et al, 1968; Barry, 1981). Indeed, work by Storm and Ørskov (1984) demonstrated that the supply of methionine, lysine, histidine and arginine were 1st, 2nd, 3rd and 4th limiting amino acids respectively of rumen microbial protein for growth in sheep. Similar conclusions have been reached by previous workers (Schelling et al, 1968; Reis et al, 1972; Barry, 1981).

Therefore, because the ruminant relies on microbial protein for the majority of the supply of amino acids for maintenance and/or growth, in order to increase the availability of amino acid supply the following observations have been made. Increased growth in lambs has been obtained through the infusion of amino acids post ruminally as described above, by either making them rumen-protected (Schelling et al, 1968; Mowat et al, 1972; Oke et al, 1986; Rogers et al, 1986; Wright et al, 1988) or through the use of rumen non-degradable protein sources, such as fishmeal supplementation for lambs fed high quality pasture (Poppi et al, 1988). The later study by Poppi et al (1988), therefore suggests that the supply of amino acids to the small intestine may be limiting growth on highly degradable forages.

Experiments involving the infusion of casein with or without amino acids (Schelling et al, 1968; Barry, 1981), have shown increased nitrogen retention or increased live-weight gains. Other

investigations to determine the order of the essential limiting amino acids for either microbial protein synthesis (Storm and Ørskov, 1984), or for nitrogen balance studies (review by Armstrong and Annison, 1970) point towards the need for a study of comparative post-ruminal infusion of the most limiting amino acids and casein and their effect on nitrogen retention of lambs consuming fresh pasture.

Responses in nitrogen retention have been obtained with protein supplements to lambs on high quality pasture (Poppi *et al.*, 1988), indicating amino acid deficiency, and Storm and Ørskov (1984) have established the amino acids limiting rumen microbial protein production. Therefore there exists the need to establish if post-ruminal amino supplements, as used by Storm and Ørskov (1984), will give responses in nitrogen retention comparable to that obtained from infusions of casein protein of similar magnitude.

Chapter 2.

MATERIALS AND METHODS

2.0 Animals and surgical preparation

Four wether and four cryptorchid Dorset Down x Coopworth lambs of average liveweight 38 ± 3.3 kg were obtained and subsequently housed indoors in individual metabolism cages for the duration of the experiment. Each lamb was weighed every 10 days throughout the experiment, and was removed every 20 days to a larger pen for a rest period of 24 hours. Prior to the start of the experimental trial, each lamb had been fitted with rumen and duodenal cannulae (Cruickshank, 1988).

2.1 Timing of the experiment

The experiment started at the beginning of February 1989 at the Johnstone Memorial laboratory, Lincoln College, and continued until the end of April 1989.

2.2 Feeding

A pasture of predominately 60% ryegrass (Lolium sp) and 30% white clover (Trifolium repens) was set aside for the whole experiment, with soil moisture levels maintained through irrigation. The daily feed allowance was gathered with the use of a portable 4-stroke Gravely sickle mower with minimum emulsion of the herbage. In a preliminary period of 7 days, the lambs were adjusted to ad libitum intake of pasture. Pasture allowances thereafter were restricted to that of the animal with the lowest intake; i.e $0.47 \text{ kg fresh herbage/kgW}^{0.75}/\text{d}$, and the individual daily herbage allowance were adjusted every ten days according to changes in liveweight. Harvested herbage was weighed into large numbered plastic bags daily and the lambs were fed four times a day from monday to friday; approximately 10:00, 13:00, 16:30 and 21:30, and three times a day during the weekend; approximately 10:00, 14:00 and 21:30. Daily herbage allowances were stored in a cool room at 5°C between feeds.

2.3 Digestibility trial

In order to determine the digestibility of the feed, a seven day digestibility trial was performed. Daily feed offered and feed refused samples were weighed, as were daily faecal excretions, with samples taken from each for dry matter (DM), organic matter (OM), neutral detergent fibre (NDF) and nitrogen (N) determination. All samples, except those for DM analysis were stored in a freezer at -15°C until bulking as described below for chemical analysis. DM, OM, NDF and N digestibility of the feed offered was accordingly calculated as follows;

$$\frac{(\text{DM/OM/NDF/N})_{\text{Intake}} - \text{Faecal}(\text{DM/OM/NDF/N})}{(\text{DM/OM/NDF/N})_{\text{Intake}}}$$

$$(\text{DM/OM/NDF/N})_{\text{Intake}}$$

2.4 Digesta flow

Digesta flow was estimated by the double marker technique of Faichney (1975). Markers Chromium ethylene diamine tetra- acetic acid (Cr-EDTA) and Ytterbium acetate (Yb) were continuously infused into the rumen using portable infusion pumps ('Siropump', Everest Electronics, South Australia). Plastic containers (Vaxipac, 200ml, ICI Tasman) were used as infusion reservoirs. Infusion continued for four days to allow the marker concentration in the rumen to reach equilibrium.

Rumen and duodenal digesta samples were then collected every 4 hours over the next 2 days, such that 12 samples were obtained at 2 hour intervals on a theoretical 24 hour day. Rumen samples were acidified using 10% HCl, and a sample of whole duodenal digesta was taken and the remaining digesta was immediately phase separated at 2000 r.p.m for 2 minutes and the supernatant retained. All rumen and duodenal digesta samples were frozen until bulking for ammonia and N determination as described below.

Daily faecal excretions, feed offered and refused samples were collected and stored as for the digestibility trial above. Neutral detergent fibre content was determined on all the samples collected from both the digestibility and digesta flow trials. The method involved the boiling of weighed sub-samples in a detergent for one hour prior to drying and ashing in an air-forced oven and a muffle furnace respectively (see Appendix, part A1 for detailed methods).

Calculations as used by Faichney (1975) were used to determine the flow of non-ammonia nitrogen (NAN) from the marker, nitrogen and ammonia concentrations in the rumen and duodenal digesta samples, with the Cr-EDTA corrected for 2% absorption prior to the duodenum. Assuming a high proportion of the NAN supply is from rumen microbial protein (RMP), and using values of 0.80 for the proportion of RMP that is amino acid nitrogen (AAN) (Storm and Ørskov, 1983) and the true digestibility value of 0.85 for RMP (Storm *et al.*, 1983), the supply of absorbed AAN can be calculated as follows:

$$\text{absorbed AAN} = \frac{\text{NAN flow}}{\text{Proportion of AAN in total N} \times \text{true digestibility}}$$

(Proportion of AAN in total N)(true digestibility)

2.5 Experimental design and treatments

The experimental design was based on an 8x8 latin square. The eight experimental treatments were duodenal infusions of water (control), four levels of casein infusions (3,6,9 and 12 g casein protein/kgW^{0.75}/d) and three levels of amino acid infusion (see Appendix table T1). The amino acids infused were methionine, lysine, histidine and arginine and were present in the same proportions as found in casein (Fraser, 1988). Amino acid infusions were such

that the quantities infused were equivalent to those in 3,6 and 9g casein/kgW^{0.75}/d). The last amino acid treatment level was not used as it was hypothesised that it was unlikely that a response would be obtained from this high level of infusion. The treatments were randomly allocated to each of the eight lambs over the eight replicates (see Appendix Table T1).

2.6 Infusion procedure

2.61 Preparation of infusates:

A 10% casein solution was prepared a day in advance by mixing lactic casein powder with 0.53%Na₂CO₃ (Sodium carbonate) in batches of 7kg (Macleod et al, 1982). The prepared casein solution was stored at -5°C, each batch being sampled for later nitrogen determination. The individual daily requirement was weighed into 5 litre plastic bottles and the infusate made up to 2 kg with cold water. The weighed amino acid mixtures were added to similar plastic bottles containing 2 kg of cold water and were shaken well to completely solubilize the mixture. All the infusion bottles were stored at -5°C until required.

2.62 Infusion of infusates:

The duodenal infusates were pumped from the numbered bottles to each lamb via 2mm infusion lines and a peristaltic pump adjusted to pump 2kg water in 23 hours.

2.7 Experimental procedure for infusion trial

The lambs were fed 0.67kg fresh herbage/kgW^{0.75}/d as used for the digesta flow trial above. Each treatment period lasted for 5 days, and quantitative collections of feed offered, feed refusals, faeces and urine excreted were made over the last 4 days of each treatment period. Nitrogen balance was calculated from intake of nitrogen in herbage consumed and casein or amino acids duodenally

infused, and excretions of nitrogen in urine and faeces over the last three days only of each treatment period. A daily sub-sample for dry matter, botanical and chemical analysis was taken from the samples to be bulked. Botanical composition of the feed offered was determined for % ryegrass, % clover and % weed periodically.

2.8 Sample bulking

2.81 Digestibility trial;

Faeces: The collections from each lamb were mixed in a bucket, and 20% of the total weight taken as a representative bulked and thoroughly mixed sample. Sub-samples were taken for dry matter determination and chemical analysis.

Feed offered and refused: Representative samples from each collection was taken to 20% of the total weight. Sub-samples were similarly taken as for the faeces for dry matter determination and chemical analysis.

2.82 Digesta flow trial;

Bulking of the feed offered, feed refused and faeces was as above. Individual rumen and duodenal whole and supernatant digesta collections were proportionately bulked over the two day period.

2.83 Infusion trial;

Bulking of feed offered, feed refused and faeces was as for the digestibility trial. Ten percent of the daily urine output from the respective collections were mixed and bulked for 3 of the 4 collection days.

All the bulked sub-samples were stored in a freezer for later chemical analysis.

2.9 Analytical methods

The nitrogen content of all samples was determined using the Kjeldahl Technique with a Kjeltic distillation unit (Tecator, Hoganas, Sweden).

Marker concentrations, Cr and Yb, in the rumen and duodenal whole and supernatant digesta samples were determined using atomic absorption spectrometry (Shimadzu 2200). The method is detailed in the appendix, section A3.

2.10 Statistical analysis

The data was analysed by analysis of variance to partition animal, period and treatment effects of the respective periods. Least significant differences at the 5% significance level were calculated for the nitrogen retention mean values using Student's t-test.

Chapter 3.

RESULTS**3.0 Digestibility**

From the 7 day in vivo digestibility trial, the mean animal organic matter, nitrogen, neutral detergent fibre and dry matter digestibilities were 0.77 ± 0.07 , 0.75 ± 0.03 , 0.78 ± 0.02 and 0.77 ± 0.02 respectively.

3.1 The loss of nitrogen across the rumen

Mean forage nitrogen intake (FONI) values, flow of non-ammonia nitrogen (NAN) at the duodenum, and % FONI arriving at the duodenum as NAN (%FINAN), for each animal are presented in table 1. The values for %FINAN for animals 61 and 70; 129.5 and 100 % respectively, (table 1.) were not used in the overall mean calculation because of inconsistent marker infusions over the measurement period. The overall values are, therefore, means of the remaining six lambs.

Table 1. Mean animal forage nitrogen intake (FONI), non-ammonia nitrogen flow at the duodenum (NAN) and % FONI arriving at duodenum as NAN (%FINAN) for lambs consuming fresh ryegrass/white clover, (g/kgW^{0.75}/d).

Animal	NAN flow (gNAN/kgW ^{0.75} /d)	FONI(gFNI/kgW ^{0.75} /d)	%FINAN
61	1.99*	1.55*	129.5*
62	1.49	2.17	68.8
64	1.64	2.15	76.1
67	1.37	2.07	66.3
70	1.74*	1.70*	100.0*
71	1.40	2.25	62.17
72	1.37	2.21	61.9
73	1.50	2.22	67.5
Mean	1.46 ± 0.10	2.18 ± 0.06	67.13 ± 5.22

* Not included in mean

3.2 Liveweight gain

The mean daily animal liveweight gains were estimated with periodic weighing for the duration of the digestibility, digesta flow and the duodenal infusion trials. For the digestibility and digesta flow trial the mean daily liveweight gain (dLWG) was 148 ± 85 g/d. The mean dLWG over the infusion period was 195 ± 170 g/d. Individual liveweight gains are presented in Appendix table T3.

3.3 Nitrogen balance

Table 2. presents treatment mean values of the forage nitrogen intake (FONI), nitrogen from the infusion of casein and amino acids, total nitrogen intake, nitrogen excretions and nitrogen retention (NR), (all $\text{mgN/kgW}^{0.75}/\text{d}$). Individual mean values are detailed in appendix, table T2.

Both the animal and period effect on FONI was highly significant ($P < 0.01$), whereas the treatment effect on FONI was not significant. Urinary nitrogen excretion (UNE) was significantly ($P < 0.01$) affected by the infusion treatment effect, increasing as TNI increased relative to the basal water infusion (BWI). Furthermore, the period and animal effects on UNE were both significant ($P < 0.01$ and $P < 0.05$), respectively.

Faecal nitrogen excretion (FNE) increased as the total nitrogen input increased, but the infusion treatment effect was not significant. However, the period and animal effects on FNE were significant ($P < 0.01$ and $P < 0.05$, respectively).

Relative to the BWI, NR increased as nitrogen input from all the infusion treatments increased, despite the associated increase

in UNE, with both the period and treatment effects being highly significant ($P < 0.01$). The animal effect on NB was not significant. The NB resulting from treatments B through E were consistently higher than the corresponding NR from treatments F through H, reflecting the same pattern with the total nitrogen input. The NR resulting from treatments B through E were significantly different from treatment A, but only treatments B and C were significantly ($P < 0.05$) different from each other. However treatments F through H were not significantly (LSD 5%) different from each other.

3.4 Efficiency of utilisation of absorbed amino acid nitrogen for nitrogen retention

The relationship between NR ($\text{gN/kgW}^{0.75}/\text{d}$) and estimated truly absorbed amino acid nitrogen (TabsAAN, $\text{gAAN/kgW}^{0.75}/\text{d}$) is presented in figure 1.. TabsAAN was estimated by using the calculated individual %FINAN values and the assumptions by Storm and Orskov (1983) and Storm *et al* (1983) as outlined in section of the Materials and Methods. Table 3. presents the regression analysis from the amino acid and casein infusion treatments, with the slope ('b') of the regression line being the coefficient of efficiency of utilisation of absAAN for NR (BVaan). The BVaan from the duodenal infusion of the 4 amino acids for NR was 0.56 ± 0.16 . Regression analysis only of the 0,3 and 6g casein/ $\text{kgW}^{0.75}/\text{d}$ infusion levels (treatments A,B and C) improved the BVaan from 0.38 ± 0.08 to 0.64 ± 0.01 .

Figure 2. depicts the response in NR to changes in TabsAAN as in figure 1., but with the calculated equivalent quantity of AAN from the 4 AA for each casein infusion level replacing the corresponding estimated casein AAN supply.

Figure 3. presents similar diverging curves as for figure 2., but with the AAN supplied from the forage (using the individual calculated %FINAN values) omitted.

Table 2. Mean values for nitrogen inputs, excretions and retention (all mgN/kgW^{0.75}/d) for lambs consuming fresh ryegrass/white clover pasture and receiving duodenal infusions of water, casein or amino acid.

TREATMENT	NITROGEN INPUT (All mgN/kgW ^{0.75} /d)			N EXCRETION		N RETENTION	
	FORAGE	CASEIN	AA	TOTAL URINE	FAECES		
A	2311	-	-	2311	1190	690	431a
B	2285	395	-	2680	1389	655	636ab
C	2270	793	-	3064	1508	711	845bc
D	2330	1191	-	3520	1579	740	902c
E	2314	1565	-	3880	2180	770	930c
F	2267	-	104	2371	1164	661	546a
G	2261	-	207	2468	1207	676	586a
H	2309	-	309	2617	1329	672	617a
Standard error of difference				71	45	86	

Different alphabetical notation for NR represents significant differences (P<0.05).

A water (control)

B 3g casein/kgW^{0.75}/d

C 6g casein/kgW^{0.75}/d

D 9g casein/kgW^{0.75}/d

E 12g casein/kgW^{0.75}/d

F 4 amino acids equivalent to B

G 4 amino acids equivalent to C

H 4 amino acids equivalent to D

Table 3. Mean animal regression analysis of TabsAAN (from duodenal AA or casein supplementation and calculated individual %FINAN) on NR for lambs consuming fresh ryegrass/white clover (gAAN/kgW^{0.75}/d, gN/kgW^{0.75}/d), where 'b' and 'c' in the equation y=bx + c correspond to the BVaan and the y intercept, respectively.

TREATMENTS*	REGRESSION EQUATIONS		r ²
A through E	y = 0.38(SE 0.08)x	+ 0.10(SE 0.08)	0.89
F through H	y = 0.56(SE 0.16)x	- 0.12(SE 0.04)	0.85
A,B and C	y = 0.64(SE 0.01)x	- 0.24(SE 0.00)	1.00
C,D and E	y = 0.13(SE 0.02)x	+ 0.63(SE 0.01)	0.98

* Refer to treatments in table 1.

Figure 1. Nitrogen retention ($\text{gN}/\text{kgW}^{0.75}/\text{d}$) response to the duodenal infusion of casein or amino acids for lambs consuming fresh ryegrass/white clover pasture. Absorbed AAN ($\text{gAAN}/\text{kgW}^{0.75}/\text{d}$) supplied from the duodenal infusion of the casein or amino acids supplementing that derived from the consumed forage (assuming a 33% loss of nitrogen across the rumen).

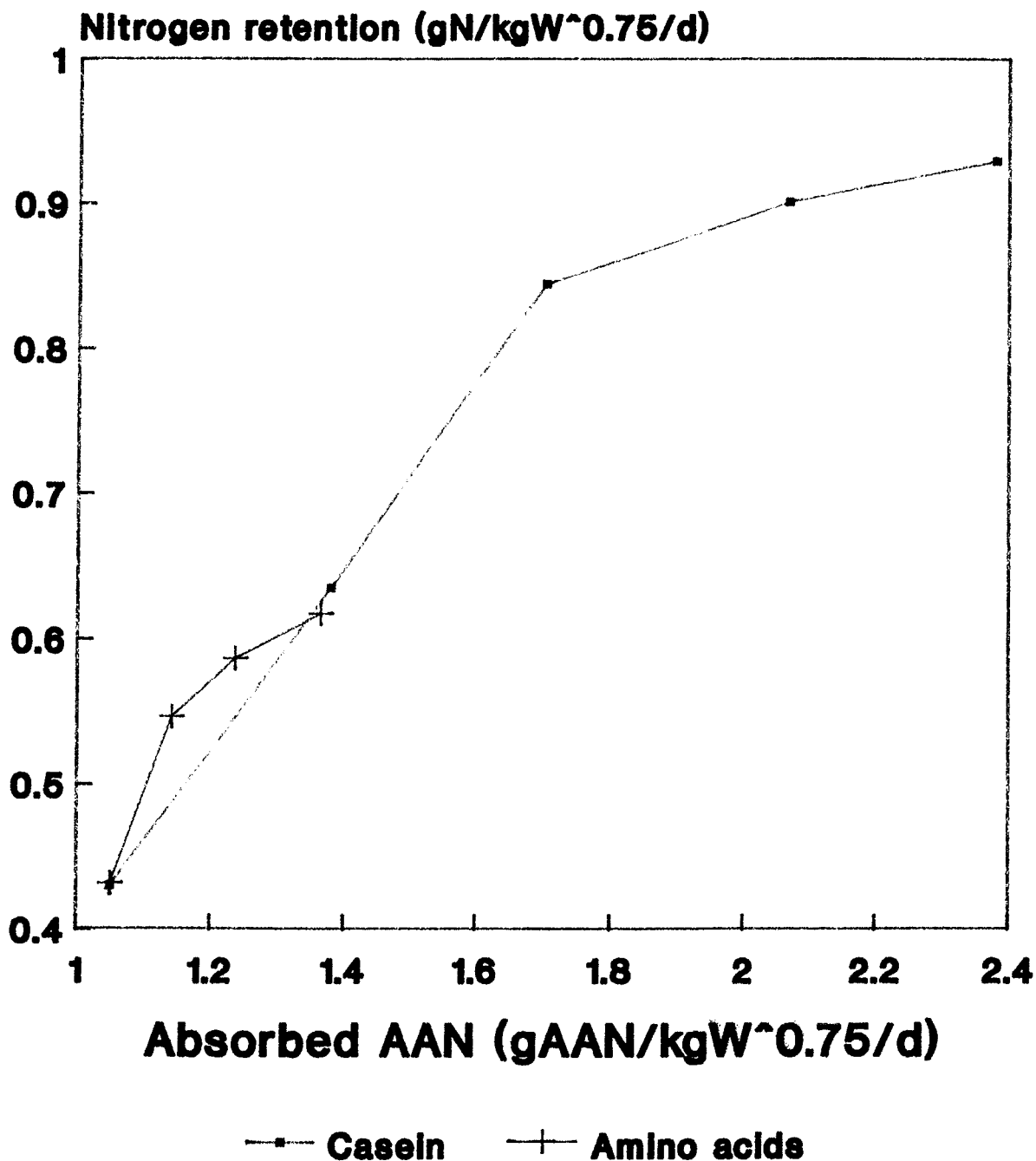


Figure 2. Nitrogen retention ($\text{gN}/\text{kgW}^{0.75}/\text{d}$) response to the duodenal infusion of casein or amino acids for lambs consuming fresh ryegrass/white clover pasture. Absorbed AAN as for figure 1., but with the AAN supplied from the infused casein estimated as that derived from the equivalent quantity of AAN contributed to by the 4 amino acids contained in the casein ($\text{gAAN}/\text{kgW}^{0.75}/\text{d}$).

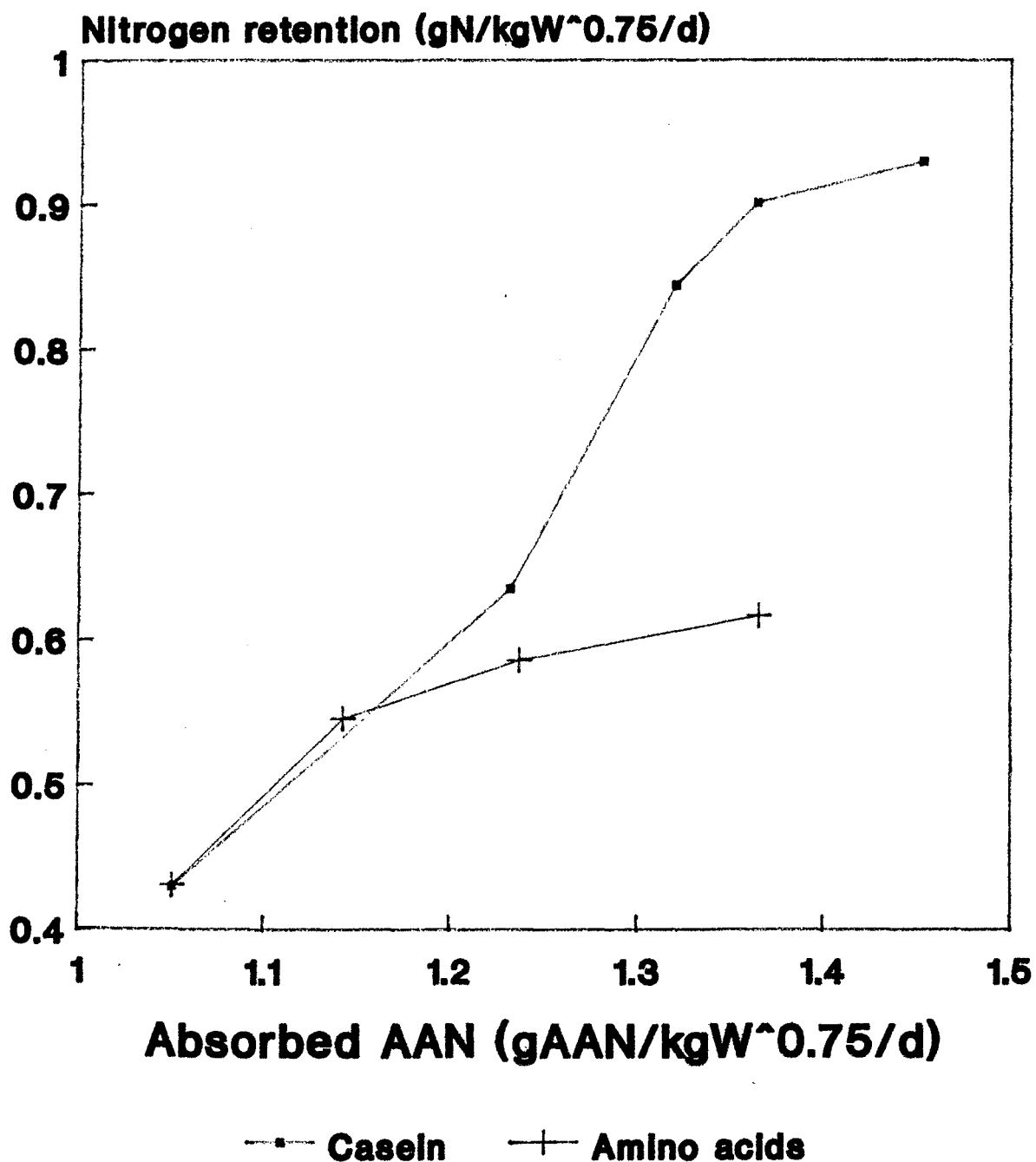
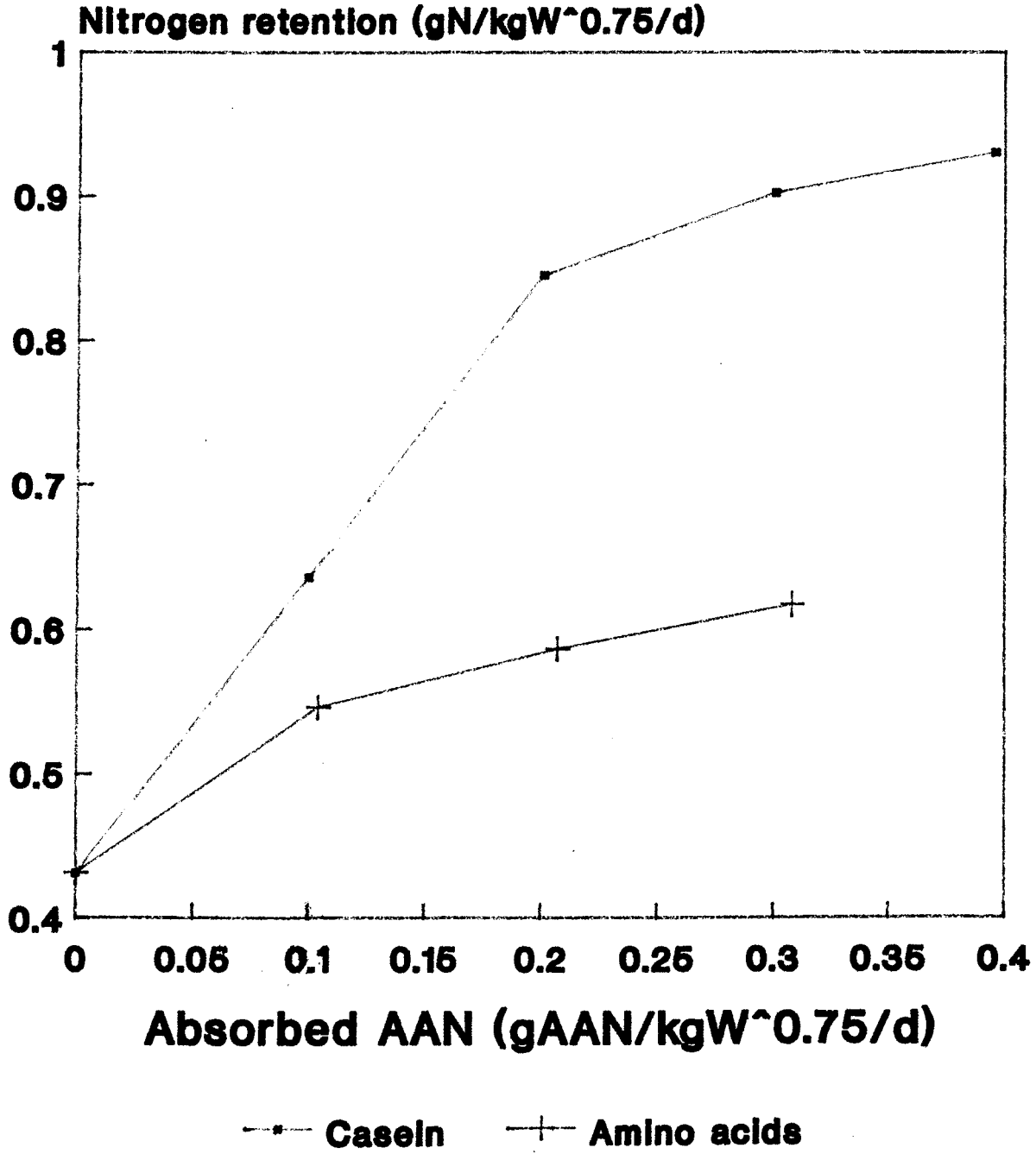


Figure 3. Nitrogen retention ($\text{gN}/\text{kgW}^{0.75}/\text{d}$) response to the the duodenal infusion of casein or amino acids for lambs consuming fresh ryegrass/white clover pasture, but with the AAN absorbed from the consumed forage having being subsequently ommited. Absorbed AAN supplied from the infused casein as for figure 2.



Chapter 4.

DISCUSSION

4.0 Experimental errors

The average loss of nitrogen on collection of urine at pH values below 2 has been demonstrated to be 0.97% with ambient temperatures between 15 and 18°C. Duncan (1966) suggested that the Kjeldahl method of nitrogen determination does not completely measure all forms of nitrogen, and therefore together with the errors associated with the urine collection, the values obtained for the nitrogen balance data in this study have an associated error of approximately 5%.

The usefulness of the latin square design is supported by observations by Phillips and Walker (1978). They concluded that the 8 x 8 change-over design best minimises carry-over effects and can be effectively used to estimate the AA requirement with fewer animals and with shorter dietary periods than with conventional balance experiments.

4.1 Nitrogen balance

The main observation of importance pertaining from the analysis of variance (ANOVA) is the highly significant ($P < 0.01$) effect of the treatments on the nitrogen retention (NR). Significant increases ($P < 0.05$) in NR were obtained when the basal water infusion (treatment A) was supplemented with 3g casein/kgW^{0.75}/d (treatment B), 6g casein/kgW^{0.75}/d (treatment C), 9g casein/kgW^{0.75}/d (treatment D) and 12g casein/kgW^{0.75}/d (treatment E). However, only treatments A,B and C were significantly different from each other ($P < 0.5$). Therefore these results clearly demonstrate that duodenal infusion of casein of at least 6g/kgW^{0.75}/d can significantly increase NR for lambs consuming fresh ryegrass/white clover.

The non significant response in NR resulting from the duodenal infusion of the 4 amino acids (AA) to levels equivalent to that contained in the corresponding treatments B,C and D is in contrast to the finding by Storm and Ørskov (1984) that they are the first four limiting AA of rumen microbial protein. However a non significant response in NR to an increased supply of AA was also observed by Poppi *et al* (1988) with dietary supplementation of rumen protected methionine and lysine for lambs consuming fresh white clover. Furthermore, Schelling and Hatfield (1968) observed the response in NR from the abomasal infusion of the same 4 AA (but with the inclusion of phenylalanine) was less, although still significant ($P < 0.01$), than the abomasal infusion of all the essential AA for lambs receiving an ad lib purified urea diet. However the results of this study suggest that there exists little

benefit in terms of a response in NR from the duodenal supplementation of a combination of the AA lysine, arginine, histidine and methionine to levels equivalent to that contained in the respective casein infusion treatments B,C and D for lambs receiving fresh ryegrass/white clover.

Non-essential AA could have been involved in the superior NR response observed with the casein infusion over that obtained with the AA infusion (Schelling and Hatfield, 1968), with nitrogen from the deamination of essential AA possibly being used in non-essential AA synthesis (Asplund, 1986). Therefore because casein contains a relatively high proportion of non-essential AA, and has a desirable essential limiting AA profile, the quality of casein as a protein source could be underestimated (Asplund, 1986). It follows that the 4 essential AA duodenally supplemented could have been utilised to a lesser extent owing to possible deamination to synthesise non-essential AA. However there still exists the possibility of there being the potential to increase the NR of lambs grazing fresh ryegrass/white clover pasture through increasing the duodenal AAN supply of a combination of limiting essential AA, incorporating either those used in this study or other limiting essential AA.

4.21 Forage nitrogen intake, faecal and urine nitrogen excretions:

Both the animal and period effects on the forage nitrogen intake (FONI) were highly significant, due in part presumably to variations in the weather and associated animal behaviour. Forage of a relatively high moisture content would decrease the available dry matter, due to the method of allocation of the herbage

allowance based on metabolic body weight, and consequently would have been a major component in the observed variation in the FONI and rate of weight live-weight gain. Conversely, the treatment effect on FONI was not significant. Therefore the response in NR to the increased supply of nitrogen to the duodenum would presumably be due to higher levels of absorbed nitrogen consequently resulting in nitrogen utilisation for protein deposition being greater, and not due to an associated higher FONI.

Since urine nitrogen excretion (UNE) increased highly significantly ($P < 0.01$), but faecal nitrogen excretion (FNE) showed a non significant response, respectively, to an increased duodenal nitrogen supply, it could be assumed that the increased supply of nitrogen to the duodenum coincided with an increase in absorption of nitrogen from the small intestine. Furthermore, it can be concluded that the variation in FNE was due mainly to an animal effect ($P < 0.01$), and partly due to a period effect ($P < 0.05$). However, the period effect on UNE was also highly significant ($P < 0.01$) and therefore caution is needed in that not all of the increase in UNE was due to the associated higher quantity of nitrogen absorbed from the duodenum as the supply of nitrogen increased. Any explanations for the likely cause of the increase in UNE would include that it was most probably due to the level of nitrogen absorbed from the small intestine being above the animals requirement for maintenance and growth. Hence the excretion of the excess nitrogen into the urine as urea (e.g. 40% of the nitrogen from the $6\text{g casein/kgW}^{0.75}/\text{d}$ infused was excreted in the urine). An increase in body protein turnover as a result of the increased supply and utilisation of the essential AA could be a further component of the observed increase in UNE.

The significant ($P < 0.01$) period effect on the NR could presumably be a result of a similarly significant ($P < 0.01$) period effect on both the FONI and UNE with some periods being associated with a combination of either a high or low FONI or UNE respectively. As expected the animal effect on NR was non significant, despite there being a significant ($P < 0.01$) effect on FONI and FNE indicating that animals that consumed larger quantities of forage had associated higher faecal outputs.

4.2 Evidence of limiting amino acids from a forage based diet

Egan (1984) in a review of the factors affecting the nitrogen requirement for ruminants reported observations by Hennessey (1984) that for low nitrogen roughages, where the supply of digestible protein/MJME is less than 6, it would appear that specific AA limit intake and subsequent NR. Indeed, with diets of mature ryegrass (chopped, or ground and pelleted), infusions of mixtures of AA into the small intestine have led to improvements in NR and in appetite only when methionine, threonine and isoleucine were included in the mixture (Fennesy, 1976 in Hennessey, 1984). It follows that if the protein synthesis is restricted by the supply of a limiting AA, then increasing the quantity of that limiting AA should increase protein synthesis, stimulating demand for the other essential AA (Wakeling *et al.*, 1970).

Consequently, because in this study the casein duodenal infusion supplied all the limiting essential AA, their resulted a larger response in NR as the supply of nitrogen increased, compared to that obtained from the duodenal infusion of the four AA. Barry (1981) observed a similar response of increasing NR to abomasal infusion of casein, measured by a comparative slaughter technique.

Although a non-significant response in NR resulted from an increased supply of the four duodenally infused limiting essential AA, the significant ($P < 0.1$) increase in NR from infusion of all the essential limiting AA (i.e. casein infusion) supports observations by Barry (1981). He argued that the digestion products from fresh ryegrass/white clover pasture are deficient in one or more essential AA. Furthermore, Reis and Schinckel (1964) observed that the deficiency in the supply of sulphur AA from forage based diets restricts not only wool growth, but protein deposition in the wool-free body as well. However the results of this study contrast the findings by Reis and Schinckel (1964) by clearly demonstrating that the sulphur AA are not singly limiting protein deposition in only the wool free body.

4.3 Amino acid nitrogen supply

Dietary crude protein (N x 6.26) concentration in this study was found to be 188g/kgDM, which is comparable to that found in the diets of Barry (1981) and Ørskov *et al* (1971). Furthermore the mean animal NAN flow of 1.46gNAN/kgW^{0.75}/d (table 3.) is similar to that observed by Cruickshank *et al* (1985) (although for somewhat heavier liveweight lambs) with lambs consuming fresh ryegrass/white clover pasture.

The mean conversion of FONI to NAN of 67%, calculated from the digesta flow trial, is only slightly less than the 70% estimated by Barry (1981) for growing lambs on fresh ryegrass/white clover pasture. The primary cause of this nitrogen loss is presumably due to excessive ammonia formation in the rumen as a result of rapid fermentation of the forage by the rumen microbes. The subsequent use of this estimation of nitrogen loss across the

rumen in determining the amino acid nitrogen (AAN) supplied by the consumed forage assumed that it varied little between animals and that it would remain relatively constant for the duration of the experiment. Both these assumptions can be viable owing to the relatively small variations between animals and the relatively short time period involved. However, the possibility of substantial errors in estimation of the NAN supply to the duodenum should not be ignored.

Using the mean NAN flow of $1.46\text{gNAN/kgW}^{0.75}/\text{d}$ and a 33% loss of nitrogen across the rumen, then the level of nitrogen supplementation needed to replace this loss for a 45kg lamb will be approximately 37.9gN. However, the maximum NR response occurred when $793\text{mgN/kgW}^{0.75}/\text{d}$ was duodenally infused, and therefore an approximately 36% reduction in the nitrogen losses across the rumen is needed to achieve a significant increase in NR, the consequences of which for future plant breeding will be discussed in a later section.

Furthermore, using the A.R.C. (1980) regression equations for body composition, and assuming an empty bodyweight of 33.5kg and a liveweight of 40kg, then the 6g casein/kgW^{0.75}/d supplementation level (corresponding to a protein retention of 84g) would increase the daily liveweight gain (as a component of protein, water and fat gain) by approximately 692g. If this liveweight gain were to be attainable, then there would be a considerable reduction in the dietary costs associated with the fattening of lambs, and hence increased productivity.

The predicted plane of nutrition for the animals was restricted to that of the lowest level of intake for lambs of $0.47\text{kg fresh herbage/kgW}^{0.75}/\text{d}$ (Cropper, 1987) for the duration of the investigation. The following assumptions were used to calculate the $2.625\text{gAAN/kgW}^{0.75}/\text{d}$ supply estimated needed to

achieve the maximum theoretical potential NR of $0.7\text{gN/kgW}^{0.75}/\text{d}$ (Cropper, 1987); The efficiency of utilisation of absorbed AAN for NR was 0.4, with $0.35\text{gN/kgW}^{0.75}/\text{d}$ used for maintenance requirements. It follows that the highest level of supplemental casein needed to achieve this AAN supply, given an estimated $1.2\text{gAAN/kgW}^{0.75}/\text{d}$ derived from the forage (assuming an intake of $0.47\text{kg fresh herbage/kgW}^{0.75}/\text{d}$, with a DM% of 15.7, a nitrogen content of 32% and with 25% loss of nitrogen across the rumen), was $12\text{gCasein/kgW}^{0.75}/\text{d}$. However, owing to the greater than expected loss of N across the rumen (33% compared to 25%), there was consequently an under-estimation in the supply of absorbed AAN from the forage, amounting to on average approximately $0.2\text{gAAN/kgW}^{0.75}/\text{d}$.

The greater than expected loss in nitrogen across the rumen may have been due to the high solubility of the protein leading to a greater proportion of nitrogen escaping microbial protein synthesis, owing to rapid ruminal fermentation (Little *et al*, 1967). However there is a dilemma in that even with the less than expected supply of absorbed AAN from the forage ($0.2\text{gAAN/kgW}^{0.75}/\text{d}$), the highest duodenal casein supplementation of $12\text{g/kgW}^{0.75}/\text{d}$ resulted in a NR of $0.93\text{gN/kgW}^{0.75}/\text{d}$, which is somewhat greater than the predicted theoretical potential of $0.7\text{gN/kgW}^{0.75}/\text{d}$ (Cropper, 1987). Therefore it could be argued that the efficiency of utilisation of either the absorbed forage AAN and/or casein AAN for NR was greater than first expected.

4.4 Nitrogen retention response to the duodenal casein and amino acid supplementation

The experiment was designed in such a way that for each supplementation level, the level of nitrogen supplied by the infusion of AA mixture was equivalent to that supplied by the corresponding casein infusion level. However, fig 1. demonstrates

the the quantity of absorbed AAN with the casein infusion was somewhat higher than that with the AA infusions. Using the assumption that the total proportion of the four AA in casein is 0.2841 (Fraser, 1988), the equivalent quantity of AAN supplied by the four AA in the casein infusion was able to be determined for each supplementation level (figure 2.).

At each level of supplementation, the lambs receiving the casein consumed on average 0.071kgDM more forage compared to the lambs receiving the infusion of the four AA. The resulting higher forage intake with the casein supplementation consequently increased the supply of AAN to the small intestine by approximately 0.09gAAN/kgW^{0.75}/d. This could be argued to be in part due to the increase in metabolizable energy (ME) supplied from the casein above that supplied by the AA mixture stimulating a higher forage intake (in actuality a reduction in feed refused), resulting to some degree from a higher relative absolute AAN utilisation (Macrae *et al*, 1985).

The random allocation of treatments to the various periods should have decreased any period effect on DM intake, however, there was a significant ($P<.01$) period effect on FONI (table 2.). Therefore it could be argued that the lambs receiving the casein infusion could have had a higher FONI due to contrasting environmental conditions as well due to a ME stimulus compared to those receiving the AA infusion, although the latin square design should have minimised this.

In order to investigate the NR response from AA supplementation only, the NR results are examined in relation to the level of AAN supplied by the equivalent quantity of AAN derived from the AA mixture contained in the casein (Figure.3.). NR was found to increase to a greater extent for the casein than for the appropriate AA infusion as the supply of AAN from the AA

mixture increased, and consequently reached a higher plateau (due presumably to some limiting factor). At the asymptote of each curve, the level of absorbed AAN with the casein infusion was higher than with the AA infusion (approximately 0.2 versus 0.1 gAAN/kgW^{0.75}/d, respectively). Furthermore, because the supply of AAN from the four AA infused and that supplied by the infused casein is approximately the same, then the diverging curves (fig 2.) clearly demonstrates that the limiting essential AA which were contained in the casein and not incorporated in the AA mixture, would have most likely resulted in the observed higher NR. Therefore the smaller response in NR from the AA mixture would most likely be due to the absence of the other six limiting essential AA, thus limiting protein deposition and associated NR. Consequently to achieve maximum responses in NR the combination of the infused AA mixture may need to be altered, through incorporation of one or more of the remaining six limiting essential AA.

4.5 Efficiency of utilisation of the absorbed amino acid nitrogen for subsequent nitrogen retention

Because most practical diets contain sufficient micro-nutrients, it is the quantity and pattern of absorbed AA and the amount of ATP produced which usually determine the capacity of a diet to promote protein synthesis (Black *et al.*, 1973). Therefore regression of the AAN from the forage, and from the casein or AA duodenal supplementation against the associated NR was used to estimate the coefficient of efficiency of utilisation of the absorbed AAN for nitrogen retention (hereafter referred to as the biological value (BV_{AAN}) of the absorbed AAN).

By only regressing the first three casein infusion levels against NR, the BV_{AAN} supplied from the casein and forage for NR was 0.64±0.01 (table 3.), and contrasts with a BV_{AAN} of 0.8 as

reported in the A.R.C. (1984) from intragastric studies by Storm and Ørskov (1983b) for lambs consuming fresh forage. This is unexpected, as up to 85% of AAN supplied to the duodenum from the consumed forage is RMN (Agricultural Research Council, 1980).

Regression analysis of the absorbed AAN supplied from the AA infusions and consumed forage produced a value of BVaan of 0.56 ± 0.16 (table 3.) which is similarly substantially less than that estimated by Storm and Ørskov (1983b) and reported in the A.R.C. (1980).

Owing to the odd number of AA treatments, it was not possible to divide them evenly into two sections for regression analysis. If this was possible then the value obtained of 0.56 for the BVaan with the AA infusion could have been more comparable to that observed with the casein infusion. Furthermore, as the supply of AAN to the duodenum increased, UNE significantly increased ($P < 0.01$) for all the treatments, indicating that it was not fully utilised for NR. This could possibly be due in part to a deficiency of dietary ME, especially in light of an assumed adequate supply of all the essential limiting AA from the casein supplementation. Consequently there was a lower utilisation of the increased supply of AAN from the small intestine for subsequent NR.

4.6 Energy balance

Under conditions of inadequate protein absorption, NR is influenced by liveweight. However the relationship between NR and N absorption of lambs of different liveweights is parallel (Black and Griffiths, 1974). Consequently because the lambs were on a restricted plane of nutrition, then the supply of nitrogen above that obtained in the diet when dietary ME may have been limiting, would not have been utilised to as a greater extent than if there was an adequate dietary ME supply.

4.7 The influence of a variable energy supply on the utilisation of absorbed nitrogen

The extra ME supply with the lambs receiving the casein supplementation, compared to those receiving the AA supplementation, could have produced the observed higher BVaan for casein compared to that for the AA. This extra ME could have resulted in a stimulation in the efficiency of utilization of ME of the forage leading to a greater incremental absorption of AAN (Macrae *et al*, 1985), hence the observed higher liveweight gain and DM intake. Furthermore, the extra ME in the form of AA absorbed from the small intestine with the casein infusion could have markedly increased protein as a proportion of total energy deposited, with an associated increase in the incremental heat production, but would have resulted in only small differences in the rate of total energy deposition, and in Kpg (Barry, 1981).

Extra glucogenic AA absorbed from the small intestine (Storm and Orskov, 1983b), which provides increased reducing equivalents (NADPH) and glycerol phosphate necessary for the conversion of acetate into fatty acids, could have resulted in a higher efficiency of utilisation of ME supplied by the forage for the somewhat limited fat deposition (Macrae *et al*, 1985). However, the positive energy balance obtained with the highest casein infusion levels of 9 and 12g/kgW^{0.75}/d coincides with the plateau for the NR resulting from the increased supply of AAN. This illustrates that the extra energy supplied from the highest casein infusion level was not fully utilised in increasing NR, and some limiting factor(S) may be apparent.

The inadequate availability of protein absorbed from the small intestine with the lambs receiving the duodenal amino acid supplementations may have influenced the efficiency with which the

VFA's that were absorbed from the rumen were utilised as an energy source (Macrae et al, 1985 and Storm and Ørskov, 1983b). Therefore the ME and associated protein deficit, supplied by the consumed forage and the AA mixture infused, for maintenance and growth could have resulted in the observed lower BVaan. It follows that the NR response from the increased supply of AAN to the duodenum with the AA infusion could have been significant if there was a greater quantity of ME supplied in the diet resulting in a high BVaan.

4.8 Increasing amino acid nitrogen supply: factors influencing the subsequent nitrogen retention response

The estimated ME deficit from the forage, below that required for maintenance and growth, of up to 3MJME/d is not substantial compared to the total ME requirement of approximately 14MJME/d. Furthermore the general NR response to increased duodenal AAN supply is in reality a curve, and therefore the BVaan from the casein and forage for NR being only 0.64 could be due in part to that part of the curve observed from the experimental data being near the asymptote. Therefore if the lambs were on a lower plane of nutrition, with a decreased availability of dietary nitrogen, then the observed response in NR to the increased supply of AAN from the casein and AA could have been substantially higher as a result of an increase in the BVaan. It follows that if there had been sufficient dietary ME, then the NR response from the 4 AA could have been as high as that from the casein, considering they both have comparable BVaan.

It is evident from this study that the non significant response in NR to the duodenal supplementation of the 4 AA could be due to a number of contributing factors. However, even with a lower than expected BVaan, the increased supply in nitrogen as a

result of the casein duodenal supplementation caused a significant response in NR. This finding clearly confirms the observation by Barry (1981) and Poppi *et al* (1988) that the digestible products from the consumption of fresh ryegrass/white clover are deficient in the supply of protein and/or one or more limiting essential AA.

4.9 The need for further research

Further research is suggested to evaluate whether the replacement of one or more of the 4 AA infused with other limiting essential AA will result in a response in NR comparable to that observed with the infusion of casein, or if the deficit in ME from the consumed forage was limiting the BVaan and hence response in NR from the increased supply of limiting essential AA to the duodenum, or if the lambs were actually receiving too much nitrogen from the forage.

Any means whereby high quality protein and perhaps AA supplementation can be provided to the host by avoiding rumen destruction coupled with rumen microbial growth from non-protein-nitrogen would offer considerable potential for reducing the dietary protein cost in ruminants (Jacobson, 1969). If a desirable AA mixture is found in future research, or if the one used in the present study is subsequently demonstrated to be correct, then plant genetic engineering could utilise the condensed tannins contained in plants to rumen-protect limiting essential AA to some degree (Barry, 1981). This would increase the quantity of the desirable undegraded plant protein arriving at the duodenum.

Furthermore, any method that increases the AA absorption from the small intestine or that manipulates the endocrine system to produce more glucose could have the potential to significantly

increase the productivity of ruminants grazing pasture (Clark, 1975). This would lead to the potential to significantly increase nitrogen retention of lambs from a solely forage based diet such as in New Zealand, and hence substantially increase lamb production from pasture.

Chapter 5.

CONCLUSIONS

The consumption of highly digestible forages, such as fresh ryegrass/white clover, has been shown to be limiting in one or more essential AA for NR in growing lambs (Barry, 1981). This present study clearly demonstrated that there was a substantial loss of nitrogen across the rumen (33%), the consequences of which enabled a significant ($P<0.5$) NR response to occur as a result of duodenal supplementation of casein of upto $6\text{g/kg}^{0.75}/\text{d}$ to lambs consuming fresh pasture. Therefore it follows that the protein supplied from the consumption of fresh ryegrass/white clover by lambs could be limiting potential NR and hence production.

The BVaan supplied by the casein and forage for nitrogen retention was 0.64 ± 0.01 , contrasting to the 0.80 reported in the A.R.C. (1984), possibly due in part presumably from a deficit in ME or more due to an inappropriate supply of individual AA ratios.

It can be argued that with a fresh herbage diet, rapidly growing young ruminants demanding a high protein content diet are disadvantaged due to the supply of RMP to the small intestine for subsequent absorption limiting the potential production.

Contrary to the suggestion by Barry (1981), the present study did not show a limiting supply of one or more limiting essential AA, as duodenal supplementation of the four limiting essential AA did not achieve a significant ($P<0.5$) response in NR. A deficit in available ME to utilise this increased AA supply (Black and Griffiths, 1974) is suggested as a possible reason for the disappointing response, possibly contributed to by plane of

nutrition ($0.47\text{kg fresh herbage/kgW}^{0.75}/\text{animal/d}$) being too restrictive. Nevertheless, over the period of the NR response, the BVaan supplied from the AA and forage for NR was somewhat higher than that observed for the corresponding casein infusion, at 0.56 ± 0.16 , and consequently closer to that obtained by Storm and Ørskov (1983b).

However it would be more plausible to suggest that the AA mixture used in this present study may not have been desirable, owing to the quantity of those AA supplied from the RMP arriving at the small intestine consequently not limiting protein deposition (assuming the observed ME deficit was not a factor). This view is supported by the finding of a diverging curve when an equivalent quantity of AAN supplied by the four AA contained in the casein and from the AA infusions, respectively, was plotted against the corresponding NR (figure 3.)

Liveweight gains could be significantly increased for lambs consuming fresh herbage if the nitrogen losses across the rumen can be reduced (i.e. through the use of plant tannins protecting AA from rumen degradation), or if the supply of a relatively small number of limiting essential AA to the small intestine for absorption could be increased.

Chapter 6.
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Chapter 7.
APPENDIX

7.0 Table T1. Latin square experimental design.
Note: Cas=Casein; AA=amino acids; x g/kgW^{0.75}/d.

PERIOD	ANIMAL							
	64	67	71	70	72	73	61	62
1	A Basal	B 3gCas	C 6gCas	D 9gCas	E 12gCas	F 3gAA	G 6gAA	H 9gAA
2	B 3gCas	E 12gCas	F 3gAA	G 6gAA	H 9gAA	A Basal	C 6gCas	D 9gCas
3	F 3gAA	D 9gCas	A Basal	B 3gCas	C 6gCas	H 9gAA	E 12gCas	G 6gAA
4	C 6gCas	G 6gAA	H 9gAA	E 12gCas	F 3gAA	B 3gCas	D 9gCas	A Basal
5	G 6gAA	A Basal	D 9gCas	H 9gAA	B 3gCas	C 6gCas	F 3gAA	E 12gCas
6	D 9gCas	F 3gAA	B 3gCas	A Basal	G 6gAA	E 12gCas	H 9gAA	C 6gCas
7	H 9gAA	C 6gCas	E 12gCas	F 3gAA	D 9gCas	G 6gAA	A Basal	B 3gCas
8	E 12gCas	H 9gAA	G 6gAA	C 6gCas	A Basal	D 9gCas	B 3gCas	F 3gAA

7.1 **Table T2.** Mean individual nitrogen input from the casein and amino acid supplementation and forage nitrogen intake, nitrogen excretions and balance. (all mgN/kgW^{0.75}/d). N=Nitrogen.

ANIMAL		TREATMENT							
		A	B	C	D	E	F	G	H
CASEIN N	64	0	398	801	1203	1567			
	67	0	366	789	1202	1592			
	71	0	402	751	1201	1578			
	70	0	401	789	1162	1602			
	72	0	400	802	1180	1561			
	73	0	400	801	1201	1603			
	61	0	396	811	1194	1594			
	62	0	395	802	1182	1426			
	MEAN	0	395	793	1191	1565			
AMINO ACID N	64					106	212	319	
	67					106	213	317	
	71					106	208	319	
	70					104	210	316	
	72					106	212	318	
	73					96	209	308	
	61					105	197	319	
	62					106	194	252	
					MEAN	104	207	309	
TOTAL N	64	2244	2956	2796	3389	4259	2587	2376	2955
	67	2074	2541	3153	3709	4055	2166	2077	2934
	71	2462	2448	2949	3309	4296	2585	2972	2208
	70	2104	2817	3465	3357	3503	2603	2697	2393
	72	2808	2564	3261	3640	3689	1998	2349	2805
	73	2391	2352	2822	3992	3742	2258	2619	2787
	61	2535	3187	8277	3190	4056	2229	2360	2430
	62	1866	2575	2790	3576	3438	2544	2293	2426
	MEAN	2311	2680	3064	3520	3880	2371	2468	2617

7.3 Table T2. continued.

FORAGE	64	2244	2558	1995	2186	2692	2480	2163	2637
N	67	2074	2176	2363	2507	2462	2060	1864	2618
	71	2462	2046	2197	2108	2717	2479	2764	1889
	70	2104	2417	2675	2195	1901	2498	2487	2077
	72	2808	2163	2459	2461	2127	1892	2137	2486
	73	2391	1951	2022	2792	2139	2162	2410	2479
	61	2535	2791	2465	1996	2462	2124	2163	2111
	62	1866	2180	1988	2394	2012	2438	2098	2174
MEAN		2311	2285	2270	2330	2314	2267	2261	2309
URINE	64	1306	1290	1656	1681	1994	1124	1180	1503
N	67	885	1317	1964	2225	2161	1294	1458	1627
	71	1069	1377	1102	1932	2582	1005	1223	1405
	70	1081	1358	1604	1784	2250	1219	1001	1200
	72	1154	1216	1647	1960	2052	993	1130	1085
	73	1207	1457	1365	1876	1988	1137	1426	1202
	61	1654	1692	1497	1852	2331	1217	1110	1430
	62	1162	1406	1227	1719	2081	1326	1121	1177
MEAN		1190	1389	1508	1879	2180	1164	1207	1329
FAECAL	64	694	769	725	684	864	647	706	870
N	67	497	541	618	591	801	479	577	663
	71	664	588	614	660	783	682	650	617
	70	794	826	889	684	773	808	647	803
	72	744	839	879	795	586	783	718	676
	73	648	480	722	892	905	654	819	672
	61	952	661	690	825	773	562	673	559
	62	527	538	550	787	672	671	615	516
MEAN		690	655	710	740	770	661	676	672
N	64	244	897	415	1023	1401	815	489	583
RET	67	692	684	571	893	1092	392	42	645
	71	729	482	1232	717	931	899	1099	186
	70	229	634	971	889	479	576	1046	389
	72	910	509	735	885	1050	222	501	1044
	73	535	415	735	1225	849	468	374	913
	61	-71	834	1090	514	953	450	577	441
	62	178	631	1013	1069	686	548	556	732
MEAN		431	636	845	902	930	546	586	617

7.4 **Table T3.** Individual lamb liveweights (kg) at 7 to 13 day intervals over the entire experimental period.

ANIMAL	DATE (Liveweight in kg)							
	20/1	31/1	10/2	23/2	6/3	16/3	28/3	3/4
64	37.9	38.6	39.3	40.9	43.4	45.5	51.2	-
67	39.5	37.6	40.0	42.2	44.8	44.5	49.1	48.4
71	34.0	33.7	35.4	36.7	37.6	38.6	43.8	38.4
70	39.1	39.3	41.1	42.0	45.3	45.0	50.7	50.1
72	40.7	37.3	39.7	40.6	43.4	44.5	48.7	49.7
73	32.5	33.1	33.9	34.4	36.1	38.7	42.8	44.3
61	39.7	38.8	42.2	42.4	45.8	45.8	51.5	50.6
62	42.2	38.7	40.9	42.8	44.6	44.8	48.4	47.2

7.5 ANALYTICAL METHODS

7.51 A1:Ammonia:

The ammonia concentration was determined for the urine and faeces bulked sub-samples and for the rumen, whole digesta, and supernatant duodenal digesta bulked sub-samples using a Kjeltic 1300 distillation unit (Tecator, Hoganas, Sweden), according to the method proposed by Davidson *et al.*, (1970). The urine and faeces samples were thawed out quickly, and after use returned to the freezer to prevent excessive loss of ammonia. For liquid samples, 10ml was weighed into the digestion tubes (Tecator, Hoganas, Sweden) and for the faeces, 0.5g was similarly weighed into the digestion tubes. All samples were prepared in duplicate, with 20ml of A.R grade ammonium sulphate, a pinch of bumping granules, 5g of 19:1 K₂SO₄:CuSO₄ catalyst, and 5ml hydrogen peroxide being added to each tube prior to digestion. The digestion was performed with a 'Kjeltic digestion system' (Tecator, Hoganas, Sweden) at 350°C for 30 minutes, then 415°C for

one and a half hours. 70mls of 'nanopure' water was added after allowing the samples to cool. Included in the digestion procedure was two tubes devoid of any samples (blanks), and a sample of freeze dried and finely ground lucerne of known ammonia content (control).

Just prior to distillation, 25mls of saturated sodium tetra- borate from a tilt measure was added to each tube. The distillation unit used approximately 0.03N of hydrochloric acid, which was standardised, and 40g/l boric acid indicator. Ammonia recovery was determined using ammonium sulphate which was digested, and added to 'nanopure' water, and related to ammonia sulphate not digested.

7.52 A2: Nitrogen determination:

Total nitrogen was determined on freeze-dried finely ground feed offered and feed refused bulked sub-samples, and on previously frozen faeces, urine, rumen and duodenal whole digesta and supernatant bulked sub-samples using the distillation unit as described above and the Kjeldahl Method (Davidson *et al*, 1970). 20ml urine, 0.5g refusals and feed offered, and 1g faeces were taken for analysis. For digestion, the same procedure was followed as for the ammonia determination, but 20ml A.R grade concentrated sulphuric acid was used in place of the ammonium sulphate.

The distillation procedure was the same as for the ammonia determination, except saturated sodium tetra-borate was not added

prior to distillation, and caustic soda was automatically added to the samples just prior to distilling to increase the pH to facilitate the release of the nitrogen.

Any duplicates, including the control, differing in nitrogen content from each other by more than 5% were repeated, for both the ammonia and nitrogen determinations. The distillation unit automatically removed the blank titration from each result, and an ammonia recovery was calculated every two weeks as above.

Nitrogen content was calculated on a dry matter basis.

7.53 Percentage dry and organic matter and ash determination

Percentage dry matter, organic matter and ash were determined on all samples in duplicate by placing weighed quantities of the ground feed offered and feed refused and previously frozen faeces bulked sub-samples into porcelain crucibles prior to placing in an air-forced oven at 105⁰C for 48 hours. The crucibles were then re-weighed, and placed in a furnace at 550⁰C for 9 hours, and the final weight recorded. For the urine, the procedure was the same except 10 ml of the urine sample was weighed into 20ml scintillation vials.

Percentage dry matter, organic matter and ash for the rumen and duodenal whole and supernatant digesta samples were determined by weighing of 20ml into 20ml scintillation vials prior to drying in the oven and furnace as described above, and was a preparation step for the marker concentration determination detailed below.

7.54 A3:Marker concentration

Individual concentrations of the Cr-EDTA and Yb markers in the whole digesta and supernatant duodenal samples were determined by atomic absorption spectrometry. A 'Shimadzu AA670' unit with a nitrous-acetylene flame was used at 346.4nm to increase precision. The lamp was allowed 4 hours to warm up prior to use.

20ml of 25% A.R grade nitrate (HNO_3) and hydrochloric acid (HCl) were added to the scintillation viles prepared as for the dry matter determination procedure described above. The Diluted HNO_3 and HCl mixtures were prepared with the addition of 167ml HNO_3 and 83ml HCl to 1 litre of 'nanopure' water. The scintillation vials were then shaken with plastic caps (with aluminium liners removed) for one hour, and left overnight so as to let the ash settle. The super-natant was then decanted off into 20ml plastic vials. Faeces collected when no marker was being infused (blank) were also analysed, and 5 standards of each of the two markers of concentration range 0-200mg/l were prepared.

7.55 A4:Neutral detergent fibre

Neutral detergent fibre content was determined in duplicate on faeces, feed offered and feed refused bulked samples from the digestibility and digesta flow trials. Glass beakers containing 50ml of detergent and the weighed sample were heated on hot plates with the steam being captured and recycled through cooling bulbs on top of the beakers. After one hour of boiling, with acetate added to prevent the samples boiling over, the liquid was pored into fibreglass strainers attached to a water forced vacuum. Boiling

hot water was added until foam formation ceased, followed by 10 mls acetate. The samples were then oven dried at 105⁰C for 24 hours prior to furnacing at 550⁰C for 9 hours to determine the % neutral detergent fibre content.

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