ESSENTIAL AMINO ACID REQUIREMENTS FOR GROWTH IN WOOLLED SHEEP

by

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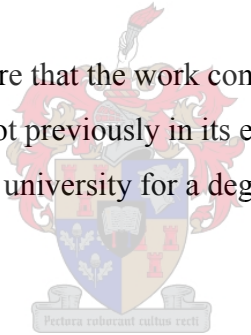
DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.



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ABSTRACT

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This project consisted of five studies. The objectives were to determine the essential amino acid (AA) requirements of growing woolled lambs (Merino and Dohne Merino) and the essential AA profile of duodenal digesta pre-dominantly derived from microbial protein. The limiting essential AA`s in high rumen degradable protein (RDP) diets to growing lambs, where microbial protein is the primary source of AA`s, were also identified.

The first study determined the essential AA profile of duodenal protein on a high rumen degradable diet and evaluated the impact of dietary RDP concentration and source [true RDP *vs.* non-protein nitrogen (NPN)] on the AA composition of supplied in the duodenum. The first trial in this study evaluated the effects of increasing true RDP levels on the essential AA composition of duodenal protein primarily derived from rumen microbes. The lambs had free access to wheat straw and fresh water. The daily RDP supplements were administered in two equal doses into the rumens through rumen cannulas at 07:00 and 19:00. Duodenal digesta was extracted with 6h intervals through T-type cannulas, inserted proximally to the common bile duct. Sampling time was advanced 2h every day to obtain duodenal samples on every even hour of a 24h period after three days. As expected, deficient RDP limited the supply of essential AA`s in the

duodenum. When the true RDP supplements increased, the duodenal flow of essential AA`s also increased concomitantly, but appeared to level off at the higher RDP levels. Despite the positive quantitative effects of true RDP supplementation on AA supply to the duodenum, the AA profile in the duodenum was unaltered. Consequently, the essential AA profile of duodenal protein of sheep receiving high RDP diets, where microbial protein is the primary source of AA`s in the duodenum, is relatively constant and insensitive to dietary RDP concentration.

In the second trial the effects of RDP source (true RDP vs. NPN) on the essential AA profile of duodenal protein on high RDP diets were evaluated by substituting increasing amounts of urea for true RDP in isonitrogenous teatments. Higher NPN increments reduced the daily supply of essential AA`s in the duodenum. In corroboration of the first trial, the AA profile of the duodenal protein was very constant, irrespective of the RDP source. Since microbial protein is the major source of duodenal AA`s on high RDP diets, this study supports the view that microbial protein has a relatively constant AA profile, but microbial protein yield varies according to several rate limiting factors in the rumen. A constant microbial AA profile allows accurate estimates of microbial essential AA supply in the small intestine if microbial protein production and fluid and particulate outflow rates from the rumen can be accurately predicted. This allows the development of more accurate undegradable protein (UDP) supplementation strategies, based on the essential AA requirements of animals.

In the second study growing male Merino and Dohne Merino lambs were slaughtered at different weights and body condition scores. The digesta was removed from the stomachs and intestines and every organ or body part were weighed to determine the whole empty body (WEB) composition. The WEB was partitioned into the carcass, internall offal (stomachs, intestines, organs and blood) and external offal (head, feet, skin and wool). No differences were apparent in the proportional weight distribution of similar body components of the same breed at different ages. In a comparison between breeds, the proportional weight contributions of the carcasses from both breeds to the WEB weight were remarkably similar at both slaughtering stages. The Dohne Merino lambs had proportionally larger internal offals and smaller external offals than the Merino lambs at both slaughters. Unless the essential amino acid compositions of the internal and external offals were identical to the carcass, the dissimilarities in weight and protein allocation to

these two components within the WEB`s of Merino and Dohne Merino lambs imply a distinct WEB essential AA composition for each breed.

The apparent digestibilities of dry matter (DM), crude protein (CP), energy, acid detergent fibre (ADF), neutral detergent fibre (NDF), fat and ash did not differ between Merino and Dohne Merino lambs. Energy retention was also similar for the two breeds, but the Merino lambs retained considerably more N than the Dohne Merino lambs. This may also impact on the respective amino acid requirements of the lambs. Since the Merino lambs utilise N more efficiently, they may have potentially lower essential amino acid requirements to achieve a similar growth rate.

The WEB essential AA compositions of growing Merino and Dohne Merino lambs were determined in the third study. Based on the ideal protein concept, the WEB essential AA profile was accepted as representative of the AA requirements for growth. The use of a single body part as a representation of the WEB AA profile was also evaluated. Differences in the proportional weight and protein contribution of the three body components (carcass, internal offal and external offal) of the two breeds strongly suggested that the WEB AA composition of the breeds would differ, because of likely differences in the AA profiles of these components. The essential AA profiles of the carcasses from the two breeds were surprisingly similar. However, the essential AA compositions of the internal offal and external offal differed substantially from each other, as well as from the carcass. In addition, the internal offal and external offals of each breed had characteristic essential AA profiles. Inevitably, the WEB essential AA profiles of Merino and Dohne Merino lambs differed considerably. Only the leucine and phenylalanine concentrations in the WEB`s of Merino and Dohne Merino lambs did not differ. Significant differences in the concentrations of eight essential AA`s implied that the two breeds have different AA requirements for growth. The different AA compositions of the internal and external offal within each breed also illustrated that the use of a single body component, like the carcass, as a predictor of WEB essential AA composition contains considerable inaccuracies.

The essential AA index indicated that the duodenal protein, primarily derived from rumen microbes, provided approximately 81 % of the qualitative AA requirements of growing lambs. During periods of sufficient availability of very low-quality forage, as the diet in

this study simulated, microbial protein is not able to support maximum growth. The first two limiting AA`s (histidine and methionine) could not even support daily growth rates of 100 g/d. This is very low and stresses the need for effective undegradable AA supplementation under these conditions.

Chemical scores identified histidine as the first limiting AA in high RDP diets (predominantly microbial protein), followed by methionine, leucine, arginine and phenylalanine. However, the requirements for histidine and arginine are frequently over estimated and these AA`s should actually be considered semi-essential, which could render methionine, leucine and phenylalanine the first three limiting AA`s to growing lambs receiving high RDP diets. Because of the limitations of static measurement systems for the determination of AA requirements, a more comprehensive evaluation method was introduced for determination of the limiting AA`s in duodenal protein of lambs on high RDP diets, in the fourth study.

The fourth study focused on the identification of limiting AA`s to growing lambs being limit-fed a high RDP diet. The diet consisted primarily of soybean hulls, for its' low rumen UDP content. Microbial protein production was calculated as 13 % of total digestible nutrient intake and complementary AA supplements prepared to simulate the WEB AA profile, determined in the previous study, in the small intestine. To eliminate the influence of the rumen on the AA supplements, the latter were infused into the abomasums via flexible tubing. Each essential AA was in turn removed from the control treatment (simulating the WEB composition) and the effect on N retention measured. When methionine or the branched-chain amino acids (BCAA`s) were removed from the infusate, N retention of the lambs was reduced. Consequently, methionine and at least one of the BCAA`s limited growth performance of young lambs when microbial protein was the predominant source of AA`s.

The concomitant increased plasma concentrations of total AA`s when methionine or the BCAA`s were removed from the infusate corroborates the effects on N retention, since it indicates that AA utilisation was reduced when these AA imbalances were introduced. Amino acid imbalances had no effect on apparent DM, organic matter (OM) or NDF digestion, but N digestibility was reduced.

The final study verified whether the BCAA's were co-limiting the growth of lambs, or if any single BCAA was responsible for the limitation. Again the WEB AA profile of growing lambs was simulated in the small intestine via abomasal infusions to lambs receiving a soybean hull-based diet. Leucine, isoleucine and valine were individually or simultaneously removed from the infusate and the impact on N retention measured. On an individual basis valine had the largest negative impact on the efficiency of N utilisation. However, the simultaneous removal of the BCAA`s resulted in the lowest N retention, suggesting that valine might be limiting, but the three BCAA`s are more likely to be co-limiting in diets to growing lambs where microbial protein is the primary source of AA`s. Once again, neither DM, OM or NDF digestibility were affected by the AA imbalances. Nitrogen digestibility was, however, negatively affected by AA imbalances.

This project succeeded in establishing the essential AA profile of duodenal protein in sheep receiving high RDP diets. The WEB essential AA compositions of growing lambs from two prominent sheep breeds in South Afica were then determined and the duodenal essential AA profile evaluated against the calculated AA requirements. Finally, the AA`s that limit growth in diets where microbial protein is the predominant source thereof were identified. These results contribute to the current knowledge of AA requirements in growing lambs, and highlight areas for future research, as discussed in the General Conclusion.

SAMEVATTING

ESSENSIËLE AMINOSUUR BEHOEFTES VIR GROEI IN WOLSKAPE

deur

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Hierdie projek het uit vyf studies bestaan. Die doel was om die essensiële aminosuurbehoeftes van groeiende wolskaaplammers (Merino en Dohne Merino), sowel as die essensiële aminosuurprofiel van duodenale proteïen op hoë rumendegradeerbare proteïen diëte, waar mikrobeproteïen die primêre bron van aminosure is, te bepaal. Terselfdertyd is die volgorde van beperkende aminosure in hoë rumendegradeerbare proteïendiëte vir groeiende lammers ook bepaal.

Die eerste studie het die essensiële aminosuursamestelling van duodenale proteïen, op hoë rumendegradeerbare proteïen diëte, bepaal en ook die invloed van verskillende rumendegradeerbare proteïen konsentrasies en -bronne (rumendegradeerbare proteïen *vs.* nie-proteïenstikstof) op die aminosuursamestelling in die duodenum geëvalueer. In die eerste proef in hierdie studie is die effek van toenemende ware rumendegradeerbare proteïenvlakke op die aminosuursamestelling van duodenale proteïen ondersoek. Die rumendegradeerbare proteïenaanvullings is daagliks in gelyke hoeveelhede om 07:00 en 19:00 deur rumenkannulas in die rumens toegedien. Duodenummonsters is met 6h-intervalle uit T-tipe kannulas, wat voor die gesamentlike galbuis in die duodenums geplaas is, geneem. Die monsternemingstyd is elke dag met 2h aangeskuif, sodat `n duodenummonster op elke gelyke uur uit `n 24h-periode na drie dae geneem is. `n Rumendegradeerbare proteïentekort het die lewering van essensiële aminosure in die

duodenum betekenisvol benadeel. Met stygende rumendegradeerbare proteïenaanvullings het die daaglikse vloei van essensiële aminosure na die duodenum dienooreenkomstig toegeneem. Dit blyk egter dat die aminosuurvloei afplat by hoër rumendegradeerbare proteïenaanvullings. Ongeag die invloed van toenemende rumendegradeerbare proteïenvlakke op die kwantitatiewe lewering van aminosure in die duodenum, het die aminosuurprofiel in die duodenum onveranderd gebly. Gevolglik is die aminosuursamestelling van duodenale proteïen, wat hoofsaaklik vanaf rumen mikrobes afkomstig is, relatief konstant en ongevoelig vir rumendegradeerbare proteïenkonsentrasie in die diëet.

In die tweede deel van die eerste studie het alle behandelings gelyke hoeveelhede N bevat, maar ware rumendegradeerbare proteïen is met toenemende hoeveelhede nie-proteïenstikstof vervang om die invloed van rumendegradeerbare proteïenbron op die aminosuursamestelling in die duodenum te ondersoek. Stygende nie-proteïenstikstofvlakke het die daaglikse vloei van aminosure, wat primêr van mikrobe-oorsprong is, na die duodenum verlaag. Ter ondersteuning van die eerste proef was die aminosuurprofiel van die duodenale proteïen weereens baie konstant, ongeag die oorsprong van die rumendegradeerbare proteïen. Hierdie resultate ondersteun die standpunt dat mikrobeproteïen oor `n relatief konstante aminosuurprofiel beskik, maar mikrobeproteïen produksie sal na gelang van verskeie faktore in die rumen wat die groeitempo van die mikrobes bepaal, varieer. Indien mikrobeproteïenproduksie en die uitvloeitempo van beide die vaste stof- en die vloeistoffases uit die rumen akkuraat voorspel kan word, maak `n konstante mikrobe-aminosuurprofiel akkurate voorspellings van mikrobe essensiële aminosuur lewering in die duodenum moontlik. Gevolglik kan meer akkurate strategieë met betrekking tot nie-degradeerbare proteïenaanvullings, gebaseer op die essensiële aminosuurbehoeftes van skape, ontwikkel word.

In die tweede studie is groeiende Merino and Dohne Merino ramlammers op verskillende lewende massas en kondisiepunte geslag. Die maag- en derminhoud is verwyder en elke orgaan en liggaamsdeel is geweeg om die leë liggaamsmassa te bepaal. Die leë liggaam is verdeel in die karkas, interne afval (spysverteringskanaal, organe en bloed) en eksterne afval (pote, kop, vel en wol). Daar was geen verskille in die proporsionele massabydrae van ooreenstemmende liggaamsdele tussen lammers van dieselfde ras op verkillende ouderdomme (lewende massas en kondisiepunte) nie. In `n vergelyking tussen rasse, was

die proporsionele massabydrae van die karkasse op beide slagstadiums verbasend eenders. Die interne afvalle van die Dohne Merino lammers was egter proposioneel swaarder en die eksterne afvalle ligter as vir die Merino lammers by beide massas. Indien die essensiële aminosuursamestelling van die interne en/of eksterne afval dus van die karkas verskil, impliseer dit dat elke ras oor `n unieke leë liggaam aminosuursamestelling beskik, as gevolg van die verskille in die massa en proteïen verspreiding tussen die verskillende liggaamskomponente.

Daar was geen verskille in die waarskynlike verteerbaarheid van droë materiaal, ruproteïen, energie, suurbestande vesel, neutraalbestande vesel, vet en as tussen die twee rasse nie. Energieretensie was ook dieselfde, maar die Merino lammers het N beduidend meer doeltreffend benut as die Dohne Merino lammers. Dit mag `n verskil in die aminosuurbehoeftes van die twee rasse tot gevolg hê, omdat `n meer effektiewe N-benutting waarskynlik `n laer aminosuurbehoefte verteenwoordig om `n ooreenstemmende groeipeil te handhaaf.

In die derde studie is die leë liggaam essensiële aminosuursamestelling van groeiende Merino en Dohne Merino lammers bepaal. Die leë liggaam essensiële aminosuurprofiel is na aanleiding van die ideale proteïenbeginsel aanvaar as verteenwoordigend van die aminosuurbehoefte vir groei. `n Evaluasie vir die gebruik van `n enkele liggaamskomponent om aminosuurbehoeftes vir groei te voorspel is ook gedoen. Verskille in die proporsionele massabydrae van die verskillende liggaamskomponente (karkas, interne afval en eksterne afval) het `n sterk aanduiding gebied dat die leë liggaam aminosuurprofiele tussen die twee rasse sou verskil, vanweë moontlike verskille in die aminosuursamestellings van bogenoemde komponente onderling of tussen ooreenstemmende komponente tussen die rasse. Die aminosuursamestellings van die karkasse van beide rasse was verrassend eenders. Die essensiële aminosuursamestellings van die interne en eksterne afvalle het egter merkwaardig van mekaar, asook van die karkas verskil. Hierdie twee komponente het ook `n onderskeidende aminosuursamestelling vir elke ras getoon. Die leë liggaam aminosuursamestelling van Merino en Dohne Merino lammers het gevolglik van mekaar verskil. Slegs die leusien- en fenielalanienkonsentrasies in die leë liggaam samestelling van Merino en Dohne Merino lammers het nie van mekaar verskil nie. Betekenisvolle verskille in die konsentrasies van agt essensiële aminosure lewer egter onbetwisbare bewyse dat die leë

liggaam essensiële aminosuursamestellings, en dus die behoefte vir groei, tussen rasse van dieselfde spesie kan verskil. Die kenmerkende aminosuursamestellings van die interne en eksterne afval binne elke ras het ook aangetoon dat die gebruik van `n enkele liggaamskomponent om aminosuurbehoeftes vir groei te voorspel tot aansienlike foute aanleiding sal gee.

Die essensiële aminosuurindeks het aangetoon dat duodenale proteïen, wat hoofsaaklik vanaf rumen mikrobes afkomstig is, ongeveer 81 % van die kwalitatiewe aminosuurbehoefte vir groeiende lammers voorsien. Die diëet in hierdie studie het toestande waar volop lae-kwaliteit weiding beskikbaar is, voorgestel. Die resultate het aangedui dat daar onder soortgelyke toestande noemenswaardige essensiële aminosuurtekorte vir optimale groei van jong lammers bestaan. Die eerste twee beperkende aminosure (histidien en metionien) kon nie `n daaglikse groeitempo van 100 g/d handhaaf nie. Dit is baie laag en beklemtoon die behoefte vir doeltreffende nie-rumendegradeerbare aminosuuraanvullings onder soortgelyke toestande.

Chemiese tellings van die aminosuur konsentrasies in duodenale proteïen op hoë rumen degradeerbare diëte het histidien as die eerste beperkende aminosuur vir groeiende lammers geïdentifiseer, gevolg deur metionien, leusien, arginien en fenielalanien. Die behoeftes vir histidien en arginien word egter gereeld oorskat en hierdie aminosure behoort eerder as semi-essensiëel beskou te word. Dit impliseer dat metionien, leusien en fenielalanien die eerste drie beperkende aminosure in hoë rumendegradeerbare proteïendiëte vir groeiende lammers kan wees. As gevolg van die beperkings van statiese analitiese metodes vir die bepaling van aminosuurbehoeftes, is `n meer omvattende evaluasiemetode in die volgende studie aangewend om die beperkende aminosure in duodenale proteïen vir groeiende lammers op hoë rumen degradeerbare diëte te identifiseer.

In die vierde studie is die volgorde van beperkende aminosure vir groeiende lammers wat beperkte voeding van `n hoë rumendegradeerbare proteïendieet ontvang het, bepaal. Weens die vereiste hoë rumendegradeerbare proteïeninhoud het die diëet hoofsaaklik uit sojaboondoppe bestaan. Mikrobeproteïenproduksie is bereken as 13 % van die totale verteerbare voedingstofinname en essensiële aminosuuraanvullings saamgestel om die leë liggaam aminosuurprofiel, soos in die voorafgaande studie bepaal, in die duodenum te

verskaf. Om die degraderingsinvloed van die rumen op die aminosuuraanvullings uit te skakel, is laasgenoemde met behulp van elastiese pypies direk in die abomasum toegedien. Die essensiële aminosure is beurtelings uit die aanvulling verwyder en die effek op N-retensie gemeet. Wanneer metionien of die vertaktekettingaminosure uit die indrupping verwyder is, het die N-retensie van die lammers verlaag. Gevolglik beperk metionien en ten minste een van die vertaktekettingaminosure die groei van lammers wat hoë rumendegradeerbare proteïendiëte ontvang.

Die ooreenstemmende styging in die totale plasma aminosuurkonsentrasie wanneer metionien of die vertaktekettingaminosure uit die aanvulling verwyder is, bevestig die negatiewe effek op N-retensie. Verhoogde plasma-aminosuurvlakke dui `n verlaagde benutting van aminosure aan. Aminosuurwanbalanse in die duodenum het ook N-vertering aansienlik verlaag, maar het geen invloed op die vertering van droë materiaal, organiese materiaal of neutraalbestande vesel gehad nie.

Die doel van die laaste studie in hierdie projek was om vas te stel watter vertaktekettingaminosuur(e) die groei van jong lammers, waar mikrobeproteïen die primêre bron van aminosure was, beperk het. Die lammers het weereens beperkte hoeveelhede van `n sojaboondop-gebaseerde diëet ontvang en die leë liggaam aminosuurprofiel in die duodenum nageboots met behulp van abomasale aminosuurindruppings. Leusien, isoleusien en valien is individueel of gesamentlik uit die aanvulling verwyder en die effek op N-retensie gemeet. Op `n individuele basis het die verwydering van valien die grootste verlaging in N-retensie tot gevolg gehad. Die gesamentlike verwydering van die vertaktekettingaminosure het egter die laagste N-retensie veroorsaak. Gevolglik is die vertaktekettingaminosure gesamentlik beperkend in groeiende lammers wat hoë rumendegradeerbare proteïendiëte gevoer word. Op `n individuele basis blyk dit egter dat valien die grootste impak op N-retensie het wanneer mikrobeproteïen die primêre bron van aminosure is. Weereens het aminosuurwanbalanse in die dunderm N-vertering benadeel, maar die vertering van droë materiaal, organiese materiaal en neutraalbestande vesel was onveranderd.

Hierdie projek het die essensiële aminosuurprofiel van duodenale proteïen in skape waar rumen degradeerbare proteïen die primêre bron van aminosure is, bepaal. Die leë liggaam essensiële aminosuursamestellings van groeiende lammers uit twee prominente

skaaprasse in Suid-Afrika is vervolgens vasgestel en teenoor die duodenale aminosuurprofiel geëvalueer. Laastens is daardie aminosure wat groei in lammers beperk, waar mikrobeproteïen die primêre bron van aminosure is, geïdentifiseer. Hierdie resultate verbreed die huidige kennis van aminosuurbehoeftes in groeiende lammers en lei tot die identifikasie van toekomstige navorsingsgeleenthede, soos bespreek in die Algemene Gevolgtrekking (p. 124).

To Noreen

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My friends and family, for your interest and loyal support.

LIST OF ABBREVIATIONS

AA - amino acid
AAN - amino acid nitrogen
ad lib - ad libitum
ADF - acid detergent fibre
Arg - arginine
BCAA - branched-chain amino acid
BCVFA - branched-chain volatile fatty acid
CP - crude protein
DM - dry matter
EAA - essential amino acid
His - histidine
Ile - isoleucine
Leu - leucine
Lys - lysine
MCP - microbial crude protein
Met - methionine
N - nitrogen
NDF - neutral detergent fibre
NH_3 - ammonia
NPN - non-protein nitrogen
OM - organic matter
Phe - phenylalanine
RDP - rumen degradable protein
TEAA`s - total essential amino acids
Thr - threonine
Trp - tryptophan
UDP - undegradable protein
Val - valine
VFA - volatile fatty acid
WEB - whole empty body

RESEARCH CONTRIBUTIONS FROM THIS PROJECT BY DATE OF COMPLETION

Scientific Publications

1. A.V. Ferreira & J. van E. Nolte, 2002. The effect of increasing rumen degradable protein levels on the essential amino acid composition and flow to the duodenum of Dohne Merino sheep fed wheat straw. Wool Technology and Sheep Breeding. 50(4):541-546.
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CHAPTER 1

General Introduction

The Crude Protein System

Animals use most of their required nitrogen (N) for protein synthesis. Most of the N available in feeds is also present as protein, which explains why the N requirements of animals, as well as the N status of feedstuffs are stated in terms of protein (McDonald *et al.*, 1995a). Until recently the crude protein (CP) system has been used, and is still used for certain species, to calculate the protein needs of animals and the protein content of feedstuffs from their respective N contents. The CP system is based on two assumptions; firstly that all N is present as protein, and secondly that all protein contains 16 % N. From there the well known formula for the calculation of protein content:

CP (g/kg) = N (g/kg) x 6.25

A prominent limitation of the CP system is that all N is certainly not contained as protein, since some lipids, amines, amides, purines, pyrimidines, nitrates, alkaloids and most members of the vitamin B complex also contain N (McDonald *et al.*, 1995b). Secondly, all protein does not contain 16 % N and thirdly, although Kjeldahl N analysis includes most forms of N, nitrites, nitrates and some cyclic N compounds require special techniques for their recovery (McDonald *et al.*, 1995a). Further constraints of the CP system include the unpredictable variation in faecal N content and the assessment of dietary non-protein nitrogen (NPN) utilisation (Ørskov, 1992a). Even if dietary NPN was 100 % digestible, it would result in the production of some indigestible microbial N and NPN. The use of a constant digestibility for urea is equally difficult to accept, since the digestibility will depend on the amount of NPN incorporated into micro-organisms and their digestibility (Ørskov, 1992a). Despite these restrictions, the average protein conversion factor of 6.25 in the CP system is justified, since it actually expresses protein requirements of animals in terms of N and also eliminates confusion and inefficiency in feeding (McDonald *et al.*, 1995a).

A further very important limitation of the CP system is that it does not consider the protein quality [amino acid (AA) profile] of either the feed or the animal`s requirement. Crude protein gives an indication of the N content of a feed but not of its real value to the animal. This implies that the CP or N needs of an animal might be met but individual AA deficiencies may still exist. It is generally accepted that the ruminant is able to synthesise sufficient quantities of the nonessential AA`s to meet its requirements. The essential AA`s (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) are most likely to be deficient from time to time. Egan & Black (1968) stated that arginine is synthesised at considerably lower rates than the nonessential AA`s, rendering the synthetic capabilities of the ruminant incapable to satisfy the excessive need for arginine by lactating mammary tissue (Egan *et al.*, 1970). In corroboration, Merchen & Titgemeyer (1992) reported that microbial protein, the primary source of the ruminant`s protein synthesising abilities, may be unable to meet the high producing animal`s needs for essential AA`s. Animals receiving such imbalanced AA profiles will not produce to their full genetic potential, but will be restricted to the maximum response allowed by the first limiting AA, given that no other nutrients are limiting. Supplementing the first limiting AA will render the second limiting AA first limiting as a result of an altered AA profile (Schingoethe 1991; Coetzee *et al.* 1995), which will then restrict animal performance. Therefore, the protein requirement of an animal in a specific production stage should be expressed in terms of its daily need for individual essential AA`s. This should allow the development of feed formulation models that predict the expected production of the animal from the feed quality and quantity.

Alternative systems for protein evaluation

As a result of the limitations of the CP system to accurately predict animal performance and the progress that has been made in understanding the protein requirements of ruminants, several new systems for protein evaluation have been proposed. All the systems have a remarkably similar concept, namely a separation of host animal protein nutrition from that of microbial nutrition. These systems include the rumen degradable protein/undegradable protein (RDP/UDP) system of Britain, the AAT-PBV sytem used in Scandinavia, the absorbed protein (AP) system of the USA, the PDIE-PDIN system of France (Ørskov, 1992a) and the Dutch DVE/OEB system (Tamminga *et al.*, 1994). The

British system is based on a separation of dietary protein into RDP and UDP fractions. The Scandinavian system is based on AA absorption and protein balance in the rumen, while the absorbed protein system of the USA is calculated from protein degradability and microbial protein supply.

To illustrate the similarities between these systems, the French and Dutch systems are discussed in more detail. The French PDIE-PDIN system calculates protein adequacy for rumen microbes and digestible protein supply to the small intestine. The PDIE fraction represents maximum digestible protein availability if all the fermentable energy is used for microbial protein synthesis. The PDIN component gives the maximum digestible protein available if all the degradable protein is incorporated into microbial proteins. The animal`s protein requirement is given as the protein needed to be digested in the intestine (PDI). When PDIE exceeds PDIN, a higher degradable protein source like urea could be used to increase PDIN. If PDIN is greatest, a less degradable protein source or additional fermentable energy should be supplemented.

$PDIE = (UF \times FK_{UF}) + (MP_E \times FK_{MF})$

$PDIN = (UF \times FK_{UF}) + (MP_N \times FK_{MF})$

Where:

UF = undegraded feed proteins

FK_{UF} = digestibility in the small intestine of UF

MP_N = microbial protein that could be synthesised from degraded protein

MP_E = microbial protein that could be synthesised from fermentation of digestible organic matter

FK_{MF} = digestibility of microbial protein in the small intestine

In the Dutch system DVE reflects the sum of digestible true feed protein and digestible true microbial protein in the small intestine. The digestible true protein in the small intestine is also corrected for endogenous protein losses. The OEB-value expresses the balance or imbalance between the potential microbial protein synthesis from available RDP or from available fermentable energy in the rumen. When OEB is positive, surplus N in the rumen will be lost and rumen degradable N should either be reduced or fermentable energy should be increased. A negative OEB-value indicates surplus

fermentable energy in the rumen. Therefore, the degradable N in the rumen should be increased to achieve optimum microbial protein production.

DVE = DVBE + DVME – DVMFE
OEB = MREN – MREE

Where:

DVBE = Undegraded feed protein digested in and absorbed from the small intestine as amino acids

DVME = Microbial protein digested in and absorbed from the small intestine as amino acids

DVMFE = Endogenous losses resulting from digestion

MREN = Microbial protein that could be synthesised from degradable protein

MREE = Microbial protein that could be synthesised from fermentable energy

Nitrogen utilisation in the ruminant

Figure 1 illustrates the fate of N in the rumen. Protein can be divided into rumen degradable and rumen undegradable fractions. The degradable fraction is degraded to peptides, AA`s and ammonia by bacteria, protozoa and fungi in the rumen. Amino acids are the building blocks of proteins. When AA`s are linked together proteins are formed. The energy cost of linking AA`s together during protein synthesis is relatively small, especially when these AA`s are present in the required proportions. When some AA`s have to be synthesised while others are deaminated, the efficiency of protein synthesis will be reduced considerably (McDonald *et al.*, 1995c). Peptides and AA`s can be directly incorporated into microbial protein, while excess AA`s are degraded to ammonia. The direct incorporation of AA`s and peptides into microbial protein has a much lower energy cost than protein synthesis from ammonia (Nolan *et al.*, 1976) and increases microbial growth efficiency (Baldwin & Allison, 1983). It appears that RDP is essential for maximum microbial protein synthesis. Russell *et al.* (1992) reported that cellulolytic bacteria utilise ammonia as chief N source and sugar and starch degrading bacteria require AA`s and peptides as well.

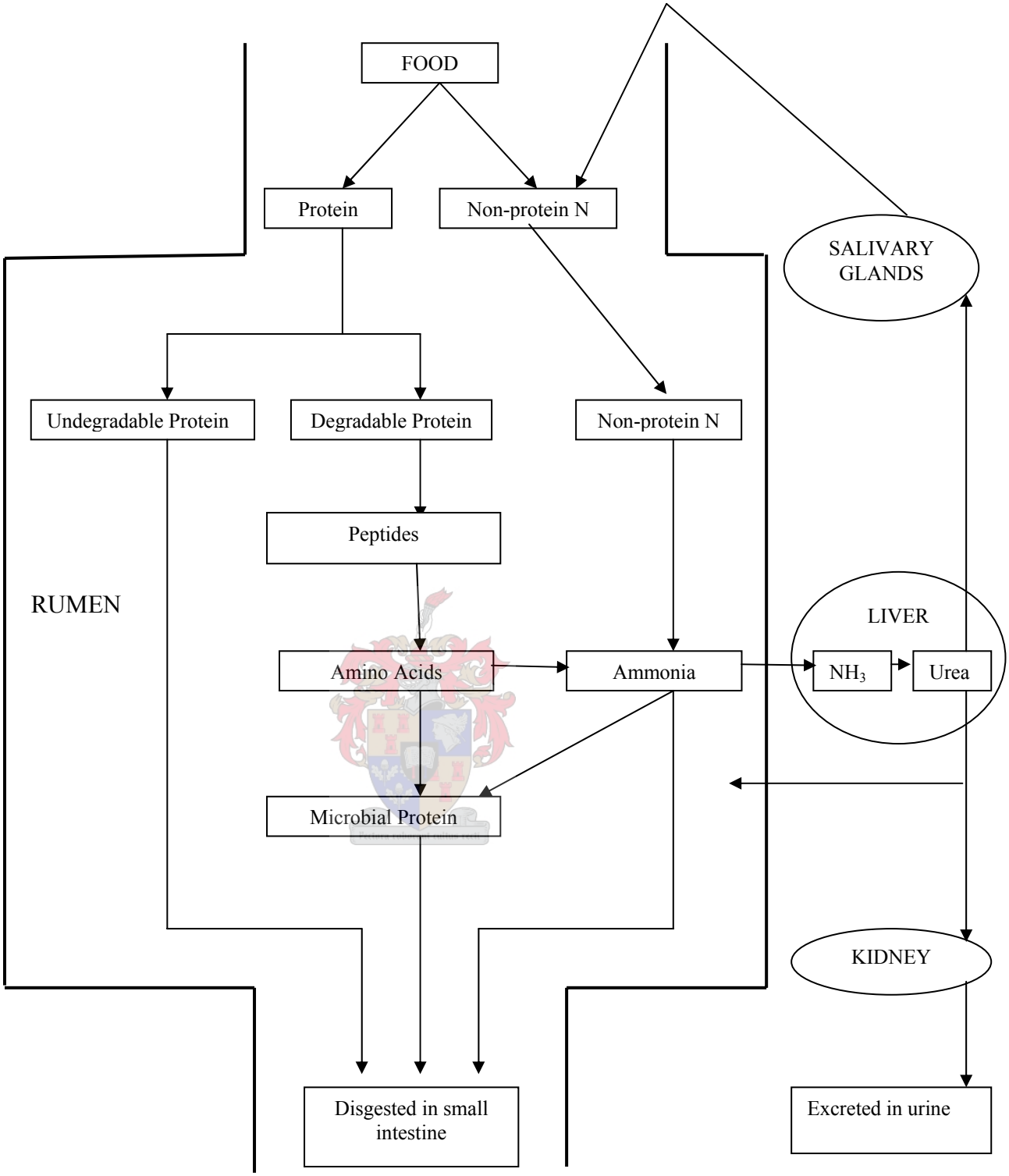

Figure 1 Digestion and metabolism of nitrogenous compounds in the rumen (McDonald *et al.*, 1995d)

The ammonia supplied in the rumen from the degradation of AA`s and/or NPN is primarily used for microbial protein synthesis (Figure 1). The rest of the ammonia is absorbed through the rumen wall and transported to the liver where it is converted to urea.

The urea is then recirculated to the rumen via saliva or passive transportation across the rumen wall, where it serves as an ammonia source to the rumen microbes for microbial protein synthesis. The urea that is not recirculated to the rumen is excreted in the urine, which requires energy and increases the maintenance requirements of the animal. It is therefore important to synchronise the rate of N and energy supply in the rumen to allow maximum microbial growth efficiency. A small amount of ammonia may pass through the forestomachs to the intestines and will be used for microbial protein synthesis in the caecum. This microbial protein will not serve as a source of AA`s to the host animal, since it cannot be digested and absorbed, but will be excreted in the faeces (Ørskov, 1992b).

Microbial protein and UDP pass from the rumen to the small intestine where it is broken down into AA`s and peptides and absorbed. Klemesrud *et al.* (2000) maintained that the supply of AA`s from microbial protein as well as dietary escape protein must be considered when evaluating the supplementation of specific AA`s to maximise the efficiency of protein utilisation. Since microbial protein is the cheapest protein source available to ruminants and supplies from 40 to 80 % of the intestinal protein supply to the ruminant (Owens & Bergen, 1983; Sniffen & Robinson, 1987) the objective in any livestock production system should always be to first optimise microbial protein production before supplementing additional UDP. Care should also be taken not to over supply RDP, since surplus ammonia will be absorbed, converted to urea in the liver and recirculated to the rumen or excreted in the urine, with a subsequent increased maintenance energy requirement. Merchen & Titgemeyer (1992) reported that microbial protein may not always provide in all the AA requirements of high producing animals. A need for specific rumen by-pass AA`s may therefore exist under certain conditions. In order to optimise animal production it is imperative to identify and quantify the required rumen undegradable AA`s.

Some discrepencies exist in literature about the AA profile of microbial protein. Leibholz (1972), Storm & Ørskov (1983) and Cecava & Parker (1993) reported constant AA profiles for microbial protein, irrespective of dieatary alterations. In contrast, Clark *et al.* (1992) and Rulquin *et al.* (1998) indicated differences in bacterial AA composition due to species, separation and analysis methodology or nutritional factors like roughage to concentrate ratio`s or feed intake. The AA profile and quantity delivered in the

duodenum from microbial protein obviously impact on the source and amount of undegradable AA`s required for optimum production. More research on the AA composition of microbial protein is required to assess whether dietary alterations only impact on the quantity of microbial protein produced, or also on the quality. In order to have diets that provide the ideal AA balance and quantities in the duodenum we need to know which AA`s microbial protein supplies in the duodenum and also in what quantities. This implies that during certain production stages like growth, the last six weeks of gestation and early lactation when microbial protein may not provide in the animal`s need of all the essential AA`s, supplemental rumen undegradable AA`s may be required.

Since UDP escapes rumen degradation and provides AA`s directly in the small intestine (Figure 1), it could correct AA imbalances, even in diets adequate in metabolisable protein, if it contained a high content of the limiting AA`s. However, the AA content of dietary protein is not always representative of UDP because all AA`s are not degraded at equal rates (Chen & Ørskov, 1994) or to the same extent (Webb & Matthews, 1994). More research on the undegradable AA content of feedstuffs is required to establish a data bank for utilisation in formulation models. The success of free AA supplementation in correcting intestinal AA imbalances depends on the quantities of the supplement, simultaneity of AA appearance in the intestine, digestion of the dietary protein, size and fequency of the meals and the presence and proportions of non-protein components in the meal (Rolls, *et al.*, 1972). In addition, only methionine has been successfully protected against rumen degradation, which implies post-ruminal supplementation of the other essential AA`s with obvious practical limitations. Merchen & Titgemeyer (1992) suggested that several AA`s are often co-limiting in ruminants. Fraser *et al.* (1990) reported that casein supplements to lambs receiving a freshly cut ryegrass/white clover (60:30) pasture improved N balance to a much larger extent than a mixture of methionine, lysine, histidine and arginine, the first limiting AA`s for whole empty body (WEB) growth in lambs according to Storm & Ørskov (1984), in similar proportions as found in casein. The efficiency of utilisation of the AA infusion was approximately 1.0 and the casein 0.68, indicating that the AA infusion alleviated an AA deficiency to some degree, but not enough to cause significant responses in nitrogen balance. Therefore, some other AA`s might have been limiting as well (Fraser *et al.*, 1990). In support of these findings Schingoethe (1991) reported that supplementation of the first limiting AA rendered the

second limiting AA first limiting, as a result of an altered AA profile (Coetzee *et al.*, 1995).

The available AA quantity and profile could be estimated relatively accurately if the amounts and AA profiles of the daily microbial and UDP flow to the small intestine were known, since these are the major AA suppliers to the small intestine. However, endogenous protein must also be considered in the AA supply to the small intestine. Endogenous N is derived from non-food substances entering the intestine, such as saliva, bile, gastric and pancreatic secretions and cells sloughed off the mucous membrane of the gut (McDonald *et al.*, 1995a). MacRae *et al*. (1979) found that 2.2 to 2.8 g from a total gain of 3.3 to 3.7 g non-ammonia N/day was derived from non-urea endogenous non-ammonia N. According to the NRC (2001) endogenous N accounts for 9 to 12 % of non-ammonia N and is calculated (g N/d) as 1.9 x dry matter intake (kg/d). The ARC (1980) calculated dermal loss (g/d) as 0.1125 x $kgW^{0.75}$, while McDonald *et al.* (1995e) reported the daily endogenous N loss for ruminants as 350 mg N/kg $BW^{0.75}$. A study with dairy cows revealed that endogenous N secretions represented between 10 and 20 % of duodenal N flow (Demers *et al.,* 1999). Although the type of diet, the N sources used in the diet and the N level of the diet affect total N recirculation, endogenous N may form a significant part of the total available N to the animal and the AA contribution from endogenous N should be considered in the calculation of intestinal AA supply.

Xing-Taihan *et al.* (2001) reported that the forestomach may play an important role in peptide absorption in ruminants and may be regulated by dietary protein degradability. In contrast, Webb & Matthews (1994) reported that the main site for AA absorption in sheep is the ileum, while some AA`s are equally efficiently absorbed from the jejunum. Very little, if any, absorption appears to take place in the duodenum and the rumen (Webb & Matthews, 1994). Therefore, it appears that the objective of protein nutrition should be to manipulate protein supply and digestion to provide the optimum AA quantity and profile in the small intestine. Sloan (1997) stated that AA nutrition has a quantitative and qualitative component that are both essential for maximum performance. Protein flow from the rumen to the intestine is the main determinator of the protein quantity available for absorption. If a large flow of a poor quality protein results in a greater supply of an essential AA in the small intestine than a smaller flow of a higher quality protien, the former would be preferable (Wallace, 1994). The protein quality or AA profile is

determined by the various sources of AA`s, e.g. microbial protein, undegradable dietary protein and endogenous protein, of which the contribution and impact to available AA`s have been discussed earlier.

The ideal protein concept as basis for evaluation of amino acid requirements

The ideal protein refers to the supply of absorbed AA`s in a proportion that gives maximum utilisation efficiency (Chen & Ørskov, 1994). If the AA profile available for absorption in the small intestine does not render maximum utilisation efficiency, the imbalances must be corrected by supplementing rumen UDP`s with high concentrations of the limiting AA`s. In this way protein utilisation can be maximised by meeting the animal`s requirement for AA`s without overfeeding them. Rolls, *et al.* (1972) suggested that the supplementation of limiting AA`s in an AA deficient diet might delay the appearance of plasma AA peaks, which generally occur 1 to 2 hours after protein ingestion, as a result of an increased removal of AA`s for protein synthesis. This proofs that balanced AA profiles are utilised more efficiently. However, AA supplementation in ruminants is complicated by the variable nature of the amount and quality of the AA supply to the small intestine, the inconsistent protein degradability and AA contents of protein supplements and the changing requirement of the animal. Kung & Rode (1996) reported that the translation of tissue-level AA requirements to dietary AA requirements in ruminants is very complex, due to the impact of rumen nitrogen metabolism on the quality and quantity of protein reaching the duodenum. Further, Chen & Ørskov, (1994) stressed that the optimum AA compostion is specific to a particular type of production. The ideal AA profile may thus vary for maintenance, tissue growth, wool growth, milk production etc. (Chen & Ørskov, 1994). Another complicating factor is that the gastrointestinal tissues metabolise more than 30 % of the absorbed AA`s on a net basis (Berthiaume *et al.,* 2001), suggesting that differences may exist between the AA profile in the duodenum and the profile reaching the portal blood circulation. In corroboration, Tagari & Bergman (1978) suggested that the gastrointestinal tract exerts a selective and preferential use of essential AA`s during absorption, which may indeed result in large differences between the duodenal and portal AA compositions.

Fuller (1996) recognised that the profile of AA`s required for body protein accretion was closely correlated to the AA composition of the WEB protein. Therefore, in terms of

growth, the essential AA composition of the WEB could serve as an ideal example of the AA`s required for body protein accretion (Fuller, 1996). Chen & Ørskov (1994) also argued that the AA requirements for tissue maintenance are possibly similar to that needed for tissue growth, since protein turnover primarily takes place in tissue. The AA profile of the WEB protein can thus serve to predict the ideal protein required in the small intestine (Fuller, 1996).

In contrast, Bergen (1979) argued that static measurements may not be representative of an animal`s AA status, since it does not consider the flux of AA`s in and out of free AA pools. The AA deposition and turnover rate may differ between various tissues, which may have a significant impact on the accurate determination of AA requirements. However, for this study the static AA status of the WEB is considered representative of the ideal protein requirements for growing lambs.

Amino acid profile

Since microbial protein may not always provide in the requirement for all the essential AA`s (Merchen & Titgemeyer, 1992) and the dietary UDP content, as well as the AA profile thereof, may differ between diets, AA deficiencies could occur quite frequently in high producing animals. Harper (1964) reported the inability of the body to compensate for even a slight deficiency of a single AA as the reason why the AA balance is so critical and organisms so sensitive to alterations in dietary AA profiles. In the case of an AA imbalance, there will be a waste of the limiting AA via catabolism and excretion, concomitant with the excretions of surplus amino acids, resulting in increased AA deficiencies and retarded growth (Rudolfo & Pearson, 1962). When one or more AA`s are deficient, the other AA`s are rendered relatively excessive in terms of the AA profile. Waldroup *et al.* (1976) showed that excess AA`s might impair feed intake and growth rate. Abebe & Morris (1990) maintained that surplus protein cannot be ignored in least-cost diet formulation, but needs to be balanced by increasing the specification for critical AA`s via specifying AA requirements as a proportion of the protein and not as a proportion of the diet. It is generally accepted that AA imbalances reduce appetite via altered plasma and tissue AA profiles (Waldroup *et al.*, 1976). The mechanism by which the altered AA profile affects the appetite regulating centres is still unknown. Harper & Rogers (1965) suggested the anabolic theory by which a surplus of AA`s stimulates

synthesis or suppresses breakdown of protein in the liver, resulting in more of the limiting AA being retained in the liver in the imbalanced than the control group. The plasma concentration of the limiting AA is thereby reduced, leading to an altered AA profile and subsequent depressed feed intake. The anabolic response to AA imbalances appears to be triggered by a homeostatic response to prevent undue losses of the limiting AA when an incomplete AA mixture is added to a diet limiting in the AA not supplemented (Waldroup et al., 1976). Lewis & D`Mello (1967) and Nesheim *et al.* (1972) reported that excess levels of some AA`s might increase the catabolism of other AA`s, resulting in a disturbed free AA profile in the plasma and tissues and a subsequent depression of growth rate and feed intake.

Amino acid balance in practice

It is clear that AA deficiencies may sporadically occur, especially in high producing animals like ewes in late gestation or early lactation and young growing lambs. Since sheep are farmed with in a wide variety of climates and environmental conditions throughout the world, which in many cases are too harsh for cattle farming because of the limited availability of good quality forages, sheep may be particularly succeptible to AA deficiencies. In such extreme conditions, where the average annual rainfall is too low to maintain quality grazing throughout the year, and frequent periodic and long term droughts occur, even more pressure is exerted on the animals to maintain a high level of production and reproduction despite the nutritional deficiencies in the grazing. Nitrogen (N) is generally viewed as the first limiting nutrient for ruminants grazing low quality forage (Kempton & Leng, 1979), resulting in a relatively inactive rumen microbial population and subsequent low fermentation of the ingested forage. Kartchner (1980) reported that responses to supplemental protein are usually observed when the CP content of the forage is less than 6 to 8 %, which is very likely in the environmental conditions described above. As a result of the limited available N to the rumen microbes the fibrous diets may be poorly digested and subsequently lead to a reduced feed intake, which may result in an energy deficiency to the animal, manifested in retarded production rates. Since energy is the main driving force of microbial fermentation and yield (Balch, 1967; Henning *et al.,* 1993), it is likely that high producing animals in harsh environmental conditions will experience AA deficiencies, primarily because of the limited available microbial protein. It is also highly unlikely that endogenous protein and the expected

small amount of dietary UDP available from low quality forages will supply all the limiting essential AA`s of high producing animals in the small intestine. Strategic supplementation procedures should therefore be implemented to correct intestinal AA imbalances via rumen undegradable AA supplementation.

Objectives

The purpose of this study is to:

- Determine the AA profile of duodenal protein, primarily derived from rumen microbes, and the impact of dietary protein level and source on the essential AA profile and flow to the duodenum.
- Determine the production efficiency, carcass yield and WEB composition of Merino and Dohne Merino lambs.
- Determine the essential AA composition of individual body components (carcass, internal offal and external offal) to establish whether any single component was representative of the AA composition of the WEB.
- Calculate the essential AA composition of the WEB from the individual components.
- Calculate the chemical scores of the essential AA`s in the duodenum of lambs receiving high RDP diets, where microbial protein is the predominant protein source, and determine their order of limitation.
- Calculate the daily supplemental essential AA needs, according to the chemical score of microbial protein.
- Identify the limiting essential AA`s in diets low in UDP by measuring N-retention when one AA is omitted, in turn, from a post-ruminal essential AA supplement formulated to complement microbial protein and simulate the WEB essential AA profile in the duodenum.

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CHAPTER 2

The effect of rumen degradable protein level and source on the duodenal essential amino acid profile of sheep[1]

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Abstract

Two trials were conducted to determine the effects of rumen degradable protein (RDP) level and source on the duodenal essential amino acid (AA) composition of Dohne Merino wethers. The animals had *ad libitum* access to wheat straw [32 g crude protein (CP)/kg DM; 742 g neutral detergent fibre (NDF)/kg DM] and water. In the first experimental treatments casein provided 0, 40, 80, 120 and 160 g supplemental RDP/d. In experiment two, urea-nitrogen replaced 0, 25, 50, 75 and 100 % of the true protein in the isonitrogenous treatments. Expressing essential AA concentration as a percentage of duodenal protein indicated that increasing RDP levels tended to decrease arginine, but significantly increased tryptophan concentrations. Histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine and total essential AA concentrations were not affected by dietary RDP level. Increasing the urea-N content of RDP supplements significantly decreased isoleucine, leucine, lysine, phenylalanine, tryptophan, valine and total essential AA concentrations. Histidine and threonine proportions also tended to decrease with higher non-protein N levels. When the essential AA profile is expressed in relation to lysine, the concentrations of histidine, leucine, phenylalanine and threonine decreased significantly. Arginine and valine showed a decreasing trend and tryptophan increased significantly as RDP levels were raised. The largest differences appeared between 0 and 40 g RDP supplementation, while the AA profiles for the rest of the treatments remained relatively constant. Substituting urea for true RDP induced a variable response in arginine and isoleucine, but did not affect any of the other AA`s. Increasing RDP intakes significantly increased the duodenal flow of every essential AA, except arginine, and showed a strong tendency to increase total essential AA flow as well. Replacing true RDP with urea significantly reduced the flow of methionine and

tryptophan and also tended to decrease lysine availability in the duodenum. Individual AA and total essential AA flow to the duodenum were also numerically decreased by a minimum of 34 % as urea-N was increased. It appears that the qualitative duodenal essential AA profile in sheep fed low-quality forages is relatively insensitive to RDP level or source. The daily essential AA flow to the duodenum seems to be compromised by urea substitution for true RDP.

Keywords: Amino acid, microbial protein, rumen degradable protein, urea, casein

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Introduction

Sheep often subsist on low quality roughages, such as crop residues and dry forages, for considerable parts of the year. Nitrogen (N) is generally considered to be the first limiting nutrient for ruminants grazing low quality forages (Kempton & Leng, 1979; Freeman *et al*., 1992; Mawuenyegah *et al*., 1997). When N requirements are met, microbial growth will be enhanced and subsequently rumen fermentation as well. This implies that the structural carbohydrates (cellulose and hemicellulose) will be more extensively fermented. The fermentation of carbohydrates provides most of the energy supply to rumen microbes and is the main driving force behind a productive rumen microbial population (Balch, 1967; Henning *et al*., 1993). The growth rate of rumen microbes is also greatly affected by the availability of ammonia, peptides and amino acids (AA`s) (Kang-Meznarich & Broderick, 1981; Argyle & Baldwin, 1989), but the effects of protein source on rumen microbial growth rate are limited when energy deficient diets are fed (Balch, 1967). When carbohydrate availability allows growth, 66 % of the non-structural carbohydrate microbial protein originates from peptides and 34 % from ammonia. When carbohydrates are lacking all peptide N is converted to ammonia (Russell *et al*., 1992). Sugar and starch degrading bacteria require ammonia and AA`s or peptides for growth, while cellulolytic bacteria utilise ammonia as the chief N source (Russell *et al*., 1992). Bryant & Robinson (1962) indicated that 82 % of rumen bacteria can grow with ammonia as the only N source, 25 % would not grow unless ammonia was present and 56 % could utilise either ammonia or AA`s. In contrast, Carro & Miller (1999) reported that both structural and non-structural carbohydrate fermenting bacteria

could utilise ammonia as well as pre-formed AA`s as an N source. It seems that ammonia is acknowledged as the primary N source for the growth of rumen microbes (Nolan, 1975; Aharoni *et al*., 1991), but that AA`s and peptides also play an important role in the N supply to rumen micro-organisms. Stimulating rumen microbial growth via urea supplementation holds considerable financial benefits in terms of the cost of the protein supplement, but may be inferior to natural protein in terms of animal performance (Helmer & Bartley, 1971).

Intestinal protein primarily consists of undegraded dietary protein, microbial protein (40 to 80 %; Owens & Bergen, 1983; Sniffen & Robinson, 1987) and endogenous protein (9 to 12 % of non-ammonia N; NRC, 2001). Therefore, the efficiency of microbial growth and the AA composition of rumen microbes are decisive to the quantitative and qualitative AA supply in the intestines and the subsequent animal performance. Firkins *et al.* (1986) reported on the ability of the microbial population to increase the ratio of essential AA`s to non-essential AA`s, and thus improve the protein quality entering the duodenum. The objective of this study was to determine: (a) whether the duodenal essential AA profiles, predominantly derived from microbial protein of sheep fed a low-protein, roughage diet supplemented with casein, are affected by rumen degradable protein (RDP) concentration (experiment 1), and (b) the effect of substituting urea for true protein (casein) on the essential AA profile in the duodenum (experiment 2).

Material and Methods

Five Dohne Merino wethers fitted with rumen and duodenal cannulas, proximally to the common bile duct, were used in a 5 x 5 Latin square to evaluate the effects of increasing RDP levels on the duodenal essential AA profile. The animals with an average live weight of 60.0 kg (± 2.87 kg) were individually housed in metabolism cages and had *ad libitum* access to wheat straw (Table 1) and water. The wheat straw was chopped into 50 mm pieces with a hammer mill and offered at 140 % of the average intake of the previous five days for each individual animal. Experimental treatments provided supplemental true RDP at 0, 40, 80, 120 and 160 g RDP/d respectively. The RDP in the form of calcium caseinate (900 g CP/kg; 100 % rumen degradable) was dissolved in 1.2 L water, divided into two equal portions and administered intraruminally at 07:00 and 19:00 just before offering the forage. To prevent possible trace and macro mineral deficiencies, a

mineral premix (260 g NaCl/kg, 160 g Ca/kg, 80 g P/kg, 120 g S/kg, 0.04 mg I/kg, 0.01 mg Co/kg, 2.5 mg Mn/kg and 2.2 mg Zn/kg) was formulated according to the NRC (1985) and the mineral content of wheat straw. This supplement was infused intraruminally with the morning supplements at 19.05 g/wether/d. The N:S ratio was manipulated to be below 10:1 and peaked at 0.35 % of DM intake. That is below the maximum tolerable level of 0.4 % for dietary sulphur from sodium sulphate (NRC, 1985).

Table 1 Chemical composition of wheat straw and calcium caseinate on a dry matter basis

Item	Wheat straw	Casein	Urea	Maize starch
	g/kg DM			
Dry matter	935	891	993	903
Ash	43	29	0	0
Crude protein	32	900	290	11
Neutral detergent fibre	742	0	0	1
Acid detergent fibre	536	0	0	0
Fat	9	2	7	5

Animals were allowed to adapt to the diet and their specific supplements for the first 14 days of each period. From day 15 to 18 samples of the wheat straw were taken at each feeding time to form one representative sample for each period. Duodenal samples (± 50 mL) were taken at 6-h intervals via the T-type duodenal cannula during days 16 to 18, and frozen immediately. Sampling time was advanced two hours each day to obtain samples on every even hour of a 24-h period. At 06:00 and 15:00 on day 19 the rumens of the sheep were completely emptied, the contents weighed and immediately replaced via the rumen cannula. The rumen volume was used to calculate daily duodenal flow. According to Owens & Goetsch (1986) passage rate was taken as 50 % of the average fluid dilution rate over all five treatments to calculate duodenal flow. Fluid dilution rate was measured by infusing 15 mL Cr-EDTA (360 mg Cr/15 mL; Uden *et al.*, 1980) into various ruminal sites at 06:00 on day 20. Twenty mL ruminal fluid for analysis of Cr concentration, was extracted at 0, 3, 6, 9, 12, and 24 h and frozen immediately.

Ruminal fluid was extracted by a specially developed device. This apparatus consisted of the casing of a 5-mL syringe, inserted into an *in situ* degradation bag with 50 µ diameter

pores. The syringe had 25 pores with a diameter of 3 mm each and the open end was blocked with a rubber stopper. A 200 mm long rubber tube was connected to the nozzle of the syringe and passed through the cork of the rumen fistula. The tube was connected externally to a small tap that could be attached to a 60 mL syringe to extract ruminal fluid. Merits for using this apparatus are that the tap is only opened during extraction and thus conditions in the rumen are kept anaerobic and constant. In addition, rumen fluid obtained in this manner is clean and free of large feed particles.

The five dietary samples were ground with a Scientec Hammer Mill (Peter Rassloff, Instruments & Services (PTY) Ltd.) to pass a 1-mm screen and combined into one composite sample. Dry matter (DM) content of the feed was determined by drying the samples for 24 hours in a convection oven at 100 °C, where after the samples were ashed at 500 °C for 5 h to determine organic matter (OM) content (AOAC, 1995). The feed samples were also analysed for acid detergent fibre (ADF; AOAC, 1995) and neutral detergent fibre (NDF; Van Soest & Wine, 1967). The duodenal samples were lyophilised, equivalent quantities from every sample pooled for each wether within each period and finely ground with a Scientific Apparatus sample mill (Arthur H. Thomas Co., Philadelphia, PA). Duodenal and dietary samples were analysed for N content with a Leco Auto Analyser (Model FP 428) and AA composition was determined with a Beckman System 7300 high performance analyser after 22 h of acid hydrolysis (6 N HCl) at 110 °C (AOAC, 1995). Chromium concentration was measured with a radial emission, inductively coupled plasma spectrometer (Liberty Series II) after ruminal fluid samples were diluted (1:2 dilution). Fluid dilution rate was determined by regressing the natural logarithm of Cr concentration on sampling time (Warner & Stacy, 1968).

Streptomyces griseus protease incubation was used to determine the protein degradability of wheat straw and casein at 48 h (Krishnamoorthy *et al.*, 1983; Broderick, 1994). Passage rate used in the calculations was taken as 50 % of the average fluid dilution rate (Owens & Goetsch, 1986) over all five treatments.

After the data were analysed for differences using ANOVA, multiple comparisons of means, using the t-test, were performed (SAS, 1994).

For the second trial, 25 Dohne Merino wethers were used in a randomised block design. Isonitrogenous treatments provided 3.30 g RDP/kg $BW^{0.75}$ for optimal feed intake and digestion (Nolte *et al.*, 2003), but respectively contained 0, 25, 50, 75 and 100 % urea-N. Further experimental and sampling procedures, chemical and statistical analyses were similar to experiment one.

Results and Discussion

The protein degradability of wheat straw after incubation of 48 hours was determined as 66 %. Due to the slow rumen turn over of fibrous diets (Del Curto *et al.*, 1990; Hannah *et al.*, 1991) and the quick fermentation of casein and urea, the protein degradability of the RDP supplements was estimated to be almost complete. Therefore, the essential AA composition of the duodenal digesta in these experiments was essentially derived from microbial protein.

Tryptophan concentration increased ($P<0.01$) and arginine tended to decrease ($P = 0.06$) with increasing RDP supplements (Table 2), suggesting that the AA composition of rumen bacteria might be slightly altered by nutritional factors. Supporting data by Clark *et al.* (1992) and Rulquin *et al.* (1998) indicated differences in bacterial AA composition due to species, separation and analysis methodology or nutritional factors such as roughage to concentrate ratios or feed intake. Supplementing true RDP to sheep consuming N deficient diets increased feed intake (Del Curto *et al.*, 1990; Matejovsky & Sanson, 1995), as well as digestible organic matter intake, microbial efficiency and daily microbial N flow to the duodenum (Nolte *et al.*, 2003). This may lead to changes in the physiological state of bacterial cells, altering their cell wall to protoplasm ratio and, thus, the AA proportions (Rodriguez *et al.*, 2000). An increased feed intake is usually also associated with higher rumen outflow rates and since protein increases somewhat slower than bacterial cell mass, an improved bacterial growth rate implies a moderate decrease in the protein content of bacteria (Rodriguez *et al.*, 2000). However, a reduced protein content in rumen bacteria does not necessarily imply a decreased supply of microbial protein to the duodenum, since the effect of an increased feed intake, as observed by Nolte (2000) and Nolte *et al.* (2003), on daily microbial N flow to the duodenum may override the lower microbial protein concentration.

Table 2 The effect of increasing rumen degradable protein levels and different rumen degradable protein:non-protein nitrogen ratios on the essential amino acid composition in the duodenal digesta of sheep fed low-quality forage diets

Amino acid	Supplemental RDP[1] (g/d)					SEM[2]	P
	0	40	80	120	160		
	Essential AA[3] (g/100 g CP[4])						
Arginine	8.02	10.70	5.52	5.90	3.92	0.81	0.06
Histidine	1.20	1.16	1.14	1.15	1.10	0.02	0.75
Isoleucine	3.73	3.76	3.69	3.65	3.57	0.06	0.88
Leucine	5.72	5.61	5.50	5.40	5.30	0.09	0.67
Lysine	5.91	6.05	5.93	5.83	5.68	0.11	0.89
Methionine	0.70	0.72	0.73	0.64	0.70	0.01	0.29
Phenylalanine	3.46	3.36	3.32	3.24	3.18	0.05	0.53
Threonine	3.70	3.76	3.62	3.51	3.42	0.07	0.51
Tryptophan	1.46	1.71	1.96	1.96	2.08	0.06	<0.01
Valine	4.69	4.62	4.50	4.45	4.36	0.07	0.64
TEAA`s[5]	38.59	41.45	35.92	35.73	33.30	1.06	0.13
	Supplemental N from urea (%)						
	0	25	50	75	100		
	Essential AA[3] (g/100 g CP[4])						
Arginine	5.54	5.71	3.79	4.63	8.57	0.63	0.11
Histidine	1.29	1.62	1.14	1.04	0.93	0.08	0.11
Isoleucine	4.02	3.54	3.64	3.33	2.97	0.12	0.03
Leucine	5.85	5.44	5.43	4.98	4.44	0.17	0.05
Lysine	6.69	6.94	6.12	5.51	4.84	0.25	0.04
Methionine	0.79	0.70	0.69	0.65	0.55	0.03	0.15
Phenylalanine	3.41	3.53	3.37	2.51	2.57	0.15	0.04
Threonine	3.85	3.61	3.60	3.38	2.98	0.11	0.09
Tryptophan	1.54	1.48	1.45	1.25	1.09	0.05	0.02
Valine	4.89	4.53	4.54	4.12	3.65	0.14	0.02
TEAA`s[5]	39.69	37.08	33.76	31.40	32.59	1.04	0.05

[1]Rumen degradable protein

[2]Standard error of mean

[3]Essential amino acids

[4]Crude protein

[5]Total essential amino acids

However, increasing supplemental RDP levels did not affect histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, valine or total essential AA concentrations in the duodenums of sheep fed a basal diet of wheat straw ($P \geq 0.29$). The reduced protein content of bacteria caused by increased microbial growth rates (Rodriguez *et al.*, 2000), therefore appears to be more applicable to the nonessential AA concentrations. Since eight of the 10 essential AA`s, as well as the total essential AA concentration were not affected by supplemental RDP level, the qualitative duodenal essential AA profile appears to be relatively constant. Leibholz (1972), Storm & Ørskov (1983) and Cecava & Parker (1993) also reported constant AA profiles for microbial protein. Despite conflicting reports in literature the results from the present study suggest a reasonably constant essential AA profile for microbial protein, irrespective of dietary RDP level.

From Table 2 it is evident that substituting non-protein nitrogen (NPN) for true protein on an isonitrogenous basis has a more pronounced effect on the essential AA concentrations in the duodenum than the true RDP level. Increasing the supplemental N from urea decreased isoleucine, leucine, lysine, phenylalanine, tryptophan, valine and total essential AA concentrations in the duodenum ($P \leq 0.05$) and tended to reduce threonine concentrations ($P \leq 0.09$). The decrease in branched-chain amino acids (BCAA`s) is supported by the reduced branched-chain volatile fatty acid (BCVFA) concentrations, reported by (Nolte, 2000), when substituting urea for true RDP, since BCAA`s serve as precursors to BCVFA`s (Russell & Sniffen, 1984). The duodenal histidine and methionine concentrations for the 100 % urea-N treatment were also 28 and 30 % lower than for the 100 % true RDP supplement. Maeng & Baldwin (1976a) also indicated that urea alone resulted in lower bacterial growth rates than a combination of urea and AA`s. Since almost all the essential AA`s, except for arginine, which showed a variable response, tended to decrease as urea-N was substituted for true RDP, these results suggest that the duodenal essential AA profile is not qualitatively affected by protein source, but essential AA availability may be quantitatively reduced when true RDP is replaced by non-protein N. Nolte (2000) reported that increasing urea-N levels in isonitrogenous RDP supplements did not affect daily microbial N flow to the duodenum. Therefore, the reduced essential AA concentrations associated with increased urea levels suggest a decreased proportion of essential AA`s:nonessential AA`s in microbial protein. This may limit animal production and be part of the reason why natural protein is generally accepted as superior to NPN in terms of animal production (Helmer & Bartley, 1971).

According to Zhang *et al.* (1986) the NPN content in the body varies with increasing body weight. Amino acid comparisons between animals of different body weights may therefore be inaccurate. To eliminate the influence of NPN and obtain a clearer picture of the AA profile, essential AA`s can be expressed relative to lysine as an ideal protein ratio (Table 3). Lysine is chosen as a reference for ideal protein for several reasons: (1) Lysine and methionine are generally considered limiting in most ruminant diets (Rulquin & Vérité, 1996), (2) Analysis of lysine content in feedstuffs is straight forward (Mack *et al*., 1999), (3) Lysine is only used for body protein accretion (Mack *et al*., 1999), (4) Lysine and methionine have frequently been studied as potential limiting AA`s under a variety of conditions, providing a large body of information (Rulquin & Vérité, 1996).

The comparison of essential AA:Lysine ratios (Table 3) shows that histidine, leucine, phenylalanine and threonine decreased as supplemental RDP increased ($P \leq 0.04$), while arginine and valine tended to decrease ($P \leq 0.08$). For most of these AA`s the largest decrease was between 0 and 40 g supplemental RDP where after the essential AA ratios showed little variation. Isoleucine and methionine were unaffected ($P \geq 0.35$) as the supplemental protein level was raised. In contrast to the other essential AA`s the tryptophan:lysine ratio increased ($P<0.01$) as RDP supplements increased, suggesting that tryptophan synthesis might be dependant on peptides and AA`s. The initial decrease in the ratios of essential AA`s to lysine between the control treatment and 40 g supplemental RDP/d probably resulted from enhanced microbial growth rates (Argyle & Baldwin, 1989; Nolte *et al.*, 2003) that lead to reduced protein concentrations in rumen microbes (Rodriguez *et al.*, 2000). Little variation in the duodenal essential AA profile occurred from 40 to 160 g RDP/d, which is indicative of a relatively constant essential AA profile of microbial protein, irrespective of dietary alterations.

When urea-N was substituted for true RDP on a N-equivalent basis, only the relative arginine ($P = 0.04$) and isoleucine ($P = 0.09$) concentrations appeared to be affected. Arginine showed a substantial increase at the 100 % urea-N supplement and isoleucine seemed to decrease at the 25 % urea-N treatment. For the other treatments these two AA`s were relatively constant. The other essential AA`s were not affected by treatment ($P \geq 0.22$), indicating that the duodenal essential AA profile, predominantly derived from

Table 3 The effect of increasing rumen degradable protein levels and different rumen degradable protein:non-protein nitrogen ratios on the duodenal essential amino acid:lysine ratio of sheep fed low-quality forage

Amino acid	Supplemental RDP[1] (g/d)					SEM[2]	P
	0	40	80	120	160		
	Essential AA[3] (g/100 g CP[4])						
Arginine	133.59	180.11	93.26	100.91	66.99	13.85	0.08
Histidine	20.34	19.24	19.29	19.67	19.31	0.14	0.04
Isoleucine	63.45	62.23	62.21	62.60	62.87	0.32	0.76
Leucine	97.11	92.89	92.70	92.62	93.24	0.53	0.02
Lysine	100	100	100	100	100		
Methionine	12.08	12.01	12.40	10.95	12.37	0.25	0.35
Phenylalanine	58.83	55.76	56.01	55.58	55.94	0.35	<0.01
Threonine	62.75	62.16	61.12	60.20	60.28	0.30	<0.01
Tryptophan	24.98	28.48	32.98	33.57	36.66	0.96	<0.01
Valine	79.77	76.59	75.79	76.20	76.72	0.49	0.07
	Supplemental N from urea (%)						
	0	25	50	75	100		
	Essential AA[3] (g/100 g CP[4])						
Arginine	86.36	90.16	61.86	81.78	181.27	14.52	0.04
Histidine	19.19	22.27	18.73	18.91	19.19	0.52	0.23
Isoleucine	60.12	52.85	59.55	60.54	61.30	1.04	0.09
Leucine	87.61	81.32	88.90	90.57	91.80	1.57	0.32
Lysine	100	100	100	100	100		
Methionine	11.72	10.53	11.31	11.86	11.39	0.36	0.85
Phenylalanine	51.30	50.83	56.24	45.41	53.06	1.79	0.43
Threonine	57.58	54.43	58.97	61.42	61.58	1.10	0.26
Tryptophan	23.21	21.98	23.61	22.78	22.54	0.47	0.88
Valine	73.21	67.49	74.48	74.82	75.53	1.14	0.22

[1]Rumen degradable protein

[2]Standard error of mean

[3]Essential amino acids

[4]Crude protein,

microbial protein, is relatively constant, irrespective of protein source. Purser & Buechler (1966) also reported little variation in the AA composition of 22 strains of pure cultures of rumen organisms, specifically selected for their differing substrate utilisation

characteristics. Supporting evidence by Bergen *et al.* (1968) and Martin *et al.* (1996) also indicated that the AA composition of rumen micro-organisms was independent of dietary changes. In contrast, Clark *et al.* (1992) reported variation in the AA profile of rumen bacteria.

The daily flow of arginine to the duodenum was not affected by supplemental RDP level ($P = 0.14$; Table 4). The flow of the other essential AA`s to the duodenum increased as RDP level was raised ($P \leq 0.04$) and total essential AA flow tended to increase ($P = 0.07$). It appears that the essential AA profile of rumen microbes is independent of dietary influences (MacRae & Reeds, 1980; John, 1984), but the quantitative essential AA supply from microbial protein to the duodenum is improved by RDP supplementation when sheep are fed low quality forages. The ability of true RDP supplementation to enhance the daily flow of essential AA`s to the duodenums of sheep consuming low quality roughage diets is indicative of a more active rumen microbial population and subsequent increased microbial efficiency and higher microbial N flow to the duodenum (Nolte *et al.*, 2003). In addition, protein supplementation to ruminants offered low quality forages (< 60 to 80 g CP (CP)/kg; Kartchner, 1980) increased feed intake (DelCurto *et al.*, 1990; Matejovsky & Sanson, 1995), forage organic matter intake and digestible organic matter intake (Nolte *et al.*, 2003). The increased flow of essential AA`s to the duodenum therefore appears to result from the combined effects of an increased forage intake that might have caused a higher rumen outflow rate, which, together with the improved microbial efficiency resulted in an increased flow of microbial N to the small intestine. In contrast, Nolte *et al.* (2003) reported no response of RDP supplementation on fluid dilution rate, which implies that the increased duodenal flow of microbial N in RDP supplemented sheep consuming low-quality, N deficient roughage diets probably originated from an improved microbial efficiency, stimulated by the supply of peptides, AA`s and ammonia to the rumen microbes.

Sniffen & Robinson (1987) stated that a number of factors, such as the amount and degradability of dietary protein, could impact on rumen microbial growth rate. Although higher microbial growth rates tend to decrease essential AA concentrations (Rodriguez *et al.*, 2000) the increased numbers and activity of the rumen microbial population improves the quantitative essential AA availability at the duodenum. This is in agreement with

Cecava & Parker (1993) who also found that protein source has a larger effect on the quantity of AA`s presented to the small intestine than on the AA profile.

Table 4 The effect of increasing rumen degradable protein levels and different rumen degradable protein:non-protein nitrogen ratios on daily essential amino acid flow to the duodenum of sheep fed low-quality forage

Amino acid	Supplemental RDP[1] (g/d)					SEM[2]	P
	0	40	80	120	160		
	Essential AA[3] (g/d)						
Arginine	3.59	7.36	4.73	5.79	2.82	0.62	0.14
Histidine	0.47	0.77	1.00	1.09	0.95	0.07	0.04
Isoleucine	1.45	2.50	3.25	3.47	3.08	0.24	0.03
Leucine	2.20	3.74	4.85	5.13	4.57	0.35	0.04
Lysine	2.32	4.02	5.22	5.55	4.92	0.38	0.04
Methionine	0.26	0.48	0.64	0.60	0.61	0.04	0.03
Phenylalanine	1.35	2.24	2.91	3.07	2.75	0.21	0.04
Threonine	1.44	2.50	3.20	3.33	2.96	0.23	0.04
Tryptophan	0.53	0.88	1.20	1.25	1.10	0.08	0.03
Valine	1.82	3.08	3.94	4.21	3.75	0.28	0.04
TEAA`s[4]	15.43	27.57	30.94	33.50	27.49	2.18	0.07
	Supplemental N from urea (%)						
	0	**25**	**50**	**75**	**100**		
	Essential AA[3] (g/d)						
Arginine	2.67	3.14	1.98	2.05	3.28	0.28	0.47
Histidine	0.62	0.89	0.61	0.48	0.38	0.06	0.13
Isoleucine	1.95	1.86	1.96	1.54	1.22	0.11	0.14
Leucine	2.84	2.85	2.92	2.30	1.83	0.16	0.14
Lysine	3.23	3.75	3.30	2.54	1.99	0.23	0.10
Methionine	0.38	0.36	0.36	0.30	0.22	0.02	0.05
Phenylalanine	1.67	1.90	1.84	1.19	1.06	0.13	0.11
Threonine	1.86	1.91	1.94	1.56	1.22	0.11	0.17
Tryptophan	0.74	0.78	0.76	0.58	0.45	0.04	0.05
Valine	2.37	2.40	2.45	1.90	1.50	0.14	0.13
TEAA`s[4]	19.86	19.86	18.12	14.45	13.16	1.17	0.22

[1]Rumen degradable protein

[2]Standard error of mean

[3]Essential amino acids

[4]Total essential amino acids

Substituting urea for true protein on an isonitrogenous basis decreased the flow of methionine and tryptophan to the duodenum (P = 0.05) and also tended to decrease the daily duodenal flow of lysine (P = 0.10). The duodenal flow of isoleucine, leucine, phenylalanine, threonine and valine was also numerically decreased between 34 and 39 % when 100 % urea-N was substituted for true RDP (P ≥ 0.11). Subsequently, the total essential AA flow to the duodenum was also numerically reduced as supplemental NPN contributions increased. Hume (1970) and Maeng *et al.* (1976) reported increased microbial protein yields when true protein N replaced urea-N in purified diets or *in vitro* studies. This decrease in essential AA flows to the duodenum as urea-N increased might be attributed to a reduced supply of AA`s, peptides and BCVFA`s from rumen proteolysis. These fermentation products are required for or stimulatory of microbial growth (Bryant & Robinson, 1962). When urea is substituted for true protein more ammonia and less AA`s, peptides and BCVFA`s are formed in the rumen, which may decrease microbial growth (Hume, 1970; Maeng & Baldwin, 1976b), resulting in reduced essential AA supplies to the duodenum.

Generally, urea is inferior to true protein sources in terms of N retention for various kinds of ruminants (Helmer & Bartley, 1971). In contrast, Cruz Soto *et al.* (1994) stated that the benefits of pre-formed AA`s on microbial growth cannot be realised when growth rate is limited by energy availability, as on high cellulose diets. Carro & Miller (1999) contradicted these findings by observing increased microbial efficiencies and microbial N flows to the duodenum in the presence of pre-formed AA`s on high fibre diets, corroborating that N is the first limiting nutrient on low-quality, roughage diets. A combination of true RDP and NPN seemed to most efficiently alleviate the N deficiency in low-quality roughage diets (Nolte, 2000).

Conclusion

The results from the present study suggest that the qualitative essential AA profile in the duodenum of sheep fed low-quality forages is to a large extent independent of dietary protein level or source. From a quantitative perspective it appears that true RDP has a higher stimulatory effect on microbial essential AA flow to the duodenum than NPN. In corroboration, various studies conducted with animals on widely differing diets have also

indicated remarkable similarities in the essential AA profiles of microbial protein. This implies that the postruminal essential AA supply from microbial protein can be predicted for animals utilising a wide range of different diets. The available essential AA`s can thus be compared to the essential AA requirements for specific production stages and imbalances accordingly corrected via undegradable protein supplements.

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CHAPTER 3

Production efficiency, empty body composition and carcass yield of Merino and Dohne Merino lambs[1]

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Abstract

Morphological data and carcass yields of 12 Merino and 13 Dohne Merino male lambs were determined at two body condition scores and live weights. Five lambs of each breed were slaughtered at a body condition score of 2.4. The remaining lambs were offered a feedlot diet until a body condition score of 2.8 and slaughtered. Merino lambs had larger external offals and smaller internal offals than Dohne Merino lambs. Individual feed and water consumption, digestibility, efficiency and growth of these lambs were also investigated. Generally, no large differences in the efficiency of feed utilisation occurred. Nitrogen retention for the Merino lambs was higher than for the Dohne Merinos. The latter excreted considerably more urinary N than the Merino lambs. This study indicates that Merino lambs utilised protein more efficiently than Dohne Merino lambs, which might imply that Merinos would perform better in harsh conditions with low-quality, N-deficient forages. It also suggests that Merino lambs may have lower essential amino acid requirements at a corresponding growth rate. The morphological differences between these breeds suggest that their whole empty body (WEB) amino acid (AA) compositions may differ, and subsequently their AA requirements for growth as well.

Key words: growth, energy retention, nitrogen retention, water, body composition

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Introduction

Sheep and goats comprise a significant part of the wealth of African society (Peacock, 1996) and are often the predominant source of meat and milk. These flocks are kept in a variety of ecological areas with different climates and vegetation. At times, these animals survive in environments too harsh for cattle (El Khidir *et al*., 1998), effectively utilising large parts of hostile agricultural land. In such hostile environments, where feed and water are limited, it is essential that animals utilise the available nutrients very efficiently. Those animals that have the highest utilisation efficiency of ingested nutrients will probably have higher voluntary feed intakes and improved performance in terms of production and reproduction. The different abilities of various sheep breeds to ingest and utilise low-quality forages may contribute to the reasons for some farming areas being dominated by certain breeds.

In any livestock production system the key to success is to raise as many offspring as possible. Quite often lambs are raised on poor quality forage, resulting in higher mortality rates, slower growth rates and reduced reconception of the ewes in the following mating season. In such conditions lambs may have to be weaned earlier and finished in feedlots. The high performance conditions in feedlots require a high nutrient utilisation efficiency, which directly affects the conversion of feed to product and profitability criteria such as average daily gain, feed conversion ratio and dressing percentage. Since the primary objective in feedlots is mutton production, the body and carcass compositions of different breeds also have to be investigated. McDonald *et al*. (1995) reported that the composition of empty body weight gain of growing animals could vary significantly between species, breed or sex. Clearly the distribution of weight in the empty body impacts on profitability, since some cuts, like the loin and buttock, are of higher value. The specific organs and/or body parts where animals put on most of their weight during feedlot finishing will thus directly affect their profitability.

Therefore, the efficiency of nutrient utilisation and the whole empty body (WEB) and carcass compostions of Merino and Dohne Meriono lambs were evaluated in this study. The information obtained in this study may also be usefull in interpreting the amino acid (AA) requirements of the two breeds. The purpose of this study was to measure the efficiency of nutrient utilisation and the WEB and carcass composition of Merino and Dohne Merino lambs during feedlot finishing.

Material and Methods

Experiment One

Six wethers, fitted with rumen cannulas, were used to determine the *in situ* dry matter and crude protein (CP) degradability (Weakly *et al.,* 1983) of a pelleted feedlot diet (Table 1). The lambs were fed at 07:00 and 19:00 and had ad libitum access to the diet and water. With each feeding a small dietary sample was collected to ensure a representative sample for proximate analysis. Approximately 5 g (DM basis)of the diet, ground to pass through a 2 mm screen, were placed into dacron polyester bags (179 x 90 mm) with a pore size of 60 µm. According to Weakly *et al.* (1983) the N content of dacron bags is <0.5 % and therefore negligible in degradability calculations. The bags were prepared in duplicate, tightly closed with drawstrings and attached to a clip on the inside of the cannula stopper with 250 mm lenghts of nylon lines. The bags were kept in thorough suspension with 80 g stainless steel weights and were incubated in the rumens for 0, 1, 2, 3, 5, 6, 12, 24, 36 and 48 hours (Mehrez & Ørskov, 1977). After removal from the rumen all the bags, including the controls (0 h), were rinsed in a washing machine for 45 minutes.

The particulate flow rate of the diet was determined with the same animals used for the degradability study. Wheat straw was treated with Cr_2O_3 (Udén *et al.,* 1980) and tested for dry matter degradation by placing a dacron bag containing 5 g of the chromium-mordanted wheat straw for 24 h into the rumens of the animals. The average DM degradation of 2.69 % illustrated significant resistance against rumen degradation and the Cr-treated wheat straw was approved for determination of the particulate flow rate of the diet. Sixty grams of Cr-mordanted wheat straw was hydrated and added to the rumen contents of each animal via the rumen cannula. Faecal samples were collected at 6, 9, 12, 18, 36, 48, 60, 84 and 108 hours (Löest, 1995) after adding the treated straw to the rumens and frozen until analysis was performed.

The diet sample was ground to pass a 1 mm screen and analysed for dry matter (DM), CP, gross energy, crude fibre, fat, ash (AOAC, 2003), acid detergent fibre (ADF; Van Soest, 1963), neutral detergent fibre (NDF; Van Soest and Wine, 1967), Ca and P

Table 1 Physical and chemical composition of the feedlot diet

Item	Contents (%)
Physical composition[1]	
Maize meal	56.00
Gluten 60	1.92
Gluten 20	1.92
Soya oilcake	5.33
Lucerne	8.50
Wheat straw	14.17
Molasses	9.36
Limestone	1.04
Salt	0.68
Ammonium chloride	1.00
Vit/Min premix[2]	0.08
Chemical composition[3]	
Dry matter	92.00
Metabolisable energy [4] (MJ/kg)	12.10
Crude protein	14.40
Undegradable protein[5]	5.10
Fat	4.40
Crude fibre	13.00
Acid detergent fibre (ADF)	11.17
Neutral detergent fibre (NDF)	21.97
Calcium	0.69
Phosphorus	0.28
Effective rumen degradability	
Dry matter	78.21
Crude protein	74.67
Kd[6] (dry matter)	0.045
Kd[6] (crude protein)	0.042
Kr[7]	0.021

[1] On an as is basis
[2] A standard mineral (macro and micro) and vitamin supplement formulated by Saldanha Feedmills according to the NRC (1985)
[3] On a dry matter basis
[4] Metabolisable energy (ME) = Digestible energy (DE) x 0.82 (NRC 1985)
[5] Based on laboratory values determined by Saldanha feedmills
[6] Kd = Rate constant for disappearance of dry matter and protein from the rumen incubated polyester bag
[7] Kr = Rate constant for passage of undegraded particulate dry matter or protein from the rumen.

(Watson, 1994). Nitrogen content was analysed according to the combustion method (Method 990.03, AOAC, 2003) on a FP-428 Nitrogen and Protein Determinator (Leco Corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085-2396). Gross energy was determined by adiabatic oxygen bomb calorimetry (CP500 calorimeter), crude fibre, ADF and NDF analyses were performed with an Ancom Fibre Analyser (220) and fat was analysis was performed with a Tecator Soxtec System (HT 1043).

Rumen degradability of DM and protein were determined according to the procedures described by Miller *et al.* (1980) and Stern and Satter (1984).

Effective DM and protein degradability was estimated according to the following formula (Miller *et al.,* 1980):

D = a + (100-a)(Kd) / (Kr + Kd)

where:
- D = Effective degradability
- a = Proportion of dry matter or protein disappearance at t = 0
- Kd = Rate constant for disappearance of dry matter or protein from the polyester bags. Kd is thus equal to the slope of the natural logarithm of the residue in the dacron bags regressed against time.
- Kr = Rate constant for passage of undegraded dry matter or protein from the rumen. The average chromium concentration in the faeces of consecutive sampling times was used to determine Kr (Hartnell and Satter, 1979)

The faeces samples for determination of particulate flow rate were dried for 96 h at 50 ºC, air-equilibrated and ground to pass a 1 mm screen. These samples were then prepared for Cr analysis as described by Williams *et al.* (1962) and analysed with an atomic absorption spectrophotometer (Varian Spectra>AA 300/400). Rumen particulate flow rate was calculated according to Hartnell and Satter (1979).

Experiment Two

Twelve male Merino and 13 male Dohne Merino lambs were randomly allocated into individual pens (1m x 2m) in a ventilated, enclosed barn with a slatted floor and continuous lighting. One Merino lamb died of an unknown cause shortly before the

experiment commenced. Five lambs of each breed were selected according to their body condition score (±2.4) and slaughtered. Body condition score was used to indicate slaughter, in order to ensure comparable physiological ages between the breeds. The average live weight of the slaughtered Merino and Dohne lambs were 28.7 (±1.2 kg) and 29.7 (±2.2 kg) respectively. After being exsanguinated the lambs were weighed again and blood volume calculated by difference. The head, skin and wool, feet, lungs and trachea, heart, heart fat, diaphragm, liver, spleen, stomachs with contents, intestines with contents, intestinal fat, kidneys, kidney fat, reproductive organs and warm carcass of each lamb were weighed. The stomachs and intestines were emptied and washed with water to obtain the weight of the contents and empty stomachs and intestines. The empty body mass (live weight excluding stomach and intestinal contents) was calculated by summing all the organs and body components. The individual organs and body components were expressed as a percentage of the empty body weight to disallow the influence of rumen fill effects on the proportional body composition. The blood, lungs and trachea, heart, heart fat, diaphragm, liver, spleen, kidneys, kidney fat, gastro-intestinal tract and abdominal fat were summed to calculate the internal offal. The external offal consisted of the skin, wool, head and feet.

The carcasses were stored overnight at 4 ˚C and weighed the next morning to determine the moisture loss. The carcasses were then divided into two replicas by cutting along the median. Both sides were divided into commercial cuts according to the South African Meat Board`s chart for retail mutton cuts, namely neck, breast and shank, thick rib, rib, loin, flanck and buttock (Hoffmann, 2000) and each cut weighed separately.

The remaining seven Merino and eight Dohne Merino lambs were adapted for 14 days to the same pelleted feedlot diet as in experiment one (Table 1), where after the lambs had *ad libitum* access to the feed and water. The lambs were fed twice daily at 07:00 and 19:00 and weighed weekly to determine the average daily gain for each lamb. Orts and refused water were removed with every morning feeding to calculate voluntary feed and water intakes, as well as the feed conversion ratio (kg feed / kg live weight gain) and water efficiency (kg water / kg live weight gain) for each lamb. The lambs received 8 L of clean, fresh water with every morning feeding. The average water evaporation during the experimental period was determined and voluntary intakes corrected accordingly.

During the last week of the experiment faeces and urine were collected to determine nutrient digestibilities and N retention. Faeces were collected twice daily at 07:00 and 19:00 and urine with the morning feedings. To prevent volatilisation of ammonia from the urine 20 mL of urine preservative (80 g potassium dichromate and 20 g mercuric chloride dissolved in 1 L distilled water) was added each morning to the urine collection jugs. Daily sub-samples of 10 % from the faeces and urine of each lamb were composited and frozen until chemical analyses. Methane gas production was calculated as 8 % of the gross energy intake (McDonald *et al.,* 1995). Nitrogen excretion was corrected for metabolic faecal nitrogen (MFN) and endogenous urinary nitrogen (EUN) and calculated according to McDonald *et al.* (1988):

MFN = 5 g N/kg dry matter intake

EUN = 0.18 g N/kg $BW^{0.75}$/d

N retention (g N/kg $BW^{0.75}$/day) = $[N_{intake}–(N_{faeces} – MFN) – (N_{urine} – EUN)]/BW^{0.75}$/days

When the lambs reached the required condition (body score of ±2.8) they were electrically stunned, slaughtered and processed in a similar manner than the first lambs. The average final body weights of the Merino and Dohne Merino lambs for the second slaughter were 43.6 (±0.8 kg) and 42.4 (±1.4 kg) respectively.

The faeces from the digestibility and N balance collections was dried at 50 ºC for 96 h, air-equilibrated, ground through a 1 mm screen and analysed for DM, CP, gross energy, fat and ash (AOAC, 2003). Acid detergent fibre and NDF were analysed according to the procedures described by Van Soest (1963) and Van Soest and Wine (1967). The urine and faeces samples were also analysed for N and gross energy content (AOAC, 2003). All nitrogen analyses were performed according to the combustion method (Method 990.03, AOAC, 2003) on a FP-428 Nitrogen and Protein Determinator (Leco Corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085-2396) and gross energy was determined by adiabatic oxygen bomb calorimetry (CP500 calorimeter).

Analysis of variance was performed on the data, according to the GLM procedure of SAS (2000) with main effects for breed and body condition score where applicable.

Results and Discussion

The growth parameters of Merino and Dohne Merino lambs are presented in Table 2. The initial body weight of the Dohne Merino lambs was higher than for the Merinos ($P=0.03$), which explains the differences in body weight gain and cumalative feed intake between the two breeds ($P<0.01$), since the Merino lambs were fed for a longer period. However, daily feed intake parameters for the two breeds were similar ($P\geq0.20$). Although average daily gain (ADG) and feed conversion ratio (FCR) did not vary significantly ($P\geq0.14$), the ADG of the Merinos was 7 % higher and the FCR 14 % more efficient than for the Dohne Merino lambs. Since Merinos are generally considered better wool producers but poorer mutton producers than Dohne Merinos, these results were somewhat surprising. Malik *et al.* (1996) found that breed does influence daily feed intake, but not ADG or FCR. Age, however, has a pronounced effect on daily feed intake, ADG and FCR (Malik *et al.,* 1996). Age was not expected to influence results in the present study, since the lambs were within 3 weeks of age from each other. Both Merino and Dohne Merino lambs are considered inferior to South African Mutton Merino lambs in terms of growth potential and feed conversion efficiency. However, the ADG and FCR of these two breeds correspond well with that of South African Mutton Merino lambs (281 g/d and 5.56 kg feed/kg body weight gain) receiving a similar type of diet at an equivalent live weight (Sheridan *et al.,* 2003). Although the diet in the present study was essentially similar to that used by Sheridan *et al.* (2003), the relatively high maize content of the diet in the present study (Table 1) could be expected to lead to an increased ruminal proportion of propionate and decreased acetate (Bergman, 1990), due to the high starch levels. This increase in glucogenic precursors reduces the need for gluconeogenesis from glucogenic AA`s, which increase growth rate and feed conversion efficiency via an improved protein accretion rate (MacRae & Lobley, 1986).

The higher cumalative water intake (Table 2) of the Merino lambs ($P=0.03$) is attributed to a longer trial period than the Dohne Merinos, following their lower initial body weight. In contrast to their daily feed intake, the Dohne Merino lambs had higher daily water intakes than the Merino lambs ($P<0.01$). The daily water intakes of the Merinos and Dohne Merinos were 11.5 % and 13.4 % of their live body weights respectively, which correspond well with normal water intakes that vary between 10 and 20 % of live body weight (Adegbola & Obioha, 1984). Various factors, such as the environment (e.g.

temperature), production status and breed (Quick & Dehority, 1986) and sex (Rathore, 1987) affect voluntary water intake. Since dry matter and water intakes are linearly related to each other (MacFarlane & Howard, 1972; Silanikove, 1987) and the former was similar for the two breeds in this study, the Dohne Merino appears to have a higher water requirement than the Merino. This is substantiated by a 9 % poorer water efficiency (L water/kg body weight gain) for the Dohne Merino lambs. In contrast, the numerically higher water consumption (L/kg feed intake) of the Merino lambs is explained by their slightly lower daily feed intakes and marginally better FCR. The water consumption for both breeds (Merino = 3.00; Dohne Merino = 2.84) correspond well with the 2.56 and 3.05 L/kg feed intake for Dorpers and South African Mutton Merinos respectively, reported by Schoeman & Visser (1995). It appears that the Merino consumes more water relative to dry matter intake, less water relative to live body weight and converts ingested water more efficiently into product.

Table 2 Feed and water efficiency parameters (LS Mean) of Merino and Dohne Merino lambs receiving a feedlot diet

Parameter	Merino	Dohne Merino	SEM	P
Initial body weight (kg)	28.71	32.31	0.87	0.03
Final body weight (kg)	43.64	42.38	0.44	0.16
Body weight gain (kg)	14.93	10.06	0.81	<0.01
Cumalative feed intake (kg)	72.56	54.61	2.83	<0.01
Daily feed intake (kg)	1.44	1.50	0.02	0.20
Daily feed intake (g/kg $BW^{0.75}$/d)	97.75	99.65	1.82	0.62
ADG (g)	297.69	277.75	13.45	0.48
FCR[1] (kg feed/kg body weight gain)	4.87	5.64	0.26	0.14
Cumalative water intake (kg)	216.12	154.13	9.20	<0.01
Daily water intake (L)	4.08	4.97	0.17	<0.01
Daily water intake (L/kg $BW^{0.75}$/d)	0.28	0.33	0.01	<0.01
Water consumption (L/kg feed intake)	3.00	2.84	0.10	0.43
Water efficiency (L/kg body weigth gain)	14.57	15.93	0.77	0.40

[1]FCR = Feed conversion ratio

Water turnover is related to metabolic rate (Fairall & Klein, 1984; Silanikove, 1989) and the higher water requirement of the Dohne Merino lambs can thus be explained by

evolution and the adaptation strategies of the two breeds (Ferreira *et al.*, 2002). Dohne Merinos were developed in, and still predominantly inhabits many of the higher rainfall grazing areas in South Africa. Merinos are kept in a greater variety of evironments, of which arid regions form a significant part. The lower daily water intake of 0.17 L/kg $BW^{0.75}$ by Sudan desert lambs (Mousa & Elkalifa, 1992) in comparison to 0.28 and 0.33 L/kg $BW^{0.75}$ for Merino and Dohne Merino lambs respectively, accentuates the impact of evolution and adaptation mentioned by Ferreira *et al.* (2002). This suggests that the Merino is more suited for arid farming conditions than the Dohne Merino, since production under extensive conditions requires the sheep to cover great distances each day in search of food and water.

The morphological data of Merino and Dohne Merino lambs slaughtered at two different ages and body condition scores are presented in Table 3. The live weight between the breeds at corresponding body condition scores did not differ, but the older Merinos had higher empty body weights than the corresponding Dohne Merino lambs (38.05 *vs.* 36.06 kg). The only variable in the calculation of empty body weight is the stomach and intestinal digesta. Therefore, the numerically lower (12.2 %) proportional stomach and intestinal digesta of the Merino lambs might have been responsible for the difference in empty body weight. Another contributing factor might have been that the Merino lambs had a 20.8 % higher proportional external offal and only 9.4 % lower proportional internal offal than the Dohne Merino lambs, despite the similarity in the warm and cold carcass weights of the two breeds. The larger external offal of the Merino lambs resulted from their relatively heavier heads (7.43 *vs.* 6.00) and skin/wool (16.30 *vs.* 13.23). Converting the data presented by Cronjé & Weites (1990) to proportional weight of different body components to empty body mass shows that the lambs in the present study had slightly lower proportional carcass weights, remarkably smaller external offals and larger internal offals than the South African Mutton Merino lambs used in their study.

Neither breed nor body condition influenced the moisture loss of Merino and Dohne Merino lamb carcasses (Table 3). Dressout, calculated as the percentage carcass weight (excluding kidneys and kidney fat) relative to live weight, was similar for both breeds at an equivalent body condition. The dressing percentages of the heavier lambs (43.8 and 44.6 %) correspond very well with the reported 44.9 % for lambs of similar weight reported by Al-Sabbagh *et al.* (1996). Despite similar proportional carcass weights

relative to the WEB, for both breeds at both conditions, the older Dohne Merino lambs had a higher dressout than the younger lambs of both breeds. This can be attributed to the lower proportional stomach and intestinal digesta of the older Dohne Merino lambs, resulting in a relatively larger empty body than for the younger lambs. Subsequently, the magnitude of the increase in the carcass as a percentage of live weight instead of WEB weight is lower for the older lambs in comparison to the younger ones. For the same reason carcass weight as a percentage of empty body weight was not influenced by breed or body condition. Cold carcass weight as a percentage of empty body weight was lower than reported for Mutton Merinos and Boer Goats (Sheridan *et al.* 2003), probably resulting from heavier external offal components of the Merino and Dohne Merino, since both breeds are good wool producers.

The younger Dohne Merino lambs had higher proportional blood volumes than the Merino lambs at both ages. The older Dohne Merinos also had a 30.5 % higher blood volume than the highest blood content of the Merinos. Since blood contain high AA concentrations, this may have a remarkable effect on the WEB essential AA profiles of the two breeds. The hearts of the older lambs contributed significantly less to the empty body weight than for the younger lambs, indicating a higher growth priority for the essential organs during the early growth stages. The younger lambs did not have any fat accumulation around their hearts, suggesting that the available energy was efficiently utilised for growth of the skeleton and essential organs. Reasons for the larger lungs and trachea of the Merino lambs at a body condition score of 2.8 are unclear. The proportional weights of the diaphragm and spleen did not differ between breed or condition. The kidneys of the Merino lambs at a body condition of 2.4 were proportionally larger than for any of the other lambs. The empty bodies of the older Merino and Dohne Merino lambs contained more perirenal fat (15.6 and 14.2 g/kg cold carcass weight respectively), than the younger Dohne Merino lambs (3.6 g/kg cold carcass weight). The Merino lambs seem to start depositing fat at an earlier age than the Dohne Merino lambs, since their perirenal fat content at a body condition score of 2.4 was already 10 g/kg dressed cold carcass in comparison to 3.6 g/kg for the Dohne Merino lambs. The proportional mesentery fat content of the younger lambs (12.1 and 15.5 g/kg cold carcass weight for the Merino and Dohne Merino respectively) was substantially

Table 3 Body components (LS Means) of Merino and Dohne Merino lambs at 30 and 40 kg live weight

CarcassComponent	BCS[1]=2.4		BCS=2.8		SEM
	Merino	Dohne	Merino	Dohne	
Live weight (kg)	28.70[b]	29.72[b]	43.64[a]	42.38[a]	1.41
Empty body weight[2] (kg)	23.14[c]	24.02[c]	38.05[a]	36.06[b]	1.38
Warm carcass[3] (kg)	11.82[b]	12.30[b]	19.11[a]	18.91[a]	0.72
Cold carcass[4] (kg)	11.52[b]	11.96[b]	18.53[a]	18.49[a]	0.70
Moisture loss (% of warm carcass)	2.54[a]	2.79[a]	3.06[a]	2.24[a]	0.13
Dressout (%)[5]	41.21[b]	41.29[b]	43.81[ab]	44.62[a]	0.46
Dressout (%)[6]	40.14[b]	40.16[b]	42.47[ab]	43.62[a]	0.46
Proportional distribution					
Empty body	100	100	100	100	
Blood	4.29[b]	6.53[a]	4.32[b]	5.64[ab]	0.27
Heart	0.60[a]	0.62[a]	0.52[b]	0.53[b]	0.01
Heart fat	0[b]	0[b]	0.27[a]	0.32[a]	0.03
Liver	2.83[a]	2.23[b]	2.37[b]	2.77[a]	0.07
Lungs and trachea	1.85[b]	1.97[b]	2.41[a]	1.92[b]	0.07
Diaphragm	0.51[a]	0.66[a]	0.74[a]	0.48[a]	0.04
Spleen	0.25[a]	0.26[a]	0.22[a]	0.22[a]	0.02
Kidneys	0.49[a]	0.41[b]	0.41[b]	0.38[b]	0.01
Perirenal/Kidney fat	0.50[ab]	0.18[b]	0.76[a]	0.73[a]	0.07
Stomach and Intestines: Full	30.19[ab]	32.86[a]	21.72[c]	25.49[bc]	1.14
Stomach and Intestines: Empty	9.23[c]	11.70[a]	8.31[c]	10.22[b]	0.29
Stomach and intestinal digesta	20.97[a]	21.16[a]	13.40[b]	15.26[b]	0.96
Mesentery fat	0.60[c]	0.77[c]	1.84[a]	1.37[b]	0.12
Skin/Wool	14.50[ab]	12.30[b]	16.30[a]	13.23[b]	0.43
Head	8.56[a]	7.19[b]	7.43[b]	6.00[c]	0.21
Feet	3.72[a]	3.60[a]	2.94[b]	2.85[b]	0.09
Reproductive organs	0.80[a]	0.65[a]	0.92[a]	0.88[a]	0.05
Internal offal	22.13[b]	25.82[a]	23.08[b]	25.48[a]	0.41
External offal	26.78[a]	23.10[b]	26.68[a]	22.09[b]	0.54
Warm carcass	51.09[a]	51.09[a]	50.24[a]	52.44[a]	0.38
Cold carcass	49.80[a]	49.67[a]	48.70[a]	51.26[a]	0.41

[1]Body condition score

[2]Consists of the whole body excluding stomach and intestinal digesta

[3]Warm carcass weight immediately after slaughter

[4]Cold carcass weight after hanging overnight

[5]Warm carcass relative to live weight

[6]Cold carcass relative to live weight

[a,b,c]Row means with common superscripts do not differ (P>0.05)

lower than for the older lambs (37.8 and 26.7 g/kg cold carcass weight for the Merinos and Dohne Merinos respectively). A higher lipogenic activity in the internal fat depots of the younger lambs might have been responsible for the lower kidney, heart and mesenteric fat of the lighter lambs (Ingle *et al.,* 1972a; Ingle *et al.,* 1972b). The differences in morphological data between Merino and Dohne Merino lambs are consistent with the results of Riley *et al.* (1989) that the proportional weights of specific offal items differ between breeds.

The lower proportional weights of the Merino lambs` stomachs and intestines at both body conditions are responsible for their lower internal offal. When the internal offal is included in the meat producing potential of total usable product (Riley *et al.* 1989), the average for the Merino and Dohne Merino lambs were 73.3 and 77.4 % respectively. The heavier heads at both ages and also the larger skin/wool of the Merino lambs at the higher body condition score are responsible for the larger proportional external offal of the Merinos. Wool has a significant effect on the total financial yield of woolled sheep. When skin/wool is also incorporated into the usable product, the average for Merino and Dohne Merino lambs were 88.7 and 90.2 % respectively.

There were no differences in the weights of the neck and breast/shank of the groups (Table 4). The necks of both breeds in the present study formed a significantly larger part of the carcass (6.39 to 6.82 %) than the Merino lambs (2.7 %) reported by Scales *et al.* (2000). This difference could possibly be attributed to gender differences, since only male lambs were slaughtered in the present study, while Scales *et al.* (2000) used lambs of both sexes. The lambs in the present study were also three to four months younger than those used by Scales *et al.* (2000). The thick rib of the older Dohne Merino lambs was lighter and the flank heavier than for all the other lambs. The ribs of the older lambs were heavier than for the younger lambs. The older Merino lambs had heavier loins than the other lambs. The buttocks of the younger Dohne Merino lambs were 8 % heavier than for the corresponding Merino lambs, whose did not differ from the older lambs of either breed. Surprisingly, the higher priced cuts (loin and leg of lamb) were not heavier for the Dohne Merino. Merinos are considered higher wool producers than Dohne Merinos and are therefore expected to have lower carcass yields. This was not the case in the present study.

Table 4 Carcass yields (LS Means) of Merino and Dohne Merino lambs at different ages and body conditions

Carcass Component	BCS[1]=2.4		BCS=2.8		SEM
	Merino	Dohne Merino	Merino	Dohne Merino	
Cold Carcass (kg)	11.52	11.96	18.53	18.49	0.70
Proportional composition (%)					
Neck	6.82[a]	6.47[a]	6.59[a]	6.39[a]	0.15
Breast/Shank	16.36[a]	14.86[a]	16.13[a]	15.04[a]	0.23
Thick Rib	17.72[a]	16.63[ab]	15.98[ab]	14.18[c]	0.44
Rib	7.20[b]	6.99[b]	8.39[a]	9.22[a]	0.23
Loin	7.49[b]	6.98[b]	9.33[a]	8.08[b]	0.24
Flank	10.24[b]	11.19[b]	10.58[b]	12.52[a]	0.23
Leg of Lamb	33.45[b]	36.06[a]	33.16[b]	34.25[b]	0.32

[1]Body condition score

[a,b,c]Row means with common superscripts do not differ (P>0.05)

No differences in the apparent digestibility of any nutrient were observed (Table 5). This indicates that Merino and Dohne Merino lambs digested the available diet with similar efficiency and suggests that the composition of the microbial population of these two breeds was relatively similar when fed the same diet. This would allow the prediction of microbial AA supply to the small intestine for different breeds on similar kinds of diets, which has remarkable consequences in practical feed formulation. However, the efficiency of absorption and metabolisation of nutrients were not necessarily similar for the two breeds. Therefore, the energy (Table 6) and nitrogen retentions (Table 7) for both breeds were also determined.

During the period when energy and N retention were determined the Dohne Merino lambs had a higher daily dry matter and energy intake than the Merino lambs (P<0.01; Table 6). Consequently, more easily fermentable carbohydrates were available for fermentation in the rumens of the Dohne Merino lambs, resulting in a higher methane gas production (P<0.01). Higher feed intakes usually lead to increased passage rates and reduced rumen

Table 5 Apparent nutrient digestibilities (LS Means) of a feedlot diet by Merino and Dohne Merino lambs

Item (%)	Merino	Dohne Merino	SEM	P
Dry matter	72.42	72.20	1.02	0.92
Crude protein	68.33	68.21	1.23	0.96
Energy	73.08	72.62	0.98	0.83
Acid detergen fibre	31.93	27.68	2.70	0.45
Neutral detergent fibre	30.65	30.45	2.65	0.97
Fat	88.63	87.40	0.60	0.32
Ash	52.96	50.87	2.04	0.63

retention times, resulting in decreased digestibilities. Faecal energy excretion for the Dohne Merino lambs was only numerically higher than for the Merino lambs. This suggests that energy digestibility was not significantly affected by the higher daily feed intakes of the Dohne Merino lambs, as confirmed by the results in Table 5. The high degradability and expected rapid fermentability of the diet (Table 1) probably explains the lack of a response in nutrient digestibility. Rapidly fermentable diets require a shorter rumen retention time for maximum catabolism. Therefore, the digestibility of the feedlot

Table 6 Energy metabolism (LS Means) of Merino and Dohne Merino lambs fed a feedlot diet

Item	Merino	Dohne Merino	SEM	P
Dry matter intake (g/day)	1244.70	1428.76	37.20	<0.01
Gross energy intake (MJ/day)	22.55	25.89	0.67	<0.01
Faecal energy (MJ/day)	6.13	7.10	0.34	0.16
Urinary energy (MJ/day)	0.66	0.86	0.04	0.01
Methane gas production (MJ/day)	1.80	2.07	0.05	<0.01
Total energy excreted (MJ/day)	8.59	10.04	0.40	0.07
Faecal energy (% of energy intake)	26.97	27.38	0.99	0.85
Urinary energy (% of energy intake)	2.92	3.35	0.16	0.18
Total energy excreted (% of energy intake)	37.88	38.73	0.93	0.67
Energy retention (MJ/kg $BW^{0.75}$)	0.96	1.00	0.02	0.38
Energy retention (% of energy intake)	62.12	62.27	0.93	0.67
Dietary ME content (MJ/kg)	11.25	11.10	0.17	0.67

diet by the Dohne Merino lambs was not lower than for the Merino lambs, despite the higher feed intake of the Dohne Merinos. Interestingly, urinary energy excretion was higher for the Dohne Merino lambs (P=0.01), suggesting that the absorbed nutrients were metabolised less efficiently than by the Merino lambs. Urinary energy excretion as a percentage of energy intake was 15 % higher for the Dohne Merino lambs. McDonald *et al*. (1995) also reported that higher feed intakes reduce the metabolisability of feed energy. However, the efficiency of energy retention and the calculated ME content of the diet did not differ between these breeds (P≥0.38).

The higher daily nitrogen intake of the Dohne Merino lambs (Table 7) is consistent with their dry matter intake (P<0.01; Table 3). Faecal nitrogen excretion as a percentage of N intake was similar for the two breeds (P=0.95), suggesting a relatively similar nitrogen digestion, as seen in Table 5. The Dohne Merino lambs excreted much more urinary and total nitrogen than the Merinos (P<0.01), which might indicate an excessive ammonia production within the gut (Giraldez *et al.*, 1997; Cole, 1999). Scott (1975) also reported that ammonium is a primary carrier of hydrogen in the urine of ruminants fed high concentrate diets and might be required for renal and systemic buffering (Galyean, 1996). Since mammals have no endogenous urease, excretion is the only means of removing urea from the body (Nolan, 1993). Therefore, the higher urinary nitrogen levels of the Dohne Merino lambs indicate an inferior nitrogen utilisation and/or recirculation (Ørskov, 1992; Nolan, 1993) in comparison to the Merino lambs, resulting in a lower nitrogen retention (P<0.01). Although various factors such as rumen pH, ammonia concentration, type of energy substrate (Lindberg, 1987) and the balance between rumen ammonia concentration and fermentable carbohydrate supply (Cronjé, 1992) impact on microbial population dynamics, the similarity in faecal N excretion and digestibility parameters between the two breeds, as well as the higher urinary N excretion of the Dohne Merino lambs indicate that the difference in N retention between the breeds was not caused by differentiation in rumen microbial processes, but variation in metabolisation efficiency parameters after absorption. The Merino seemed to have a more efficient post-absorptive N utilisation.

Table 7 Nitrogen retention (LS Means) of Merino and Dohne Merino lambs fed a feedlot diet

Item	Merino	Dohne Merino	SEM	P
Dry matter intake (kg/day)	1244.70	1428.76	37.16	<0.01
N intake (g/day)	26.91	30.89	0.80	<0.01
Faecal N (g/day)	8.57	9.82	0.45	0.17
Urinary N (g/day)	7.16	14.52	1.10	<0.01
Total N excreted (g/day)	15.73	24.34	1.30	<0.01
Faecal N (% of N intake)	31.85	31.67	1.24	0.95
Urinary N (% of N intake)	26.53	47.24	3.26	<0.01
Total N excreted (% of N intake)	58.38	78.92	3.17	<0.01
N retention (g/kg $BW^{0.75}$/day)	1.38	1.04	0.06	<0.01
N retention (% of N intake)	74.56	53.53	3.20	<0.01

N = nitrogen

The essential AA requirements of these two breeds may also differ considerably, which might have caused the diet used in this study to provide a duodenal essential AA profile that was closer related to the specific AA requirements of the Merino than the Dohne Merino lambs. Titgemeyer & Merchen (1990) stated that differences in the genetic growth capacity of animals from the same species to deposit lean tissue would alter estimates of AA requirements. Despite methionine, lysine, histidine and arginine being reported as first limiting for WEB growth in lambs (Storm & Ørskov, 1984), casein supplements to lambs receiving a freshly cut ryegrass/white clover (60:30) pasture improved N balance to a much larger extent than a mixture of these AA`s in similar proportions as found in casein (Fraser *et al.,* 1990). The efficiency of utilisation of the AA infusion was approximately 1.0 and the casein 0.68, indicating that the AA infusion alleviated an AA deficiency to some degree, but not enough to cause significant responses in nitrogen balance. Therefore, some other AA`s might also have been limiting (Fraser *et al.,* 1990), suggesting that a dissimilarity in AA requirements between Merino and Dohne Merino lambs contributed to the variation in nitrogen retention.

Conclusion

These results indicate that Merino lambs utilised ingested N more efficiently than Dohne Merino lambs. Energy metabolism, apparent digestibilities, feed and water utilisation

parameters and carcass yields were relatively similar for the two breeds. The differences in the relative weights of the internal and external offals of the breeds may imply different WEB AA compositions for these breeds. These data may help clarify possible differences in the WEB AA composition of growing Merino and Dohne Merino lambs.

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CHAPTER 4

The essential amino acid requirements for growing Merino and Dohne Merino lambs[1]

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Abstract

Ten male Merino and Dohne Merino lambs of equal body condition score had ad libitum access to a feedlot diet [14.4 % crude protein (CP); 12.1 MJ metabolisable energy/kg] and fresh water. Five lambs of each breed were slaughtered at a body condition score of 2.4 and a corresponding live weight of 28.7 kg and 29.7 kg for the Merino and Dohne Merino lambs, respectively. The five remaining lambs of each breed received the feedlot diet until a body condition score of 2.8, at respective live weights of 43.2 kg and 41.9 kg for the Merino and Dohne Merino lambs, when they were also slaughtered. The digesta was removed from the stomachs and intestines and the whole empty body (WEB) separated into the carcass, internal offal and external offal. The weight, protein concentration and amino acid (AA) composition of each component were recorded and the WEB essential AA composition subsequently calculated. Remarkable differences occurred in weight distribution, as well as the protein contents of these body components between the breeds. The AA compositions of the carcasses of the two breeds were relatively similar, but prominent differences were detected in the AA contents of the other body components. The chemical scores and essential AA indexes of the duodenal protein from lambs receiving high rumen degradable (RDP) diets were determined, to assess the extent to which intestinal protein supplied in the essential AA requirements of growing lambs when microbial protein was the primary source of intestinal AA`s.. Based on the essential AA profiles of the WEB`s of the lambs and of duodenal protein from high (RDP) diets, the daily essential AA deficiencies in the intestinal protein supply were also calculated. These results illustrated that the duodenal protein supply was unable to qauntatively provide in the essential AA requirements of growing lambs, when microbial protein was the major source of AA`s.

Keywords: Amino acid, microbial protein, whole empty body, growth, lambs

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Introduction

The maximum genetic capacity for protein deposition of an animal depends on age, live weight, physiological state, the ratio of energy and protein supply and the amino acid (AA) composition of the available protein (Tamminga & Verstegen, 1996). Cole & Van Lunen (1994) also suggested that the efficiency of protein utilisation is not only dependent on the amount of protein, but the balance of absorbed AA`s is the most important single factor affecting the efficiency of protein utilisation for production of meat and other products. In corroboration, Lapierre *et al*. (2000) reported increased utilisation of absorbed AA`s for protein synthesis when the profile of the limiting AA`s was improved. The AA profile available for absorption clearly plays a significant role in the efficiency of protein utilisation. A deficiency of a single AA will inhibit the responses to those in adequate supply (Cole & Van Lunen, 1994), while AA`s in excess to the limiting AA`s will be degraded and excreted, with a subsequent waste of energy and nitrogen (Cromwell, 1996). Therefore, it is essential that the ideal AA profile must be supplied in the small intestine to achieve optimum performance. Chen & Ørskov (1994) and Boisen *et al.* (2000) stated that the ideal protein represents the perfect ratio among individual AA`s that allow maximum utilisation efficiency and optimal performance.

Various methods have been proposed to determine the ideal essential AA requirements of animals. The ARC (1981) proposed that lean meat was representative of the ideal AA balance. Hussein *et al.* (1991) and Fuller (1996) suggested that the AA profile of the whole empty body (WEB) could serve as an indication of the required essential AA profile. In ruminants, the translation of tissue-level AA requirements to dietary AA requirements is very complex, due to the impact of rumen nitrogen metabolism on the quality and quantity of protein reaching the duodenum (Kung & Rode, 1996). Merchen & Titgemeyer (1992) reported that both the profile and the levels of essential AA`s supplied to the small intestine are rate-limiting factors for tissue synthesis, and are therefore of critical importance for maximum performance (Sloan, 1997). Accurate estimates must therefore be made of both the quality and quantity of microbial essential

AA`s, dietary undegradable essential AA`s and endogenous essential AA`s supplied to the small intestine. Since the ruminant receives 40 to 80 % of its daily AA requirements from microbial protein (Sniffen & Robinson, 1987), the degree to which microbial protein supplies in the qualitative and quantitative requirement of each essential AA, has significant implications on animal performance and supplementation strategies.

The first objective of this study was to establish possible differences in weight, protein and essential AA distribution in the whole empty bodies of Merino and Dohne Merino male lambs, in order to assess whether the essential AA composition of any body component was representative of the WEB essential AA profile of growing lambs. Since information on the WEB AA composition of growing lambs seems to be limited (MacRae *et al*., 1993; Ferreira, *et al.*, 1999a; Jurgens, 2002), the WEB essential AA compositions of finishing male Merino and Dohne Merino lambs were also determined. Finally, the order of limiting AA`s in duodenal protein, mainly derived from rumen microbes, and the extent to which this protein supply provides in the essential AA requirements for growing lambs were also established, since microbial protein is one of the major sources of AA`s to ruminants.

Material and Methods

Ten male Merino and Dohne Merino lambs each were randomly allocated into individual pens (1 m x 2 m) in a ventilated, enclosed barn with a slatted floor and continuous lighting. The lambs were adapted to a feedlot diet (Table 1) for 14 days, where after five lambs of each breed were selected according to their body condition score (±2.4), electrically stunned and slaughtered. Body condition score was used to indicate slaughter, in order to ensure comparable physiological ages between the breeds. The average live weight of the slaughtered Merino and Dohne lambs were 28.7 (±1.2 kg) and 29.7 (±2.2 kg) respectively. The five remaining lambs of each breed had *ad libitum* access to the feedlot diet that was offered twice daily at 07:00 and 19:00. These lambs were slaughtered at a body condition score of 2.8 with average final live weights of 43.2 (±0.8 kg) and 41.9 (±1.4 kg) for the Merino and Dohne Merino lambs, respectively. The lambs at both slaughters were weighed immediately before slaughter, electrically stunned, exsanguinated and weighed again. Blood volume was calculated by difference. A 500 mL blood sample was collected during exsanguination and frozen until later AA analysis.

Table 1 Physical and chemical composition of the feedlot diet

Item	Contents (%)
Physical composition[1]	
Maize meal	56.00
Gluten 60	1.92
Gluten 20	1.92
Soya oilcake	5.33
Lucerne	8.50
Wheat straw	14.17
Molasses	9.36
Limestone	1.04
Salt	0.68
Ammonium chloride	1.00
Vit/Min premix[2]	0.08
Chemical composition[3]	
Dry matter	92.00
Metabolisable energy [4] (MJ/kg)	12.10
Crude protein	14.40
Undegradable protein[5]	5.10
Fat	4.40
Crude fibre	13.00
Acid detergent fibre (ADF)	11.17
Neutral detergent fibre (NDF)	21.97
Calcium	0.69
Phosphorus	0.28
Effective rumen degradability	
Dry matter	78.21
Crude protein	74.67
Kd[6] (dry matter)	0.045
Kd[6] (crude protein)	0.042
Kr[7]	0.021

[1] On an as is basis
[2] A standard mineral (macro and micro) and vitamin supplement formulated by Saldanha Feedmills according to the NRC (1985)
[3] On a dry matter basis
[4] Metabolisable energy (ME) = Digestible energy (DE) x 0.82 (NRC, 1985)
[5] Based on laboratory values determined by Saldanha feedmills
[6] Kd = Rate constant for disappearance of dry matter and protein from the rumen incubated polyester bag
[7] Kr = Rate constant for passage of undegraded particulate dry matter or protein from the rumen.

The gastro-intestinal tract of each lamb was emptied and washed to remove all digesta for the determination of the WEB (live mass excluding the stomach and intestinal contents) weight. Each organ and body part was weighed and the WEB divided into the carcass (warm carcass weight), internal offal (metabolic organs, empty gastro intestinal tract and blood) and external offal (head, feet, skin and wool). The carcasses were hung overnight at 4 °C and divided into two equal halves by cutting longitudinally through the median. The right halves, the internal and external offals were frozen at -20 ºC and ground separately through a carcass mill. A 1 kg representative sample was collected from each component and fat extracted from a sub-sample with di-methyl ether (AOAC, 2003). The fat free components were frozen again at -10 ºC, lyophilised, and ground to pass a 1 mm screen. The blood samples were also lyophilised and proportionally added to the internal offal. These three components were analysed for Kjeldahl nitrogen (N) according to the AOAC (2003). Essential AA analysis on each component was done with a Beckman System 7300 AA analyser after 22 h of acid hydrolysis (6 *N* HCl) at 110 °C (AOAC, 2003). The essential AA composition of the WEB was calculated by proportionally summing the essential AA compositions of the carcass, internal and external offal for each lamb.

Analysis of variance was performed on the data, according to the GLM procedures of SAS (2000). The main effects were breed, carcass, internal offal and external offal. Weight and protein contribution were the response variables.

Results and Discussion

The contribution of the carcass to the total WEB weight did not differ between Merino and Dohne Merino lambs (P=0.25; Table 1). However, the Merino lambs had a smaller relative internal offal and a larger relative external offal weight than the Dohne Merino lambs (22.5 vs. 25.4 %; 26.71 vs 22.83; P<0.01). The larger external offal of Merino lambs might indicate a higher wool contribution. It is likely that the AA compositions of these components will differ from each other, which suggests that a single body component may not be representative of the WEB AA composition.

Table 2 The whole empty body compositon and proportional protein contribution (LS Means ± SEM) of the carcass, internal offal and external offal to the whole empty body of Merino (35.95 ±7.25 kg) and Dohne Merino (35.8 ±6.1 kg) lambs (n=10)

Item	Merino	Dohne Merino	SEM	P
	Whole Empty Body Composition (%)			
Whole Empty Body	100	100		
Carcass	50.75	51.79	0.44	0.25
Internal Offal	22.54	25.38	0.46	<0.01
External Offal	26.71	22.83	0.57	<0.01
	Protein Contribution (%)			
Whole Empty Body	100	100		
Carcass	46.89	49.29	0.58	0.03
Internal Offal	24.27	26.93	0.59	0.01
External Offal	28.84	23.79	0.68	<0.01

The relative protein content of the Merino carcasses was lower (P=0.03) than for the Dohne Merino lambs (Table 1), suggesting that the Dohne Merino carcasses might have higher protein concentrations, since the relative weights of the carcasses from the two breeds did not differ. These values correspond well with results of MacRae *et al.* (1993), but were considerably lower than for goats (Ferreira; unpublished data). In contrast, Early *et al.* (2001) reported from a comparison between Omani and crossbred Suffolk sheep that empty body protein deposition was not affected by genotype. The Dohne Merino lambs had higher protein values in the internal offal, but lower in the external offal than the Merino lambs (P≤0.01), which are consistent with the suggestion that the Merino lambs might have a larger wool contribution in the external offal. Although the energy requirement for fibre growth is generally not a limiting factor, since it represents less than 10 % of the basal metabolic rate in sheep (Black & Reis, 1979; Black, 1987), it may have a significant impact on sulphur AA requirements. MacRae *et al.* (1993) reported that wool accounts for more than 80 % of total empty body cysteine retention. Since methionine can be converted to cysteine via transsulfuration (Campbell *et al.*, 1997) Merino lambs may have a higher methionine requirement than Dohne Merino lambs. In Angora goats receiving diets with 10 % CP rumen protected, biologically available methionine supplements enhanced growth rate and nitrogen retention (Souri *et al.*, 1997). The relative partitioning of dietary nitrogen to mohair nitrogen remained at approximately

40 %, suggesting that methionine were limiting in both fleece and non-fleece tissues and that relative partitioning was not affected by altered supply (Souri *et al.*, 1997).

It is evident that the carcass AA composition of Merino and Dohne Merino lambs is remarkably similar, differing only in histidine and lysine concentrations (Table 3). Internal offal between the two breeds displayed more differences and was only similar for arginine, lysine, threonine and valine. The external offal displayed differences in the concentrations of arginine, leucine, phenylalanine and threonine. In contrast to Smith (1980), who maintained that the body AA compositions of different animal species are relatively similar, these results suggest that the WEB AA composition may even differ between breeds of the same species.

For the Merino lambs the phenylalanine and valine concentrations did not differ between the carcass and external offal, and were lower than in the internal offal (Table 3). Leucine concentrations in the carcass and external offal and threonine levels in the internal and external offal of Dohne Merino lambs were similar. All the other AA concentrations between the components within each breed displayed remarkable differences. The lower methionine concentrations in the external offal of both breeds support the lower incidence of sulphur-containing AA`s in connective than contractile tissue reported by Lawrie (1991). According to MacRae *et al.* (1993) the gastro-intestinal tract contains relatively high concentrations of methionine, lysine and threonine. This is supported by the highest lysine and threonine concentrations occurring in the internal offals of both breeds in the present study. However, the carcass methionine concentrations were significantly higher than for the internal offals in both breeds. These results imply that different body components have characteristic AA profiles, confirming earlier suggestions to this effect by Bikker *et al.* (1994) and Fuller (1996). This variation in the AA profiles of different body components emphasises the limitations of using a single body component as a predictor of the ideal protein requirement for WEB growth and opposes the proposal by the ARC (1981) that the AA composition of lean meat could serve as an example of the AA balance of the ideal absorbed protein. In corroboration, Boisen *et al.* (2000) reported that endogenous protein and hair, which contributes to maintenance AA requirements, are considerably higher in

Table 3 The essential amino acid concentrations (g essential AA[1]/100 g crude protein) of the carcass, internal offal and external offal of Merino (35.95 ±7.25 kg) and Dohne Merino (35.8 ±6.1 kg) lambs (LS Means ± SEM) (n=10)

EAA	Merino Carcass	Merino Internal Offal	Merino External Offal	Dohne Merino Carcass	Dohne Merino Internal Offal	Dohne Merino External Offal	SEM
Arginine	6.91^{c}	5.09^{d}	9.24^{a}	6.92^{c}	5.33^{d}	8.77^{b}	0.21
Histidine	2.19^{d}	5.02^{a}	1.19^{e}	2.42^{c}	4.53^{b}	1.14^{e}	0.20
Isoleucine	3.86^{a}	2.04^{d}	3.07^{b}	4.04^{a}	2.27^{c}	3.00^{b}	0.10
Leucine	6.90^{d}	11.25^{a}	7.96^{c}	7.21^{d}	10.79^{b}	7.22^{d}	0.23
Lysine	7.14^{c}	9.09^{a}	4.01^{d}	7.62^{b}	8.78^{a}	3.73^{d}	0.28
Methionine	1.98^{a}	1.40^{c}	0.74^{d}	2.06^{a}	1.57^{b}	0.74^{d}	0.07
Phenylalanine	3.54^{cd}	5.84^{a}	3.66^{c}	3.63^{c}	5.59^{b}	3.40^{d}	0.13
Threonine	3.76^{b}	4.56^{a}	3.36^{c}	3.85^{b}	4.58^{a}	4.55^{a}	0.07
Tryptophan	1.04^{c}	1.67^{a}	0.75^{d}	0.95^{c}	1.33^{b}	0.77^{d}	0.05
Valine	4.68^{bc}	7.82^{a}	5.04^{b}	4.32^{c}	7.63^{a}	4.84^{b}	0.19

[1]Essential amino acid

^{abcd} Row means with common superscripts do not differ (P>0.05)

sulphur AA`s and threonine than muscle protein. Therefore, predicting AA requirements from muscle protein alone, will underestimate the requirement for sulphur AA`s. Although the carcass, of which the essential AA composition is remarkably similar for the two breeds, is the main site for protein deposition, it contributes less than 50 % of the total WEB protein (Table 2). The AA profiles of the remainder of the WEB differ from that of the carcass, as well as between breeds. Therefore, the WEB will present a more accurate estimate of the AA requirements for growth, but might differ between breeds. In addition, Löest *et al.* (1997) and Ferreira *et al.* (1999a) argued that because the carcass was responsible for only approximately 57 % of the live weight gain and 47.8 % of the incremental nitrogen deposition in growing South African Mutton Merino lambs, it might not be representative of the essential AA requirements for WEB growth in lambs.

Fuller (1996) recognised that the profile of AA`s required for body protein accretion was closely correlated to the AA composition of the WEB protein. Therefore, in terms of growth, the essential AA composition of the WEB could serve as an ideal example of the

AA`s required for body protein accretion (Fuller, 1996). Hussein *et al.* (1991) also defined the AA composition of whole body protein as the ideal AA profile that will allow maximum utilisation of dietary protein. Chen & Ørskov (1994) argued that the AA requirements for tissue maintenance are possibly similar to that needed for tissue growth, since protein turnover primarily takes place in tissue. The AA profile of

Table 4 The whole empty body essential amino acid concentration (g essential AA[1]/100 g crude protein) of male Merino (35.95 ±7.25 kg) and Dohne Merino (35.8 ±6.1 kg) lambs (LS Means ± SEM) (n=10)

EAA	Merino	Dohne Merino	SEM	P
Arginine	7.12	6.93	0.05	0.03
Histidine	2.56	2.67	0.02	<0.01
Isoleucine	3.24	3.35	0.02	<0.01
Leucine	8.16	8.13	0.05	0.67
Lysine	6.74	7.04	0.07	<0.01
Methionine	1.52	1.64	0.02	<0.01
Phenylalanine	4.09	4.08	0.02	0.87
Threonine	3.83	4.20	0.05	<0.01
Tryptophan	1.11	1.01	0.01	<0.01
Valine	5.48	5.29	0.07	0.04

[1]Essential amino acid

the WEB protein can thus serve to predict the ideal protein required in the small intestine (Fuller, 1996). While such a concept has probably been best developed in pigs, it is equally applicable to other species (Cole & Van Lunen, 1994). In contrast, Bergen (1979) argued that static measurements might not be representative of an animal`s AA status, since it does not consider the flux of AA`s in and out of free AA pools.

The present study contradicts the findings of Smith (1980) who stated that the body AA composition of different animal species is remarkably similar, since even breeds seem to differ (Table 4). Except for leucine and phenylalanine, the level of every essential AA in the WEB of Merino and Dohne Merino lambs differed ($P \leq 0.05$; Table 4).

According to Zhang *et al.* (1986) the non-protein nitrogen content in the body varies with increasing body weight. To eliminate the influence of non-protein nitrogen and obtain a clearer picture of the AA profile, essential AA`s can be expressed relative to lysine as an ideal protein ratio (Table 5). Lysine is chosen as a reference for ideal protein for several reasons: (1) Lysine and methionine are generally considered limiting in most ruminant diets (Rulquin & Vérité, 1996), (2) Analysis of lysine content in feedstuffs is straight forward (Mack *et al*., 1999), (3) Lysine is only used for body protein accretion (Mack *et al*., 1999), (4) Lysine and methionine have frequently been studied as potential limiting AA`s under a variety of conditions, providing a large body of information (Rulquin & Vérité, 1996).

Table 5 The whole empty body essential amino acid concentration (%) of male Merino (35.95 ±7.25 kg) and Dohne Merino (35.8 ±6.1 kg) lambs relative to lysine (LS Means ± SEM) (n=10)

EAA	Merino	Dohne Merino	SEM	P
Arginine	105.73	98.60	4.55	<0.01
Histidine	38.05	38.05	1.07	0.99
Isoleucine	48.07	47.72	1.39	0.37
Leucine	121.14	115.59	3.34	<0.01
Lysine	100	100		
Methionine	22.57	23.30	0.87	<0.01
Phenylalanine	60.68	58.05	1.72	<0.01
Threonine	56.90	59.67	1.77	<0.01
Tryptophan	16.40	14.34	1.26	<0.01
Valine	81.40	75.09	4.42	<0.01

The WEB essential AA profiles of Merino and Dohne Merino lambs display substantial differences, since only histidine and isoleucine did not differ (P≥0.37; Table 5). The higher relative methionine requirement of the Dohne Merino lambs was unexpected, since it is generally accepted that Merino`s produce more wool. Since wool contains high levels of cysteine (MacRae *et al.,* 1993), which can be supplied by methionine via transsulfuration (Campbell *et al.*, 1997), high wool yields are associated with high methionine requirements. However, Nolte & Ferreira (2004a) indicated that Dohne Merino lambs have higher internal offal weights than Merino`s and internal offal contains

high methionine concentrations (MacRae *et al*., 1993). This might explain the higher methionine requirement observed for the Dohne Merino lambs.

From Tables 4 and 5 it is clear that breeds can vary extensively in their WEB essential AA profiles. The differences in the weight contribution (Table 2) and AA concentrations of various body components (Table 3) between breeds support the view of Titgemeyer & Merchen (1990) that differences in the genetic capacity of animals to deposit lean tissue would alter estimates of AA requirements.

Table 6 The whole empty body essential amino acid profiles (%) of various sheep breeds relative to lysine

EAA	Merino	Dohne Merino	SA Mutton Merino[1]	Dorper[2]
Arginine	106	99	119	107
Histidine	38	38	69	36
Isoleucine	48	48	48	50
Leucine	121	116	131	109
Lysine	100	100	100	100
Methionine	23	23	55	24
Phenylalanine	61	58	80	59
Threonine	57	60	72	57
Tryptophan	16	14	-[3]	11
Valine	81	75	80	73

[1]Data form Ferreira *et al*. (1999a)

[2]Data from Jurgens (2002)

[3]Not determined

Table 6 clearly illustrates that although the concentrations of some individual AA`s in the WEB of various sheep breeds are remarkably similar, some large differences also exist. This entails that maximum growth rates could only be achieved if breed specific supplements are formulated on intestinal AA concentrations. Even then the differences in the flux of AA`s in and out of various tissues and AA pools might still result in under estimates of AA requirements.

The chemical scores (Table 7), that can be used to determine the limiting amino acids for WEB growth, indicate that the isoleucine and tryptophan concentrations in the duodenum were in excess of the growth requirements for both Merino and Dohne Merino lambs. The other essential AA`s, except threonine which also met the requirement of the Merino lambs, were supplied in inadequate concentrations to support optimal growth. The duodenal AA supply, primarily derived from microbial protein, was first-limiting in histidine, followed by methionine, leucine, arginine and phenylalanine for both breeds. Ferreira *et al.* (1999b) determined the order of the most limiting AA`s for growing South African Mutton Merino lambs as histidine, methionine, threonine and arginine. Storm & Ørskov (1984) reported methionine, lysine, histidine and arginine as the first through fourth-limiting AA`s in microbial protein for the growth of Suffolk x (Finnish Landrace x Dorset Horn) lambs. In growing Dorper lambs fed a high rumen degradable protein (RDP) diet, Jurgens (2002) determined arginine and histidine as the first and second limiting AA`s. The chemical scores indicated that the other essential AA`s were over supplied in the duodenums of the Dorper lambs.

Since histidine and arginine were limiting in four and methionine in three of these studies, these AA`s should be considered limiting to growing lambs receiving high RDP diets. However, histidine requirements are often over estimated because histidine is found as non-protein dipeptides, carnosine and serine in large endogenous reservoirs (Zinn *et al.*, 2000). Since arginine is a semi-essential AA that can be synthesised from glutamine, its requirement is also regularly over predicted (Boisen *et al.*, 2000). If histidine and arginine are sufficiently supplied by microbial protein, methionine, leucine and phenylalanine appear to be the first three limiting AA`s for growing Merino and Dohne Merino lambs. Given the high order of limitation of lysine for the growth of lambs reported by Owens *et al.* (1973) and Storm & Ørskov (1984), the high chemical scores of lysine in the duodenal AA supply of both breeds in the present study were unexpected. In support, Ferreira *et al.* (1999b) also did not find lysine limiting for growing South African Mutton Merino lambs.

Table 7 The chemical scores[1] and essential amino acid index[2] (LS Means ± SEM) of predominantly microbial protein for growing male Merino (35.95 ±7.25 kg) and Dohne Merino (35.8 ±6.1 kg) lambs

EAA	Merino	Dohne Merino	SEM	P
Arginine	77.84	80.00	0.57	0.06
Histidine	50.21	48.11	0.43	0.01
Isoleucine	124.17	119.92	0.76	<0.01
Leucine	71.65	71.98	0.43	0.72
Lysine	99.33	95.24	0.94	0.03
Methionine	52.16	48.43	0.56	<0.01
Phenylalanine	83.44	83.59	0.50	0.88
Threonine	100.46	91.82	1.25	<0.01
Tryptophan	139.99	153.35	1.97	<0.01
Valine	89.26	92.91	1.12	0.11
EAA Index	82.20	81.13	0.52	0.16

[1]The proportion of an individual amino acid in duodenal protein, mainly from microbial protein, relative to whole empty body protein

[2]The proportion of the total essential amino acids in duodenal protein, mainly from microbial protein, relative to whole empty body protein

Clearly, microbial protein, which was the major source of AA`s, is unable to supply all the essential AA`s in the required quantities (Merchen & Titgemeyer, 1992). Consequently, some AA`s may be in excess while others are deficient, causing an imbalanced AA supply. McDonald *et al*. (1995) reported that the synthesis of some AA`s, while others undergo deamination, reduces the efficiency of protein synthesis, which emphasises the importance of providing a balanced AA profile in the small intestine. Deficient AA`s may cause obvious constraints on animal performance, like retarded body and wool growth or reduced milk production, with obvious detrimental effects on over all production rate. Surplus AA`s can also restrict animal performance, since it will be de-aminated to supply ammonia, which must be detoxified by ureagenesis or glutamine synthesis with a consequently increased maintenance energy requirement of the animal. According to Milano & Lobley (2001) any overflow of ammonia that escapes periportal ureagenesis stimulates perivenous glutamine synthesis. Branched-chain amino acids are degraded to restore glutamate pools that are depleted during glutamine synthesis induced by hyperammonaemia (Leweling *et al.*, 1996). Requirements for isoleucine,

leucine, and valine may thus increase on high protien diets. Reynolds (1992) and Parker *et al.* (1995) corroborated that ammonia detoxification leads to elevated catabolism of AA`s, since additional AAN is required for ureagenesis.

The essential AA index of duodenal protein displayed an overall AA deficiency for both breeds (Table 7), when microbial protein was the major essential AA supplier. Animal performance might be even more limited than indicated by the essential AA index, due to the measure of constraint by the first limiting AA (Chen & Ørskov, 1994). Post-ruminal supplementation of the limiting AA`s is therefore required for optimal animal performance.

From Table 8 it is evident that the essential AA supply was unable to support a growth rate of 250 g/d in Merino and Dohne Merino lambs when fed high RDP diets. The intestinal protein supply to the Merino lambs was deficient in all the essential AA`s, while for the Dohne Merino lambs only the tryptophan requirement was met, and even exceeded by 8 %. Histidine showed the largest deficit of 64.7 % and 66.4 % for Merino and Dohne Merino lambs, respectively. Assuming the duodenal AA profile is representative of microbial protein, an additional 217 g and 231 g microbial protein is required daily by the Merino and Dohne Merino lambs, respectively, to correct their histidine deficiencies and achieve a growth rate of 250 g/d. This implies an additional requirement of almost twice the daily microbial protein production of 118 g, calculated as 13 % of the total digestible nutrient (TDN) intake (NRC, 1996) for a 35 kg lamb that consumes 4 % of its body mass, on an as-is basis, of a typical feedlot diet (720 g TDN/kg). Almost three times more microbial protein therefore needs to be synthesised to supply in the histidine requirements of Merino and Dohne Merino lambs growing at 250 g/d. In support of the trends observed in the chemical scores (Table 7) methionine displayed the second largest deficit of 62.5 % for the Merino lambs and 66.2 % for the Dohne Merinos. This was followed by a deficiency in leucine of approximately 50 %, arginine 45 % and phenylalanine 41 % for both breeds. To correct these deficiencies much more microbial protein than the daily synthesis of 118 g is required. Even for phenylalanine, that was the fifth limiting AA according to the chemical scores, an additional 83 g microbial protein is needed, which entails a microbial protein synthesis rate of approximately 1.7 times the average daily synthesis.

Because of the limitations in the AA profile and quantitative supply of microbial protein, the maximum average daily gain (ADG) allowed when microbial protein was the sole source of AA`s, would be 88 g/d for Merinos and 84 g/d for the Dohne Merinos. At this growth rate histidine would become limiting. When an undegradable protein (UDP) source, high in histidine, sufficiently relieves this deficiency, methionine would become limiting at a correspondinge growth rate of 91 g/d and 85 g/d for the Merino and Dohne Merino lambs, respectively. Schingoethe (1991) and Coetzee *et al.* (1995) reported that supplementing the first limiting AA would render the second limiting AA first limiting as a result of an altered AA profile. In this manner leucine, arginine and phenylalanine would become limiting, as each next limiting AA was sufficiently supplemented, at corresponding ADG`s of 126 g/d, 138 g/d and 146 g/d respectively.

The degree of limitation by the most limiting AA`s from Table 8 appears very severe. It is also well known that commercial feedlots regularly achieve growth rates in excess of 250 and even 300 g/d. These feedlot diets generally contain only 30 to 40 % UDP, which renders the attribution of the improved production rates to better-quality post-ruminal AA profiles from the inclusion of undegradable AA`s questionable. This suggests that the microbial production in the calculations in Table 8 was underestimated. When optimum rumen conditions exist, the AA contribution of microbial protein in the lower digestive tract may actually be substantially higher than estimated in this study. The diet used to determine the microbial protein AA profile could also be a further reason for the low microbial protein production values. The driving force behind a productive rumen microbial population is a sufficient energy supply (Henning *et al.,* 1993 & Balch, 1967). The diet used in the study by Nolte & Ferreira (2005) to determine the AA profile of duodenal protein, primarily from rumen microbes, contained low energy levels to represent low-quality forage and therefore, may have limited AA concentrations in the duodenum. Consequently, the AA supply from microbial protein may have been underestimated. However, the vast majority of commercial sheep herds in the summer rainfall regions of South Africa lamb from March to April, implying that these lambs would reach the finishing stage during winter, when the available forage is similar to the diet used. The AA deficiencies as calculated in Table 8, therefore, highlights the dietary restrictions of grazing lambs during periods of limited, low-quality forage availability and stresses the need for efficient protein and/or energy supplementation strategies. These results should however, be interpreted with great care for lambs under feedlot conditions,

in which case a totally different basal diet could improve the amount of microbial protein synthesised.

Since feedlot lambs regularly grow over 300 g/d, thus requiring even more AA`s, this illustrates the inability of microbial protein synthesis to supply all the essential AA`s in the required profile (Merchen & Titgemeyer, 1992). McDonald *et al.* (1995) reported that protein synthesis might be reduced when some AA`s are over supplied while others are deficient. Inter relationships among AA`s may also affect an altered utilisation efficiency of related AA`s when the corresponding AA is over or under supplied. This demonstrates the significance of supplying a balanced AA profile in the small intestine to optimise AA absorption and metabolisation and that an UDP source, high in the limiting AA`s and low in the excess AA`s, might be required. Ferreira *et al.* (2002) also corroborated the need for post-ruminal AA supplementation by reporting increased nitrogen retentions when lambs were abomasally infused with AA`s.

Table 8 The essential amino acid requirements of Merino and Dohne Merino lambs for an average growth rate of 250 g/d and the microbial protein[2] and undegradable essential amino acids needed to meet these requirements

EAA[1]	EAA comp: MCP[2] (g/100 g protein)[3]	EAA: required Merino (g/d)[4]	EAA: required Dohne Merino (g/d)[4]	MCP required: Merino (g/d)[5]	MCP required: Dohne Merino (g/d)[5]	Estimated MCP deficiency: Merino (g/d)[6]	Estimated MCP deficiency: Dohne Merino (g/d)[6]	EAA required as UDP[7]: Merino (g/d)[8]	EAA required as UDP: Dohne Merino (g/d)[8]	Maximum ADG[9] from MCP: Merino (g/d)[10]	Maximum ADG[9] from MCP: Dohne Merino (g/d)[10]
Arg	5.54	11.97	11.65	215.96	210.23	98.03	92.30	5.43	5.11	136.52	140.24
His	1.29	4.31	4.49	335.01	349.38	217.07	231.44	2.79	2.98	88.01	84.39
Ile	4.02	5.44	5.63	135.35	140.16	17.42	22.22	0.70	0.89	217.83	210.36
Leu	5.85	13.71	13.66	234.56	233.61	116.62	115.67	6.82	6.76	125.70	126.21
Lys	6.69	11.32	11.82	169.25	176.76	51.32	58.83	3.43	3.93	174.20	166.80
Met	0.79	2.55	2.75	322.34	347.37	204.41	229.43	1.62	1.82	91.47	84.88
Phe	3.41	6.87	6.86	201.49	201.10	83.56	83.16	2.85	2.84	146.33	146.62
Thr	3.85	6.44	7.05	167.38	183.23	49.44	65.29	1.90	2.51	176.15	160.91
Trp	1.54	1.86	1.69	120.25	109.69	2.31	-8.24	0.04	-0.13	245.19	268.79
Val	4.89	9.21	8.88	188.34	181.57	70.41	63.64	3.44	3.11	156.55	162.38

[1]Essential amino acid

[2]Duodenal crude protein pre-dominantly derived from microbial protein

[3]From Nolte & Ferreira (2005)

[4]Calculated as Y = (X/100)*Z, where Y = EAA requirement for a growth rate of 250 g/d; X = a crude protein requirement of 168 g/d to allow an average daily gain of 250 g/d (NRC, 1985); Z = the whole empty body amino acid composition for each breed (Nolte & Ferreira, 2004b).

[5]Calculated as Y = X/(Z/100), where Y = MCP required (g/d); X = individual amino acid required (g/d); Z = concentration of individual amino acid in MCP (g/100 g protein)

[6]Calculated as Y = X - Z where Y = estimated MCP deficiency; X = calculated MCP requirement and Z = estimated MCP production. Z is calculated as 13 % of the total digestible nutrient (TDN) intake (NRC, 1996), for an average daily voluntary feed intake of 1.26 kg DM of a diet with a TDN content of 720 g/kg (DM-basis) = 117.94 g/d

[7]Undegradable protein

[8]Calculated as Y = X – (Z/100*A), where Y = essential amino acid A required as UDP; X = essential amino acid requirement to allow a growth rate of 250 g/d; Z = estimated MCP production; A = concentration of essential amino acid A in MCP

[9]Average daily gain

[10]Calculated as Y = [(X/100)*Z]/A*250, where Y = maximum ADG supported by essential amino acid B produced by MCP, X = estimated daily MCP production; Z = concentration of essential amino acid B in microbial protein and A = requirement for essential amino acid B to allow an ADG of 250 g/d

Conclusion

Significant differences exist in the weight distribution and protein contents of various body components between growing Merino and Dohne Merino lambs. Since these body components also vary in their essential AA compositions a single body component would not be representative of the WEB essential AA profile. This study indicates that the WEB presents the most accurate estimate of the AA requirements for growth. Further, this study also shows that the WEB essential AA profiles differ between breeds, which entails that more breed specific research is required to establish AA requirements for every breed to improve their protein utilisation efficiency. The chemical scores illustrated that histidine, methionine, leucine, arginine and phenylalanine were the most limiting AA`s when microbial protein was the major source of AA`s to growing Merino and Dohne Merino lambs. Since histidine and arginine are considered semi-essential amino AA`s, for reasons discussed earlier, methionine, leucine and phenylalanine may be the first three limiting AA`s for growing Merino and Dohne Merino lambs. This warrants further research on the effect of different post-ruminal AA profiles on the efficiency of N utilisation to validate the order of limiting AA`s to growing lambs receiving high RDP diets.

Finally this study illustrated that, under these conditions, microbial protein did not provide the required profile or quantity of AA`s in the small intestine to support a growth rate of 250 g/d by male Merino and Dohne Merino lambs. Hence, rumen undegradable AA supplements are required to correct AA deficiencies and support optimal production. Further research on the AA supply from microbial protein under feedlot conditions, the efficiency of absorption and metabolisation of different essential AA`s, the response of lambs to the supply of the ideal AA profile in the small intestine, protection of AA`s against rumen degradation and the development of models to predict dietary AA adequacy and undegradable AA requirements and estimate subsequent response levels is justified.

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CHAPTER 5

Limiting amino acids for growing sheep fed a soybean hull-based diet[1]

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Abstract

Two 6 x 6 Latin squares were performed with Rambouillet wethers to evaluate the effects of the amino acid (AA) profile on N retention and plasma AA concentrations. The animals were limit-fed (630 g DM) a high rumen degradable soybean hull-based diet in two equal portions, twice daily. Continuous ruminal infusions of acetate (41 g/d) and propionate (14 g/d) and abomasal infusions of dextrose (75 g/d) provided additional energy without affecting microbial protein synthesis. Treatments 2 to 5 also provided similar amounts of the nonessential AA`s L-glutamate and L-glycine. Treatments were continuously infused into the abomasum. In experiment 1 the wethers were provided 1) water, 2) a mixture of 12 AA in a profile that complimented microbial protein to simulate the whole empty body (WEB) composition in the small intestine, 3) 12 AA with methionine removed, 4) 12 AA with lysine removed, 5) 12 AA with histidine removed and 6) 12 AA with threonine removed. Blood samples were collected 3h after feeding on day 7. Retained N, as well as the efficiency of N retention decreased when methionine was removed. Treatments in experiment 2 were 1) water, 2) a mixture of 12 AA, 3) 12 AA with the branched-chain amino acids (BCAA`s) removed, 4) 12 AA with arginine removed, 5) 12 AA with phenylalanine removed and 6) 12 AA with tryptophan removed. Retained N decreased when BCAA were removed from 12 AA. Methionine and at least one BCAA are limiting for growing wethers receiving soybean hull-based diets.

Key words: Sheep, Amino acid, Degradable protein, Nitrogen retention

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Introduction

The efficiency of protein utilisation is not only dependent on the amount of protein, but also on the amino acid (AA) profile available for absorption (Cole & Van Lunen, 1994; Schwab, 1996). A deficiency of a single AA will inhibit the responses to those in adequate supply (Cole & Van Lunen, 1994), while excessive AA`s in comparison to the limiting AA`s will be degraded and excreted, with a subsequent waste of energy and nitrogen (Cromwell, 1996). Excess AA`s may also have a negative effect on feed intake and productive animal performance (Robinson *et al.*, 2000). D`Mello (1994) reported that the intake of AA`s in quantities and profiles that are disproportionate to those required for optimum tissue utilisation might result in adverse effects that range from depressions in growth, feed intake and nutrient utilisation to acute neurological aberrations and death. Factors such as the nutritional status and age of the animal, the degree of dietary disproportions of the AA`s and intrinsic properties and the metabolic fate of individual AA`s determine the precipitation of these effects (D`Mello, 1994). Therefore, the ideally absorbed AA supply must be available in the small intestine for optimal animal performance. Chen & Ørskov (1994) and Boisen *et al.* (2000) stated that the ideal protein represents the perfect ratio among individual AA`s that allow maximum utilisation efficiency and optimal performance. The utilisation efficiency of the ideally absorbed amino acid nitrogen (AAN) should therefore be one (Fraser *et al.*, 1991).

When ruminants are fed diets low in ruminally undegradable protein (UDP), microbial protein is the predominant source of AA`s for small intestinal absorption (Merhchen & Titgemeyer, 1992). However, Merchen & Titgemeyer (1992) also suggested that microbial protein might not always be able to supply in the requirements for the entire essential AA`s of high producing animals. Identification of the limiting AA`s in microbial protein and supplementation of these AA`s to by-pass the rumens of animals receiving highly degradable diets, may improve animal production considerably. The classical method for identifying limiting AA`s for the whole animal involves supplementing the diet with individual AA`s (Reis *et al.*, 1990). The converse, which has proved useful both in identifying and quantifying AA requirements in animals, is a deletion approach (Storm & Ørskov, 1984). Storm & Ørskov (1983) determined the efficiency of absorption of AAN, with rumen micro-organisms as the sole source of protein, by maintaining growing lambs by intragastric infusion and measuring the response in retained N to an increasing supply of microbial protein (Storm *et al.*,

1983a,b). From these results the supplemental microbial protein requirement was calculated to achieve a theoretical efficiency of absorption for microbial protein of one. By removing each individual AA in turn from this ideal AA mixture, the order of limiting AA`s for growing lambs was determined as methionine, lysine, histidine and arginine (Storm & Ørskov, 1984). The most important inadequacy of the methods these authors used is that the lambs were maintained by sole intragastric infusion, which, although very precise, may alter the metabolism of absorbed AA`s as a result of the atrophy of the gastrointestinal tissues (Ørskov *et al.*, 1979). Literature indicates some discrepancies in the sequence of limiting AA`s for growing sheep (Storm & Ørskov, 1984; Ferreira *et al.*, 1999; Nolte & Ferreira, 2004a). Therefore, the aim of this study was to establish the order of limiting essential AA`s in duodenal protein, pre-dominantly derived from rumen microbes, of growing sheep receiving a high rumen degradable protein (RDP) diet, in a series of N balance studies.

Material and Methods

The Institutional Animal Care and Use Committee of New Mexico State University approved procedures for this study.

Experiment 1

This experiment was conducted with two wethers (31.6 kg average BW) to determine the protein degradability of the basal diet, as well as the rumen pH, rumen ammonia-N and volatile fatty acid (VFA) concentrations. The animals were adapted to the diet (Table 1) for 7 days prior to incubation of the dietary samples in the rumen. The lambs were fed 630 g dry matter (DM)/d in two equal portions at 08:00 and 20:00, to correspond with experiments two and three, and had free access to fresh water. The diet was ground to pass a 1mm screen and 2 g was placed in each dacron bag (25 x 80 mm; 53 µm pore size). Dacron bags were prepared in duplicate and heat sealed, before it was placed in large polyester mesh bags (200 x 350 mm) and incubated in the rumens of the lambs for 0, 2, 4, 6, 8, 12, 16, 24, 48 and 72 h. Since the diet was formulated to be highly rumen degradable, shorter intervals were used between each removal of the dacron bags for the first 16 h. All the bags were inserted into the rumens at time 0 and the various incubation

times achieved by extracting the bags at the appropriate time. The control bags (0 h) were not incubated, but were subjected to the same rinsing procedures as the rest. All the bags

Table 1 Diet composition on a DM-basis

Item	% of diet DM
Ingredient	
Soybean hulls	79.60
Lucerne hay	15.00
Molasses	3.50
Salt	0.20
Urea	0.35
Sodium bicarbonate	0.50
Elemental sulphur	0.05
Vitamin/Mineral/Trace mineral mixture[1]	0.80
Chemical Analysis	
Dry matter	91.00
Organic matter	91.10
Crude protein	15.00
ADF	41.30
NDF	55.40
Ca	1.10
P	0.33
Mg	0.31
K	1.30
Protein Degradability (%)	
Fraction A[2]	26.67
Fraction B[3]	58.51
Fraction C[4]	14.82
Protein degradation rate (%/h)	2.45

[1]Composition: Ca (14 to 16.8 %), P (≥11 %), NaCl (11 to13.2 %), Mg (≥0.5 %), K (≥0.1 %), Cu (5 to 7 ppm), Se (≥15ppm), Zn (≥1980 ppm), Vit A (≥660 000 IU/kg), Vit D (≥165 000 IU/kg), Vit E (≥1 320 IU/kg).

[2]N that washed out of the dacron bags at t=0, and represents the rapidly degradable N fraction

[3]N that remained in the bags at the various time intervals (excluding t=0 and t=72), minus fraction C. B represents the potentially degradable N

[4]The N remaining in the bags after completion of the incubation period, which represents the undegradable N fraction

were subjected to 10 cold water rinses, of three minutes each, in a commercial washing machine (Coblentz *et al*, 1997). Immediately after rinsing the bags were dried at 55 ˚C for 24 h and air-equilibrated. The air-dry residues were weighed and analysed for N. The protein content was fractionised according to the NRC (1985). Fraction A is the protein that was assumed to be rapidly and completely degraded. This was the N portion that rinsed out of the *in situ* bags without being incubated. Fraction C represents the UDP fraction, which was left in the bags after the incubation period was completed. Fraction B represents the potentially degradable protein and is calculated by difference (B = 100 % - A – C). The protein degradation rate (K_d) was calculated by regressing the natural logarithm of the protein remaining in the *in situ* bags, after fraction C was subtracted, against time. The slope of this regression curve equals protein degradation rate (Mathis *et al.*, 2001).

Table 2 Rumen pH, rumen ammonia-N levels and volatile fatty acid concentrations in lambs receiving the basal soybean hull-based diet at 700 g/d (air dry basis)

Parameter	Concentration
Rumen pH	6.49
Rumen ammonia-N (mg/L)	18.91
Volatile fatty acids (m*M*)	
Acetate	59.72
Propionic acid	12.71
Butyrate	4.53
Isobutyrate	0.68
Valerate	0.63
Isovalerate	0.63
Acetate:Propionate	4.7:1

After the 72 h incubation samples were extracted from the rumens, the lambs were given 4 days to rest and recover constant rumen conditions before starting with the rumen pH, ammonia-N and VFA sampling (Table 2). From 08:00 to 18:00 on day 8 ruminal contents were collected on every even hour to yield samples at 0, 2, 4, 6, 8, and 10 h. The rumen contents were strained through cheesecloth to obtain a 100 mL rumen fluid sample and the pH was immediately recorded with an automated pH probe. Ammonia-N and VFA samples were prepared by adding 8 mL of the strained rumen fluid to viles

containing 2 mL of a 25 % (w/v) m-phosphoric acid solution. These samples were thoroughly mixed and immediately frozen until analysis.

Experiment 2

Six ruminally cannulated Rambouillet wethers [36 ± 3.3 kg initial body weight (BW)] were housed in individual metabolism crates in a room with continuous lighting to evaluate the effects of various post ruminal AA infusions on N retention and plasma AA concentrations. Wethers were adapted to the basal diet (Table 1) for 14 days before initiation of the treatments. The lambs had free access to fresh water and received 630 g DM/d (1.7 % of body weight BW) of the basal diet (Table 1) in equal portions at 12-h intervals (at 08:00 and 20:00). The diet was formulated to be highly rumen degradable and provided low amounts of dietary AA`s to the small intestine.

The experimental design was a 6 X 6 Latin square with 7-d periods that allowed 3 d for adaptation to abomasal infusions and 4 d for urine and faecal collections. The short adaptation periods were considered sufficient, since ruminants adapt rapidly to post ruminal nutrient infusions (Hovell *et al.*, 1983; Moloney *et al.*, 1998). Daily continuous infusions of 41 g acetate and 14 g propionate into the rumen, as well as 73 g glucose into the abomasum supplied additional energy to the lambs, to prevent energy from being first limiting, without affecting microbial protein production. Although Hovell *et al.* (1983) indicated that ruminants preferentially utilise AA`s for protein deposition instead of oxidation for energy; they can serve as gluconeogenic precursors under conditions of energy restriction. The supplemented energy would prevent the use of AA`s for gluconeogenesis in the liver and the concentrations of the glucogenic AA`s would therefore indicate their utilisation for protein deposition. Amino acids known to be gluconeogenic in sheep are alanine, glutamate, glutamine, glycine, serine and threonine (Obitsu *et al.*, 2000). The VFA infusions were gradually increased from 3 days before commencing with treatments to allow the rumen microbial population to adapt to the treatments and prevent burning the rumen wall. Ruminal infusions were achieved by putting flexible tubing through the rumen cannulas. Abomasal infusions were accomplished by extending similar flexible tubing through the rumen and reticulo-omasal orifice into the abomasums. The tubing was secured in the abomasums by attaching a rubber flange (3 cm diameter) to its end. The increased glucose concentrations may have

affected AA metabolisation by stimulating insulin secretion (Balcells *et al.,* 1995; Piccioli Cappelli *et al.,* 1997), which might increase AA uptake to peripheral tissues (Brockman *et al.,* 1975; Ahmed *et al.,* 1983; Wester *et al.*, 2000). Since energy is the main driving force behind microbial protein synthesis (Henning *et al.,* 1993) and only a limited amount of VFA`s could be supplemented in the rumen without damaging the rumen wall, the authors agreed on the abomasal glucose infusion.

Table 3 Ruminal and abomasal infusions (g/d)

Item	Treatment[1]					
	GLC	**12 AA**	**- MET**	**- LYS**	**- HIS**	**- THR**
Essential AA						
L-Isoleucine	0	1.8	1.8	1.8	1.8	1.8
L-Leucine	0	7.9	7.9	7.9	7.9	7.9
L-Valine	0	4.4	4.4	4.4	4.4	4.4
DL-Methionine	0	1.8	-	1.8	1.8	1.8
L-Lysine	0	6.6	6.6	-	6.6	6.6
L-Histidine	0	3.1	3.1	3.1	-	3.1
L-Threonine	0	3.1	3.1	3.1	3.1	-
L-Arginine	0	6.4	6.4	6.4	6.4	6.4
L-Phenylalanine	0	3.6	3.6	3.6	3.6	3.6
L-Tryptophan	0	0.4	0.4	0.4	0.4	0.4
Nonessential AA						
L-Glycine	0	5.6	5.6	5.6	5.6	5.6
L-Glutamate	0	10.9	10.9	10.9	10.9	10.9
Volatile Fatty Acids						
Acetate	41	41	41	41	41	41
Propionate	14	14	14	14	14	14
Glucose	73	73	73	73	73	73

[1]GLC = no AA`s, only VFA`s and glucose; 12 AA = a mixture of 10 essential AA`s, two Nonessential AA`s, VFA`s and glucose; -MET = Methionine removed from 12 AA; -LYS = Lysine removed from 12 AA; -HIS = Histidine removed from 12 AA; -THR = Threonine removed from 12 AA

To ensure that N was not limiting and that treatments were isonitrogenous, except for the N lost in the removed AA, L-glycine and L-glutamate were included in the abomasal infusates. Treatments (Table 3) were continuous abomasal infusions of a solution (500 g/d) containing: 1) only glucose **(GLC),** 2) a mixture of 10 essential AA`s, 2 nonessential

AA`s (glycine and glutamate) and glucose **(12 AA)**, 3) 12 AA with methionine removed **(-MET)**; 4) 12 AA with lysine removed **(-LYS)**; 5) 12 AA with histidine removed **(-HIS)** and 6) 12 AA with threonine removed **(-THR)**. The deletion approach would thus evaluate the impact of different AA profiles on the efficiency of N utilisation and establish the order of limiting AA`s in high rumen degradable protein (RDP) diets to growing lambs. According to Titgemeyer & Merchen (1990) the successful measurement of AA requirements are dependant of 1) the knowledge of the basal flow of AA`s disappearing from the small intestine, 2) a deficiency in the AA of interest and 3) other nutrients must meet or exceed the animal's requirements. When other nutrients are limiting responses will only take place to the level allowed by these limitations.

Twelve g of a 6 *N* HCL solution was mixed with 250 g water and the AA`s were dissolved in the following order: Isoleucine, Leucine, Valine, Methionine, Histidine, Lysine, Threonine, Phenylalanine, Arginine, Tryptophan and Glycine. The L-Glutamate was dissolved separately in an alkaline solution of 80 g water and 10 g NaOH (50 % solution) and added to the first solution. Finally 73 g Dextrose was dissolved in the AA solution and water added to bring the total infusate to 500 g/d with a final pH of 3.8. The profile of the 10 essential AA`s was based on the whole empty body (WEB) essential AA composition of growing lambs reported by Nolte & Ferreira (2004b).

From day 4 to 7 of each period total urine and faecal samples, for calculation of N retention, were collected daily at 08:00. The total daily faecal collection for every sheep was frozen and compounded by period. These samples were dried in a forced-air oven at 55 ˚C and ground to pass a 1 mm screen. Daily urine samples for individual lambs were sub-sampled to 2 % and pooled for the 4 collection days within each period. Urine aliquotes were immediately frozen until N analysis. Fifty mL of a 6 *N* HCL solution was used to prevent ammonia loss from the urine during collection. Orts were collected and frozen at the end of each period. Composite feed, orts and faecal samples were analysed for DM, OM, N (AOAC, 2003) and NDF (Van Soest and Wine, 1967) to calculate nutrient digestibilities. Nitrogen retention was calculated from N analyses (LECO FP-528, LECO Corporation, St. Joseph, MI) on the feed, orts, faeces and urine.

At 23:00 on day 7 of each period blood was extracted from the jugular vein into vacuum tubes containing sodium heparin as anticoagulant. The blood was immediately chilled on

ice and then centrifuged (Sorvall RT6000B, Heraeus Instruments, South Plainfield, NJ, H-100B rotor) at 1 500 x *g* for 20 min. The plasma was extracted with pasteure pipettes and frozen to be analysed for AA concentrations. Amino acids were analysed by gas chromatography (Chen *et al.*, 2002).

Data were analysed using the MIXED procedure of SAS (2000) with effects for period and treatment and lamb as a random effect. The LS Means option was used and treatment means were also compared to the 12 AA treatment with individual t-tests. Differences were considered significant when $P < 0.05$.

Experiment 3

Table 4 Ruminal and abomasal infusions (g/d)

Item	Treatment[1]					
	GLC	**12 AA**	**- BCAA**	**- ARG**	**- PHE**	**- TRP**
Essential AA						
L-Isoleucine	0	1.8	-	1.8	1.8	1.8
L-Leucine	0	7.9	-	7.9	7.9	7.9
L-Valine	0	4.4	-	4.4	4.4	4.4
DL-Methionine	0	1.8	1.8	1.8	1.8	1.8
L-Lysine	0	6.6	6.6	6.6	6.6	6.6
L-Histidine	0	3.1	3.1	3.1	3.1	3.1
L-Threonine	0	3.1	3.1	3.1	3.1	3.1
L-Arginine	0	6.4	6.4	-	6.4	6.4
L-Phenylalanine	0	3.6	3.6	3.6	-	3.6
L-Tryptophan	0	0.4	0.4	0.4	0.4	-
Nonessential AA						
L-Glycine	0	5.6	5.6	5.6	5.6	5.6
L-Glutamate	0	10.9	10.9	10.9	10.9	10.9
Volatile Fatty Acids						
Acetate	41	41	41	41	41	41
Propionate	14	14	14	14	14	14
Glucose	73	73	73	73	73	73

[1]GLC = no AA`s, only VFA`s and glucose; 12 AA = a mixture of 10 essential AA`s, two nonessential AA`s, VFA`s and glucose; -BCAA = Branched chain AA`s removed from 12 AA; -ARG = Arginine removed from 12 AA; -PHE = Phenylalanine removed from 12 AA; -TRP = Tryptophan removed from 12 AA

Experimental procedures, chemical and statistical analyses were identical to experiment two, only the treatments differed (Table 4).

Results and discussion

The rumen fermentation data presented in Table 2 clearly illustrate that the basal diet maintained favourable conditions for microbial protein synthesis in the rumen. The average rumen pH over a 12 h period was 6.49, which corresponds extremely well with the generally accepted optimum rumen pH of 6.5. The rumen ammonia-N concentration (18.9 mg/L) was slightly lower than expected on a high RDP diet. However, Slyter *et al.*, (1979) concluded that rumen ammonia-N concentrations between 20 and 50 mg/L were sufficient to allow maximum rumen microbial growth. Since this was a high fibre low starch diet, evident from the high proportional acetate levels; (Table 2) the high DM degradability (Table 2) indicates highly rumen fermentable fibres. Consequently, it appears that the rumen microbes utilised the available ammonia extremely efficiently. The rumen ammonia-N levels could also have been depressed by the fact that the lambs were limit-fed.

From Table 5 it is clear that the AA profile available for absorption had no effect on faecal N excretion. Since the GLC treatment contained only 60 % of the N in the 12 AA treatment, the similarity in faecal N excretion suggests a reduced N digestibility in the GLC treatment, which was indeed the case (61.5 vs. 75.7). This was somewhat unexpected, since Heger & Frydrych (1989) suggested that the level of intake of an AA relative to its requirement has a remarkable influence on its efficiency of utilisation. Due to the dilution of maintenance requirements the utilisation efficiency of AA`s by rats increased when supplied at 30-60 % of requirements. Supplementation above 30-60 % of requirements decreased the efficiency of utilisation due to diminishing returns (Heger & Frydrych, 1989). It seems therefore unlikely that the lower N content in the GLC treatment would be responsible for the reduced N digestibility, which suggests that the differences in AA profiles between the GLC and 12 AA treatments induced these differences. In contrast, N digestibility did not differ when a single AA was removed from the 12 AA treatment. The treatments from which only a single AA was removed was formulated to be deficient in the removed AA only, while the GLC treatment was

calculated to be deficient in all 10 essential AA`s. Whether the lack of a response in N digestibility could be attributed to the degree of limitation induced by the essential AA profile of each treatment is unclear, but it appears that AA profile does influence N digestibility.

The apparent dry matter, organic matter and NDF digestibilities were not significantly affected by treatment and averaged 74.5, 76.9 and 74.8 % respectively (Table 5). This suggests that post ruminal AA profiles may affect N digestibility, but has no influence on the digestibility of these other nutrients. Supplementation of AA`s in the rumen may however, have beneficial effects in nutrient digesstion as well. Bull & Vandersall (1973) indicated that sulphur-containing AA supplementation to urea-containing diets could improve rumen metabolism and nutrient digestion rate by an enhanced rumen fermentation rate (Clark & Petersen, 1988).

Urinary N excretion for the GLC treatment was significantly lower than for the 12 AA infusion (Table 5). Obitsu *et al.* (2000) found that abomasal glucose infusion in sheep reduced urea synthesis and urinary N excretion. These authors argued that glucose absorption from the small intestine might contribute to an increased AA flow to peripheral tissues and subsequently reduce urinary N excretion. However, in the present study all treatments received similar amounts of glucose. The lower urinary N excretion for the GLC treatment was probably because N intake and digestibility for the GLC treatment were significantly lower than for the 12 AA treatment, but faecal N excretion was similar. Less N was thus available for metabolisation, as illustrated by the lower retained N for the GLC treatment (Table 5).

When methionine was removed from the 12 AA treatment the daily urinary N excretion increased 26 %, stressing the value of methionine in the metabolisable protein supply to growing sheep. These results suggest that the subsequent imbalances in the AA profile when methionine was removed from the 12 AA infusate resulted in a reduced metabolisation of the remaining AA`s and a resultant higher urinary N excretion. A reduced urinary N excretion and resultant improved N retention suggest that N was more efficiently utilised at tissue level (Meissner & Todtenhöfer, 1989). Cole & Van Lunen (1994) suggested that the balance of absorbed AA`s is the most important single factor

Table 5 The effects of removing methionine, lysine, histidine or threonine from postruminal infusions of 10 essential AA`s on nitrogen balance and diet digestibility of growing lambs

Item	Treatment[1]						SEM
	GLC	12 AA	-MET	-LYS	-HIS	-THR	
Nitrogen (g/d)							
Dietary	14.32	15.50	14.71	15.95	15.34	14.08	
Infusion	0	8.42	8.26	7.43	7.58	8.07	
Total intake	14.32	23.92	22.97	23.38	22.92	22.15	
Faecal	5.39	5.79	6.00	5.88	5.95	5.54	0.39
Urinary	6.22**	9.81	12.36**	9.56	8.79	10.68	0.77
Retained	2.71**	8.21	4.62**	7.94	8.19	5.82*	0.88
Retained Efficiency[2]	16.11**	34.83	20.10**	33.89	35.43	25.82	4.11
Diet digestibility (%)							
Dry matter	75.79	74.01	73.66	75.47	74.41	73.91	1.30
Organic matter	77.92	76.55	76.29	77.72	76.65	76.06	1.34
NDF	76.06	74.51	74.73	75.57	74.00	74.15	1.70
N	61.51**	75.72	73.84	74.79	74.00	74.50	1.42

[1]GLC = no AA`s, only VFA`s and glucose; 12 AA = a mixture of 10 essential AA`s, two nonessential AA`s, VFA`s and glucose; -MET = Methionine removed from 12 AA; -LYS = Lysine removed from 12 AA; -HIS = Histidine removed from 12 AA; -THR = Threonine removed from 12 AA

[2]Retained Efficiency = (N Retained / N intake)*100

**Different from 12 AA treatment (P≤0.05)

*Tend to be different from 12 AA treatment (P≤0.10)

affecting the efficiency of protein utilisation and a deficiency of a single AA would inhibit the responses to those AA`s in adequate supply. Froidmont *et al.* (2000) also indicated that the supply of a limiting AA and the corresponding positive interaction in the utilisation of whole available AA`s for protein synthesis could result in biological values higher than one. In support of the observed importance of methionine in protein metabolisation in the present study, Storm & Ørskov (1984), Ferreira *et al.* (1999) and Nolte & Ferreira (2004a) also reported methionine as the first or second limiting AA to growing sheep receiving a variety of diets. Urinary N excretion did not differ from the 12 AA treatment when lysine, histidine or threonine were removed from the infusate, which indicate that the requirements for these AA`s are more readily met on highly degradable protein diets where microbial protein is the primary source of AA`s.

Although the total N intake in the GLC treatment (Table 5) was only 40 % lower than in the 12 AA treatment (14.32 *vs.* 23.92 g/d), retained N in the GLC treatment was 67 % lower than for the 12 AA treatment (2.71 *vs.* 8.21 g/d). This suggested that the lambs receiving the better AA balanced treatment utilised N more efficiently. The lower N retention efficiency of the GLC treatment (16.11 *vs.* 34.83 g/d) corroborates the improved efficiency of N utilisation when the imbalances in the post-ruminal AA supply were corrected. Because Heger & Frydrych (1989) reported that the AA supply relative to its requirement affects the utilisation efficiency of individual AA`s, one would expect the lower N intake in the GLC treatment to improve N retention efficiency. This was certainly not the case in the present study where the efficiency of N retention increased by 116 % (16.11 to 34.83 g/d) when AA deficiencies were corrected, despite the increased total N intake associated with the AA supplementation. This indicates that the AA profile of metabolisable protein plays a critical role in protein utilisation and animal performance. Lapierre *et al*. (2000) also reported an increased utilisation of absorbed AA`s for protein synthesis when the profile of the limiting AA`s was improved. The lower efficiency of N retention in the unsupplemented animals also corroborates the findings of Merchen & Titgemeyer (1992) that microbial protein may not always provide in all the AA requirements of high producing animals.

When methionine was removed from the 12 AA infusion N retention decreased 44 % (Table 5). The N loss as a result of the removal of methionine from the 12 AA infusate was only 4 %, which stresses the value of methionine in the efficiency of protein utilisation in highly degradable protein diets. Since the WEB of woollen breed lambs between 30 and 40 kg live weight contain 11.4 % N (DM basis; Nolte & Ferreira, unpublished data) the 12 AA treatment would allow an average daily gain of 72 g/d, assuming that retained N was directly incorporated into protein deposition. When methionine was removed the lambs would gain body weight at a rate of 41 g/d, illustrating the effect of a reduced efficiency of protein utilisation caused by a methionine deficiency. These results corroborate the high order of limitation of methionine to growing lambs by various other reports (Storm & Ørskov, 1984; Ferreira *et al.*, 1999; Nolte & Ferreira, 2004a). Methionine may be especially important to woollen breed sheep, because of the high cysteine content of wool. Sherlock *et al.* (2001) reported that methionine supplementation improved fibre length, without increasing fibre diameter of

sheep receiving lucerne pellets [17 % crude protein (CP)]. Campbell *et al.* (1997) also reported that in addition to protein accretion, methionine might be used for other functions as well, such as a donor of methyl groups or a precursor of cysteine. The methyl group donor, S-adenosylmethionine, an intermediate product formed during the conversion of methionine to homo-cysteine, is involved in more than 100 reactions in the body (Lobley, 1992). Wiese *et al.* (2003) also indicated that methionine supplements to sheep receiving *ad libitum* high quality diets reduced the fat cover over the loin and decreased flavour intensity in Merino lambs. The improved N retention in comparison to the GLC treatment was probably due to only methionine being imbalanced, while more AA`s might have been over or under supplied when no AA`s were infused (GLC treatment).

When threonine was removed from the 12 AA infusion, the retained N tended to decrease (Table 5). Ferreira *et al.* (1999) reported threonine as one of the most limiting AA`s for growing lambs. Storm & Ørskov (1984) and Nolte & Ferreira (2004a) did not find threonine as one of the four most limiting AA`s in growing sheep. Retained N efficiency displayed a similar pattern, with only the GLC and –MET infusions that had significantly lower values than the 12 AA treatment. Again the significance of methionine in protein deposition in growing sheep is highlighted, since it was the only AA from Table 5 that significantly affected N retention when it was removed from the 12 AA infusate. These results should be interpreted with great care, since Rolls *et al.* (1972) indicated that the degree of success of free AA supplementations to correct AA imbalances depends on more than the addition of the correct quantities of the supplements. The simultaneity of appearance of AA`s from the supplement and from digestion of the protein is affected by the composition of the specific protein given, the size and frequency of the meals and the presence and proportions of non-protein in the meal (Rolls *et al.*, 1972). Since the basal diet in the present study contained low levels of dietary UDP, microbial protein was the predominant AA source in the small intestine. It is assumed that the quantity of microbial protein synthesised, the AA profile and the digestibility of the microbial protein of the same species at similar ages being fed the same basal diet at similar daily feed intakes are equal. The variables mentioned by Rolls *et al.* (1972) were therefore, supposed to be similar in all treatments, except for the specific AA removed from the 12 AA infusate. It is thus reasonable to attribute the observed changes to the altered AA profiles in the various treatments.

The N balance and digestibility data when the BCAA`s, arginine, phenylalanine or tryptophan was removed from the 12 AA treatment are presented in Table 6. Similar to the previous experiment, AA profile had no effect on faecal N excretion, suggesting a lower N utilisation, since N intake was much lower than for the 12 AA infusion. Urinary N excretion however, was lower in the GLC than the 12 AA treatment. The lower urinary N excretion for the GLC treatment could possibly be attributed to the lower N intake, implying less N was available for metabolisation and subsequently less N were excreted. Removing tryptophan from the 12 AA infusion caused the urinary N excretion to

Table 6 The effects of removing the BCAA`s, arginine, phenylalanine and tryptophan from postruminal infusions of 10 essential AA`s on nitrogen balance and diet digestibility of growing lambs

Item	Treatment[1]						SEM
	GLC	12 AA	-BCAA	-ARG	-PHE	-TRP	
Nitrogen (g/d)							
Dietary	16.19	16.19	16.19	16.19	16.19	16.19	
Infusion	0	8.42	6.86	6.37	8.12	8.37	
Total intake	16.19	24.61	23.05	22.55	24.3	24.56	
Faecal	5.52	5.49	5.82	5.33	5.67	5.35	0.23
Urinary	8.37**	10.54	10.55	10.03	10.80	12.14*	0.64
Retained	2.32**	8.53	6.69**	7.21*	7.84	7.03*	0.70
Retained Efficiency[2]	13.36**	34.23	29.02	31.95	32.25	28.74	3.28
Diet digestibility							
Dry matter	77.15	76.51	75.78	77.93	76.35	78.11	1.08
Organic matter	79.42	78.85	77.88	80.43	78.63	80.25	1.03
NDF	78.31	77.75	76.19	78.65	76.59	78.64	1.19
N	65.52**	77.69	74.77**	76.38	76.67	78.24	0.96

[1]GLC = no AA`s, only VFA`s and glucose; 12 AA = a mixture of 10 essential AA`s, two nonessential AA`s, VFA`s and glucose; -BCAA = Branched chain AA`s removed from 12 AA; -ARG = Arginine removed from 12 AA; -PHE = Phenylalanine removed from 12 AA; -TRP = Tryptophan removed from 12 AA

[2]Retained Efficiency = (N Retained / N intake)*100

**Different from 12 AA treatment (P≤0.05)

*Tend to be different from 12 AA treatment (P≤0.10)

increase, suggesting that tryptophan might be limiting in the microbial protein of growing lambs receiving highly degradable diets. Cole & Van Lunen (1994) reported that a deficiency of a single AA will inhibit the responses to those in adequate supply. The removal of tryptophan also indicated the value of a well-balanced AA profile in the efficiency of N utilisation.

The lower retained N for the GLC treatment (Table 6) should probably be attributed to the AA profile of the unsupplemented treatment that contained more AA deficiencies (Cole & Van Lunen, 1994). The reduction of 22 % in N retention when the branched-chain amino acids (BCAA`s) were removed from 12 AA, as well as the strong tendencies for a lower retained N when arginine and tryptophan were removed from 12 AA indicates the significance of the AA profile in protein metabolisation. The efficiency of N retention was also numerically reduced by 15 % when the BCAA`s were removed from the 12 AA infusate and 61 % in the GLC treatment where no AA`s were supplemented to correct deficiencies. This indicates that the AA profile of microbial protein is unable to supply in all the AA requirements of growing lambs (Merchen & Titgemeyer, 1992) and also strongly suggests that at least one of the BCAA`s are limiting production when microbial protein is the predominant source of metabolisable protein. Assuming that N retention could be directly converted into protein deposition, a BCAA deficiency of the magnitude created in this experiment would reduce the daily growth rate by 18 % (72 *vs.* 59 g/d). In addition, N digestibility of the GLC animals was significantly lower than the 12 AA treatment. Heger & Frydrych (1989) reported that AA`s were utilised more efficiently when ingested at levels lower than their requirement. Since the total N intake of the GLC treatment was much lower than for any of the other treatments, these animals should have had the highest efficiency of N retention. The explanation why this was not the case must be in the AA profiles of the treatments, since the GLC treatment received no AA supplements, its AA profile was more imbalanced than the supplemented treatments. This indicates that AA deficiencies in the metabolisable AA profile will seriously impede the efficiency of protein utilisation and animal performance.

The effects of removing methionine, lysine, histidine or threonine form the 12 AA infusion on plasma AA concentrations are presented in Table 7. The plasma concentrations of leucine, lysine, methionine, threonine and valine were significantly lower in the GLC treatment that received no AA infusions, compared to the postruminal

Table 7 The effects of removing methionine, lysine, histidine or threonine from postruminal infusions of 10 essential AA`s on plasma AA concentrations of growing lambs

Item	Treatment[1]						SEM
	GLC	12 AA	-MET	-LYS	-HIS	-THR	
Essential AA (μ*M*)							
Arginine	-	-	-	-	-	-	
Histidine	81.25*	111.76	83.25*	109.86	40.37**	102.17	10.73
Isoleucine	68.64	77.70	85.39	73.08	80.66	79.43	6.16
Leucine	72.20**	157.79	170.73	151.44	157.61	173.54	12.12
Lysine	86.27**	176.36	157.57	53.67**	174.94	169.74	18.25
Methionine	14.02**	34.18	10.90**	37.46	34.06	36.34	2.66
Phenylalanine	21.75	30.69	33.32	57.58**	38.24	32.08	5.03
Threonine	33.12**	106.60	109.81	91.27	79.71	25.15**	11.57
Tryptophan	39.79	42.30	37.04	42.70	39.43	39.04	3.28
Valine	122.31**	262.82	293.05	276.94	270.75	277.58	17.44
Total essential AA`s	539.34**	1004.30	981.05	893.98	915.78	939.17	59.87
Nonessential AA (μ*M*)							
Alanine	148.68	123.57	124.92	100.99	106.68	136.55	12.35
Aspartate	5.64	8.21	5.57	5.37	5.23*	4.65*	
Asparagine	57.97*	142.10	139.42	105.87	130.45	192.74	28.51
Cysteine	18.96	26.95	14.01*	16.92	24.76	13.51*	4.68
Glutamate	151.56	143.99	118.45	97.02	162.69	131.64	18.24
Glutamine	1667.51	1464.78	1499.04	1351.41	1299.52	1166.58	160.17
Glycine	685.80	768.87	1147.51**	712.15	761.88	575.99**	84.46
Ornithine	47.88**	110.65	110.70	89.75	107.59	102.07	10.64
Proline	74.29	68.13	77.50	63.03	60.35	66.44	5.84
Serine	128.82	166.63	293.61**	128.75	158.39	112.89	27.07
Tyrosine	51.52	47.74	43.88	53.59	48.80	46.74	5.67
Total nonessential AA`s	3038.63	3083.03	3574.61	2724.84	2866.34	2561.23	247.32
Total	3577.97	4122.03	4555.66	3618.83	3782.12	3535.10	274.31

[1]GLC = no AA`s, only VFA`s and glucose; 12 AA = a mixture of 10 essential AA`s, two nonessential AA`s, VFA`s and glucose; -MET = Methionine removed from 12 AA; -LYS = Lysine removed from 12 AA; -HIS = Histidine removed from 12 AA; -THR = Threonine removed from 12 AA

[2]Retained Efficiency = (N Retained / N intake)*100

**Different from 12 AA treatment (P≤0.05)

*Tend to be different from 12 AA treatment (P≤0.10)

supply of the ideal protein in the 12 AA treatment. Histidine concentrations also tended to be lower for the GLC than the 12 AA treatment. The remainder of the essential AA concentrations for the GLC treatment were also numerically lower than for the 12 AA treatment. Total essential AA concentrations in the 12 AA infusion were also significantly higher than for the GLC treatment. Amino acid concentrations in the systemic blood represent a balance of various processes, such as absorption, catabolism and protein synthesis. The lower plasma AA levels in the GLC treatment do not indicate a higher utilisation of AA`s for protein synthesis, since the efficiency of N retention was significantly higher for the supplemented diets (Table 5), but may be the result of the lower N intake in comparison to the supplemented diets.

Removing methionine from the 12 AA treatment reduced methionine concentrations in the blood plasma (Table 7). This may indicate that less methionine was absorbed from the small intestine and lower levels of methionine were available for metabolisation, or that the lower availability of methionine resulted in an increased utilisation efficiency (Heger & Frydrych, 1989). However, AA imbalances may result in a reduced efficiency of AA utilisation, as observed in the efficiency of N retention (Table 5). It is thus unlikely that the efficiency of utilisation of an AA would increase when it was removed from the ideal AA profile. Histidine concentrations in the jugular vein also tended to decrease when methionine was removed. The tendency to reduce cysteine concentrations by removing methionine from the 12 AA infusion corresponds with the interaction between these two AA`s. An increased glycine plasma concentration when methionine was removed corroborates the existence of an interrelationship between these two AA`s, suggested by D`Mello & Lewis (1970). It appears that a methionine deficiency inhibits the utilisation of glycine. Serine is used in the conversion of homocysteine to cysteine during transsulfuration (Campbell *et al.*, 1997). Therefore, when methionine was removed from the 12 AA infusion, plasma cysteine levels tended to decrease and serine concentrations increased. When lysine was removed from the 12 AA infusion phenylalanine concentration in the jugular vein increased. Fisher & Shapiro (1970) also observed increased plasma amino nitrogen levels as a result of AA imbalances. The increased plasma AA concentrations induced by AA imbalances suggest that the utilisation of some AA`s is reduced when the AA profile is imbalanced. Supplementation of the most limiting AA reduced plasma AA concentrations (Fisher & Shapiro, 1970),

indicating a higher efficiency of utilisation. Unfortunately arginine concentrations could not be analysed, but based on the reported antagonism between arginine and lysine (D`Mello & Lewis, 1970) a corresponding increase in plasma concentrations of the former could be expected with decreased lysine infusions. O`Dell & Savage (1966) indicated that the arginine-lysine antagonism might be reciprocal. Since keratin contains about 10 % arginine, a lysine induced arginine deficiency may depress fibre production, as seen with Angora goats (Sahlu & Fernandez, 1992). Removing histidine and threonine from the 12 AA infusion had only minor effects on the jugular concentrations of a few nonessential AA`s, but no essential AA`s were affected (Table 7).

From Table 8 it is clear that except for isoleucine, threonine and tryptophan, all the essential AA`s were deficient in the jugular vein of sheep receiving the GLC treatment compared to the 12 AA infusion. This underscores the inability of microbial protein to always supply in the AA requirements of high producing animals (Merchen & Titgemeyer, 1992; Nolte & Ferreira, 2004a). The AA deficiencies in microbial protein are intensified by the high metabolic acitivity of the gastrointestinal tract. A study by Berthiaume *et al.* (2001) revealed that the gastrointestinal tissues metabolise more than 30 % of the absorbed AA`s on a net basis.

When the BCAA`s were removed from the 12 AA infusion plasma concentrations of histidine, methionine and threonine were significantly higher than for the 12 AA treatment (Table 8). This may indicate that the utilisation of these AA`s for protein deposition was reduced when some, or all of the BCAA`s were removed from the ideal AA profile in the abomasum. Since the metabolisation of the BCAA`s (leucine, isoleucine and valine) is limited in the liver, their concentration in the extra hapatic blood circulation reflects protein uptake from the gut (Harper *et al.*, 1984). Froidmont *et al.* (2000) also highlighted the significance of essential AA profiles in the efficiency of protein utilisation by confirming that digestible AA`s could have biological values higher than one. In their study Froidmont *et al.* (2000) reported an 11-fold increase in N retention when 1.6 g methionine was supplemented to double-muscled Belgian Blue bulls. These authors contributed this response to the positive interaction between the supply of a limiting AA and the utilisation of the available AA`s for protein synthesis. The unaffected isoleucine concentration in the jugular vein when leucine, isoleucine and

Table 8 The effects of removing the BCAA`s, arginine, phenylalanine or tryptophan from postruminal infusions of 10 essential AA`s on plasma AA concentrations of growing lambs

Item	Treatment[1]						SEM
	GLC	12 AA	-BCAA	-ARG	-PHE	-TRP	
Essential AA (μ*M*)							
Arginine	-	-	-	-	-	-	
Histidine	74.67**	116.03	144.53*	147.55*	112.88	112.60	11.22
Isoleucine	74.13	119.29	72.79	75.96	60.47**	81.45	19.74
Leucine	75.09**	144.88	56.12**	146.47	130.65	164.79	9.40
Lysine	67.97**	141.73	172.69	141.39	117.91	146.52	15.42
Methionine	13.54**	32.36	39.42**	38.56**	27.82*	36.06	1.90
Phenylalanine	12.35**	44.29	39.89	40.52	18.77**	34.03	8.27
Threonine	39.28	60.75	111.68**	71.89	74.93	75.43	10.35
Tryptophan	32.19	42.18	46.74	40.93	34.82	41.41	4.75
Valine	131.14**	241.10	117.56**	260.29	225.57	257.24	17.43
Total essential AA`s	516.15**	942.60	801.42**	965.79	803.80**	947.95	47.07
Nonessential AA (μ*M*)							
Alanine	136.06	108.83	173.18**	120.40	98.74	130.73	14.81
Aspartate	4.59*	2.40	10.43**	4.35*	3.74	5.31**	0.76
Asparagine	84.63	100.59	88.17	121.51	113.87	151.25**	14.78
Cysteine	5.64	9.73	28.17**	10.47	14.08	18.89	5.38
Glutamate	165.74*	142.80	164.03*	150.72	122.51*	142.27	10.68
Glutamine	1417.32	1274.20	1718.79*	1532.26	1220.81	1241.20	164.95
Glycine	635.91	559.11	804.86**	837.19**	642.28	681.79	85.97
Ornithine	52.95**	90.55	125.08**	56.37**	86.20	101.64	8.84
Proline	74.70**	59.93	84.44**	69.90*	55.33	71.78**	3.74
Serine	136.93	115.92	136.08	128.15	99.72	104.82	15.27
Tyrosine	56.69	47.85	82.79**	69.15**	24.37**	61.03	7.10
Total nonessential AA`s	2744.79	2511.91	3416.01**	3071.19	2481.65	2656.99	219.18
Total	3260.94	3454.51	4217.43**	4036.97	3285.45	3604.94	234.84

[1]GLC = no AA`s, only VFA`s and glucose; 12 AA = a mixture of 10 essential AA`s, two nonessential AA`s, VFA`s and glucose; -BCAA = Branched chain AA`s removed from 12 AA; -ARG = Arginine removed from 12 AA; -PHE = Phenylalanine removed from 12 AA; -TRP = Tryptophan removed from 12 AA

[2]Retained Efficiency = (N Retained / N intake)*100

**Different from 12 AA treatment (P≤0.05)

*Tend to be different from 12 AA treatment (P≤0.10)

valine were removed suggests that isoleucine concentrations in microbial protein were not limiting the growth of young lambs. Nolte & Ferreira (2004a) calculated that the daily microbial protein deficiency to allow an average daily gain of 250 g/d for isoleucine was much smaller than for leucine or valine (±20 g vs. 115 g and 67 g respectively). The lower total essential plasma AA concentration with the removal of the BCAA`s, despite the increased concentrations of histidine, methionine and threonine, can possibly be attributed to the quantitative effects of the removal of the BCAA`s from the infusate.

Plasma concentrations of most nonessential AA`s (Table 8) were increased when the BCAA`s were removed from the 12 AA infusate. Removing the BCAA`s also significantly increased the total nonessential, as well as the total AA concentrations. The general increase in plasma AA concentrations when the BCAA`s were removed from the 12 AA infusion might indicate a reduced utilisation of various other AA`s for protein deposition when one or a combination of 2 or all 3 BCAA`s are in deficit.

Removing arginine from the 12 AA treatment increased methionine and tended to increase histidine concentrations in the jugular vein (Table 8). Interestingly, lysine concentrations were not affected. Based on the reported reciprocal antagonism between these two AA`s (O`Dell & Savage, 1966; D`Mello & Lewis, 1970) higher lysine concentrations were expected. Again a number of nonessential AA`s showed increased plasma concentrations. Only ornithine had a lower concentration. Total essential, total nonessential and total AA concentrations were not significantly affected by removing arginine from the 12 AA infusate, but showed numeric increases. When phenylalanine was removed, isoleucine decreased, methionine tended to decrease and total essential AA concentration also decreased significantly. The nonessential AA`s showed minor effects, with tyrosine decreasing significantly and glutamate tending to decrease. The response in tyrosine was expected, based on the interrelationship between phenylalanine and tyrosine. Total nonessential and total AA concentrations both showed marginal numeric decreases. The removal of tryptophan had no effect on essential AA concentrations, neither on total essential, total nonessential or total AA concentrations. Only aspartate and asparagine concentrations were increased, again stressing the value of a well-balanced AA profile on the efficiency of protein utilisation.

Conclusion

For wethers limit-fed soybean hull-based diets, where microbial protein is the predominant source of AA`s, methionine and at least one BCAA are limiting growth. The limitations imposed by these AA deficiencies reduced the utilisation of plasma AA`s for protein synthesis and subsequently resulted in a lower efficiency of N retention. This clearly illustrates the impact of the essential AA profile on the efficiency of protein utilisation and highlights the need for a well balanced essential AA profile in the intestines to optimise animal performance. Further research is required to establish which BCAA`s are co-limiting with methionine in growing sheep being fed highly protein degradable diets.

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CHAPTER 6

Limiting branched-chain amino acids for growing sheep fed a soybean hull-based diet[1]

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Abstract

The effects of limiting branched-chain amino acids (BCAA`s) on the efficiency of nitrogen (N) utilisation were evaluated in a 6 x 6 Latin square. Rambouillet wethers (46 ±1.3 kg BW) had *ad libitum* access to fresh water, but were limit-fed (800 g/d) a high rumen degradable soybean hull-based diet in two equal portions. To prevent energy deficiencies without affecting microbial protein production, acetate (41 g/d) and propionate (14 g/d) were administered into the rumens and glucose (73 g/d) into the abomasums. Treatments were abomasal infusions (500 g/d) of 1) of glucose (GLC), 2) a mixture of 10 essential amino acids (AA`s), two nonessential AA`s (glutamate and glycine) and glucose (12 AA) that complimented microbial protein to simulate the whole empty body (WEB) essential AA composition in the small intestine, 3) 12 AA with leucine removed (-LEU), 4) 12 AA with isoleucine removed (-ILE), 5) 12 AA with valine removed (-VAL), and 6) 12 AA with leucine, isoleucine and valine removed (-BCAA). Retained N, as well as the efficiency of N retention was reduced when valine or the entire BCAA`s were removed from the infusate. This indicates that the three BCAA`s were co-limiting, but on an individual basis valine was most likely to be deficient in the duodenums of growing lambs receiving high rumen degradable protein (RDP) diets.

Key words: Branched-chain amino acid, leucine, isoleucine, valine, nitrogen retention

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Introduction

The fates of absorbed amino acids (AA`s) include increased protein gain, the synthesis of specific metabolites (e.g. Neurotransmitters and glucose) and oxidation to waste products (mainly CO_2 and urea). For efficient use of metabolisable protein by growing lambs the ideal AA profile must be supplied in the small intestine. The ideal protein represents the perfect ratio among individual AA`s that allows maximum utilisation efficiency and optimal performance (Chen & Ørskov, 1994; Boisen *et al.,* 2000). Deficient AA`s will inhibit animal response (Cole & Van Lunen, 1994), while excess AA`s will be degraded and excreted ($CO_2$ and urea), with a subsequent waste of energy and nitrogen (Cromwell, 1996). Amino acid catabolism usually occurs within the liver, which plays a vital role in maintaining AA homeostasis. Sufficient AA`s must enter the peripheral circulation to support the maintenance and growth requirements of protein stores, while AA over-supply may have deleterious metabolic consequences (Hargreaves & Pardridge, 1988). Therefore, AA supply and use should be balanced to support maximum efficiency of metabolisable protein utilisation and animal performance.

When high rumen degradable protein (RDP) diets are fed microbial protein is the predominant source of AA`s. Owens & Bergen (1983) and Sniffen & Robinson (1987) reported that microbial protein supplies from 40 to 80 % of the AA`s available for absorption in the small intestine. However, microbial protein is not always able to support the AA requirements of high producing animals (Merhchen & Titgemeyer, 1992). These AA imbalances (deficiencies and/or excesses) in microbial protein should be identified and correctional supplementation strategies implemented to improve animal performance.

The branched-chain amino acids (BCAA`s; iso-leucine, leucine and valine) comprise approximately 35 % of the indispensable AA`s in muscle proteins (Harper *et al.*, 1984), rendering the BCAA supply highly significant in the efficiency of protein turn-over. These AA`s also appear to play an important regulatory role in tissue protein synthesis and degradation (Harper *et al.*, 1984). Through their role in the control of brain AA concentrations, the BCAA`s are involved in the synthesis of AA-derived neurotransmitters (Harper *et al.*, 1984). Branched-chain AA`s are also important

precursors of the branched-chain volatile fatty acids (BCVFA`s; iso-butyric acid, iso-valeric acid and valeric acid; Broderick *et al.*, 2002) and play and important role in ammonia detoxification during AA excess. Lobley *et al.* (2001) stated that the liver is not capable of complete and instantaneous prevention of hyperaminoacidaemia. During periods of over-supply excess AA`s must accumulate in body free pools or be catabolised in non-liver tissues (Lobley *et al.*, 2001), the latter resulting in elevated ammonia productions. The two major mechanisms for ammonia detoxification are ureagenesis and glutamine synthesis. Any overflow of ammonia that escapes periportal ureagenesis stimulates perivenous glutamine synthesis (Milano & Lobley, 2001). Branched-chain amino acids are degraded to restore glutamate pools that are depleted during glutamine synthesis induced by hyperammonaemia (Leweling *et al.*, 1996). Thus, excess AA`s may induce an increased BCAA degradation and potential deficiency, underscoring the significance of a well balanced AA supply. Nolte *et al.* (2004) illustrated that methionine and at least one BCAA limited the growth of young lambs receiving high RDP diets. The objective of this study was to identify which BCAA were limiting in diets with a high RDP content.

Material and Methods

The Institutional Animal Care and Use Committee of New Mexico State University approved procedures for this study.

Six ruminally cannulated Rambouillet wethers (46 ± 3 kg) were used in a 6 x 6 Latin square to evaluate the effects of different post ruminal BCAA profiles on nitrogen (N) retention and plasma AA concentrations. The periods consisted of 3 days adaptation and 4 days for collections. Since ruminants adapt rapidly to post ruminal nutrient infusions (Hovell *et al.*, 1983; Moloney *et al.*, 1998), the short adaptation periods were considered sufficient. The lambs were individually housed in metabolism crates in an enclosed barn with continuous lighting and had free access to fresh water. From two weeks before the trial started the lambs were adapted to the soybean hull-based diet (Table 1) to allow the microbial population to adjust. During the trial the diet was offered at 0.8 kg/d (dry matter (DM) basis), in two equal portions at 07:00 and 19:00. The diet was formulated to contain low levels of rumen undegradable protein (UDP) so that microbial protein would be the predominant source of AA`s in the small intestine.

Table 1 Diet composition on a DM-basis

Item	% of diet DM
Ingredient	
Soybean hulls	79.60
Lucerne hay	15.00
Molasses	3.50
Salt	0.20
Urea	0.35
Sodium bicarbonate	0.50
Elemental sulfur	0.05
Vitamin/Mineral/Trace mineral mixture[1]	0.80
Nutrients	
Dry matter	91.00
Organic matter	91.10
Crude Protein	15.00
Acid detergen fibre	41.30
Neutral detergent fibre	55.40
Ca	1.10
P	0.33
Mg	0.31
K	1.30
Protein Degradability (%)	
Fraction A[2]	26.67
Fraction B[3]	58.51
Fraction C[4]	14.82
Protein degradation rate (%/h)	2.45

[1]Composition: Ca (14 to 16.8 %), P (≥11 %), NaCl (11 to13.2 %), Mg (≥0.5 %), K (≥0.1 %), Cu (5 to 7 ppm), Se (≥15ppm), Zn (≥1980 ppm), Vit A (≥660 000 IU/kg), Vit D (≥165 000 IU/kg), Vit E (≥1 320 IU/kg).

[2]N that washed out of the dacron bags at t=0, and represents the rapidly degradable N fraction

[3]N that remained in the bags at the various time intervals (excluding t=0 and t=72), minus fraction C. B represents the potentially degradable N

[4]The N remaining in the bags after completion of the incubation period, which represents the undegradable N fraction

To prevent energy from being first limiting, and without altering microbial fermentation, volatile fatty acids (VFA`s; 41 g acetate and 14 g propionate daily) were continuously

infused into the rumens of the lambs and glucose into the abomasums (75 g/d). Infusions into the rumen were made by putting flexible tubing through the rumen cannulas. Abomasal infusions were achieved by extending a similar tube through the rumen and the reticulo-omasal orifice. The tubes were secured in the abomasums with rubber flanges (3 cm diameter) attached to their ends. Treatments were continuous abomasal infusions of a solution (500 g/d) containing: 1) only glucose (**GLC**), 2) a mixture of 10 essential AA`s, 2 nonessential AA`s (glutamate and glycine), and glucose (**12 AA**), 3) 12 AA with Leucine removed (**-LEU**), 4) 12 AA with Isoleucine removed (**-ILE**), 5) 12 AA with Valine removed (**-VAL**), and 6) 12 AA with Leucine, Isoleucine and Valine removed (**-BCAA**). The compositions of the treatments are shown in Table 2.

Table 2 Rumen and abomasum infusions (g/d)

Item	Treatment[1]					
	GLC	**12 AA**	**- LEU**	**- ILE**	**- VAL**	**- BCAA**
Essential Amino Acids						
L-Isoleucine	0	2.0	2.0	-	2.0	-
L-Leucine	0	8.2	-	8.2	8.2	-
L-Valine	0	4.7	4.7	4.7	-	-
DL-Methionine	0	2.0	2.0	2.0	2.0	2.0
L-Lysine	0	7.0	7.0	7.0	7.0	7.0
L-Histidine	0	3.4	3.4	3.4	3.4	3.4
L-Threonine	0	3.3	3.3	3.3	3.3	3.3
L-Arginine	0	6.6	6.6	6.6	6.6	6.6
L-Phenylalanine	0	3.7	3.7	3.7	3.7	3.7
L-Tryptophan	0	0.5	0.5	0.5	0.5	0.5
Nonessential amino acids						
L-Glycine	0	6.0	6.0	6.0	6.0	6.0
L-Glutamate	0	11.0	11.0	11.0	11.0	11.0
Volatile Fatty Acids						
Acetate	41	41	41	41	41	41
Propionate	14	14	14	14	14	14
Glucose	75	75	75	75	75	75

[1]GLC = no AA`s, only VFA`s and glucose; 12 AA = a mixture of 10 essential AA`s, two nonessential AA`s, VFA`s and glucose; -LEU = Leucine removed from 12 AA; -ILE = Isoleucine removed from 12 AA; -VAL = Valine removed from 12 AA; -BCAA = Branched chain AA`s removed from 12 AA

Twelve g of a 6 *N* HCL solution was mixed with 250 g water and the AA`s were dissolved in the following order: isoleucine, leucine, valine, methionine, lysine, histidine, threonine, phenylalanine, arginine, tryptophan and glycine. The glutamate was dissolved separately in an alkaline solution of 80 g water and 10 g NaOH (50 % solution) and added to the first solution to bring the final pH of the abomasal infusates to 3.8. Finally 75 g Dextrose was dissolved in the AA solution. The AA profile of the 12 AA treatment was based on the (WEB) AA composition of lambs reported by Nolte & Ferreira, (2004a) and the calculated microbial essential AA supply (Nolte & Ferreira, 2004b).

From day 4 to 7 of each period total urine and faecal samples, for calculation of N retention, were collected daily at 07:00. The total daily faecal collection for every sheep was frozen and combined by period. These samples were dried in a forced-air oven at 55 ˚C. Daily urine samples for individual lambs were sub-sampled to 5 % and pooled for the 4 collection days within each period. Urine aliquots were immediately frozen until N analysis. Fifty mL of a 6 *N* HCL solution prevented ammonia loss from the urine during collection. Orts from each lamb within every period were collected and frozen at the end of each period. Composite feed, orts and faecal samples were ground to pass a 1 mm screen and analysed for DM, organic matter (OM), N (AOAC, 2001) and neutral detergent fibre (NDF; Van Soest and Wine, 1967) to calculate nutrient digestibilities. Nitrogen retention was calculated from N analyses on the feed, orts, faeces and urine (LECO FP-528, LECO Corporation, St. Joseph, MI).

At 10:30 on day 7 of each period blood was extracted from the jugular vein into vacuum tubes containing sodium heparin. The blood was immediately chilled on ice and then centifuged (Sorvall RT6000B, Heraeus Instruments, South Plainfield, NJ, H-100B rotor) at 1500 x *g* for 20 min. The plasma was extracted with pasteure pipettes and frozen to be analysed for AA concentrations by gas chromatography (Chen *et al.*, 2002).

Data were analysed using the MIXED procedure of SAS (2000) with effects for period and treatment and lamb as a random effect. The LS Means option was used and treatment means were also compared to the 12 AA treatment with individual t-tests.

Results and discussion

Table 3 illustrates that dietary protein intake tended to increase when the BCAA`s were simultaneously removed from the infusate. This suggests that the induced AA imbalance by removing the BCAA`s stimulated voluntary feed intake in an effort to correct the imbalances. Feed intakes from the other treatments did not differ from the 12 AA treatment. The 12 AA treatment had the lowest numerical feed intake, suggesting that these lambs experienced the smallest AA imbalances and only ingested what was needed from the diet. Alternatively, it could also suggest that some AA`s were over supplied in the infusate, which depressed voluntary feed intake (Robinson *et al.*, 2000). Total N intake only differed from the 12 AA treatment when all the AA`s were removed from the infusate (GLC treatment).

Table 3 The effects of removing leucine, isoleucine, valine or the entire branched-chain amino acids from postruminal infusions of 10 essential amino acids on nitrogen balance and diet digestibility of growing lambs

Item	Treatment[1]						SEM
	GLC	12 AA	-LEU	-ILE	-VAL	-BCAA	
Nitrogen (g/d)							
Dietary	17.16	15.83	17.77	16.71	16.92	17.90*	
Infusion	0	8.87	7.99	8.64	8.31	7.22	
Total intake	17.16**	24.70	25.76	25.35	25.23	25.12	
Faecal	6.73	6.86	7.44	6.81	6.89	8.43**	0.60
Urinary	6.99**	9.89	10.94*	11.29**	11.56**	9.94	0.46
Retained	3.44**	7.95	7.38	7.27	6.79*	6.75*	0.55
Retained Efficiency[2]	19.21**	32.20	28.74	27.87	27.03*	26.78*	2.07
Diet digestibility (%)							
Dry matter	76.30	75.40	73.86	75.93	76.02	71.88	1.53
Organic matter	78.77	77.56	76.05	78.08	78.36	74.13	1.56
NDF	81.02	78.67	77.50	79.93	80.23	76.38	1.82
N	60.52**	72.77	71.15	73.49	72.89	66.39**	1.77

[1]GLC = no AA`s, only VFA`s and glucose; 12 AA = a mixture of 10 essential AA`s, two nonessential AA`s, VFA`s and glucose; -LEU = Leucine removed from 12 AA; -ILE = Isoleucine removed from 12 AA; -VAL = Valine removed from 12 AA; -BCAA = Branched-chain AA`s removed from 12 AA

[2]Retained Efficiency = (N Retained / N intake)*100

**Different from 12 AA treatment (P≤0.05)

*Tend to be different from 12 AA treatment (P≤0.10)

Faecal N excretion increased 23 % from 6.86 g/d for the 12 AA treatment to 8.43 g/d when all three BCAA`s were removed from the infusate (Table 3). Lambs receiving the –BCAA treatment excreted 34 % of their total N intake in faeces in comparison to 28 % by the lambs receiving the 12 AA supplement. The faecal N excretion of the –BCAA treatment was also substantially higher than when the BCAA`s were individually removed (27 to 29 %). It is not clear why AA absorption was not affected when the BCAA`s were individually deficient in the small intestine, but from this data it is apparent that faecal N excretion only increased, and absorption thus decreased, when the BCAA`s were simultaneously removed from the infusate. The reduced AA absorption by the –BCAA lambs resulted in a substantial protein loss of approximately 10 g/d and highlights the interrelationships between AA`s and the need for a well balanced AA profile supplied in the small intestine.

The high proportion of total N intake excreted in the faeces by the GLC lambs (39 %) corroborates the impact of a well balanced duodenal AA profile on N utilisation, since the better balanced AA profile of the 12 AA treatment remarkably improved N utilisation efficiency (Table 3). Because AA`s are absorbed across the intestinal wall by an active transport mechanism, an imbalanced duodenal AA profile, resulting from excess AA`s, may potentially reduce the absorption of other AA`s (Scrimgeour, 1994). Similarly, high arterial AA concentrations may reduce AA absorption and/or enhance AA utilisation by tissues of the portal drained viscera (Lobley *et al.*, 2001). Either or both these mechanisms would result in reduced AA levels across the portal drained viscera. Removal of the BCAA`s from the infusate probably rendered other AA`s relatively in excess, which may have inhibited the absorption of various essential and, potentially, nonessential AA`s. The protein waste incurred from reduced AA absorption may become even more acute if the liver is unable to correct imbalances in the absorbed AA profile. Imbalanced AA profiles in the post-hepatic blood flow may reduce AA metabolism and consequently increase AA catabolism and urinary N excretion. In contrast, the absorption and use by the digestive tract of threonine, which forms a substantial part of digestive tract secretions (Mukkur *et al.*, 1985), and glycine and serine, which are involved in nucleic acid biosynthesis in the mucosal cells (Perez & Reeds, 1998), seem to be driven by metabolic needs and not concentration mediated mechanisms.

Urinary N excretion increased by 14 % and 17 % to 11.3 and 11.6 g/d, respectively, when isoleucine and valine were omitted from the infusate (Table 3). This increased urinary N excretion as a proportion of total N intake from 40 % for the 12 AA treatment to 45 % and 46 % when isoleucine and valine were removed from the infusate, respectively. The removal of leucine tended to increase urinary N excretion by 11 % to 10.94 g/d or 43 % of total N intake. This confirms that an intestinal deficiency in any single BCAA rendered the absorbed AA profile imbalanced and increased post-absorptive AA catabolism that manifested in higher urinary N excretions and potentially lower N retentions. A valine deficiency had the largest stimulating effect on urinary N excretion, indicating it was the most limiting BCAA in high RDP diets to growing lambs. The increased urinary N losses when the BCAA`s were individually removed from the infusate would result in an estimated protein waste of 6 to 10 g/d. The catabolism of surplus AA`s to urea also requires additional energy, consequently increasing the maintenance requirements of the animal and potentially inhibiting growth.

In contrast to faecal N excretion, the simultaneous removal of the three BCAA`s had no effect on urinary N excretion (9.89 g/d *vs.* 9.94 g/d for the 12 AA and –BCAA treatments respectively; Table 3). Lambs on both treatments excreted 40 % of their total N intakes. Since faecal N excretions already increased by 23 % when the BCAA`s were simultaneously removed, the AA imbalances were possibly already corrected before absorption and less AA`s were catabolised after absorption, resulting in lower urinary N excretions. Lobley *et al.* (2001) reported that the BCAA`s are inefficiently (≤15 %) extracted and metabolised by the liver. Tissues of the digestive tract may thus play an important role in maintaining BCAA homeostasis. Indeed, Goodwin *et al.* (1987) reported that the catabolic enzymes for the BCAA`s, including the rate limiting enzyme branched-chain oxo-acid dehydrogenase, appear in many tissues other than the liver, e.g. muscle, fat and tissues of the portal drained viscera. Although, in the present study, the BCAA`s were deficient and not in excess, some homeostatic AA control mechanism that acts on relative BCAA concentrations appears to be present in the digestive tissues, since faecal N excretion increased substantially when the BCAA`s were simultaneously deficient. However, when the BCAA`s were individually deficient in the small intestine, AA homeostasis appeared to be controlled by post-absorption mechanisms, since urinary N excretions were affected.

The lambs that received no AA supplements excreted 29 % (6.99 *vs.* 9.89 g/d) less urinary N than the lambs on the 12 AA treatment (Table 3). Heger & Frydrych (1989) reported that the utilisation efficiency of AA`s increased when supplied in less optimal quantities. If the AA profile of the GLC treatment was well balanced, one would thus expect the GLC treatment to render the highest N utilisation efficiency and lowest faecal and urinary N excretions. Instead, the lambs receiving the GLC treatment excreted, as a proportion of N intake, more faecal N (39 vs. 28 %; GLC *vs*. 12 AA) and similar proportions of urinary N (41 % *vs.* 40 %; GLC *vs.* 12 AA). These results indicate that the better balanced AA profile of the 12 AA treatment reduced N waste in the faeces and urine and potentially improved the efficiency of N utilisation. Consequently, the AA profile in microbial protein does not support optimum growth of young lambs.

Although retained N was only numerically reduced when leucine or isoleucine was individually removed from the infusate, the concomitant increase in urinary N excretion when each of these two AA`s were deficient in the small intestinal AA supply, suggests a negative impact on N utilisation efficiency (Table 3). The detrimental effects of an imbalanced AA profile on N utilisation were, however, more prominent when valine or the three BCAA`s were simultaneously omitted from the infusate, which tended to decrease retained N by 1.2 g/d. Since the whole empty bodies of woollen breed lambs contain 11.4 % N (DM basis; Nolte & Ferreira; unpublished data) the reduced N retention would result in a growth restriction of 10.5 g/d, if absorbed protein conversion into product (meat & wool) was 100 % efficient. The efficiency of N retention (Retained N/N intake x 100) displayed a similar pattern and also tended to decrease from 32 % to about 27 % with the simultaneous removal of the three BCAA`s or valine. Again, the removal of leucine or isoleucine only had marginal effects on N utilisation efficiency. These results clearly illustrate that AA imbalances in the post-ruminal BCAA supply could substantially decrease N utilisation by the host animal. Valine appears particularly important as a supplement to complement microbial protein, since the impact of a valine deficiency on the efficiency of N utilisation was much larger than for leucine of isoleucine and almost identical to the simultaneous removal of the entire BCAA`s (Table 3; 27.03 *vs.* 26.78). In corroboration, Löest *et al.* (2001) found that valine was limiting in high RDP diets to cattle. In pigs, Moser *et al.* (2000) also reported on the positive impact of valine on litter weight and performance. However, simultaneous removal of the three BCAA`s had the largest detrimental impact on the efficiency of N retention, which

suggests that the BCAA`s might be co-limiting. This illustrates again that some AA`s are interrelated and that the entire essential AA`s should be supplied in the required quantities to achieve optimum production.

Table 3 shows that the removal of the BCAA`s from the infusate, either individually or simultaneously, had no effects on DM, OM or NDF digestibility. The lowest digestibility for each of these nutrients was, however, achieved when the BCAA`s were simultaneously removed. The individual removal of leucine, isoleucine or valine also had no impact on N digestibility, but when the BCAA`s were simultaneously omitted N digestibility decreased from 72.8 % to 66.4 %, which suggest that the BCAA deficiency and subsequent relative excess of other AA`s rendered the small intestinal AA profile imbalanced and, thus, reduced absorption. Scrimgeour (1994) reported that excess AA`s in the small intestine may reduce the absorption of associated AA`s, because AA`s are absorbed across the intestinal wall by active transport. When no AA`s were supplemented N digestibility was only 60.5 %, which also supports the reported effects of small intestinal AA imbalances on AA absorption, since the GLC treatment had the poorest AA balance.

Tagari & Bergman (1978) stated that plasma free AA levels are frequently difficult to interpret because of the multiplicity of factors that could be involved. Studies with dogs suggested that AA`s were transported to the liver within the plasma, while removal from the liver also involved the erythrocytes (Elwyn *et al.*, 1972). Most data on portal blood AA levels only reported the plasma concentrations. If the erythrocytes make a considerable contribution to AA exchanges, then inaccurate conclusions may have been drawn. In contrast, Savary *et al.* (2001) reported that the AA`s within erythrocytes are not necessarily readily available to other tissues. Lobley *et al.* (1996) reported that isotopic enrichments of plasma and blood AA`s indicated that erythrocytes probably only play a minor role in exchanges across the ovine splanchnic tissues.

Berthiaume *et al.* (2001) also reported that the gastrointestinal tissues metabolise more than 30 % of the absorbed AA`s on a net basis, inducing substantial changes from the duodenal to the pre-hepatic AA supply. Further, the post hepatic AA supply cannot be assumed constant, irrespective of dietary protein contribution. Especially where the animals are fed once or twice daily, major fluctuations in AA supply may occur. Lobley

et al. (2001) reported that under circumstances of protein excess, the hepatic removal of N functions at a constant proportion of the supply. The absolute amount of N removed by the liver may, therefore, vary according to supply. In addition, the hepatic extraction differs for each AA. Low extractions are observed for the BCAA`s (≤15 %; Lobley *et al.*, 2001), while the removal of other essential AA`s like phenylalanine and nonessential AA`s like alanine can be nearly complete (Lobley *et al.*, 1995; Lapierre *et al.*, 2000). Because of the low hepatic extraction rates for the BCAA`s, their arterial appearance closely reflects dietary absorption, explaining their acknowledged role as regulators of peripheral tissue metabolism (Harper *et al.*, 1984; Lobley, 1998). Consequently, the peripheral BCAA concentrations may be relatively high shortly after feed intake, resulting in extra-hepatic BCAA oxidation and N waste. The liver may thus not be the exclusive site for maintaining AA homeostasis, since extra-hepatic oxidation of the BCAA`s may take place.

In contrast, Lobley *et al.* (2001) reported that, despite extra-hepatic catabolism within the pancreas of pigs (Le Floc`h *et al.*, 1999) and the kidneys of rats (Ogawa *et al.*, 1991), the oxidative and metabolic capacities of peripheral tissues to dispose of AA excess are limited in sheep. Goodwin *et al.* (1987) conversely reported that the catabolic enzymes for the BCAA`s, including the rate limiting enzyme branched-chain oxo-acid dehydrogenase, appear in many tissues other than the liver, e.g. muscle, fat and tissues of the portal drained viscera. Since >80 % of the oxo-acid dehydrogenase is already in the active form in sheep (Goodwin *et al.*, 1987), an increased BCAA catabolism would require enzyme protein synthesis. Thus, BCAA uptake and catabolism would soon reach a maximum when supply increases. This suggests that the BCAA supply may be extremely sensitive to dietary alterations, since the effect of the liver in maintaining AA homeostasis is considerably less than for the other AA`s. An imbalanced AA profile may not only detrimentally affect N retention, but also the feed intake regulatory mechanism (Harper *et al.*, 1984), potentially limiting animal performance. This emphasises the specific need for a well balanced AA profile in the small intestine.

Table 4 illustrates that an intestinal leucine deficiency increased plasma isoleucine and valine levels by 118 % and 102 %, respectively. Phenylalanine and threonine

Table 4 The effects of removing leucine, isoleucine, valine or all the branched-chain amino acids from postruminal infusions of 10 essential AA`s on plasma AA concentrations of growing lambs

Item	Treatment[1]						SEM
	GLC	12 AA	-LEU	-ILE	-VAL	-BCAA	
Essential AA (μ*M*)							
Arginine	-	-	-	-	-	-	-
Histidine	41.05	57.06	64.01	67.52	61.79	68.18	12.77
Isoleucine	48.80	44.62	97.43**	32.06	49.69	50.66	5.30
Leucine	57.36**	128.68	46.97**	135.63	136.97	42.30**	6.33
Lysine	33.12	70.66	115.34	94.81	85.58	123.22*	27.27
Methionine	13.69**	35.60	41.55	37.20	38.62	44.15*	3.34
Phenylalanine	37.66**	57.28	70.78*	58.32	61.67	71.58**	5.27
Threonine	24.37	51.69	107.58*	86.51**	69.30	87.66*	13.31
Tryptophan	18.98	23.70	29.21	24.46	24.69	29.14	5.00
Valine	118.67**	236.14	476.12**	257.16	64.03**	112.66**	23.44
Total essential AA`s	393.69**	705.43	1048.98**	796.73	592.34	629.55	76.16
Nonessential AA (μ*M*)							
Alanine	128.31**	95.65	134.50**	96.16	98.14	130.66**	9.16
Aspartate	2.04**	3.87	5.81**	5.37	6.20**	8.08**	0.63
Asparagine	56.99**	94.34	177.01**	96.90	37.71**	53.66**	13.21
Cysteine	-	-	-	-	-	-	-
Glutamate	51.54*	37.61	54.35*	50.28*	43.23	56.22*	4.88
Glutamine	455.05	344.65	449.40	388.64	398.16	441.57	60.91
Glycine	510.99	553.83	565.80	570.08	591.71	635.44	39.56
Ornithine	24.74**	49.52	77.50**	66.01	56.25	75.18**	10.13
Proline	66.04*	54.60	68.18**	64.18	60.29	75.08**	4.00
Serine	108.72**	75.30	82.95	86.14	90.17	101.47**	9.17
Tyrosine	26.74	27.96	46.19	34.90	54.88	48.93	10.52
Total nonessential AA`s	1432.73	1339.10	1663.24	1463.50	1438.82	1628.72	115.16
Total AA`s	1826.41	2044.52	2712.22**	2258.83	2031.16	2258.26	183.30

[1]GLC = no AA`s, only VFA`s and glucose; 12 AA = a mixture of 10 essential AA`s, two nonessential AA`s, VFA`s and glucose; -LEU = Leucine removed from 12 AA; -ILE = Isoleucine removed from 12 AA; -VAL = Valine removed from 12 AA; -BCAA = Branched-chain amino acids removed from 12 AA

[2]Retained Efficiency = (N Retained / N intake)*100

-Not determined

**Different from 12 AA treatment (P≤0.05)

*Tend to be different from 12 AA treatment (P≤0.10)

concentrations also tended to increase when leucine was removed from the infusate. The other AA`s were not affected, explaining why the total essential AA concentration in the plasma increased by 49 %. Various nonessential plasma AA concentrations (alanine, aspartate, asparagine, glutamate, ornithine and proline) were also elevated when leucine was deficient. The increased plasma AA levels indicate that these AA`s were less efficiently metabolised, and supports the tendency for an increased urinary N excretion, when leucine was omitted from the infusate. The removal of isoleucine only increased threonine and tended to increase glutamate concentrations in the plasma. A valine deficiency did not increase any essential AA concentrations in the plasma, but raised aspartate and asparagine levels. A leucine deficiency affected the metabolisation of more AA`s than either isoleucine or valine, but, in contrast, the removal of both isoleucine and valine enhanced urinary N excretion more than leucine. Reasons for this phenomenon are unclear and support the difficulties in interpreting plasma AA concentrations (Tagari & Bergman, 1978).

The simultaneous removal of the three BCAA`s increased plasma concentrations of phenylalanine and also tended to increase lysine, methionine and threonine levels. The nonessential AA`s alanine, aspartate, ornithine, proline and serine also increased, and glutamate concentration tended to rise when the BCAA`s were deficient in the small intestine. A BCAA deficiency had a substantial negative impact on the utilisation of various AA`s, which could potentially limit animal response. The increased plasma methionine concentration is of specific importance, since methionine has been identified as a highly limiting AA to growing lambs receiving high RDP diets (Nolte *et al.,* 2004). Increased plasma methionine levels during a BCAA deficiency indicate a reduced methionine metabolisation that may limit animal performance. Additionally, MacRae *et al.* (1993) and Lobley & Milano (1997) reported that phenylalanine and histidine are the AA`s least likely to be in excess in the peripheral blood circulation. The increased phenylalanine plasma level during a BCAA deficiency underscores the degree of imbalance when the BCAA`s are limiting. Interestingly, plasma asparagine concentration decreased, suggesting an improved utilisation efficiency of this AA when the BCAA`s are limiting.

Only the removal of leucine increased the total plasma AA concentrations significantly (Table 4). This was surprising, since the removal of leucine had no significant effect on faecal N excretion, urinary N excretion or the efficiency of N retention (Table 3). From the effects on N excretion and N retention, the individual removal of valine or the simultaneous removal of the BCAA`s from the infusate was expected to increase total plasma AA concentrations. This again corroborates the reported complexity in interpretation of plasma AA levels (Tagari & Bergman, 1978).

In the absence of any AA supplements the plasma concentrations of leucine, methionine, phenylalanine, valine, as well as total essential AA`s and various nonessential AA`s were significantly lower than for the 12 AA treatment. Despite the report of Heger & Frydrych (1989) that AA utilisation efficiency increases when supplied in suboptimal quantities, this was certainly not the case in the present study, since the GLC treatment had the lowest retained N efficiency, highlighting the value of a balanced AA profile in the small intestine. Bach *et al.* (2000) supported the value of a well balanced AA supply by illustrating that the dietary AA profile had a more pronounced effect on N metabolism of dairy cows in early lactation than dietary protein level. Alanine, glutamate, proline and serine concentrations were even higher than for the 12 AA treatment, which underscores the degree of AA imbalance that occurs in microbial protein and contributed to the poor N utilisation efficiency observed for this treatment.

Conclusion

This study clearly illustrated that a valine deficiency substantially reduced the efficiency of N utilisation. Therefore, valine appears to be limiting when microbial protein is the predominant source of AA`s to the small intestine. However, N retention was poorest when the BCAA`s were simultaneously removed from the infusate. This implies that the BCAA`s are co-limiting in high RDP diets where microbial protein is the primary source of amino acids.

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CHAPTER 7

GENERAL CONCLUSION

Currently diets for sheep are formulated on minimum crude protein (CP) levels, without giving any consideration to either the available or metabolisable amino acid (AA) contents of the diet or requirements of the animals. Various dietary, digestive and physiological factors could influence the profile of AA`s reaching the target tissues. This implies that the host animal may receive sufficient CP to supply a specific production function (maintenance, growth, gestation or lactation), but AA imbalances (deficiencies and/or excesses) may still exist. The degree of limitation by the first limiting AA will thus determine the maximum level of animal performance. Amino acid imbalances may also result in reduced feed intakes, retarded growth and even certain toxic reactions. Instead of optimising animal performance, the protein quality of diets based on the CP-system may actually subject the animal to prominent AA imbalances. These imbalances increase the maintenance energy requirements of the animal, since excess AA`s must be degraded and excreted, and consequently reduce the efficiency of nutrient metabolisation. The genetic potential of the animal is thus restricted to the maximum performance allowed by the diet. Therefore, the CP system is widely acknowledged as a proper evaluation system for providing the required N to the animal, but incapable of evaluating protein quality (AA profiles). This study was conducted to establish the essential AA requirements of growing lambs and evaluate the AA profile in the duodenum when microbial protein is the major source of AA`s.

The effect of rumen degradable protein level and source on the duodenal essential amino acid profile of sheep

A large part of sheep farming in South Africa occurs in semi-arid regions with frequent seasonal and sporadic long-term droughts, subjecting the sheep to periods of limited feed availability with low-quality forage as the predominant nutrient source. These forages regularly contain CP levels below 7 %, rendering nitrogen (N) the first limiting nutrient. Because of this severe N deficiency the rumen microbial populations of sheep under these

conditions function at sub-optimal levels. Consequently, these forages are poorly digested and the potentially available energy from cellulose and hemi-cellulose is not completely metabolised. Sheep subjected to these conditions would most probably experience AA deficiencies in the small intestine. Therefore, the first study was conducted to determine the AA profile predominantly derived from microbial protein and evaluate the effects of various rumen degradable protein (RDP) levels and, because of the cost advantage of urea, different true RDP:non-protein nitrogen (NPN) ratios on the qualitative and quantitative characteristics of the AA supply to the duodenum. Sub-optimal RDP levels limited the quantity of AA`s supplied to the duodenum, but had no effect on the AA profile. Isonitrogenous substitution of urea for true RDP (casein) also decreased the concentration of the essential AA`s in the duodenum and thus, the quantity delivered in the small intestine, but again the AA profile was unaltered.

These results show that the AA profile of duodenal protein, primarily derived from rumen microbes, is relatively constant, irrespective of the protein source. Consequently, accurate estimates of microbial protein production on a given diet, as well as fluid and particulate outflow rate from the rumen on that diet would allow equally accurate predictions of the AA supply to the small intestine. If the essential AA requirements of the animal were known for a specific production function (maintenance, growth, gestation or lactation), the deficiency and/or excess of each essential AA could be calculated and corrected by undegradable AA supplementation. Methionine is unfortunately the only synthetic essential AA that is currently successfully protected against rumen degradation, implying that natural, high rumen undegradable protein (UDP) sources are required to correct duodenal AA imbalances. With the world wide pressure to exclude animal proteins, which are the best UDP sources, from ruminant diets, the undegradable AA`s would have to be obtained from protein sources of plant origin (soya oilcake, sunflower oilcake, cottonseed oilcake, canola oilcake, prime gluten and brewers' grains) and fish meal.

In future, South Africa will probably follow the European trend to also include fish meal in the list of prohibited raw materials fed to ruminants, implying that only protein sources of plant origin will be available to supply the required undegradable AA`s in the small intestine. This creates new challenges, since these raw materials not only contain lower UDP levels than animal proteins, but contain a wide variation in the UDP levels of similar

raw materials from different sources. Obviously, variation in UDP content implies a similar variation in the RDP content, which influences microbial protein production and thus, the amount of microbial AA`s supplied in the duodenum. The supplemental requirement for undegradable AA`s will vary accordingly, which creates continuing challenges in balancing the nutrient requirements of the animal. Since analysis of UDP content is not a quick procedure that could be performed rapidly at arrival of the the raw material at the feed mill and before incorporating it into animal feeds, this might create quality assurance problems to commercial animal feed mills.

Production efficiency, empty body composition and carcass yield of Merino and Dohne Merino lambs

In the second study the production efficiencies, empty body compositions and carcass yields of growing Merino and Dohne Merino lambs were evaluated as supporting data on the essential AA requirements of these lambs. If large differences existed in the whole empty body (WEB) composition and protein distribution within the WEB between the two breeds, these differences might help clarify possible dissimilarities in the WEB essential AA compositions of the lambs. The digestion and growth performance of the Merino and Dohne Merino lambs were similar, except that the latter utilised more water, suggesting that Merino lambs are better adapted to harsh environmental conditions. The lambs metabolised ingested dietary energy with equal efficiency, but the Merino lambs had significantly higher N retentions than the Dohne Merino lambs. This suggests that the protein requirements of Merino lambs may be considerably lower than for Dohne Merino lambs, because the former utilises protein more efficiently. The proportional weight contributions of the carcasses of both breeds were remarkably similar and represented just more than 50 % of the WEB weight. The most prominent differences between the WEB compositions of these breeds were the proportionally larger external offals and smaller internal offals of the Merino lambs. Differences in the AA compositions of these components between the breeds, would imply characteristic essential AA requirements for WEB growth of each breed. This would entail that commercial feed mills would have to manufacture breed specific diets, with obvious practical limitations.

The essential amino acid requirements for growing Merino and Dohne Merino lambs

Essential AA analysis of the carcass, internal offal (blood, organs, stomachs and intestines excluding digesta) and external offal (heads, feet, skin & wool) of Merino and Dohne Merino lambs illustrated that individual body components have characteristic AA profiles. Consequently, the carcass, which represented close to 50 % of the WEB protein, does not accurately represent the AA composition of the WEB and is not recommended as a predictor of the AA requirements for WEB growth. The distinct essential AA composition of each body component in conjunction with the different weight and protein allocations to every component resulted in different WEB AA compositions for the two breeds examined. The WEB concentrations of eight from the ten essential AA`s differed between Merino and Dohne Merino lambs. Breed specific diet formulations may therefore be required to allow maximum growth, since feeding the ideal protein of one breed to another, may induce AA imbalances that might limit performance. Once again, the limitations of such a concept in commercial feed mill operations are obvious, rendering the near future commercial application of such knowledge questionable.

The chemical scores of AA concentrations in duodenal protein from high RDP diets illustrated that isoleucine and tryptophan were qualitatively in excess of requirements for both breeds. The detrimental effects of AA excesses, as discussed previously, may manifest in various forms, but seem to invariably restrict animal performance. The relative threonine concentration was also marginally in excess of the Merino lambs` requirements, but was deficient to support optimum growth of the Dohne Merino lambs. The other AA`s were all deficient in terms of the ideal essential AA profile based on the WEB AA compostion. Since AA`s are inter-related, an imbalance (excess or deficiency) in only one AA may limit the utilisation of a single or various related AA`s.

According to the chemical scores the order of the first five limiting essential AA`s in the duodenal digesta of growing woollen lambs receiving high RDP diets was histidine, methionine, leucine, arginine and phenylalanine. The high limitation of histidine and arginine must however, be interpreted with care since the requirements for these AA`s are regularly over estimated. Despite in protein, histidine is also found in non-protein endogenous substances and arginine can be synthesised from glutamine. These two AA`s are, therefore, considered semi-essential, suggesting that they are less likely to be limiting

than the other essential AA`s. Methionine, leucine and phenylalanine may thus be the first three limiting AA`s for growing woollen lambs receiving high RDP diets. The next study evaluated this observation by means of N-retention.

The ability of microbial protein to quantitatively supply in the essential AA requirements for growing, woollen lambs appeared questionable. The quantitative post-ruminal supply of essential AA`s primarily from microbial protein illustrated deficiencies for almost all the essential AA`s for both Merino and Dohne Merino lambs growing at 250 g/d. Only tryptophan slightly exceeded its requirement for the Dohne Merino lambs at this growth rate. The deficiencies of the most limiting AA`s were so severe that almost three times the estimated microbial protein production would be required to meet the animals` needs for these AA`s. This raises questions about the ability of microbial protein to provide in the AA requirements of high producing animals.

However, it is important to realise that the diet used for evaluation of the AA profile and synthesis of microbial protein simulated low-quality forages. Since fermentable energy is the driving force behind microbial protein production, the quantitative AA supply from microbial protein may increase on higher quality diets and reduce the AA deficiencies. Firstly, more substrate will be available to the rumen microbes when higher quality diets are fed, since feed intake is likely to increase. The rates of N and energy release on higher quality diets are also likely to be better synchronised, resulting in an improved efficiency of N utilisation and higher microbial protein yield. A higher feed intake and microbial protein production will also reduce the maintenance requirements of the rumen microbes, since their retention time in the rumen will decrease. It is, however, unlikely that higher quality diets would alter the order of limiting AA`s in microbial protein to growing woollen lambs, because the AA composition in the duodenum when microbial protein was the major source of AA`s was relatively constant.

Limiting amino acids for growing sheep fed a soybean hull-based diet

Although chemical scores are widely used to establish limiting AA`s in animals, and certainly improves our knowledge of amino requirements, it is based on certain assumptions that do not include the complete metabolisation process. The WEB essential AA profile is a static measurement of an animal`s AA status and does not include the flux

of AA`s in and out of free AA pools or the possible differences in turn over rate in various tissues. Therefore, the order of limiting AA`s to growing woollen lambs was re-evaluated by means of protein utilisation efficiency (N retention). The order of limiting AA`s were identified by an abomasal infusion of an AA mixture that would complement microbial protein to simulate the ideal AA profile, based on the WEB essential AA composition, in the duodenum. Each essential AA was in turn removed from the infusate and the consequent decrease in N retention measured. To prevent energy from limiting animal response and thus interfere with treatment effects, and without altering microbial protein production, VFA`s were infused into the rumens and glucose into the abomasums of the lambs. Methionine and at least one branched-chain amino acid (BCAA) was limiting growth in finishing lambs where microbial protein was the predominant source of AA`s. Histidine and arginine were not limiting the performance of growing lambs and thus confirmed that the requirements for these two AA`s are often over estimated, as previously reported. However, this may not be the case for high producing animals in different production stages like gestation of lactation, when the animal`s requirements are potentially different.

Methionine has frequently been identified as a limiting AA to ruminants in different production phases. The inclusion of raw materials with high undegradable methionine contents or rumen protected methionine will therefore, improve the available duodenal AA content and enhance performance. Once the methionine requirements are met, the second limiting AA will become limiting. From this study it was evident that at least one BCAA was limiting growth in lambs. The next study determined which of the BCAA`s were deficient in high RDP diets.

In contrast to the chemical scores, histidine, arginine and phenylalanine were not identified as limiting AA`s to growing lambs receiving high RDP diets. This confirms that chemical scores of duodenal AA concentrations do not consider post absorptive metabolisation efficiencies or differences in AA fluxes in and out of various tissues. Therefore, the evaluation of N retention as a function of the available AA profile portrays more accurate estimates of the limiting AA`s.

Limiting branched-chain amino acids for growing sheep fed a soybean hull-based diet

The reduced N retention when the BCAA`s were omitted from the abomasal infusate warranted solid grounds for further investigation into the impact on N retention when the BCAA`s were individually removed from the infusate. A similar methodology was applied as in the prior study, but the BCAA`s were only removed from the infusate on an individual basis. The removal of each BCAA numerically decreased N retention, suggesting that they might be co-limiting. A valine deficiency resulted in the largest depression in N utilisation efficiency, indicating that it was the most limiting BCAA. This was contrary to the chemical scores that indicated leucine as the highest limiting BCAA. However, supplementing rumen protected methionine and at least valine will improve the performance of growing lambs, receiving high RDP diets. Nevertheless, the largest reduction in N retention was achieved when the BCAA`s were simultaneously removed from the infusate, confirming that the BCAA`s were co-limiting. Growth performance of growing woolled lambs will thus improve when rumen protected BCAA`s are supplemented. Since methionine is the only AA that is effectively protected against rumen degradation, this provides research opportunities to develop protected BCAA`s.

Implications

The absence of an impact on the essential AA profile in the duodenum, pre-dominantly derived from microbial protein, by different RDP levels or RDP:NPN ratios confirmed a relatively constant AA profile in microbial protein. Neither increasing RDP concentrations nor higher substitution levels of NPN for true RDP influenced the AA profile in the duodenum. Deficient RDP, as well as high substitution levels of NPN for true RDP limited the amount of essential AA`s in the duodenum. Protein source may thus affect the quantity of microbial AA`s supplied to the duodenum, but it will not have any impact on the AA profile of microbial protein. The AA supply to the duodenum can thus be accurately calculated if microbial protein production and rumen outflow rate is known.

Because different body parts (carcass, internal offal and externa offal) have characteristic AA compositions, any single body component cannot be used to predict AA requirements for growth. The WEB composition should rather be used to predict essential AA requirements for growing lambs. A major limitation of the ideal protein concept, based

on the WEB AA composition, is the static measurement status of AA content. The WEB AA profile does not consider the differences in AA flux in and out of various tissues or the variation in efficiency of AA metabolisation. Although WEB AA profiles greatly improve our knowledge on the AA requirements for growth, and certainly is a huge improvement on the use of carcass AA profiles to estimate AA requirements for growth, it still contains some inaccuracies and more work on tissue specific AA turn over rates is required.

Prominent differences exist in the weight and protein distribution in the WEB of Merino and Dohne Merino lambs. Despite the relative similarity in the carcass AA compositions of the two breeds, substantial differences exist in the AA profiles of the internal and external offals. Consequently, large differences were identified in the WEB AA compositions of the two breeds. Despite these differences, chemical scores identified similar limiting AA`s (histidine, methionine, leucine, arginine and phenylalanine) in the duodenum for the two breeds when high RDP diets were fed, but the degree of limitation varied between the Merino and Dohne Merino lambs. The identification of hisitidine and arginine as limiting AA`s should be interpreted carefully, since the requirements for these two AA`s are frequently over estimated, rendering them semi-essential.

In order to consider not only the available AA composition in the small intestine, but also incorporate the post-absorptive metabolisation, the effects of single limiting AA`s in the duodenum on N retention were evaluated. The degree of reduction in N utilisation efficiency when each essential AA was removed from the abomasal infusate, identified methionine and at least one of the BCAA`s as limiting to growing lambs utilising high RDP diets. The BCAA`s were co-limiting, but on an individual basis valine was the most limiting BCAA to growing woolled lambs. Natural protein sources with high undegradable methionine and BCAA contents will therefore, improve the growth performance of lambs.

Since RDP is less expensive than UDP and microbial protein remains one of the best balanced AA profiles that could be supplied in the small intestine, optimum microbial protein production should be the first objective in protein nutrition to ruminants. Once optimum microbial protein yield is achieved, the identified AA deficiencies (methionine and the BCAA`s) can be corrected by undegradable AA supplementation from natural

raw materials or synthetic sources. This will improve the efficiency of N utilisation and alleviate the imposed limitations on animal performance to achieve the maximum genetic potential of each animal, given no other nutrients are limiting.

Supplementation of rumen protected AA`s should be carefully implemented, since sufficient supply of the first limiting AA will render the second limiting AA first limiting, which should then be supplemented. Once the requirement for the second limiting AA is met the third limiting AA will become first limiting and needs to be supplemented. Conversely, supplementation of a single AA at any given time could potentially result in excess amounts of that AA relative to the other AA`s. An over-supply of the first limiting AA will result in an imbalanced AA profile, with a consequent reduction in the N utilisation efficiency or even toxic reactions and an associated depressed performance. The limiting AA`s should, therefore, be supplemented simultaneously to correct the entire essential AA profile and enhance protein utilisation.

The day when both supply and need can be quantified in terms of individual AA`s is coming closer. When that happens we will be able to mix a cocktail of supplementary AA`s that will complement the intestinal AA supply and subsequently provide the ideal absorbed protein that will allow maximum production within the limits of the animal`s genetic potential.

Areas for future research

The ability of different tissues to increase or decrease their sensitivity to the vascular AA supply during an AA excess or deficiency may differ. In addition, the blood flow to a target tissue may also vary when AA imbalances occur. Whole empty body essential AA composition, which is a static measurement, may therefore, not be totally accurate in estimating the AA requirements for growth in ruminants. The possible differences in protein turn-over rate in various body organs and tissues, which are not considered in the WEB AA profile, are likely to influence essential AA requirements. Therefore, further research needs to investigate the AA flux in and out of different organs and/or tissues. In this way essential AA requirements for carcass growth, wool growth and other production functions can be estimated, which may result in more accurate predictions of essential AA requirements than the WEB essential AA profile.

The available AA supply to fulfill requirements has frequently been evaluated in terms of the AA`s appearing in the duodenum. However, the AA profile reaching the portal blood circulation may in fact differ substantially from that available in the duodenum. The gastrointestinal tissues selectively and preferentially utilise AA`s during absorption, resulting in altered, and possibly imbalanced, essential AA profiles delivered to the liver and peripheral tissues. An imbalanced AA profile in the duodenum may actually supply the ideal post-hepatic AA profile. To improve the efficiency of N utilisation more information is required on the changes imposed on the duodenal AA profile during absorption across the gastrointestinal wall.

Protein sources of animal origin that comprise fish meal, meat and bone meal, feather meal and blood meal are excellent AA supplements to ruminants. Their AA balance is well matched to the animal`s requirements and highly resistant to rumen degradation. A serious concern about the use of animal-based proteins is the transmission of bovine spongiform encephalopathy (BSE) via the consumption of blood, meat and bone products. The use of animal proteins is therefore prohibited in many countries. Consequently, fish meal is relatively widely used as a source of undegradable AA`s, but is very expensive in comparison to plant proteins. Despite their higher rumen degradability and poorer AA content than animal proteins, protein sources of plant origin are extensively utilised as animal feed components. Cost effective methods of protecting these AA`s against rumen degradation, without decreasing their intestinal digestibility, and/or the development of synthetic rumen protected AA`s would be extremely valuable.

Ingredient composition of the diet significantly affects the order and degree of limiting AA`s, since large differences exist between feed proteins with respect to their rumen degradation and AA profiles. In addition, the quantitative requirement for any specific AA will vary with changing dietary protein concentrations in order to maintain the ideal AA profile. Therefore, the AA requirements of ruminants should consider both the quality and quantity of dietary and microbial AA`s reaching the duodenum and would be best described as a proportion of the available essential AA content in the small intestine. Since these parameters are not constant, more research should be focused on the accurate prediction of dietary undegradable and microbial essential AA supply in the duodenum.

On the technological side the opportunity exists to develop instruments that provide a rapid, but reliable indication of the RDP and UDP content of feedstuffs, as well as the AA composition of the UDP fraction. This will allow more accurate feed formulations, since the natural variation in raw materials will be accounted for.

Despite the identified AA imbalances, it is clear that microbial protein provides an AA profile in the small intestine that is closely related to the WEB protein. Microbial protein is however, unable to provide in the quantitative needs of every AA to support optimum production. These AA imbalances could be corrected via supplementation of exogenous rumen protected AA`s and rumen microbial protein synthesis should be stimulated by ensuring optimal rumen conditions for maximum microbial growth. To what extent can microbial protein production be stimulated by manipulation of rumen conditions (pH, ammonia-N, structural and non-structural carbohydrates, physical effective NDF, dilution rate, etc.)? This opens up numerous research opportunities in the manipulation of rumen pH, the synchronisation of N and energy supply to the rumen microbes, the evaluation of N and energy sources as rumen microbial substrates, fibre type and length in the formation and maintenance of an efficient rumen mat (physical effective NDF), dilution rate etc.

A very urgent need also exists to clarify the complexity of AA supply to the gestating and lactating ewe. The AA composition of protein supplements that would complement microbial AA supply to achieve improved foetus growth, colostrum production, milk production and wool growth needs clarification. The effects of energy source and level, as well as AA composition and concentration on the viscosity of colostrum and the composition of milk and colostrum also provide challenging opportunities.

It is clear that numerous questions still need answering. A lot of work remains to be done to reach the point where we can confidently state that we fully understand N utilisation in sheep. Although this study did not provide all the answers, I believe it improved our knowledge of AA requirements and N utilisation in growing lambs.