

Amino Acid Requirements of Feedlot Cattle According to the Duodenal and Whole Empty Body Essential Amino Acid Profile

by
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SUMMARY

Amino Acid Requirements of Feedlot Cattle According to the Duodenal and Whole Empty Body Essential Amino Acid Profile

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The purpose of this study was to determine the essential amino acid requirements of beef cattle under feedlot conditions through evaluation of the duodenal and whole empty body essential amino acid compositions. To define the ideal protein required for growth, the whole empty body essential amino acid compositions of 8 beef steers (Simmental and Hereford crosses) was investigated. The amino acid composition of the components (carcass, metabolic organs and residual fraction), was pooled relative to their respective mass and protein contribution, resulting in the calculated whole empty body amino acid composition: arginine 6.81; histidine 2.69; isoleucine 4.02; leucine 6.96; lysine 7.43; methionine 2.01; phenylalanine 4.03; threonine 4.01; valine 5.30; tryptophan 0.82. Variations in profiles were found between scientific reports, either indicating that ratios change with growth and implants or possibly through genotype.

The present study's body amino acid ratios were used to estimate dietary amino acid requirements through evaluation of the duodenal essential amino acid compositions from three different maize based feedlot diets. Although there was a general increase in the biological value of protein after rumen fermentation, the duodenal essential amino acids in comparison with the whole empty body recorded deficient/unbalanced profiles of essential amino acids for growth. The chemical scores suggested that the first-to-third-limiting amino acids in the duodenal digesta of beef cattle, that received three different commercially available feedlot diets, were: histidine, lysine, methionine/arginine (Diet 1), histidine, arginine, lysine (Diet 2) and arginine, methionine, histidine (Diet 3). The

disproportionate duodenal amino acid concentrations obtained from the three diets, emphasise the necessity to enhance the intestinal delivery of amino acid profiles through different undegradable protein sources, with the objective to maximise protein utilisation and obtain the genetic potential for optimal growth in feedlot cattle.

When amino acid requirements and flows to the duodenum were simulated using the Cornell Net Carbohydrate and Protein system (CNCPS), predictions indicated that lysine amino acid flow was limiting the metabolizable allowable average daily gain in Diet 1 and 3. Predicted profiles indicated that the order of limitation was: lysine, arginine, histidine (Diet 1), lysine, arginine, histidine, methionine (Diet 2) and lysine, arginine, histidine (Diet 3). The predicted profiles were in accordance with observed duodenal values, except for methionine that was observed limiting in Diet 1 and 3; however, the sequence and extent of limitation varied. Results indicate that prediction models have potential in predicting requirements; however there are still limitations for use to accurately define requirements for particular EAA's.

From the present study, it is clear that the protein accretion was constrained by quantity and/or disproportionality of amino acids available for absorption. Further research should therefore be directed towards obtaining a more desirable array of amino acids to the lower digestive tract that is digestible, absorbable and an economically viable option for the feedlot operator.

SAMEVATTING

Aminosuurbehoefte van Voerkraalbeeste Volgens die Duodenale en Totale Leë Liggaam Essensiële Aminosuur Profiel

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Die doel van hierdie studie was om die essensiële aminosuur vereistes van vleisbeeste onder voerkraal toestande te bepaal deur die evaluering van die duodenale en totale leë liggaam essensiële aminosuursamestellings. Om die ideale proteïene wat vir groei benodig word te definieer, is 'n ondersoek ingestel na die totale leë liggaam essensiële aminosuursamestellings van 8 vleisbees osse (Simmetaller en Hereford kruise). Die aminosuursamestelling van die komponente (karkas, metaboliese organe en residuele fraksie), se massa en proteïen bydrae is gebruik om die totale leë liggaam aminosuursamestelling soos volg te bereken: arginien 6.81; histidien 2.69; isoleusien 4.02; leusien 6.96; lisien 7.43; metionien 2.01; fenielalanien 4.03; treonien 4.01; valien 5.3; triptofaan 0.82. Variasies in profiele tussen wetenskaplike verslae is gevind. Dit dui daarop dat groei en inplantings of moontlik genotipe 'n invloed op profiele kan hê.

Die huidige studie se liggaam aminosuurverhoudings is gebruik om die diëet aminosuurbehoefte te skat deur evaluering van die duodenale essensiële aminosuursamestellings van drie verskillende mielie-gebaseerde voerkraal diëte. Alhoewel daar 'n algemene toename in die biologiese waarde van die proteïen ná rumen fermentasie was, het die duodenale essensiële aminosure in vergelyking met die totale leë liggaam, ongebalanseerde profiele van essensiële aminosure vir groei getoon. Die chemiese tellings toon aan dat die eerste-tot-derde-beperkende aminosure in die duodenale inhoud van vleisbeeste wat drie verskillende kommersieel beskikbare voerkraal diëte ontvang het, soos volg is: histidien, lisien, metionien/arginien (Dieet 1),

histidien, arginien, lisien (Dieet 2) en arginien, metionien, histidien (Dieet 3). Die ongebalanseerde aminosuur konsentrasies wat in die duodenum van die drie diëte verkry is, beklemtoon die noodsaaklikheid om die intestinale lewering van aminosuurprofiële te verbeter deur verskillende nie-degradeerbare proteïen bronne te voer. Die doelwit moet wees om proteïen verbruik te optimaliseer en daardeur die genetiese potensiaal vir optimale groei in voerkraalbeeste te bereik.

Met die simulering van aminosuurbehoefte en vloeï na die duodenum van die drie standaard voerkraal diëte met behulp van die "Cornell Net Carbohydrate and Protein System" (CNCPS), het voorspellings gewys dat lisien aminosuurvloeï die metaboliseerbare toelaatbare gemiddelde daaglikse toename in Dieet 1 en 3 beperk het. Voorspelde profiële wys dat die volgorde van beperking soos volg sou wees: lisien, arginien, histidien (Dieet 1), lisien, arginien, histidien, metionien (Dieet 2) en lisien arginien, histidien (Dieet 3). Die voorspelde profiële was in ooreenstemming met die waargeneemde duodenale waardes, behalwe vir metionien wat beperkend was in Dieet 1 en 3; die volgorde en mate van beperking was egter verskillend. Resultate wys dat voorspellingsmodelle die potensiaal het om behoeftes te voorspel. Vir die akkurate definisie van behoeftes vir spesifieke essensiële aminosuure is daar egter nog beperkinge.

Uit die huidige studie, is dit duidelik dat proteïenneerlegging deur die hoeveelheid en/of oneweredigheid van geabsorbeerde aminosuure beperk is. Verdere navorsing moet dus fokus op die verkryging van 'n meer geskikte profiël van aminosuure wat verteerbaar, opneembaar en 'n ekonomiese lewensvatbare opsie vir die voerkraalbestuurder is.

DEDICATION

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LIST OF ABBREVIATIONS

AA	- Amino acid
AATISS	- Amino Acid content of tissue
ADF	- Acid Detergent Fibre
ADG	- Average Daily gain
ADIN	- Acid Detergent Insoluble Nitrogen
Arg	- Arginine
ATP	- Adenosine Triphosphate
BW	- Body Weight
CNCPS	- Cornell Net Carbohydrate Protein System
CP	- Crude protein
CW	- Cell Wall
DM	- Dry Matter
DMI	- Dry Matter Intake
EAA	- Essential Amino Acid
EAAG	- Efficiency of Amino Acid utilisation for Growth
EBM	- Empty Body Mass
EBW	- Empty Body Weight
ECP	- Endogenous Crude Protein
EQSBW	- Equivalent Shrunk Body Weight
EQEBW	- Equivalent Empty Body Weight
FSBW	- Final Shrunk Body Weight
His	- Histidine
Iso	- Isoleucine
Leu	- Leucine
LM	- Live Mass
LW	- Live Weight
Lys	- Lysine
ME	- Metabolizable Energy
Met	- Methionine
MJ	- Mega Joules
MO	- Micro-organisms

MCP	- Microbial Crude Protein
MP	- Metabolizable Protein
MPS	- Microbial Protein Synthesis
N	- Nitrogen
NCW	- Non Cell Wall
NDF	- Neutral Detergent Fibre
NP	- Net Protein
NPg	- Net Protein requirement for growth
NPN	- Non-Protein Nitrogen
NSC	- Non-Structural Carbohydrates
PB	- Protein content of empty body gain
PDI	- Protein truly Digestible in the small Intestine
Phe	- Phenylalanine
RE	- Retained Energy
RDP	- Rumen Degradable Protein
RPAA	- Metabolizable Amino Acid requirement for growth
RPN	- Net Protein required for growth
SBW	- Shrunk Body Weight
SC	- Structural Carbohydrates
SD	- Standard Deviation
SWG	- Shrunk Weight Gain
TDN	- Total Digestible Nutrients
Thr	- Threonine
Trp	- Tryptophan
UDP	- Rumen Undegradable Protein
WEB	- Whole Empty Body

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CHAPTER 1

GENERAL INTRODUCTION

All animals require amino acids which form the building blocks of proteins required for optimum growth and maintenance (Kung & Rode, 1996). Protein and amino acid requirements in growing animals are defined as an aggregate of integral components constituting whole-body protein and amino acid accretion, synthesis and secretion for endogenous uses, (maintenance, necessary enzymes, hormones, etc.), and amino acid oxidation (Chen & Ørskov, 1994; Robinson *et al.*, 1996). The availability (quantity and/or quality) of amino acids and efficiency of amino acid utilization influences protein synthesis and accretion in growing animals (NRC, 1996). Numerous factors effect both quality and proportion of essential amino acids required by the animal and supplied by the diet. Level of feed intake, anabolic agents, ionophore affects, digestibility of the diet, balance of energy substrate and amino acid absorption, sex and stage of animal growth all contribute to a dynamic function of requirement and supply (Ainslie *et al.*, 1993; Fox & Barry, 1996).

As amino acid requirements at tissue level are an important consideration (Schingoethe, 1996), various attempts have been directed towards defining an ideal pattern of absorbed amino acids that is believed to exist for maintenance and growth (NRC, 2000). Reeds (2000) define the need for essential amino acids as the product of the rate of protein deposition and the amino acid composition of the protein being deposited. Furthermore, it is recognized that the pattern of amino acids required for body protein accretion, is closely correlated to the amino acid composition of the whole body protein itself (Fuller, 1996). Therefore, in terms of growth, the essential amino acid composition of the whole empty body could serve as an ideal example of the amino acids required for body protein accretion (Fuller 1996).

Each animal has a maximum genetic capacity for protein deposition, depending on age, live weight, and physiological state. The extent to which maximum deposition can be achieved is dependent on the ratio in which energy and protein are supplied as well as the amino acid composition of the protein (Tamminga & Verstegen, 1996). However, the translation of tissue-level amino acid requirements to dietary amino acid requirements in terms of ruminants is complex due to dynamic rumen nitrogen metabolism and their

impact on the extensive microbial modification on the quantity and quality of protein reaching the duodenum (Kung & Rode, 1996). The metabolizable protein system (MP), (NRC, 1989) accounts for rumen degradation of protein and separates requirements into the needs of rumen micro-organisms and that of the animal (Wilkerson *et al.*, 1993). Metabolizable protein (the quantity of true protein or amino acids absorbed after post-ruminal digestion) is supplied by microbial protein (MCP), by-pass rumen undegradable protein (UDP) and endogenous protein (NRC, 1996).

In terms of amino acid nutrition in ruminants, there is a quantitative and qualitative component, both merged and essential for maximum performance (Sloan, 1997). Protein accretion is constrained by quality and/or proportionality of absorbed amino acids, even in diets balanced to optimize rumen fermentation and to provide protein in excess of NRC requirements (NRC 1996). Hence, both quality and proportionality of amino acid availability are important to achieve maximum energy-allowable average-daily-gain. Robinson *et al.* (1996), demonstrated that an increase in the total mass of amino acids available at the site of absorption is required to meet tissue requirements and genetic capacity for protein synthesis and deposition in young rapidly growing cattle. Furthermore, a recent surge of interest is devoted to formulating diets to provide ruminants with a desired array of amino acids in the small intestine (Merchen & Titgemeyer, 1992). The pattern of amino acids absorbed in the intestine is an important determinant of production and efficiency of feed nitrogen utilisation (Schwab 1996). In ruminants, various limiting amino acids that are considered first limiting for production and utilisation have been cited in literature: methionine (Met), lysine (Lys), and threonine (Thr) (Richardson & Hatfield, 1978); arginine (Arg), histidine (His), Lys, leucine (Leu), isoleucine (Ile), valine (Val), and the sulphur amino acids (Merchen & Titgemeyer, 1992); and Arg, His, Lys, and Met (Storm & Ørskov, 1984). The wide range of results from these studies suggests the need for further research as different amino acids are limiting and co-limiting in different feeding and physiological situations (Boisen *et al.*, 2000). According to Rulquin & Vérité (1996) the refinement of knowledge of absorbed nutrient flow is a fundamental step towards progress in nutritional research.

There are different protein systems available that predict the total amount of essential amino acids absorbed from the small intestine (ARC, 1984; NKJ, 1985; NRC, 1985; Madsen, 1985). The CNCPS (Fox *et al.*, 1992; Russell *et al.*, 1992; Sniffen *et al.*, 1992)

amino acid sub model, adopted in conjunction with the CNCPS model for Level II of the *Nutrient requirements for Beef Cattle* (NRC, 1996), was developed to predict the absolute flow of each of the essential amino acids and thus determines net requirements under different environmental conditions. The French PDI system (INRA, 1989) considers lysine and methionine requirements and expresses amino acids as a percentage of the total supply of absorbed amino acids. Information concerning the supply of and requirements for individual amino acids is however limited (Oldham, 1984; Zinn & Shen, 1998). Accurate economic projections are dependent on accurate prediction of performance, which in turn is dependent on the ability to describe and account for the variables that influence requirements for cattle (Fox *et al.*, 1988).

The improvement of the efficiency and economy of protein utilization forms an integral part of integrating essential information into existing feeding models that will improve the efficiency of dietary protein utilization (Schwab, 1996). Improving the efficiency of protein and nitrogen utilization, while striving for optimal productivity, is a matter of practical concern. Incentives for considering and enhancing profiles of absorbable essential amino acids when formulating diets for cattle include: (1) Reduced feed costs per unit of lean tissue gain. This is accompanied with improved feed conversion efficiency as the use of absorbed amino acids for protein synthesis is increased when the profile of the limiting amino acids are improved (Oddy *et al.*, 1997; Lapierre *et al.*, 2000); (2) The total quality of absorbed amino acids required for protein synthesis and other essential functions is reduced. This provides an opportunity to reduce the amount of UDP that must be fed to achieve a fine level of tissue production. Reducing UDP not only has the metabolic advantages of reduced absorption of "surplus" amino acids but also of creating "space" in the diet to meet other critical needs of ruminal fermentation (e.g., more fermentable carbohydrate for the host animal), resulting in increased dry matter intake (Christensen *et al.*, 1994; Schwab 1996; Tamminga & Verstegen, 1996).

The development of a dynamic and progressive beef industry is an essential element in sustaining South Africa's economy and in keeping agriculture viable for our growing population. Considering the limitation of natural resources (e.g., protein feeds) in South Africa, any basic and applied research focusing on developing strategies to improve the efficiency of feed utilization by beef cattle (and growth) would be a positive step towards achieving this goal. Knowledge of amino acid requirements, coupled with a sensitive

system for predicting amino acid passage to the intestine, would permit combining supplemental sources of UDP in such a way that total diet UDP, together with ruminally synthesised microbial protein, would optimise the balance of intestinal amino acids in a predictable way (Schwab 1996). Models should be developed, refined and validated so that in the future these approaches can be used to allow more accurate predictions of daily amino acid requirements (NRC, 1996).

The objective of this study is to: (a) determine the essential amino acid requirements of beef cattle through evaluation of the whole empty body essential amino acid composition in order to derive the ideal protein requirement for growth (Chapter 3): (b) to compare the essential amino acid profile of the duodenal digesta contents of three maize based feedlot diets, to detect the essential amino acid imbalances for whole empty body growth of beef cattle under South African feedlot conditions (Chapter 4, a quality approach) and (c) to evaluate the diets using available models to predict the essential amino acid requirements and identify the limiting amino acids of beef cattle receiving the three different feedlot diets (chapter 5, a quantity approach).

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CHAPTER 2

LITERATURE REVIEW: ESSENTIAL AMINO ACID REQUIREMENTS AND SUPPLY

1. ESSENTIAL AMINO ACIDS

At tissue level, protein nutrition involves amino acid metabolism that induces a cellular requirement for dietary amino acids to be incorporated into body protein (NRC, 1985). Non-essential amino acids (dispensable) are characterized by amino acids that can be synthesised within the body from metabolites of intermediary metabolism and from the surplus of amino groups (Schwab, 1996; Boisen *et al.*, 2000). Essential amino acids (indispensable) in contrast, either cannot be synthesised by animal tissues, or if it can, not in sufficient amounts to meet metabolic needs (Schwab, 1996). Figure 1 gives an overview of the synthetic pathways of amino acids, whereby it partitions the essential amino acids (EAA) as: lysine, methionine, threonine, tryptophan, isoleucine, leucine, histidine, phenylalanine, valine and arginine. A continuous supply of these EAA's, plus a sufficient supply of nitrogen (N) for synthesizing dispensable amino acids are essential for maintenance and production (Boisen *et al.*, 2000). A moderate deficiency of one or more of the non-essential amino acids generally does not affect the overall efficiency of use of amino acids for protein synthesis. However, deficiencies can occur when cystine or tyrosine is in short supply and when methionine and phenylalanine are limiting (Schwab, 1996). Methionine and phenylalanine are precursors for the synthesis of cystine and tyrosine, thus when the latter are in short supply, a deficiency of the corresponding EAA's will be induced. (Campbell *et al.*, 1997). Information concerning precursors and product conversions is essential when balancing diets for amino acids and determining when cysteine/cystine or tyrosine in undegradable protein can substitute for methionine and phenylalanine in cases of obligatory use (NRC, 2000). In many cases, methionine requirements are overestimated because assays do not accurately reflect the amounts of cystine and cysteine precursors in practical diets (Baker, 1989).

Ruminants are perceived to have no theoretical requirement for preformed protein or amino acids in diets due to the production of microbial protein (Kung & Rode, 1996). However, when microbial protein production is low, or if the requirements are high,

microbial protein may be limiting in amino acids needed by the host. Thus like all mammals, ruminants require an exogenous source of EAA's at tissue level (Buttery & Foulds, 1985; Merchen & Titgemeyer, 1992). Knowledge of amino acid requirements (amount of amino acids that must be absorbed to meet the needs for maintenance and growth) and the flow (supply) of amino acids to the small intestine is a fundamental approach towards amino acid nutrition in ruminants (Chen & Ørskov, 1994).

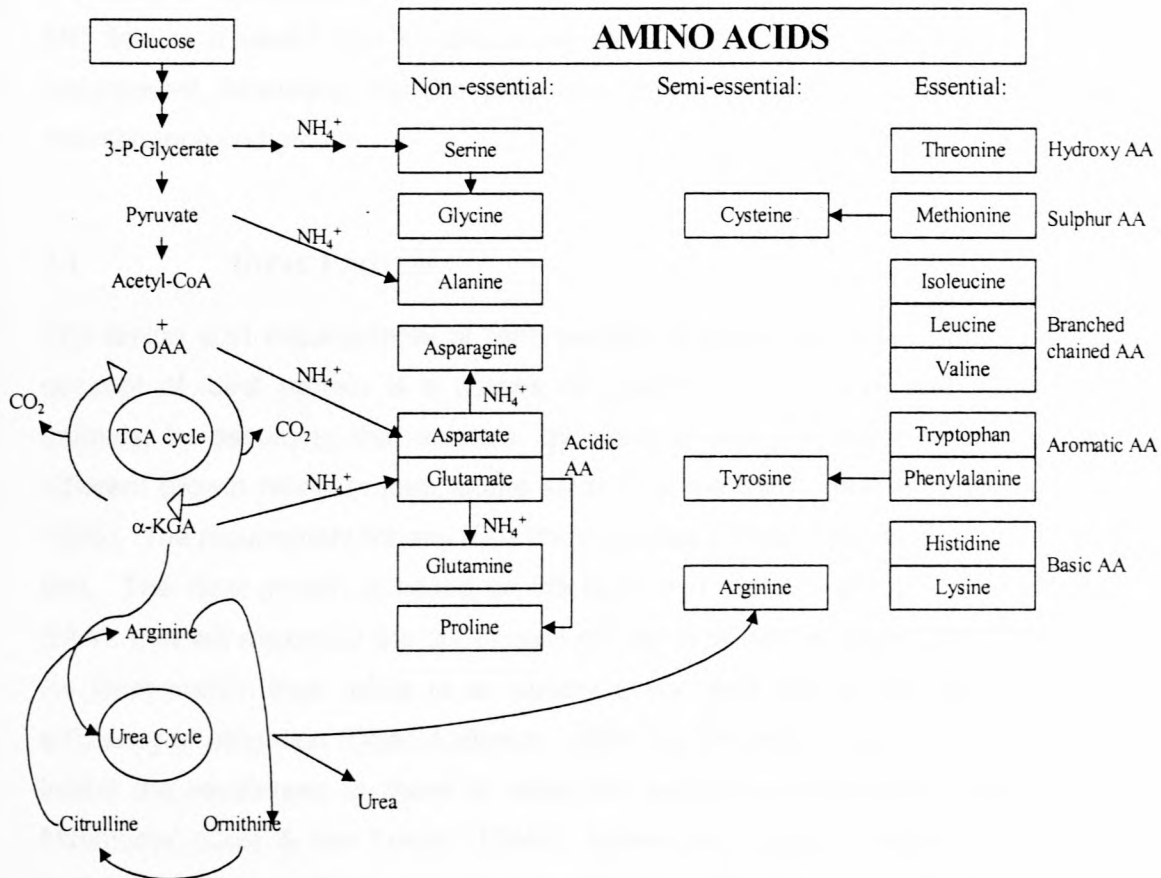


Figure 1. Essential, semi-essential and non-essential amino acids according to Boisen *et al.* (2000)

2. AMINO ACID REQUIREMENTS

The nutritive value of metabolizable protein (MP) for cattle is determined by its profile of EAA's and probably also by the contribution of total EAA's to MP (Schwab, 1996). Requirements for absorbed EAA's can be defined using the classical factorial method (O'Connor *et al.*, 1993; Schwab, 1996) or according to the ideal protein method for

specific amino acids (Rulquin & Vérité, 1993; Rulquin & Vérité, 1996). The ideal protein methods express requirements of individual EAA's as percentages of MP (Rulquin & Vérité, 1993) or as percentages of duodenal EAA's (Schwab, 1996) relative to the amino acid composition of the whole empty body. While factorial methods, which are based on theoretical grounds, express requirements in absolute flows per day. According to Schwab (1996), the determination of ideal profiles of absorbable amino acids is a prerequisite to balance diets for MP. Determining the amino acids as a percentage of MP flow is a useful tool in calculating the specific amino acid need from the MP requirement, assuming that the proportion of amino acids required is the same for maintenance and growth.

2.1 IDEAL PROTEIN

The amino acid requirements of farm animals are influenced by various factors. The concept of ideal protein is a means of specifying the amino acid requirements of animals, by assuming that animals, by virtue of sex, genotype or environment and different growth rates; require amino acids with the same relative proportions (Fuller, 1996). The requirement for any EAA therefore has a fixed proportion to the others in the diet. The ideal protein is based on the tenet that there is an optimal combination of EAA's that will maximize the conversion efficiency of dietary protein into tissue protein. An ideal protein thus refers to an dietary amino acid profile that leads to maximum efficiency of utilisation (Chen & Ørskov, 1994), as the under supply of a single EAA will inhibit the responses to those in adequate supply, as stated by Lieberg's 'Law of Minimums' (Cole & van Lunen, 1994). Boisen *et al.* (2000) further defines an ideal protein as: "The perfect ratio among individual EAA's and N required for optimal performance". Fuller (1996) describes the ideal protein as a statement of EAA requirements in proportional relationship to the requirement of lysine. Lysine is chosen as a reference for ideal protein for several reasons (Baker & Ham, 1994): (1) Methionine and lysine are generally considered limiting in most ruminant diets (Rulquin & Vérité, 1996), (2) Analysis of lysine in feedstuffs is straightforward (Baker & Han, 1994; Mack *et al.*, 1999), (3) Lysine is only used for body protein accretion, thus is not influenced by the relative proportions used for maintenance and growth (Mack *et al.*, 1999), (4) Lysine and methionine have frequently been studied as potential limiting amino acids under a variety of conditions forming a large body of information (Rulquin & Vérité, 1996).

According to Ferreira *et al.* (1999a), the ideal protein concept is applicable to the ruminant species as protein metabolism at tissue level (Iburg & Lebzién, 2000) in principle is similar to that of the ruminant and non-ruminant. The basic concept of amino acid balancing is interpreted by comparison of the amino acid flow to the small intestines with the amino acid requirements for the synthesis of tissue.

The ideal amino acid requirements for production can be obtained based on the amino acid composition and the quantity of amino acids produced (Chen & Ørskov, 1994). Thus in terms of whole growth, amino acid composition of whole body protein has been defined as an ideal example for the amino acid composition of dietary protein resulting in better amino acid utilisation (Mäntysaari *et al.*, 1989; Hussein *et al.*, 1991). The determination of the amino acid composition of body protein is an established method to balance the EAA's required for growth in mammals (ARC, 1981; Cole & Van Lunen, 1994). According to Boisen *et al.* (2000), weight, daily gain, sex and genotype, environment and health status all influence amino acid requirements. However, most changes in amino acid requirements do not lead to changes in the relative proportion of the different amino acids. Bikker *et al.* (1994) and Fuller (1996) however argue that there is a difference in the distribution of individual amino acids as a result of the differences between amino acid patterns between carcass, bone, hide and organ protein each with their unique characteristic amino acid pattern. These ratios change in absolute and relative terms during growth or with nutrition, which in turn will have an effect on whole body amino acid composition (Bikker *et al.* 1994). The ideal protein is therefore not the same for animals at all stages of maturity or all rates of production. These considerations need to be taken into account when defining ideal protein (Fuller, 1996).

2.2 FACTORIAL APPROACH

Requirements for metabolizable EAA's for growth, using factorial methods (O'Connor *et al.*, 1993), are dependent on the amino acid composition of tissue (Ainslie *et al.*, 1993) that is applied to maintenance and growth MP requirements. Factorial systems first estimate net requirements for protein and then rely on efficiency factors (transfer coefficients) to transform net requirements into metabolizable (absorbable) amounts required for maintenance and growth (Fox *et al.*, 1992; Bell *et al.*, 1995; NRC, 1996).

2.2.1 Amino acid requirements for maintenance

The amino acid requirements for maintenance are based upon the amino acid composition of tissue (Ainslie *et al.*, 1993) and depend on the prediction of MP requirement for maintenance that is, a function of non-dietary protein in the intestinal tract and the metabolic fecal N, scurf N (skin, skin secretions and hair) and urinary endogenous losses due to turnover of tissue proteins (NRC, 1985; 1996). Maintenance requirements are influenced by weight, breed type, previous nutritional treatment, level of production, tissue and external insulation, and effective ambient temperature (NRC, 1996). Therefore, in growing animals the ratio for maintenance and production changes with age and production status (Tamminga & Verstegen, 1996). According to Boisen *et al.* (2000), a tentative estimate of MP requirements can be obtained using the metabolic weight of an animal. Wilkerson *et al.* (1993) determined the MP requirements for maintenance of a 253kg steer as 3.8g MP/kg BW^{0.75} ($3.8 \times \text{BW}^{0.75}$ g/d, where body weight (BW) is expressed in kilograms), using growth as criterion. The efficiency factor used to calculate maintenance and MP requirements is 100% for metabolic fecal protein and 67% for scurf and urinary protein (Fox *et al.*, 1992).

Amino acid requirements for maintenance have not been determined directly for cattle and remain unresolved. According to Chen & Ørskov (1994) the amino acid composition required for tissue maintenance is based on the tenet that turnover of protein is mainly in tissues, thus maintenance requirements are possibly similar to that of tissue growth. However, the pattern of muscle protein is considerably lower in sulphur amino acids and threonine, compared to protein in endogenous losses and hair that contributes to maintenance requirement and therefore underestimates the respective amino acids (Boisen *et al.*, 2000).

2.2.2 Amino acid requirements for growth

Amino acid requirements for tissue growth (RPAA) are a function of the percentage of each amino acid (AATISS) in the net protein accretion (PB) and thus depend on the accuracy of predictions of protein retained (RPN) during growth ($\text{RPAA} = \text{AATISS} \times \text{RPN}$) (NRC, 1996). These values are multiplied by the net protein requirement and divided by various transfer coefficients (EAAGi) to determine absorbed amounts. Net

protein retained from accretion, and therefore required for tissue growth, has been estimated from the body composition of growing animals and is a product of weight gain (EBG) and the protein content of gain (PB), hence $(RPN = PB \times 0.01 \times EBG)$ (NRC, 1996). However, the composition of gain, proportion of protein = $0.248 - 0.0264 \times RE$ ($0.0635 \times EBW^{0.75} \times EBG^{1.097}$) and fat, changes with stage of growth (percentage of mature weight) and rate of gain. Therefore, the protein content of gain, at a particular weight, will depend on the rate of gain to an expected finished weight (NRC 1996; Tamminga & Verstegen, 1996; Tolman, 1996). Net protein required for gain (NPg, g/d) could therefore be estimated as: $SWG \times (268 - (29.4 \times (NEg/SWG)))$. The amino acid efficiency for growth (EAAG), used as a transfer coefficient to convert net requirements to metabolizable values, is estimated as a function of equivalent shrunk body weight (EQSBW), where at a given EQSBW, the EAAG is the same for all EAA's (NRC, 1996).

2.2.3 Efficiency of utilisation

The estimation of protein requirements is dependent on the efficiencies with which absorbed protein is used for specific purposes (Beever, 1996). The ARC (1984) and the NRC (1985) estimate MP content in empty body gain with efficiencies of 0.67 and 0.8 respectively, while the NRC (1996) considers the conversion of MP to net protein (NP) for gain as 0.5. The efficiency of MP use for growth is based on the biological value (the amino acid pattern of a protein source) in relation to the efficiency of use of an "ideal mixture of amino acids" for protein deposition. The proposed efficiency of utilisation of an ideal amino acid mixture is 1.00 for maintenance, and 0.85 for all other synthetic processes. Relative values of absorbed amino acid mixtures, as opposed to ideal mixtures, are 0.59 for growth. The ARC (1984) assumes an average biological value of 66% for absorbed amino acids. Biological values will also vary with the source of undegradable protein (UDP) in the diet as the relative amino acid balance defines the biological value (NRC 1996). The biological value of MCP is relatively high and strongly influences the biological value of the MP in many diets. According to Ainslie *et al.* (1993), each amino acid has its unique efficiency factor, which is depended on the biological value of the protein and the amino acid supply relative to its requirement. Therefore, amino acids differ in efficiency of absorption and utilization (Ainslie *et al.*, 1993; Parker, 2001). Furthermore, the efficiency of use for gain is not likely to be constant across body

weights (maturity) and rates of gain. The efficiency decreases with an increase in body weight (Ainslie *et al.*, 1993; Wilkerson *et al.*, 1993). The overall efficiency value (Biological value \times efficiency of use of ideal protein) is derived by the equation: $NP = 83.4 - (0.114 \times EQEBW)$. MP required for gain is therefore based on empty body composition of the gain with efficiency that varies as a function of adjusted body weight (Wilkerson *et al.*, 1993). The efficiency with which amino acids are used to meet the requirements for tissue accretion is a function of how efficiently it utilizes each amino acid, which depends on age, the proportion of each amino acid relative to its requirement, the kind of amino acid, and the ratio of dispensable to indispensable amino acids (Ainslie *et al.*, 1993).

3. PROTEIN SUPPLY

3.1 RUMEN FERMENTATION

Ruminal degradation of dietary feed carbohydrate and crude protein (CP) is a significant factor influencing ruminal fermentation and amino acid supply to the ruminant (NRC, 2000). The MP available in each feeding situation is primarily depended on the unique rates of digestion and passage of the individual feed carbohydrate and protein fractions that are fed (Sniffen *et al.*, 1992). Each feed composition is therefore described by carbohydrate and protein fractions and by their degradation rates that are used to compute the amount of structural carbohydrates (SC) and non-structural carbohydrates (NSC) available for each of the two microbial pools (SC and NSC fermenting bacteria) in the Cornell Net Carbohydrate and Protein System (CNCPS) (Sniffen *et al.*, 1992). Knowledge of ruminal kinetics is important when balancing for amino acids in diet formulation. Nutrients entering the rumen can only disappear from the rumen by two routes: by passage or by digestion (Van Soest, 1994). The extent of digestion: $kd/(kd + kp)$ or passage: $kp/(kd + kp)$, can be calculated by applying the pools of protein and carbohydrate fractions to the digestion and passage percentages (NRC, 1985). The carbohydrates fermented in the rumen determine the amount of microbial protein produced, which determines the microbial need for degradable protein (Russell *et al.*, 1992; Sniffen *et al.*, 1992). The degradable protein fractions, represents the rumen degradable protein (RDP) requirement for supporting optimal utilisation of NSC and SC to meet the respective microbial growth requirements based on carbohydrate pools

fermented. Figure 2 represents the fate of dietary CP resulting from carbohydrate and protein fermentation in the rumen.

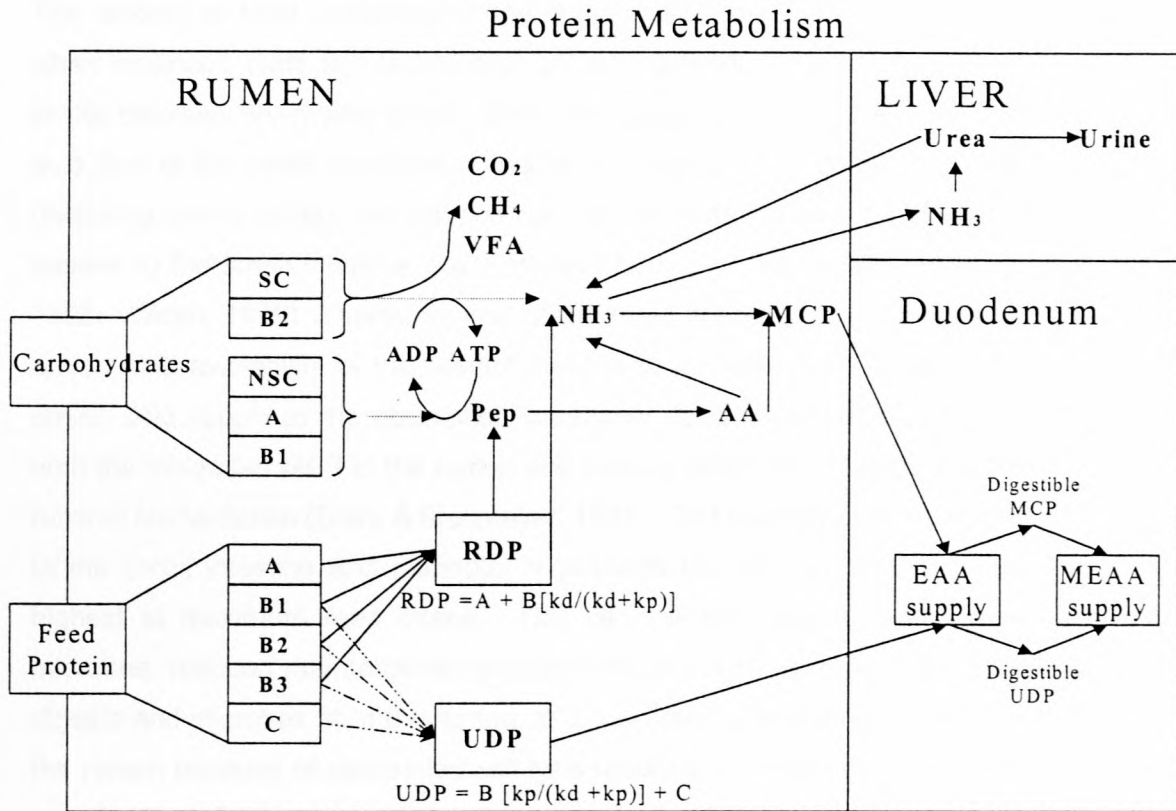


Figure 2. The metabolism of dietary protein in the ruminant (compiled from the Cornell Net Carbohydrate and Protein System)

The growth of the two microbial pools (SC-NSC) can be predicted based on the integration of rates of digestion and passage, which in turn determines the N requirements of each pool, microbial protein production and MP available from MCP. The degraded and UDP pools can also be predicted, which are used to determine N balance for each of the microbial pools, feed protein escaping undegraded and digested post ruminally, MP derived from UDP (Russell *et al.*, 1992; Sniffen *et al.*, 1992). MP is adjusted to the impacts of feed intake due to the effect of rate of passage.

3.2 DRY MATTER INTAKE

The amount of feed consumed is the nutritional factor that limits animal performance when balanced diets are fed to high producing feedlot cattle. A positive correlation exists between dry matter intake (DMI), microbial protein synthesis (MPS) and amino acid flow to the small intestines (Clark *et al.*, 1992a). The quantity of dietary nutrients (including amino acids), that escape ruminal fermentation and the amount of MCP that passes to the small intestine are increased by higher feed intake (Huntington & Prior, 1985; Volden, 1999). Therefore, one of the most important mechanisms for increasing amino acid availability of the feedlot steer is to increase feed intake. An increase in amino acid supply to the absorption sites when feed intake increased, is the result of both the increased MPS in the rumen and a larger proportion of dietary protein escaping ruminal fermentation (Clark & Klusmeyer, 1991). The quantity of microbial N that passes to the small intestine and microbial N passage per unit fermented organic matter is highest at maximum feed intake. This can be attributed to faster growth rates of microbes, reduced maintenance requirements of rumen microbes, a faster passage of digesta and microbes from the rumen, and a decreased recycling of energy and N within the rumen because of decreased cell lysis resulting in more energy and N available for growth (Clark & Klusmeyer, 1991; Dewhurst *et al.*, 2000).

3.3 METABOLIZABLE PROTEIN SUPPLY

Total MP (absorbable amino acids) required to meet maintenance and production, is a combination of microbial true protein (intestinal digested microbial amino acids) flowing to the small intestine and the digestible dietary amino acids escaping ruminal degradation (Hvelplund & Hesselholt, 1987; Sniffen *et al.*, 1992). Increasing the total amino acids available at the site of absorption is required to meet tissue requirements and genetic capacity for protein synthesis and deposition in growing cattle (Robinson *et al.*, 1996) as increased amino acid absorption is positively correlated with weight gain (MacRae & Ulyatt, 1974). Furthermore, changing the profile of absorbed amino acids from one of primarily microbial origin (winter annual forage diets; MacRae and Ulyatt, 1974), to one of dietary and microbial amino acids has also improved N balance (Gill & Beever, 1982). Therefore, it is at the site of absorption that the optimum profile must be

achieved in order to optimise protein synthesis. The EAA content of MP and flow to the duodenum of the individual digestible EAA's could be calculated by the knowing:

1. The predicted contribution of each protein fraction (microbial protein, UDP fraction of each feedstuff, and endogenous protein) because of rumen fermentation;
2. The predicted EAA composition of each fraction;
3. The digestibility coefficients assigned to microbial protein, the UDP fraction of each feedstuff, and endogenous protein; and
4. The predicted flows of MP (Wilkerson *et al.*, 1993).

According to the NRC (1989), MCP as provided by bacteria and protozoa contains 80 % true protein of which 80 % of the true MCP is digestible. Consequently, the conversion of MCP to MP is assumed to be 64 %. The UDP CP fraction is assumed to be 100 % (NRC, 1989), however the digestibility of the UDP fraction ranges from 50 to 100 %. The contribution of UDP to MP is therefore variable and dependent on feed type. The NRC (2000) assumes endogenous crude protein (ECP) consists of 50 % true protein that is 80 % digestible, hence obtaining a 40 % conversion of ECP to MP.

3.3.1 MICROBIAL PROTEIN AMINO ACID SUPPLY

Microbial protein is considered an important protein source, as they generally have a good amino acid profile (Clark *et al.*, 1992b) and supply from 50 to 90 % of the MP required by beef cattle, depending on the UDP content of the diet (NRC, 1985; AFRC, 1992). Accurate estimation of available microbial amino acids in the duodenum requires reliable estimation of the ruminal microbial yields, the amino acid composition of microbial protein and the digestibility of microbial amino acids (O'Connor *et al.*, 1993).

3.3.1.1 Microbial protein synthesis and passage

Microbial protein output to the small intestine as a result of rumen fermentation is a function of various integrated factors. Stern & Hoover (1979) and Erasmus (1992), mentioned that diet composition, frequency of feeding, feed intake, availability of N and energy, UDP and soluble and insoluble protein affect rumen dilution rate and pH, which in turn will effect MPS. The availability of specific amino acids (methionine and

cysteine), volatile fatty acids and sulphur may influence the output of microbial protein as some species of rumen bacteria have specific requirements for amino acids or possibly short peptides and branched chain volatile fatty acids (Russell *et al.*, 1983; Atasoglu *et al.*, 1999; Buttery & Foulds, 1988; Dijkstra *et al.*, 1998). According to Parker (2001), factors affecting feed values are dependent on solubility (quick degradable vs. slow degradable fractions), the rumen outflow rate and feeding level. Factors also affecting MPS (Parker, 2001) are dependent on the energy and N supply and synchronisation within the rumen.

3.3.1.2 Nutrient requirements of micro-organism

When formulating diets for ruminants, one must consider the requirements of the rumen micro-organisms (MO) and those of the host, as ruminants have two metabolic systems (Chalupa *et al.*, 1996). Optimising nutrient supply to the rumen for maximum MPS is a key element of modern diet formulation systems (Parker, 2001). The composition of the diet (availability of readily fermentable energy, peptides and amino acids) affects the proportion of microbial protein formed *de nova* (Atasoglu *et al.*, 1999).

3.3.1.2.1 Carbohydrates

The amount of MP and amino acids derived from bacterial yields, depends mainly on the rate, amount and rumen fermentability of feed carbohydrate fractions (Hoover & Stokes, 1991) as only carbohydrates or products of carbohydrate fermentation provides energy (ATP) at rates sufficient for growth of most rumen microbes (Nocek & Russell, 1988; Coomer *et al.*, 1993; Chase, 1996). The CNCPS categorizes MO into those that ferment fibre carbohydrates (cellulose and hemicellulose) and non-fibre carbohydrates (starch, pectin and sugars) (Russell *et al.*, 1992). Each SC and NSC micro-organism has its own maintenance requirements of 0.05 and 0.15 g of carbohydrates per g of micro-organism respectively. Therefore, carbohydrate type (structural vs. non-structural) may influence microbial maintenance requirements due to differences in rates of fermentation (microbial growth rate) and rate of passage and because of the effects on rumen pH (NRC, 1996). Considering that the rate of growth is proportional to the rate of carbohydrate fermentation and that fibre is fermented slower than non-fibre carbohydrates, the SC bacteria grow slower than the NSC bacteria (Russell *et al.*, 1992).

Effective NDF of diets affects calculated MP through the impacts of ruminal pH (estimated from eNDF) on degradation rate of available fibre, which in turn affects yield of fibre digestion bacteria, and the impact of diet effective NDF (as a surrogate for ruminal pH) on maximum bacterial yield (Fox *et al.*, 1992). Lipids and ensiled (fermented) sources do not provide energy for microbial growth, although microbial growth efficiency may be increased when supplemental sources of fat are fed (Nocek & Russell, 1988; AFRC, 1992).

The NRC (1996) uses carbohydrate digestion as a predictor of MPS in the rumen, as rumen available protein is related to the quantity of microbial dry matter produced per unit of carbohydrate digested (Chase, 1996). The quantity of microbial protein synthesised can be estimated from the deduction that MCP is 130g/kg of total digestible nutrients (TDN) intake ($MCP = 0.13 \times TDN$) hence the requirement for RDP is $1.18 \times MCP$ yield (NRC, 2000). According to Parker (2001), MPS is between 12.6 – 17 g/100g TDN in the diet.

3.3.1.2.2 *Microbial requirements for N substrates*

Rumen microbes can synthesise protein when provided with RDP and/or a non-protein nitrogen source (NPN) such as urea, which is first broken down to ammonia-N. Within the ruminant environment the two primary sources of N available to MO for growth yield are ammonia and amino acids (Rook & Thomas, 1983). Although most rumen bacteria can grow with ammonia as the only N source, they need some preformed amino acids and carbon skeletons along with N-urea to achieve maximal microbial growth rates (Russell *et al.*, 1992). Rumen MO as a whole population has no absolute requirement for amino acids. The bacteria requiring some amino acids may, in mixed populations, scavenge amino acids released by the degradation of protein by other species through cross-feeding. Most rumen bacteria have simple N requirements and can synthesise the majority of their amino acids from ammonia, via glutamate dehydrogenase or alanine dehydrogenase, particularly if carbon skeletons are available (Slater *et al.*, 1979; Atasoglu *et al.*, 1999). However, functional groups of bacteria exist that have different nitrogenous requirements. Bacteria that ferment cellulose and hemicelluloses (SC fermenters) grow slowly and have an absolute requirement for ammonia-N (Mackie & White, 1990; Russell *et al.*, 1992) but may however, require branched chain volatile fatty

acids (NRC, 1985), which are supplied by amino acid degradation. Bacteria that ferment sugars, starch and pectin (NSC fermenters) can use ammonia as their nitrogenous nutrient but their growth is enhanced if ruminal degradable protein (peptides and amino acids) is available (Russell *et al.*, 1992; Chalupa *et al.*, 1996). The peptide-N requirement of N in NSC-bacteria is 64%, while the ammonia-N requirement of N in SC bacteria is 100% and N in NSC bacteria is 36%.

Compared to ammonia, ruminal amino acids and peptides have been observed to increase the rate and amount of MCP synthesised (Clark & Klusmeyer, 1991; Russell *et al.* 1992; Cruz Soto *et al.*, 1994; NRC, 1996). Quantities of amino acid N increased both microbial protein production and the energetic efficiency of microbial growth (Griswold *et al.*, 1996). When a deficiency of ammonia, amino acids or peptides occurs for microbial growth, the ruminal fermentation becomes uncoupled and the microbes continue to degrade organic matter to obtain energy. Microbial protein may however not be synthesised because the availabilities of energy and N are not synchronized. MPS and the efficiency of MPS may therefore be depressed (Clark & Klusmeyer, 1991). Further evidence exists on the stimulator effect of amino acids and peptides on growth rate and yield for ruminal MO on rapidly degradable energy sources (Russell *et al.*, 1983; Cruz Soto *et al.*, 1994). However, results on slow fermented substrates have not been conclusive. Chikunya *et al.* (1996) demonstrated the stimulatory effect of peptides on microbial growth when the diet was supplied with fast degradable fibre and not in the case of slow degradable fibre. Thus, MO whose growth would be supported by amino acids would be those fermenting NSC (Russell *et al.*, 1992). Microbial requirements for N substrates can thus be affected by the basal diet (NRC, 2000), explaining variable results obtained in literature.

A lack of amino acids and peptides is, however, unlikely to be a problem in typical beef cattle diets, as most typical diets contain sufficient amounts of RDP that meets the amino acid, peptide and branched-chain amino acid requirements of MO (NRC, 1996). Finishing cattle thus require optimum levels of RDP to provide the proper balance of ammonia-N and true protein for optimal MPS. According to Chalupa *et al.* (1996), 50% of degradable protein should represent soluble protein in order to avoid deficiencies of ammonia in the rumen. The NRC (1996) regards TDN as a percentage of RDP to range from 7.1-10.9 % in order to achieve maximum gain, presumably to maximise microbial

protein. The requirement for RDP (including NPN) is considered equal to the MPS (NRC, 1996), therefore Hoover & Stokes (1991) argue that for maximum microbial growth, RDP needs to represent 14-15 % of diet dry matter as microbial growth tends to be limited when RDP is less than 10-11% diet dry matter. Components of the diet can potentially be manipulated to optimise ruminal fermentation and increase the passage of amino acids to the small intestine (Clark & Klusmeyer, 1991).

3.3.1.2.3 *N* synchronization

The efficiency of MCP synthesis is a critical factor in meeting the protein requirements of beef cattle economically (NRC, 1996). The synchronization of N and energy release within the rumen is regarded as a key factor in improving the efficiency of N utilisation by maximizing the amount of microbial protein, dietary protein and amino acids that pass to the small intestine. It is important that a readily available energy source is supplied at the same time in an amount commensurate with the microbial N requirement to sustain microbial production. Microbial protein production in growing steers has been improved by the addition of RDP to forage diets that in turn has increased intake and production (Parker, 20001). A combination of high degradable carbohydrate and high RDP that are both readily available, maximized bacterial N production (Aldrich *et al.*, 1993). The precise relationship between rates of energy and N release and overall rumen efficiency remains unresolved.

3.3.1.3 Amino acid content of microbial protein

According to Bergen *et al.* (1968), Ørskov (1982) and Storm & Ørskov (1984) the amino acid composition of isolated rumen bacteria is relatively constant. In contrast, various other researchers state that microbial protein does not have a constant amino acid composition (Clark *et al.*, 1992b; Buttery & Foulds, 1985; Hvelplund & Hesselholt, 1987; Chase, 1991; Martin *et al.* 1996). Bacteria contain average values of 62.5% CP, 21% carbohydrates, 12% fat and 4.4% ash, the values are dependant on the balance of microbial species that are regulated mainly by ingredients fed (Russell *et al.*, 1992). Amino acid N as a percentage of CP varies between 0.7 and 0.78 (Hvelplund & Madsen, 1996). The amino acid compositions of rumen bacteria are represented in Table 1. Guzzon *et al.* (1997) suggest that the variations of profiles are affected by substrate

fermented, as different microbial populations degrade SC and NSC. O'Connor *et al.* (1993) described that the amount of amino acids appearing at the duodenum, is the product of each bacterial fraction (CW and NCW) produced multiplied with the amino acid content of each fraction. It is therefore important to identify the factors responsible for variation when predicting the amino acid profile of microbial protein after feeding different diets (Boisen *et al.*, 2000).

Table 1 The amino acid content of ruminal bacteria (gAA/100g CP)

	(gAA/100g CP)									
	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val	Trp
NRC (1996) ¹⁾	5.1	2.0	5.7	8.1	7.9	2.6	5.1	5.8	6.2	-
Chase (1991) ²⁾	11.7	3.4	4.6	6.1	10.6	1.6	3.2	4.6	5.5	-
Goedenken (1990) ³⁾	6.7	1.4	5.5	8.5	7.9	2.9	5.2	6.4	6.1	1
Le Hénaff (1991) ⁴⁾	4.9	1.8	5.9	7.7	8.0	2.5	5.3	5.8	6.2	-
Cell wall ⁵⁾	3.8	1.7	4.0	5.9	5.6	2.4	4.2	3.3	4.7	1.6
Non-cell wall ⁵⁾	7.0	2.7	5.9	7.5	8.2	2.7	5.2	5.6	6.2	1.6

¹⁾ NRC (1996) values adopted from Clark *et al.* (1992b)

²⁾ Chase (1991)

³⁾ Goedenken *et al.* (1990)

⁴⁾ Le Hénaff (1991) cited by Rulquin *et al.* (1998)

⁵⁾ Average composition of cell wall and non-cell wall (O'Connor *et al.*, 1993)

3.3.1.4 Intestinal digestibility of bacterial protein

The digestion of the MP entering the small intestine is an important determinant of the amino acid fraction available for intermediary metabolism (Van Bruchem *et al.*, 1989), as the small intestine is regarded as the active site for amino acid absorption (NRC, 1985). Cell walls (CW) of bacteria contain 25% CP that is indigestible in the small intestine and excreted quantitatively in the faeces. Therefore, it is assumed that the amino acids contained in the cell wall protein are unavailable for digestion (O'Connor *et al.*, 1993). The remaining 75% of bacterial CP is digested in the small intestine, which consists of 15% nucleic acids and 60% true protein (AFRC, 1992). Only bacterial true protein is a source of metabolizable amino acids (NRC, 1996) as this represents the non-cell wall (NCW) fraction that is completely digested in the small intestine (O'Connor *et al.*, 1993). Although the composition of microbial protein may vary, the apparent intestinally digestibility of individual amino acids derived from microbial CP remained relative constant, with an average value of 85% (Schwab, 1996; Storm *et al.*, 1983; Boisen *et al.*, 2000). The NRC (1985) describes bacterial true protein (BTP) as 0.8 MCP, while 80% of

this fraction is accepted as digestible (NRC, 1985). The NRC (1996) therefore suggests that the digestible amino acid content of bacteria is 0.64, calculated as $0.8 \text{ MCP} = \text{BTP} \times 0.8$ digestibility of BTP. Storm & Ørskov (1984) calculated an utilisation level of 80% for absorbed microbial amino acids. O'Connor *et al.* (1993) describes the metabolizable amino acids available for absorption as a product of the quantity of NCW appearing at the duodenum, that is completely digested in the small intestine, with the amino acid content of this fraction.

The goal of feeding high producing ruminants should be directed towards optimising ruminal fermentation, hence maximising microbial protein production (Russell *et al.*, 1992; Hoover & Webster, 1996). This is due to the fact that microbial protein is the predominant source of absorbed protein delivered to the small intestine (NRC, 2000). In addition, microbial protein contains a near ideal balance of EAA's (Schingoethe, 1996) that reflects the need for tissue production (Hoover & Webster 1996). Furthermore, feeding and management factors that maximise rumen microbial growth will promote a ruminal environment that will maintain animal health (avoid digestive upsets such as acidosis), and enhance dry matter intake and production.

Results obtained by Mäntysaari *et al.* (1989), Merchen & Titgemeyer (1992) and Hussein *et al.* (1991) concurred that amino acid provision from microbial protein could be inadequate to fulfil the amino acid requirements to support rapid growth in steers or milk production in high producing dairy cows (NRC, 1985). Houseknecht *et al.* (1992) found that protein accretion was limited by amino acid quantity and/or quality, despite the fact that they were fed diets balanced to optimise rumen fermentation and to provide protein in excess of the current NRC (1996) requirements. Growth performance may thus be less than optimal under certain circumstances (i.e. when microbial protein production is low or if requirements are high) unless amino acids of non-microbial origin are supplied (Merchen & Titgemeyer, 1992). Based on Klofenstein *et al.* (1991), the metabolizable amino acid requirements of finishing cattle are met by microbial protein and rumen escape protein of maize. Amino acid profiles that complement rumen microbial protein suggests the potential to alter amino acid profiles absorbed from the small intestine by manipulation of diet formulations. Thus, when UDP supplements are fed, the amino acids available for absorption may be increased quantitatively and qualitatively (Keery *et al.*, 1993). Klofenstein (1993) however stresses the importance that the degradable

protein requirements for microbial growth should logically be met before a response to escape protein can be realized.

3.3.2 RUMEN UNDEGRADABLE PROTEIN

Although balancing the diet to improve the efficiency of N capture in the rumen and therefore the flow of microbial protein to the duodenum, additional UDP is needed to meet MP requirements in high producing animals for optimal production (Parker, 2001). Considering that the UDP fraction is only about 15-40% of the total protein entering the duodenum, the amino acid composition of this fraction would thus have to be markedly different from microbial protein to significantly affect the overall amino acid composition of the total protein (Van der Walt & Meyer, 1988). It is important to accurately estimate the amino acid composition of the rumen escape proteins if the amino acid requirements are to be met, but not exceeded. Parker (2001) compared rumen bacterial amino acid composition with that of duodenal protein and concluded that dietary UDP contribution could influence the overall pattern of amino acids present. According to Parker (2001) the factors that affect the feeding values of UDP include: (1) extent of rumen degradability, (2) proportion of insoluble (e.g. lignin bound) N, and (3) amino acid composition of bypass material.

3.3.2.1 Undegradable dietary protein in the small intestine

All feed sources other than NPN supplements contain a relative fraction of UDP. Estimates of the UDP fraction in feeds are often assumed constant (NRC, 1989), however the UDP value of a specific feed ingredient also varies depending upon the interaction between the rate of degradation and the rate of passage (Chase, 1991; Chase, 1996; O'Mara *et al.*, 1997; Chiou & Wu, 1999). As a result, intestinal digestibility of undegradable dietary protein varies within the same feed according to the degradability in the rumen. Considering the variable pattern of feed EAA's and its contribution to of EAA in total CP, most of the variation in the profile of EAA entering the duodenum is accounted for by the amount and the EAA composition of the UDP in the diet (Rulquin & Vérité, 1993). The NRC (1996) calculates the UDP content of a specific feed as a function of the related digestion and passage rates and the different protein pools (Sniffen *et al.*, 1992). Different dietary proteins are made up of different protein

sub-fractions that vary in amino acid composition, rates and degree of degradation and intestinal digestibility that contribute to a different EAA profile to total CP entering the small intestine (Chalupa & Sniffen, 1996; Chase, 1991; Stern *et al.*, 1994). The detergent system developed for analysis of carbohydrates in conjunction with extraction using a borate-phosphate buffer (Sniffen *et al.*, 1992) offers a system to describe protein fractions (A, B₁, B₂, B₃ & C) each with its unique degradation rates. Altering these protein fractions in diets will affect animal responses (Chalupa & Sniffen, 1996). The amino acid profile of the insoluble protein fraction is likely to be similar to the amino acid profile of the UDP fraction as this fraction is more likely to escape rumen fermentation (Tedeschi *et al.*, 2001). The availability of fractions B₁, B₂, and B₃ in UDP is based on their respective degradation rates (Roe *et al.*, 1990) and represent the potential digestible true protein. Ruminal digestion rates and passage rates of protein are integrated to predict the MP value of each feed ingredient (NRC, 1996).

3.3.2.2 Amino acid content of rumen undegradable protein

In contrast to microbial protein, there are large differences in the quality of UDP (Stern *et al.*, 1994). The amino acid profile and digestibility of the UDP fraction differs from the original feedstuffs after rumen incubation (Erasmus *et al.*, 1994a; O'Mara *et al.* 1997; Schingoethe, 1996; Chiou & Wu, 1999). However according to Crooker & Fahey (1987) and Tedeschi *et al.* (2001) there seems little difference between the EAA composition of most intact feeds and the UDP content of the relative feed. According to Chen & Ørskov (1994), the amino acids that are more resistant to ruminal degradation will be enriched in the undegraded protein flowing to the small intestine. Methionine and phenylalanine had higher resistance to rumen degradation (Chiou & Wu, 1999). According to Erasmus *et al.* (1994b), methionine degradation is dependent on the feedstuffs. Branched-chain amino acids (leucine and isoleucine) tend to have lower degradability than other amino acids (Erasmus *et al.* 1994a; Chiou & Wu, 1999) while arginine, cystine and glutamine has higher and valine, isoleucine and threonine lower, effective degradabilities compared to total amino acids (Weisbjerg *et al.*, 1996). The amino acid composition of UDP fractions thus tended to increase in isoleucine, leucine, phenylalanine, threonine, tyrosine and valine compared to the feed amino acid profiles after ruminal degradation (Hvelplund & Hesselholt, 1987). Within feeds rumen degradation influences the post ruminal provision of specific absorbable amino acids more than post ruminal digestion (Erasmus *et al.*,

1994b). The resistance of some individual amino acids to ruminal degradation varied between two of the same protein concentrates (Robinson, 1996).

3.3.2.3 Digestibility of rumen undegradable protein

The intestinal digestibility of the UDP fraction varies (Stern *et al.*, 1994; Hvelplund & Hesselholt, 1987; Weisbjerg *et al.*, 1996) but remains an important determinant of response to UDP (Calsamiglia & Stern, 1995). True digestibilities are variable depending on the acid detergent insoluble nitrogen (ADIN) content of the raw materials contributing to the UDP fraction. The CNCPS partitions UDP in intestinal available (A, B1 and B2, 100%; B3, 80%) and unavailable fractions (C), Fraction C is the insoluble acid detergent protein (Erasmus *et al.*, 1994a). Oldham (1996) describes the ARC's (1984) digestible UDP nitrogen as 0.9 (UDP – ADIN), while the NRC (1996) assumes that the UDP fraction is 80 % digestible. With the assumption that all of the proteins are equally digested, the amino acid availability for absorption parallels amino acid flow to the small intestines (Schingoethe, 1996). However, among the different undegradable protein sources, variations in digestibility of individual amino acids are found between and within feeds. With respect to individual amino acids, arginine has higher, and threonine, cystine and glycine lower, digestibility compared to total amino acids, whereas the remainder of the individual amino acids was similar to total amino acids (Hvelplund & Hesselholt, 1987). Apparent digestibility is affected by the physiological state of the animal (Coffey *et al.*, 1989). Apparent intestinal digestibilities of most EAA's were above 70% but lower for histidine, methionine and valine (Table 2). For histidine, the high content in endogenous secretions could constitute this effect (Froidmont *et al.*, 2000). Intestinal CP digestibility is used to represent individual amino acid digestibility in modern protein evaluation systems (Boisen *et al.*, 2000).

Table 2 Apparent and true digestibility of essential amino acids

Item	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Apparent digestibility (%) ¹⁾	79	66	75	75	78	60	74	68	65
True digestibility (%) ¹⁾	90	90	85	87	89	80	83	78	77

¹⁾ Apparent digestibility (%) of amino acids (Froidmont *et al.*, 2000)

²⁾ True digestibility (%) of amino acids in the small intestine of cows (Rohr & Lebzien, 1991)

Amino acid profiles that complement rumen microbial protein suggests the potential to alter amino acid profiles absorbed from the small intestine by manipulation of diet formulations. Thus, when UDP supplements are fed, the duodenal amino acid profile may be altered (Santos *et al.*, 1998; Tagari *et al.*, 1995; Merchen & Titgemeyer, 1992) and EAA's available for absorption may be increased quantitatively and qualitatively (Robinson, 1996; Keery *et al.*, 1993). Clark *et al.* (1992a) concluded that a combination of UDP sources could supplement the limiting amino acids for growth. Merchen & Titgemeyer (1992) supports the feasibility of altering intestinal amino acid profiles via protein source selection, particularly when diets based on feeds low in CP are fed which is typical of feedlot diets. Meissner & Du Plessis (1992) however reported that bypass protein obtained from typical protein sources used in South African feedlots would not significantly influence amino acid provision in the duodenum of beef cattle. Selecting UDP and rumen protected amino acid supplements with the goal of approximating the ideal profile of absorbable amino acids should enhance prediction of animal responses to changes in MP and increase considerably ones ability to fine-tune diets for amounts of UDP. According to Meissner & Du Plessis (1992) an optimal level of 35-40% UDP at 11.7 % CP is needed in feedlot diets to ensure optimal growth and efficiency. The change in amino acid composition, which may occur during incubation of proteins and the subsequent availability of amino acids in the small intestines, must thus be evaluated (Erasmus *et al.*, 1994a). Predictive models based on *in vitro* analysis have been developed that estimate the amount of individual amino acid flow to the duodenum and amino acid composition of duodenal digesta (Rulquin *et al.*, 1998; Chalupa & Sniffen, 1996).

If total absorbable amino acids limit production, any protein source high in undegradable protein may suffice to improve performance. When specific EAA's limits performance, certain protein sources may be superior in their ability to supply these amino acids, as protein sources vary greatly in the quality of individual amino acids that they supply for absorption (Merchen & Titgemeyer, 1992). It is therefore important to select supplementary protein sources that complement and not duplicate the amino acid profile of bacterial protein and are resistant to ruminal degradation, yet digestible in the small intestine.

Possible reasons for a lack of response to increased UDP is that when these sources are fed, microbial protein production in the rumen is often compromised (McCarthy *et al.*, 1989; Clark *et al.*, 1992b). The low digestibility of UDP sources in the small intestine can also reduce availability of EAA's (Erasmus *et al.*, 1994b). The variable response to UDP sources is dependent upon the ability of the amino acid balance in the proteins flowing to the duodenum to meet the requirements for growth (Parker, 2001). It is thus essential to consider the quality of UDP sources and amino acid balances when utilizing the UDP concept (Chase, 1996). In order to improve the profile of the amino acid in the duodenum:

1. Microbial protein synthesis must be maintained by including urea or a ruminally degradable protein source in the diet to provide -N and other products of protein breakdown for microbial populations in the rumen (McCarthy *et al.*, 1989).
2. Complementary protein must be resistant to ruminal degradations yet available in the small intestine (Merchen & Titgemeyer, 1992).

3.3.3 ENDOGENOUS PROTEIN

Significant amounts of endogenous protein-N (9 to 12 % of NAN) may pass to the small intestine (NRC, 2000) that may also contribute to the amino acids in the small intestines. Contributions of endogenous protein to the duodenum include; (1) mucoproteins and saliva, (2) epithelial cells from the respiratory tract, (3) cellular debris from sloughing and abrasion of the epithelial tissue of the mouth, (4) cellular debris from sloughing and abrasion of the epithelial tissue of the omasum and abomasum, and (5) enzyme secretions in the abomasum (NRC, 2000). The NRC (2000) adopted the equation to predict endogenous N (g/d) as $1.9 \times \text{DMI (kg/d)}$. The EAA composition of endogenous protein estimated by Larsen *et al.* (2000) as cited by Boisen *et al.* (2000) is represented in Table 3.

Table 3 Amino acid composition of endogenous protein (g AAN/100g AAN)

Source	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Larsen <i>et al.</i> (2000) ¹⁾	6.5	5.3	2.7	4.5	7.8	0.0	1.5	3.5	4.4

¹⁾ Amino acid composition of endogenous protein (Larsen *et al.*, 2000) cited by Boisen *et al.* (2000)

3 PROTEIN UTILIZATION

A main objective in ruminant nutrition is to improve the utilization of total N in the animal through maximising the proportion of retained N from N intake (Hvelplund & Madsen, 1996). In ruminant nutrition, one needs to look at the utilization of absorbed amino acids in the ruminant body and at the microbial utilization of the available N in the rumen. Figure 3 represents a model for N metabolism and protein utilization of MCP and UDP.

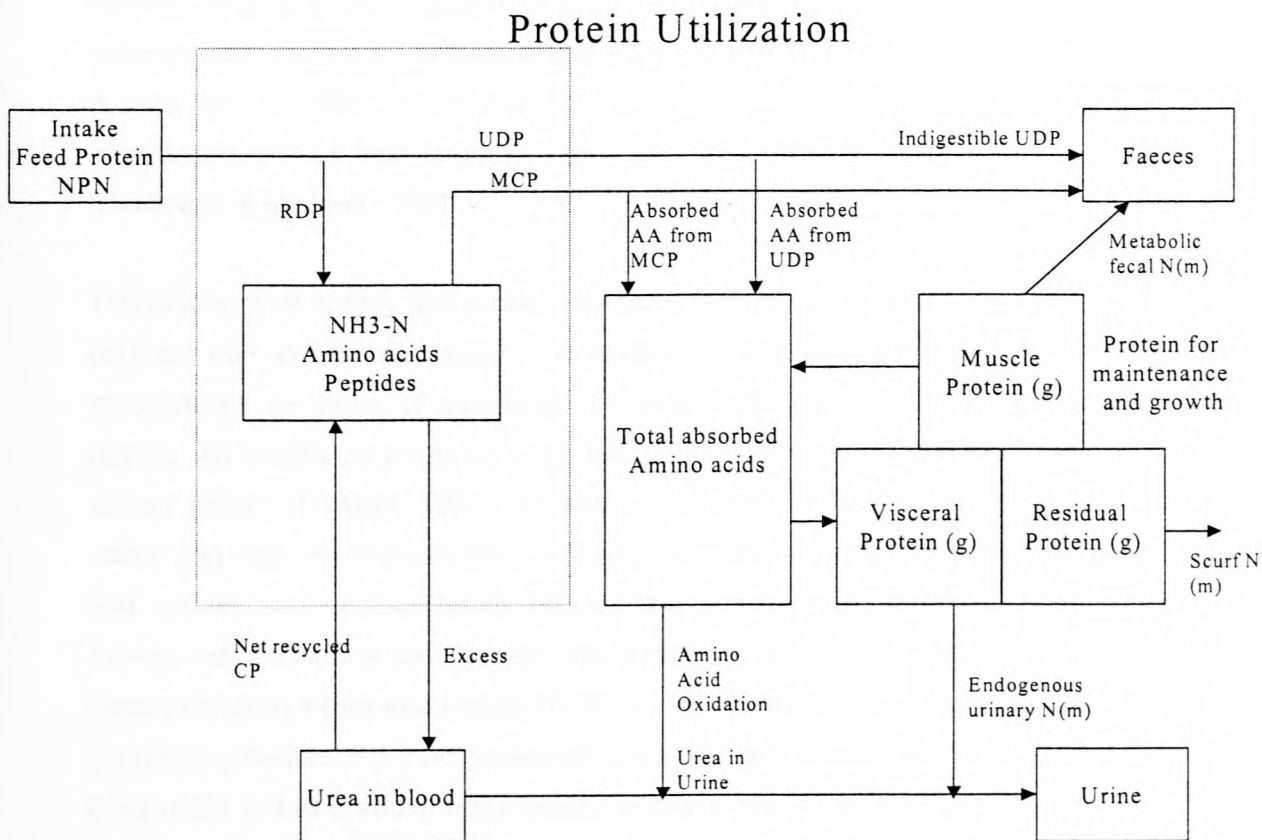


Figure 3. Model for nitrogen metabolism and utilization with the pathways influencing the magnitude of amino acids associated with maintenance and growth (Hvelplund & Madsen, 1996)

In diets where ruminal N supply exceeds the capacity of rumen microbes to synthesize microbial protein, rumen ammonia concentration increased, resulting in increased absorption of ammonia from the rumen and a reduced supply of protein to the intestine (Beever, 1996). After ammonia absorption into the blood, via the portal vein, ammonia transformation to urea occurs in the liver. In ruminants, urea can be recycled in the gut

through the saliva or through portal-drained viscera. However, 62% of urea produced by the liver is excreted in urine, which is correlated with the amount of N being absorbed as ammonia in the portal vein, resulting in a net loss of N. In addition to net N loss, ammonia imposes an increase in energy expenditure emphasizing its metabolic cost and the importance to consider the formation of this compound in the rumen when balancing a diet. Ammonia absorption should be reduced to its minimum, and this could be achieved by finding an adequate balance between dietary degradable proteins for optimal MPS and UDP bypassing the rumen combined with a good synchronization between ruminal supply of degradable sources of both energy (carbohydrates) and N (Lobley *et al.*, 1995). Absorbed amino acids in excesses of the demands and/or imbalanced profiles also contribute to N loss through urine after oxidation in the liver (Hvelplund & Madsen, 1996).

The efficiency of energy and protein use for growth is maximised when requirements for both are met, but not exceeded. The ruminant is endowed with effective detoxification mechanisms by virtue of extensive microbial metabolism of amino acids within the rumen. An imbalance would be expected to reduce the overall efficiency of utilisation of dietary protein (D'Mello, 1994). Excess dietary leucine induced a rapid fall in plasma valine and resulted in depressed utilisation of branched chain amino acids (isoleucine and valine) and excess lysine impairs the utilisation of arginine (D'Mello, 1994). Methionine can induce toxicity, resulting in reduced feed intake and performance at high dietary levels in swine and poultry (D'Mello, 1994). According to Robinson *et al.* (2000), abomasal infusion of rumen protected lysine or methionine alone or in combination on dairy cattle at 140% above requirement, reduced overall animal performance and DMI. A depressing effect of leucine on lysine uptake has been proved *in vitro* and *in vivo* (NRC, 1985). This emphasises the importance to incorporate amino acids in the correct ratios as to limit agnostic effects (Davis & Austic, 1994). These results are consistent with those of non-ruminant species, which have shown that imbalanced profiles of intestinally absorbable amino acids are associated with reduced DMI and animal performance (Robinson *et al.*, 2000). Ferreira *et al.* (1999b) stressed that several studies have focused on limiting amino acids and ignored EAA's that may have occurred in excess, resulting in a depressed amino acid and/or other nutrient utilisation.

5. LIMITING AMINO ACIDS IN GROWING CATTLE

The logic of efficiency of protein utilisation of balanced EAA's profiles, has induced the necessity to identify the EAA's that are most limiting in cattle diets when fed different feed compositions (NRC, 2000). Knowledge of the sequence of amino acid limitation as affected by diet composition provides an opportunity to improve amino acid profiles with feed supplements. Ruminants have no theoretical requirements for pre-formed protein amino acids in their diets (Kung Jr. & Rode, 1996) due to *de nova* synthesis of microbial protein. Ruminal microbial protein contains a relatively well-balanced amino acid pattern (Storm *et al.*, 1983), similar to that in tissue protein, and usually constitutes the major portion of amino acids absorbed by ruminants (Schwab, 1996). Microbial protein alone however is insufficient to supply adequate amounts of EAA's for optimal production. Richardson & Hatfield (1978) found that microbial protein is deficient in lysine, methionine, and threonine for supporting growth in Holstein steers using N retention studies. Based on N retention in steers, Klemesrud *et al.* (2000) found lysine first limiting then methionine and/or histidine the next limiting amino acids in microbial protein for growing cattle. Titgemeyer & Merchen (1990), Richardson & Hatfield (1978) and Froidmont *et al.* (2000) showed increased N retention with abomasal provision of methionine in diets where a wide variety of N sources were provided. These results confirm the assumption that metabolic amino acid requirements differ from microbial protein composition.

Most diets consumed by beef cattle contain some feed protein that escapes ruminal degradation that provides amino acids other than from microbial origin to the host. The quality of this UDP will influence which amino acid will be limiting in each feeding situation (Merchen & Titgemeyer, 1992). Lysine and methionine are regarded, as the most often limiting amino acids in ruminant nutrition (Rulquin & Vérité, 1996). The sequence of limitation is determined by their relative concentration in the UDP. Lysine has been identified as the first limiting amino acid for young post-weaned calves (Abe *et al.*, 1997) and growing cattle (Burriss *et al.*, 1976; Hill *et al.*, 1980; Abe *et al.*, 1997; Robinson *et al.*, 1998) when maize based diets provided a large fraction of the UDP (Merchen & Titgemeyer, 1992). In contrast, methionine has been cited as most limiting in diets containing less maize supplemented with soybeans (Robert *et al.*, 1999). Fenderson & Bergen (1975) demonstrated that methionine was first limiting in diets containing 80% barley, which is 80% degradable for steers. With high proportions of

RDP, the composition of amino acids reaching the duodenum will resemble that of microbial protein and will have relatively the same amino acid limitations. Evidence exists that other EAA's may be more limiting than methionine and lysine. Rohr & Lebzien (1991) found arginine and histidine limiting when comparing the intestinal amino acid supply in relation to requirements for milk production. Boisen *et al.* (2000) found leucine and isoleucine the most frequently limiting amino acids followed by methionine, lysine and threonine in 33 diets of dairy cows. Froidmont *et al.* (2000) also found phenylalanine limiting in Belgian Blue bulls when looking at plasma concentrations after they were fed hay and a concentrate low in digestible protein. Thus, when ruminants are fed diets that supply deficient quantities of absorbable protein, several EAA's may be limiting (Titgemeyer & Merchen, 1990). Methionine and lysine availability is influenced to a greater extent by diet composition than that of leucine. Tryptophan has often been ignored in ruminants because analysis is separate from other amino acids and it is difficult to analyse (Schingoethe, 1996), yet Klopfenstein *et al.* (1991) and Wilkerson *et al.* (1993) assume that tryptophan might be limiting in certain diets and may have a low digestibility. Merchen & Titgemeyer (1992) emphasizes the fact that other amino acids (particularly threonine, valine, and isoleucine) must not be overlooked as they may become limiting in certain feeding situations. The literature reviewed gives a varied account on limiting amino acids, this emphasizes that different amino acids may be limiting in different feeding situations (Boisen *et al.*, 2000).

Additional supply with protein or a complete amino acid mixture gave higher responses in protein yield than the addition of one or two limiting amino acids (Rohr & Lebzien, 1991), suggesting that several amino acids are co-limiting (Merchen & Titgemeyer, 1992). The provision of a full complement of EAA's will ensure a maximised response. Various researchers (Rohr & Lebzien, 1991; Schingoethe, 1996 and Shaver, 1991, as quoted by Erasmus, 1992) are of the understanding that a protected protein supplement that contains two to five amino acids will have a higher probability of inducing a response in animal production than a single protected amino acid. Wessels & Titgemeyer (1997) enhanced N retention in growing steers on limit-fed maize-based diets when supplemented with methionine, lysine, histidine, threonine, tryptophan, and arginine, through abomasal infusion. Optimising diets to meet the requirements of rapidly growing steers may require supplementation with combinations of amino acids rather than one or two of the most limiting amino acids (Wessels *et al.*, 1997). Approaches should be taken

to identify which amino acids are likely to be limiting and to which extent in typical growing diets for feedlot cattle.

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CHAPTER 3

ESSENTIAL AMINO ACID COMPOSITION OF THE WHOLE EMPTY BODY OF BEEF CATTLE

Abstract

The whole empty body (WEB) essential amino acid (EAA) composition of beef steers, commercially used in South African feedlots was investigated. The amino acid composition of the components (carcass, metabolic organs and residual fraction) was determined on 8 beef steers (Simmental and Hereford crosses) (200-360 kg live weight). The amino acid compositions differed between the three body components. Body component amino acid compositions (gAA/100gCP) were pooled by their respective weights and protein contribution, resulting in the calculated whole empty body amino acid composition: arginine 6.81; histidine 2.69; isoleucine 4.02; leucine 6.96; lysine 7.43; methionine 2.01; phenylalanine 4.03; threonine 4.01; valine 5.30; tryptophan 0.82. Whole-body amino acid compositions of beef steers in the present study, according to a lysine ratio, were lower in arginine, leucine and threonine and higher in valine to those noted in other scientific reports, indicating variation in amino acid values between studies and possibly genotypes. The present study's body amino acid composition could serve as an ideal example of the EAA requirements of beef feedlot steers (200 – 360 kg live weight) at tissue level, providing a desirable starting point from which the ratio of body amino acids can be used to estimate dietary amino acid requirements.

Key Words: essential amino acids; whole empty body; beef steers

Introduction

Amino acid requirement at tissue level is an important consideration (Schingoethe, 1996), as ruminants have metabolic requirements for amino acids rather than protein requirements *per se* for maintenance and production functions (Baker, 1989; Schwab, 1996b). An ideal pattern of absorbed amino acids is believed to exist for various physiological functions, including maintenance and growth (NRC, 2000). In order to determine the desired supply of essential amino acids (EAA) to optimise utilization by the host, some attempt must be made to appreciate the needs of the host (Merchen & Titgemeyer, 1990). The predetermined genetic code that regulates amino acid coupling during *de novo* protein synthesis, results in the unique sequence in which amino acids are required to synthesize specific proteins. Therefore, the amino acid composition of a specific protein remains similar every time it is synthesized (Schwab *et al.*, 1996a). The ideal protein concept is a means of specifying the host's requirement by making the assumption that the amino acids required for production are correlated to the amino acid

composition and the quantity of amino acids produced (Chen & Ørskov, 1994). Thus, in terms of whole body growth, the amino acid composition of the whole empty body (WEB) has been defined as an ideal example for the amino acid composition of dietary protein, resulting in improved amino acid utilization (Mäntysaari *et al.*, 1989; Hussein *et al.*, 1991). The requirement of the whole body represents the summation of nutrient utilization by each tissue. Each tissue bed or organ in the body has a specific function and these functions dictate the pattern of amino acid utilization. A better knowledge of the specific need for and utilization of nutrients by each tissue, will allow us to meet requirements of the whole body for optimal growth (McBride *et al.*, 1998).

Considerable attention has been devoted to the protein requirements of dairy cattle. Comparatively few studies have been reported and accurately applied on the defined requirements for particular amino acids in finishing feedlot beef cattle (Buttery & Foulds, 1988; Kung & Rode, 1996). Current amino acid requirements based on whole body protein composition are confined to Friesian calves and lightweight Holstein steers (Griffiths, 1977; Williams, 1978; Rohr & Lebzien, 1991; Ainslie *et al.*, 1993), while no research has yet been done on beef breeds commercially used in feedlots. Ferreira *et al.* (1999), emphasizes the necessity to define whole body EAA requirements of a particular breed and species, which therefore necessitates research on the WEB of finishing beef breeds commercially used in feedlots. Information on the extent of this variation is therefore essential to find a more precise amino acid requirement value (Williams, 1978). Therefore, the objective of this study was to determine the EAA requirements of beef cattle through evaluation of the whole empty body EAA composition in order to derive the ideal protein for growth.

Material and methods

Total body essential amino acid composition

To estimate the EAA composition of the WEB, eight feedlot beef steers, representing Simmental and Hereford crosses, (200-360 kg live weight) were slaughtered and dissected into separate components. The steers, being representative of those used in a commercial feedlot, received implants (Ralgro[®] and Revalor[®]). The finisher feedlot diet contained an β -antagonist (Zilmax) and an antibiotic growth stimulant (ZincBac). Slaughter procedures did not involve the removal of blood through exsanguination. The

carcasses were split along the mid ventral axis into two sides and weighed. The stomach was flushed with water and the intestines physically stripped to remove the digesta. After removal of the contents, the entire digesta-free gastrointestinal tract was weighed, including all the mesenteric, omentum, kidneyknob and channel fat. This was then combined with the individually weighed liver, heart, lungs, kidney and spleen to form a combined (visceral offal components) metabolic organ fraction. Head, feet, skin and tail were weighed and pooled together to form the residual fraction. These body components were then used to determine the WEB weights that excluded gut fill. The left half of the carcass, metabolic organs and residual fraction were frozen at -20°C, and then individually milled, mixed and milled again through a carcass mill, and thereafter milled through a 19, 13 and 5mm plate while frozen. After thoroughly mixing each individual component again, a representative sample of 2 kg of each sample was blended in a Warring blender for 30 seconds to ensure adequate homogenisation. Samples were then frozen in sealed bags and then freeze-dried. Freeze-dried samples were mixed with dry ice (prevention of fat smearing) and then milled through a 1 mm screen and stored at -10°C for later analysis.

Chemical Analysis

The carcass, metabolic organs and residual fraction were analysed, in duplicate, for crude protein with the Kjeldahl N procedure according to the methods of A.O.A.C. (1984). The amino acid compositions of the representative samples were determined with a BECKMAN SYSTEM 7300 high performance analyser after 22 h of acid hydrolysis (6 N.HCl) at 110°C according to A.O.A.C. (1984). After hydrolysis in an alkaline medium, tryptophan was analysed separately. The content of each individual amino acid was subsequently calculated on gAA/100g protein basis for each body component. To estimate the whole empty body EAA composition, the EAA composition of the carcass, metabolic organs and residual fraction were proportionally combined relative to their weight contribution to total protein in the empty body (Ainslie *et al.*, 1994).

Statistical Analysis

Regression analysis, one-way ANOVA and multiple comparisons (using Tukey's test) were performed on the data using PC SAS 6.04 (SAS Institute Inc., Cary NC). Guidelines from the SAS Procedures Guide (1998) and second edition of SAS System

for regression (1991) were followed. As there was no major effect of steer weight on the amino acid composition, the composition of the three body components (carcass, metabolic organs and residual fraction) were analysed as a completely randomised design with the least squares analysis of variance procedure of SAS (1998). Treatment contrasts were analysed by LSD only after a significant response ($P < 0.05$) was obtained.

Results and discussion

The efficiency with which an animal utilises absorbed proteins depends on how efficiently it utilises each amino acid, which, in turn, depends on body weight, the proportion of each amino acid relative to its requirement, the kind of amino acid, and the ratio of dispensable to indispensable amino acids (Ainslie *et al.*, 1993).

In Table 1, the physical composition of beef cattle with the proportional distribution of components constituting the WEB is shown. The average dressing percentage based on estimated live weight was $54.9 \pm 1.34\%$ which corresponds well with the average dressing percentage of 55% in cattle, observed by Lawrie (1998) and Ferrell & Jenkins (1998). Robelin & Geay, (1984) and Patterson *et al.* (1995), regard carcass weight as a percentage of empty body weight as being more biologically correct in expressing the dressing percentage of cattle due to the variation of gut content. Adapted data of Ferrell & Jenkins (1998) demonstrated an average dressing percentage of $62 \pm 1.0\%$ in Angus, Boran, Brahman, Hereford and Tuli breeds. These values are in accordance with the average dressing percentage of $62.0 \pm 1.5\%$ obtained in the present study. The residual fraction (head, feet, skin) contributed on average $20 \pm 1.1\%$ to the WEB weight. According to Seebeck (1967), the relative growth rates of hide, feet, and head is less than that of empty body while the tail tends to have the same growth rates as the empty body. The metabolic organs (digestive tract, liver, kidneys, heart) contributed $18 \pm 1.6\%$ to WEB weight. Variations in the relative contributions of tissues (organs) are attributed to the proportional change as a result of differences in growth rate of the parts of the body during different stages of development (Luitingh, 1962). According to Seebeck (1967), the relative weight of the liver, heart, lungs, kidneys and gut tissue decreases with increasing WEB weight, while relative spleen and blood weight remains the same.

Table 1 Proportional distribution (mean \pm SD) of live weight, carcass, whole empty body (WEB), residual fraction and metabolic organs of feedlot cattle (n = 8)

Body component	Weight (kg)	Relative weights (%) ¹⁾
Live weight (kg) ²⁾	232.5 \pm 57.2	
WEB ³⁾	204.7 \pm 50.4	100 \pm 0
Carcass	127.6 \pm 34.2	62.0 \pm 1.5
Residual fraction	40.9 \pm 9.8	20.0 \pm 1.1
Head	13.0 \pm 3.1	6.4 \pm 0.5
Feet	7.0 \pm 1.7	3.5 \pm 0.7
Skin	20.0 \pm 5.4	9.7 \pm 1.2
Tail	0.8 \pm 0.4	0.4 \pm 0.1
Metabolic organs	36.3 \pm 7.2	18.0 \pm 1.6
Organs	15.7 \pm 2.0	8.0 \pm 1.6
Liver	4.3 \pm 0.9	2.1 \pm 0.4
Heart	1.9 \pm 1.2	1.0 \pm 0.5
Kidney	1.0 \pm 0.3	0.5 \pm 0.2
Lung	6.2 \pm 1.2	3.3 \pm 1.3
Spleen	0.8 \pm 0.6	0.4 \pm 0.2
Diaphragm	1.4 \pm 0.3	0.7 \pm 0.1
GIT ⁴⁾	20.6 \pm 6.3	9.9 \pm 1.0
Fat	2.6 \pm 1.5	1.3 \pm 0.7
Stomach	8.5 \pm 2.4	4.1 \pm 0.5
Intestine	9.5 \pm 3.2	4.6 \pm 0.5

¹⁾ Percentage of body fractions relative to WEB

²⁾ Estimated Live weight where: Full body weight = EBM/(1-gut fill (0.0534 + 0.329 \times fraction NDF)) (NRC, 1996)

³⁾ Calculated as the sum of carcass, metabolic organs (excluding gut fill) and residual fraction

⁴⁾ GIT = digesta free gastrointestinal tract, including all the mesenteric, omentum, kidneyknob and channel fat

The crude protein (CP) and the proportional contribution (%) of nitrogen (N) in the carcass, metabolic organs, residual fraction and the WEB are presented in Table 2. The mean CP concentration (as-is basis) of the carcass (18.5 \pm 0.2%) and WEB (18.4 \pm 0.9%) was consistent with the observations of Ainslie *et al.* (1993) and Williams (1978) who obtained 18.83 % and 18.5 % respectively for protein concentration in the carcass and 18.6% in the WEB (Williams & Hewitt, 1979). However, the metabolic organ CP concentration (15.6 \pm 0.3%) obtained in the present study was higher than the value (12.6%) reported by Williams (1978). Hutchenson *et al.* (1997) stated that anabolic implants could moderately influence the distribution of protein between carcass and non-

Table 2 Crude protein content (%) and proportional distribution (%) (mean \pm SD) of nitrogen in carcass, metabolic organs, residual fraction and whole empty body of beef cattle (n = 8) on an as-is basis

Parameter	Carcass	Metabolic Organs	Residual Fraction	Whole empty body
Crude protein content (%)	18.5 \pm 0.2	15.6 \pm 0.3	20.7 \pm 0.2	18.4 \pm 0.9
Nitrogen Distribution (%) ¹	62 \pm 1.4	15 \pm 1.3	23 \pm 1.2	100 \pm 0.0

¹) Proportional contribution of body component protein pools relative to WEB weight

carcass components, as the protein proportion in the non-carcass tends to increase as a percentage of total empty body. According to relative weights, the carcass contributes 62 \pm 1.4 % to the WEB protein composition, organs 15 \pm 1.3%, and the residual fraction 23 \pm 1.2%. In contrast, Ainslie *et al.* (1993) adopted the ratios of 63, 12 and 25 % for carcass, metabolic organs and residual fraction respectively from lightweight Holstein steers (113-208kg). Differences can be attributed to steer genotype, plane of nutrition (Ferrell & Jenkins, 1998) and type of implant (Rumsey *et al.*, 1996; Hutchenson *et al.*, 1997) as these factors can affect the proportional contribution between carcass and non-carcass components. Consequently, the proportional change of these tissues can result in the variance in whole empty body EAA compositions in beef cattle.

The mean EAA concentrations of the carcass, metabolic organs (digestive tract, liver, kidneys, heart), residual fraction (head, feet, skin) and the WEB, are presented in Table 3. In order to obtain the whole empty body EAA composition, a weighed ratio was applied to each component relative to its contribution of total empty body protein: carcass, 62%; metabolic organs, 15%; residual fraction, 23%. As far as net amino acid requirements are concerned, the carcass represents the major site of protein deposition of 62%. The comparison of carcass, metabolic organs and residual fraction exhibited significant differences ($P < 0.05$) in the EAA concentrations. This illustrates Tamminga & Verstegen's (1996) emphasis, that different organs and tissues have different required amino acid ratios. With exception of arginine (carcass and metabolic organs) and leucine (metabolic organs), the EAA concentration of the residual fraction, was significantly ($P < 0.05$) lower in EAA's when compared to the carcass and metabolic organs.

Table 3 The amino acid composition (mean \pm SD) of carcass, metabolic organs, residual fraction and combined whole empty body of beef cattle (gAA/100 g CP) (n = 8)

Amino Acid	Carcass	Metabolic Organs	Residual Fraction	Whole empty body
Arg	6.02 ^c	7.55 ^b	8.44 ^a	6.81 ^{bc}
SD	± 0.66	± 0.41	± 0.26	± 0.44
His	3.20 ^a	2.61 ^b	1.37 ^c	2.69 ^b
SD	± 0.19	± 0.24	± 0.10	± 0.13
Iso	4.43 ^a	4.32 ^a	2.70 ^b	4.02 ^a
SD	± 0.92	± 0.13	± 0.09	± 0.57
Leu	8.12 ^a	4.32 ^d	5.58 ^c	6.96 ^b
SD	± 0.23	± 0.13	± 0.25	± 0.13
Lys	8.22 ^a	7.75 ^b	5.07 ^c	7.43 ^b
SD	± 0.35	± 0.42	± 0.25	± 0.23
Met	2.31 ^a	2.00 ^b	1.19 ^c	2.01 ^b
SD	± 0.13	± 0.06	± 0.07	± 0.07
Phe	4.18 ^b	4.78 ^a	3.11 ^c	4.03 ^b
SD	± 0.17	± 0.22	± 0.13	± 0.13
Thr	4.24 ^a	4.15 ^{ab}	3.28 ^c	4.01 ^b
SD	± 0.16	± 0.17	± 0.13	± 0.08
Val	5.50 ^b	6.38 ^a	4.06 ^c	5.30 ^b
SD	± 0.18	± 0.5	± 0.19	± 0.12
Trp	1.05 ^a	1.113 ^a	0.43 ^b	0.82 ^a
SD	± 0.39	± 0.35	± 0.04	± 0.22
Σ EAA ¹⁾	47.27 ^a	44.95 ^b	35.05 ^c	44.06
SD	± 2.24	± 2.80	± 1.37	± 1.13

^{a, b, c} Values in rows bearing different superscripts are significantly ($P < 0.05$) different

¹⁾ Total EAA composition

The carcass contained higher ($P < 0.5$) concentrations of histidine, leucine, lysine and methionine and lower ($P < 0.5$) concentrations of arginine, phenylalanine and valine when compared to the metabolic organs. The average amino acid concentrations of the carcass, organs and residual fraction also differed significantly ($P < 0.05$) with that of the WEB, emphasizing the use of WEB values for predicting amino acid requirements. The carcass contained higher ($P < 0.05$) concentrations of histidine, leucine, lysine, methionine and threonine in comparison with the WEB. The metabolic organs, contain a significantly lower ($P < 0.05$) concentration of leucine when compared to the WEB, while the residual fraction contained, in general, a lower concentration of all EAA's, except for arginine ($P < 0.5$). The main protein component of muscle connective tissue,

tendons and skin is collagen, which is a relatively poor source of EAA's except for arginine (Seifter & Gallop, 1966, as cited by Williams & Hewitt, 1979). This could be an explanation for the relatively high ($P < 0.5$) arginine concentration in the residual fraction (Table 3) as this fraction contains 43% of total body collagen (Williams, 1978). The carcass had a higher total EAA content than the metabolic organs and residual fraction (Table 3). Skeletal muscles are considered to comprise the major portion of the carcass in relation to bone (Lawrie, 1998). Given this information, Manhan & Shields (1998) hypothesised that genotypes, which develop more muscle, have higher dietary requirements for all EAA's and that higher quantities of specific EAA's could be required for muscle development.

In accordance with the study on South African Mutton Merino lambs (Ferreira *et al.*, 1999), the carcass tends not to be representative of the whole body requirements due to the variation between carcass and whole empty body EAA's. Regression equations were developed from the data to predict the whole empty body EAA composition according to the amino acid values of the carcass (Table 4). Relatively high coefficients of

Table 4 Regression equations to predict the whole empty body (WEB) essential amino acid composition (g AA/100g CP) from carcass and the coefficients of determination (R^2) between carcass and WEB

Independent Variable (X)	Dependent Variable (Y)	R^2	Regression equation $Y = b_0 + b_1X_1$
Arginine Carcass (X_1)	WEB	0.70	$Y = 3.4519 + 0.5571X_1$
Histidine Carcass (X_1)	WEB	0.82	$Y = 0.7708 + 0.5994X_1$
Isoleucine Carcass (X_1)	WEB	0.99	$Y = 1.2536 + 0.6233X_1$
Leucine Carcass (X_1)	WEB	0.86	$Y = 2.7314 + 0.5214X_1$
Lysine Carcass (X_1)	WEB	0.81	$Y = 2.4710 + 0.6027X_1$
Methionine Carcass (X_1)	WEB	0.96	$Y = 0.7778 + 0.5312X_1$
Phenylalanine Carcass (X_1)	WEB	0.83	$Y = 1.1789 + 0.6807X_1$
Threonine Carcass (X_1)	WEB	0.82	$Y = 2.1146 + 0.4462X_1$
Valine Carcass (X_1)	WEB	0.29	$Y = 2.8695 + 0.4417 X_1$
Tryptophan Carcass (X_1)	WEB	0.98	$Y = 0.2199 + 0.5762 X_1$

determination (R^2) were obtained for the prediction equations formulated. The carcass EAA composition included in the regression equations contributed significantly ($P < 0.05$) (except for valine) to the coefficients of determination when compared to that of the WEB.

Table 5 compares the EAA composition of beef steers in the present study with values of bovine tissues, carcasses and whole empty bodies cited in the literature. In the present study, the carcass EAA composition was higher in isoleucine, leucine, lysine, phenylalanine and valine than that obtained by Williams (1978) and Ainslie *et al.* (1993). Furthermore, the EAA compositions of the internal organs were higher in arginine, isoleucine and lower in leucine, lysine, phenylalanine and valine compared to the values obtained by Williams (1978). In these studies, slaughter procedures involved the removal of blood thus reducing the blood content of the carcass; this relative blood composition is then proportionally combined with the organ fraction (Williams, 1978). While in the present study, blood was not removed, which may explain the differences observed, as blood protein is high in leucine, lysine phenylalanine and valine, and lower in arginine, isoleucine and methionine (Manthan & Shields, 1998). The WEB composition would however not be compromised.

When the amino acid profile based on WEB obtained in the present study was compared to the WEB values obtained in the literature (Table 5), it was relatively higher in isoleucine, lysine, and valine. Although we cannot directly compare the carcass and organ EAA compositions with those of Ainslie *et al.* (1993) and Williams (1978) due to the contributions of blood, we cannot ignore effects of breed, nutrition and growth on muscular tissue. Ferreira *et al.* (1999) stated that different breeds have different potentials for meat production, thus differences in the proportional contribution of carcass, which is the major site of protein deposition, may influence the EAA composition of the WEB. Muscular tissue, as a percentage of carcass weight, can vary from 49 to 68% depending on breed and age (Callow, 1968). Berg (1967) as cited by Lawrie (1998), demonstrated that beef breeds (Hereford) have higher muscle to bone ratios than dairy breeds (Friesian). Considering that myosin and actin have high, well-balanced EAA profiles and are major components of muscle tissue (Williams, 1978), an increase in muscle would thus reflect an increased EAA composition of the carcass.

Table 5 Amino acid profiles of muscle, carcass, organs and whole empty body of cattle (g AA/100g CP)

	Arg	His	Iso	Leu	Lys	Met	Phe	Thr	Val	Trp
Carcass										
Carcass ¹⁾	6.0	3.2	4.4	8.1	8.2	2.3	4.2	4.2	5.5	1.1
Ainslie <i>et al.</i> (1993) ²⁾	5.3	2.0	2.4	5.1	5.9	2.4	2.6	3.4	3.0	0.5
Williams (1978) ³⁾	7.1	2.7	3.3	7.1	6.9	2.0	3.5	4.1	4.0	0.9
Organs										
Metabolic Organs ¹⁾	7.5	2.6	4.3	4.3	7.7	2.0	4.8	4.1	6.4	1.1
Williams (1978) ³⁾	5.5	3.6	2.3	9.7	7.9	1.6	5.3	4.9	5.5	0.7
Residual fraction										
Head, feet, skin & tail ¹⁾	8.4	1.4	2.7	5.6	5.1	1.2	3.1	3.3	4.1	0.3
Williams (1978) ³⁾	7.6	1.4	2.1	5.5	4.7	1.1	3.0	3.3	3.3	0.6
Whole empty body										
Whole empty body ¹⁾	6.8	2.7	4.0	7.0	7.4	2.0	4.0	4.0	5.3	0.8
Ainslie <i>et al.</i> (1993) ²⁾	5.9	2.1	2.3	5.7	5.8	2.0	3.0	3.5	3.3	0.6
Williams (1978) ³⁾	7.0	2.5	2.8	6.9	6.4	1.7	3.6	4.0	3.9	0.8
Rohr & Lebzien (1991) ⁴⁾	6.9	2.8	3.4	7.5	6.9	2.2	4.0	4.2	4.9	Nd ⁹⁾
Ainslie <i>et al.</i> (1993) ⁵⁾	6.6	2.5	2.8	6.7	6.4	2.0	3.5	3.9	4.0	Nd ⁹⁾
Selected Muscle tissue										
Lawrie (1996) ⁶⁾	6.6	2.9	5.1	8.4	8.4	2.3	4.0	4.0	5.7	1.1
Rogowski (1980) ⁶⁾	Nd ⁹⁾	Nd ⁸⁾	5.0	8.1	7.6	2.7	4.3	4.8	5.3	2.0
Hogan (1974) ⁷⁾	7.7	3.3	6.0	8.0	10.0	3.2	5.0	5.0	5.5	1.4
McCance & Widdowson (1978) ⁸⁾	6.7	1.3	5.1	8.0	9.1	2.7	4.5	4.6	5.3	1.3
Evans & Patterson (1985) ⁶⁾	6.0	3.1	7.3	5.6	6.7	2.6	5.2	4.4	5.0	Nd ⁹⁾
Mäntysaari <i>et al.</i> (1989) ⁶⁾	6.8	3.0	5.5	7.2	8.2	2.7	4.6	4.6	5.2	1.2
Rogowski (1980) ⁷⁾	Nd ⁹⁾	Nd ⁹⁾	1.7	3.7	4.5	1.0	2.1	1.5	2.1	0.1

¹⁾ Values obtained in present study on beef steers

²⁾ Carcass and WEB values determined from light weight Holstein steers

³⁾ Organ, carcass and WEB values of 8 Friesian bull calves, 70kg live weight

⁴⁾ WEB averages of Holstein steers (Rohr & Lebzien, 1991)

⁵⁾ Average WEB values of Williams (1978), Rohr & Lebzien (1991) and Ainslie *et al.* (1993)

⁶⁾ Amino acid content of selected beef muscle tissue

⁷⁾ Collagen values of Rogowski (1980) as cited by Rook & Thomas (1983)

⁸⁾ Amino acid content of selected beef muscle tissue by McCance & Widdowson (1978) as cited by Ainslie *et al.* (1993)

⁹⁾ Values not determined

In addition to the effect of genotype on muscle production, anabolic implants and β -antagonists have also been associated with increased lean muscle accretion (Bell *et al.*, 1998). In the present study, beef breeds treated with implants and β -antagonists were used in comparison with lightweight Holstein steers used in the studies of Ainslie *et al.* (1993). Thus the proportion of muscular tissue contribution to carcass and hence WEB, is probably greater. In the present study, the carcass had higher values of isoleucine,

lysine and valine that correspond well with the increased amino acid concentration of muscular tissue (Table 5). Titgemeyer & Merchen (1990) mentioned that differences in the genetic capacity of steers to deposit lean tissue will alter estimates of amino acid requirements and must be considered when making comparisons among experiments. Furthermore, the cattle used in the present study had a higher weight range (232.5 ± 57.2 kg) than Ainslie *et al.* (1993) (113-208 kg) and, as muscle percentage increases with an increase in body weight, the muscle tissue as a percentage of carcass weight increases (Berg 1968; Gaili & Nour, 1980). Kyriazakis *et al.* (1993) reported that, in pigs, WEB lysine and histidine increased during rapid muscle development. Mahan & Shields (1998) attribute the increasing lysine content of growing pigs to the higher concentrations in the carcass and blood. According to Gilka *et al.* (1989), the composition of muscle protein is genetically determined, thus it is not dependent on the conditions of growth (e.g. the quality and quantity of the diet or the health status). However, one cannot ignore the fact that the proportion of muscle and tissue (especially connective tissue) changes with increasing age (Smith, 1980). The connective tissue content of muscle decreases with increasing age, as does collagen and elastin (Wilson *et al.*, 1954), resulting in lower arginine values in the carcass. Therefore, one can assume that the EAA's associated with connective tissue would decrease, while that of muscle would increase with increasing age in the carcass, explaining the higher EAA and lower arginine values of the present study compared to that of Ainslie *et al.* (1993).

When the results of several body compositional studies in cattle are compared (Table 5), a wide difference exists between the relative (gAA/100gCP basis) amino acid compositions. The subsequent studies demonstrated that the whole body EAA concentration varies, either because of differences in breed, nutrition, age and hormones or that analytical differences for protein and amino acids occurs between laboratories (Mahan and Shields 1998). According to Zhang *et al.* (1986), the proportion of non-protein nitrogen decreases with increasing body weight. There is thus a higher content of both essential and non-EAA's per 100g CP in older cattle that could contribute to the higher EAA concentration of the present study relative to Ainslie *et al.* (1993). Consistent with this concept, the present study recorded a total EAA of 44% in CP, while Ainslie *et al.* (1993) reported a value of 39%. Thus to eliminate the differences of NPN, the EAA's can be described relative to lysine as an ideal protein ratio. The concept of ideal protein relative to lysine is a means of specifying the amino

acid requirements of animals, by assuming that animals, by virtue of sex, genotype or environment, grow at different rates and thus require amino acids in greater or lesser amounts. The animals however require the greater and lesser amounts in the same relative proportion (Fuller, 1996). Table 6, compares ideal protein values documented in literature with values obtained in the present study. When amino acids are expressed as

Table 6 Amino acid profile (relative to lysine) of ideal protein for growing cattle

Source	Whole empty body ¹⁾	Williams (1978) ²⁾	Rohr & Lebzien (1991) ³⁾	Ainslie <i>et al.</i> (1993) ⁴⁾
Lys	100	100	100	100
Arg	92	109	100	102
His	36	39	41	36
Iso	54	44	49	39
Leu	94	108	109	98
Met	27	27	32	34
Phe	54	56	58	52
Thr	54	63	61	61
Val	71	61	71	57
Trp	11	13	Nd ⁵⁾	10

¹⁾ WEB essential amino acid composition of beef steers from present study

²⁾ Average values of Friesian bull calves with an average weight of 70 kg

³⁾ Average values of Holstein steers

⁴⁾ Average values of light weight Holstein steers

⁵⁾ Values not determined

a percentage of lysine, the variation between the results of the present study and those in the literature still remains wide, however marginally smaller. According to the lysine ratio, the present study reported lower arginine, leucine and threonine concentrations compared to that reported in literature on a whole body basis and higher valine concentrations, except for Rohr & Lebzien (1991). Therefore, the relative proposed ideal protein values obtained from literature tend to differ from this study. Accordingly, Mahan & Shields (1998) and Boisen *et al.* (2000) obtained similar results, when comparing proposed ideal protein values for growing pigs.

Table 7 compares the average amino acid composition of beef carcasses and WEB, with that of sheep and pigs. The EAA concentrations of beef and sheep carcasses tend to correspond; however, variations in arginine, leucine, phenylalanine, threonine and valine occur. This is in accordance with Smith's (1980) view, that amino acid compositions of mammalian species are constant, yet small variations do exist.

Table 7 Amino acid profiles (g AA/100g CP) of carcass and whole empty body (WEB) of beef steers, sheep and pigs

	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val	Trp
Beef carcass ¹⁾	6.1	2.6	3.4	6.8	7.0	2.2	3.4	3.9	4.2	0.8
Beef WEB ²⁾	6.7	2.6	3.4	6.9	6.9	2.0	3.8	4.0	4.7	0.7
Sheep carcass ³⁾	7.1	2.6	3.5	7.3	7.0	2.0	4.1	4.4	4.6	Nd ⁶⁾
Sheep WEB ⁴⁾	7.7	4.5	3.1	8.5	6.5	3.6	5.2	4.7	5.2	Nd ⁶⁾
Pig WEB ⁵⁾	6.5	2.9	3.2	6.9	6.4	1.9	3.6	3.7	4.4	0.8

¹⁾ Average beef carcass values of the present study, Williams (1978) and Ainslie *et al.* (1993)

²⁾ Average beef WEB values of the present study, Williams (1978), Rohr & Lebzien (1991) and Ainslie *et al.* (1993)

³⁾ Average of sheep carcass values determined by MacRae *et al.* (1993) and Ferreira *et al.* (1999)

⁴⁾ Average sheep WEB

⁵⁾ Average of 9 literature studies on pig WEB (Manhan & Shields, 1998)

⁶⁾ Not determined

According to Ferreira *et al.* (1999), the WEB of species differ in EAA compositions due to the different compositions and contributions of organs and residual fractions. The lamb, cattle and pig carcasses represent 50, 55 and 75 % respectively of live weight (Lawrie, 1996). Oddy (1999) observed that phenotypic selection methods used to generate genetically distinct lines of animals, produced partitioning of protein deposition between organs (protein pools), resulting in different amino acid requirements between species. When comparing the EAA with whole empty bodies, beef tends not to correspond with sheep concentrations (Table 7). However, beef WEB had a more comparable amino acid concentration to that of the pig WEB. This was consistent with the observations of Williams (1978) where calf body EAA compositions were similar to pigs.

Conclusion

From the present study the carcass, residual fraction and metabolic organs contributed significantly to the whole empty body EAA composition. Regression equations predicting the whole empty body EAA composition from carcass values had high coefficients of determination except for valine. According to the results of the present study, the EAA composition of the WEB of beef feedlot steers differed from that of the average values of Williams (1978), Rohr & Lebzien (1991) and Ainslie *et al.* (1993) which were based on light weight Holstein steers and Friesian calves. Considering that current amino acid requirements are based on the latter, the implication of this can bare major effects on the

net requirements of beef cattle used in feedlots. Mahan & Shields (1998) hypothesised that genotypes that develop more muscle, have higher dietary requirements for all EAA's and that there could be specific EAA's required in higher quantities for muscle development than those of non-muscular development, as was demonstrated in the present study. Furthermore, considering that the carcass represents the major site of protein deposition (62%), contains the highest concentration of total EAA's, and that the carcass, as a percentage of WEB, increases with age while the internal organs decrease with age (Fendeen *et al.*, 1971; Gaili & Nour, 1979) could also contribute to different values obtained in the literature. The EAA's required for carcass muscle growth would therefore be required at higher dietary concentrations as the rate of muscle weight increases, while EAA's needed for non-carcass proteins decline with increasing live weight (Mahan & Shields, 1998). Anabolic implants, that are extensively used in feedlots, can also affect the EAA requirements, due to increased skeletal muscle accretion and therefore increase the requirements for certain EAA's associated with muscle growth. Therefore, the difference in meat production potential between breeds of cattle thus requires the establishment of amino acid requirements of specific breeds at a specific weight. The differences between species (beef, pig and sheep) EAA composition support Ferreira's *et al.* (1999) view that the amino acid compositions of animal species are not similar. In order to obtain more accurate requirements, additional work should focus on amino acid requirements compared with changes in body composition with increasing live weight of a particular breed due to the effect of nutrition, breed and implant. Amino acid requirements for gain would thus be a function of absolute and relative contributions of different tissue organs to the whole body amino acid pool during growth (MacRae *et al.*, 1993; Ainslie *et al.*, 1993). The average EAA composition of the WEB determined in the present study, could serve as an ideal example of the EAA requirements of beef feedlot steers (200 – 360 kg live weight) at tissue level that is needed for WEB tissue growth. This body amino acid composition can provide a desirable starting point from which the ratio of body amino acids can be used to estimate dietary amino acid requirements.

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ZANG, Y., PATRIDGE, I.G. & MITCHELL, K.G., 1986. The effect of dietary energy level and protein: energy ratio on nitrogen and energy balance, performance and carcass composition of pigs weaned at 3 weeks of age. *Anim. Prod.* 42: 389-395.

Abstract

The duodenal and whole-body nitrogen and energy balances of 24 weaned piglets were investigated to determine the effect of dietary energy level and protein: energy ratio on nitrogen and energy balance, performance and carcass composition. The EAA concentrations were set at three levels (high, medium and low) in comparison to three diets, including a basal energy and protein level and two nutritional modifications. The EAA concentrations were set at three levels (high, medium and low) in replicates (Diet 1, 2 and 3) and the diets were set at three levels (high, medium and low) in replicates (Diet 1 and 2) and treatments (Diet 3) relative to EAA requirements. The results indicated that the first to third weaning weeks were the most critical periods for piglets. The diets were balanced, based on maintenance (Diet 1), balanced for growth (Diet 2) and energy maintenance (Diet 3). The results were similar for Diet 1 and 2. The depression of duodenal amino acid excretion in the three diets, indicates that the basal energy and protein level was sufficient for maintenance and protein deposition.

Key Words: essential amino acids, whole body nitrogen, energy balance

Introduction

The interests of beef producers are aimed at obtaining maximum growth rate and carcass weight, while minimizing the cost of feed and the environmental impact of nitrogen excretion (Schirgoshie, 1981). The protein of absorbed amino acids is the most important determinant of growth in terms of protein production and efficiency of nitrogen utilization (Schirgoshie, 1981). Hence, the ultimate goal of beef production is directed towards obtaining a low nitrogen excretion rate in the feedlot, which is the form that is digested and which will allow absorption of the maximum amount of amino acids (Schirgoshie, 1981). However, the variation of these levels is dependent on the amount of dietary amino acid requirements of farms of animals is complex, dependent on the current nitrogen metabolism and that impact on the extensive market value of the quantity and quality of feed protein reaching the duodenum (Kung & Ross, 1980). Consequently, the amino acid composition of digesta leaving the rumen does not reflect that of the ingested diet and that nitrogen production is dependent on the extent of specific limiting amino acids (Clark et al., 1982). One of interest to evaluate the

CHAPTER 4

DUODENAL AND WHOLE EMPTY BODY ESSENTIAL AMINO ACID PROFILE OF COMMERCIALY FED FEEDLOT STEERS

Abstract

The duodenal and whole empty body (WEB) essential amino acid (EAA) compositions of beef steers receiving three different maize based commercial feedlot diets were investigated to determine limiting amino acids and imbalanced profiles. The duodenal EAA concentrations tended to be lower in histidine while higher in lysine and methionine in comparison to their diets, indicating a possible increase in biological value through microbial modification. The EAA concentration in the duodenum was significantly lower in: arginine (Diet 1, 2 and 3); histidine (Diet 1,2 and 3); lysine (Diet 1 and 3); methionine (Diet 1 and 3) and threonine (Diet 3) relative to WEB requirements. The chemical score indicated that the first to third limiting amino acids in the duodenal digesta of respective diets were: histidine, lysine, methionine (Diet 1); histidine, arginine, lysine (Diet 2); and arginine, methionine, histidine (Diet 3). The leucine concentrations were in excess in Diet 1 and 3. The disproportionate duodenal amino acid concentrations obtained from the three diets, indicate room for improvement in order to maximise nitrogen utilization and protein deposition.

Key Words: essential amino acids; whole empty body; duodenum; beef steers

Introduction

The interests of beef producers are aimed at obtaining maximum growth of the animal to produce lean meat and increase the efficiency of nitrogen utilization (Gerrits *et al.*, 1997). The pattern of absorbed amino acids in the small intestines is regarded as an important determinant of growth in terms of protein production and efficiency of feed nitrogen utilization (Schwab, 1996a). Hence the ultimate goal of beef producers is directed towards obtaining a flow of non-ammonia nitrogen to the intestinal tract in a form that is digestible and which will allow absorption of the desired array of amino acids (Schingoethe, 1996). However, the translation of tissue-level amino acid requirements to dietary amino acid requirements in terms of ruminants is complex, due to dynamic rumen nitrogen metabolism and their impact on the extensive microbial modification on the quantity and quality of feed protein reaching the duodenum (Kung & Rode, 1996). Considering that the amino acid composition of digesta leaving the rumen does not reflect that of the ingested diet and that ruminant production is dependent on the supply of specific limiting amino acids (Clark *et al.*, 1992b), it is of interest to evaluate the

relation of a specific diet in a given environment with the profile of amino acids in the duodenum (Tagari *et al.*, 1995). Tagari *et al.* (1995) regards the evaluation of the amino acids reaching the duodenum, as a fundamental approach in assessing the nutritional value of diets for ruminants. Ferreira *et al.* (1999) concluded that the designated strategy to determine the ideal protein is to determine the duodenal amino acid profile (microbial and non-degradable protein) from a specific diet, which is then compared to the whole body on a qualitative and/or quantitative basis. In this way the amino acids available for absorption, limiting amino acids and imbalanced amino acids for a specific diet can be determined.

In the quest for more rapid weight gain over shorter periods, the growing emphasis on increasing feed costs, narrowing profit margins, the increasing desire to minimise waste of dietary protein in relation to protein production, contribute to the necessity to balance diets for absorbable amino acids for more effective animal production (Schwab 1996a; Oddy *et al.*, 1997). More extensive research on the essential amino acid (EAA) requirements, particularly of lysine and methionine, has been done for dairy cattle than for beef cattle. For methodological reasons, few studies emphasise these essential amino acids in feedlot cattle bred for meat production and high ADG (Williams 1994; Zinn & Shen, 1998; Froidmont *et al.*, 2000). According to the NRC (1996) and Van Amburgh *et al.* (1998), accurate estimation of dietary protein requirements should be based on ruminal and tissue requirements in various production environments. At present, information about amino acid requirements of beef steers fed conventional feedlot diets in South Africa is limited. In order to derive an efficient feeding strategy for feedlot cattle in South Africa, further research is required on the net amino acid requirements, to establish what the optimum supply (quality and quantity) of essential amino acids for maintenance and growth (Merchen & Titgemeyer, 1992) under different feeding situations should be. This will result in more efficient meat protein synthesis and reduced output of manure nitrogen into the environment. This information will assist feedlots that produce 80% (D. Ford, South African Feedlot Association, personal communication, 2001) of the beef produced for consumption in South Africa, to be more competitive and environmentally sound. Therefore, the objective of this study was to compare the essential amino acid profile of the duodenal digesta contents of three maize based commercial feedlot diets, formulated with reasonably available raw materials, in

order to detect the essential amino acid imbalances for whole empty body (WEB) growth of beef cattle under South African feedlot conditions.

Material and methods

Ten beef steers per feedlot with slaughter weights ranging between 375-400 kg were randomly allocated from three different feedlots where they received their respective diets. The beef cattle (Hereford and Simmental crosses) and diets used, were representative of those used under feedlot conditions in different areas of South Africa (Table 1). Cattle were sent to abattoirs on full stomach just before being slaughtered. The duodenal digesta was investigated by quantitatively recovering the contents of the first meter (representing the duodenum) of the small intestine (Van der Walt & Meyer, 1988). According to MacRae (1975), cannulae may be used as an alternative; however, this method may affect the normal physiological function of the digestive tract. Representative duodenal digesta samples ($n = 10$) and their respective feedlot diets were collected and stored at $-18\text{ }^{\circ}\text{C}$ prior to analysis.

Degradation study

Digestibility of dry matter, organic matter and protein in the diets were determined by the *in vitro* technique as described by Barrell *et al.* (2000) and Calsamiglia & Stern (1995). The complete procedure is described by Tilley & Terry (1963). Rumen fluid of sheep that were on a concentrate diet was used for digestibility studies. Wilman & Adesogan (2000) obtained similar digestibility estimates using rumen fluid from sheep and cattle.

Chemical analysis

The diets were analysed for moisture, ash and crude protein (CP) according to the methods of A.O.A.C (1984). The neutral detergent fibre and acid detergent fibre were analysed using a Tetcator Fibretec System (Van Soest, 1963 and Van Soest & Wine, 1967). Duodenal samples from respective feedlot diets were analysed in duplicate for crude protein with the Kjeldahl N procedure according to the methods of A.O.A.C. (1984). The amino acid compositions of the diets and respective duodenal samples were determined with a BECKMAN SYSTEM 7300 high performance analyser after 22 h of acid hydrolysis (6 N.HCl) at 110°C according to A.O.A.C. (1984). Tryptophan was analysed separately after hydrolysis in an alkaline medium.

Statistical analysis

Regression analysis, one-way ANOVA and multiple comparisons of means (using Tukey's test) were performed on the data using PC SAS 6.04 (SAS Institute Inc., Cary NC). Guidelines would be followed from the SAS Procedures Guide (1998) and second edition of SAS System for regression (1991). The mean values used in the tables bearing different superscripts, indicate significant differences ($P < 0.05$).

Results and discussion

The amino acids available for absorption, limiting amino acids and imbalanced amino acids for a specific diet can be determined on a qualitative and quantitative basis through evaluation of the duodenal amino acid profile (Ferreira *et al.*, 1999). The physical and chemical compositions of the three standard feedlot diets under evaluation appear in Table 1. The chemical compositions of the three diets were relatively similar, except Diet 1 that had a higher CP and net energy for growth content. The *in vitro* dry matter and CP digestibility of Diet 1 was higher than that of Diet 2 and 3. The essential amino acid composition of the diets tended to differ in arginine, valine and tryptophan. Considering the relatively low concentration (g AA/100gCP) of methionine in the three maize-based diets (Table 1), it can be expected to be limiting in the present study. Methionine is regarded as one of the most often limiting amino acids in ruminant nutrition (Rulquin & Vérité, 1996). Microbial protein has been recognised to be first limiting in methionine and second limiting in lysine for nitrogen retention in growing cattle (Richardson & Hatfield, 1978). Therefore, the lower concentrations of methionine and lysine relative to the total essential amino acids in the three diets and to that of microbial protein (NRC, 1996), could cause the latter amino acids to become limiting. Lysine and methionine have been cited the most often limiting amino acids for growing cattle on a variety of maize-based diets (Schwab 1996b). Therefore, these two amino acids can be expected to become limiting in the present study. The estimation of amino acid supply to ruminants is determined more accurately from the pattern of duodenal digesta than that of the original diet (Loëst *et al.*, 1999). Table 2 gives the essential amino acid concentrations of the duodenal digesta from the respective diets and compares it with mean duodenal digesta values obtained from Rulquin & Vérité (1996).

Table 1 Physical (as fed basis) and chemical composition (dry matter basis) of the three standard feedlot diets

Physical composition (%)					
DIET 1		DIET 2		DIET 3	
Hominy Chop	27.44	Hominy Chop	18.23	Hominy Chop	30.43
Maize/dry/ground	20.00	Wheat Bran	17.68	Maize/dry/ground	41.78
Wheat bran	7.00	Corn Silage	30.94	Wheat Bran	9.91
Molasses/cane	12.34	DFG ⁴⁾	27.62	Molasses/cane	4.38
Apple Pomace	10.00	Blender ²⁾	5.53	Wheat Straw	6.90
Malt Sprouts	10.00			Blender ³⁾	6.60
Veg Fat	3.33				
Blender ¹⁾	9.89				
Chemical composition (%)					
	DIET 1	DIET 2	DIET 3		
Organic Matter	94.70	96.89	92.54		
Dry matter	87.93	77.25	84.35		
Dry matter digestibility	76.00	66.00	66.00		
Crude protein	13.00	10.54	10.95		
Crude protein digestibility	88.00	82.00	82.00		
NEg (MJ/kg DM) ⁵⁾	5.23	4.60	4.65		
Crude fibre	8.92	9.72	10.66		
Neutral detergent fiber	30.88	38.08	34.85		
Acid detergent fiber	11.96	12.42	14.01		
Ash	5.30	3.20	7.00		
Essential amino acid composition (gAA/100g CP)					
Arg	5.50	6.09	10.03		
His	2.39	3.03	2.38		
Ile	3.66	4.04	4.05		
Leu	7.35	8.62	8.57		
Lys	4.92	4.91	4.63		
Met	1.13	1.16	1.30		
Phe	4.10	4.65	4.30		
Thr	3.30	4.02	3.80		
Val	5.01	5.65	5.30		
Trp	0.99	1.42	2.17		

¹⁾ Urea, Limestone, Vit/Min, NH₃ SO₄, Zincbac, Flavomycin, Romensin and Oat hulls²⁾ Urea, Limestone, Vit/Min, Vit A, NH₃ SO₄, Salt, Romensin, Ground maize, and Tylan³⁾ Urea, Limestone, Vit/Min, Romensin, Master Sunfeed⁴⁾ DFG (Defatted maize germ) consists of 82% defatted Hominy chop, 13% Wheat bran and 5% Molasses in pellet form⁵⁾ Net energy for growth calculated according to the CNCPS

Table 2 Essential amino acid compositions (means \pm SD) of duodenal contents (n = 10) from the three standard feedlot diets (gAA/100g CP)

	Diet 1	Diet 2	Diet 3	Rulquin & Vérité (1996)			
	Duodenal 1	Duodenal 2	Duodenal 3	Mean ¹⁾	CV %	Min	Max
Arg	5.76 ^a	5.57 ^a	3.90 ^b	4.96	10.6	3.76	7.07
SD	± 0.62	± 0.27	± 0.47				
His	1.78 ^b	2.18 ^a	2.06 ^a	2.21	11.9	1.34	2.89
SD	± 0.16	± 0.10	± 0.14				
Ile	4.01 ^b	4.35 ^a	4.39 ^a	5.45	8.0	4.44	6.73
SD	± 0.16	± 0.22	± 0.21				
Leu	8.04 ^a	7.07 ^b	8.29 ^a	8.87	10.5	6.77	11.9
SD	± 0.52	± 0.71	± 0.85				
Lys	5.69 ^c	7.15 ^a	6.37 ^b	6.88	9.7	4.82	8.42
SD	± 0.48	± 0.54	± 0.82				
Met	1.70 ^b	2.05 ^a	1.5 ^c	1.97	17.6	1.27	2.99
SD	± 0.12	± 0.13	± 0.35				
Phe	4.02 ^b	4.37 ^a	4.01 ^b	5.12	7.1	4.13	6.06
SD	± 0.23	± 0.25	± 0.57				
Thr	3.88 ^a	3.95 ^a	3.59 ^b	5.32	7.0	4.36	6.16
SD	± 0.23	± 0.26	± 0.38				
Val	5.31 ^a	5.23 ^a	5.5 ^a	6.01	10.8	4.03	7.33
SD	± 0.26	± 0.28	± 0.62				
Trp	1.40 ^b	1.64 ^a	1.14 ^c	-	-	-	-
SD	± 0.37	± 0.18	± 0.12				

^{a, b, c)} Values in rows bearing different superscripts are significantly ($P < 0.05$) different

¹⁾ Mean, min and max values of duodenal amino acid concentrations calculated from Rulquin & Vérité (1996) based on cattle fed 133 diets

The essential amino acid concentrations of duodenal digesta exhibited significant differences ($P < 0.05$) in at least one diet. The duodenal digesta of Diet 1 was significantly lower ($P < 0.05$) in histidine, isoleucine and lysine in comparison to the other diets. In Diet 3, it was significantly lower ($P < 0.05$) in arginine, methionine and threonine than in Diet 1 and 2. The duodenal digesta of Diet 2 was significantly lower ($P < 0.05$) in leucine, while Diet 1 and 3 yielded significantly lower ($P < 0.05$) phenylalanine concentrations than Diet 2. Mean values obtained from literature (Rulquin & Vérité, 1996), tends to contain higher concentrations of isoleucine, leucine, phenylalanine, threonine and valine than the present study (Table 2). The essential amino acids from the duodenal digesta fell within the boundaries of the minimum and maximum values obtained in literature, except for threonine and phenylalanine in Diet 1. These examples illustrate the variation in duodenal amino acid composition from different diets. The

intestinal amino acid profile was generally assumed constant due to the smoothing effects of microbial protein on the variation (Oldham & Tamminga, 1980). However extensive literature studies based on 133 cattle diets (Rulquin & Vérité 1996), indicated that the variation (CV) of individual EAA concentrations can range from; 7-11% for lysine, arginine, phenylalanine, threonine and branched chain amino acids; 12% for histidine and 18% for methionine. According to Hvelplund & Madsen (1985) variation of 5 to 11% in duodenal contents exists within diets due to changes in the relative contribution of microbial and by-pass protein as well as each fraction's variable amino acid profiles. The variation of EAA's in diets obtained in the present study, and that of literature, may be attributed to different dietary undegradable protein amino acids. Furthermore, an additional source of variation between the three duodenal digesta EAA concentrations could be attributed to the fact that bacteria amino acid compositions can vary according to the composition of the diet, feeding frequency, passage rates and substrate fermented (Clark *et al.*, 1992b; Erasmus, 1992).

Table 3 represents a comparison of the standard diets, duodenal digesta, bacteria and whole empty body EAA compositions. The comparison between the duodenal digesta and their respective diets is an indication of the microbial modification and contribution to the duodenal amino acid flow. The duodenal EAA concentrations tended to be lower in histidine while higher in isoleucine, lysine, and methionine when compared to their respective diets. These results are in accordance with McCarthy *et al.* (1989), who indicated a decrease in the histidine concentration and an increase in the concentration of lysine and methionine (Boisen *et al.*, 2000) in the duodenal digesta, which are often limiting in ruminant nutrition (Richardson & Hatfield, 1978). According to Ferreira *et al.* (1999), rumen bacteria have the ability, through rumen fermentation, to alter the duodenal amino acid profile in comparison to the standard diet. The lower bacterial concentration of histidine and higher isoleucine, lysine, methionine concentrations relative to the three diets (Table 3), could possibly be attributed to this alteration (McCarthy *et al.*, 1989). In practice, Rulquin & Vérité (1996) found that, as the proportion of UDP:RDP in cattle diets increases e.g. maize protein, the intestinal protein composition differs from the microbial composition, which is lower in lysine, histidine and methionine content and higher in leucine and proline.

Table 3 Essential amino acid content (means \pm SD) of the standard diets, duodenal contents (n = 10) and whole empty body (n = 8)(g AA/ 100 g protein)

	Diet 1	Diet 2	Diet 3	Bacteria ¹⁾	Duodenal 1	Duodenal 2	Duodenal 3	WEB
Arg	5.51	6.09	10.03	5.1	5.76 ^b	5.57 ^b	3.9 ^c	6.81 ^a
SD					± 0.62	± 0.27	± 0.47	± 0.44
His	2.39	3.03	2.38	2.0	1.78 ^c	2.18 ^b	2.06 ^b	2.69 ^a
SD					± 0.16	± 0.10	± 0.14	± 0.13
Ile	3.66	4.04	4.05	5.7	4.01 ^b	4.35 ^a	4.39 ^a	4.02 ^b
SD					± 0.16	± 0.22	± 0.21	± 0.57
Leu	7.35	8.62	8.57	8.1	8.04 ^a	7.07 ^b	8.29 ^a	6.96 ^b
SD					± 0.52	± 0.71	± 0.85	± 0.13
Lys	4.92	4.91	4.63	7.9	5.69 ^c	7.15 ^a	6.37 ^b	7.42 ^a
SD					± 0.48	± 0.54	± 0.82	± 0.23
Met	1.13	1.16	1.3	2.6	1.70 ^b	2.05 ^a	1.50 ^c	2.01 ^a
SD					± 0.12	± 0.13	± 0.35	± 0.07
Phe	4.1	4.65	4.3	5.1	4.02 ^b	4.37 ^a	4.01 ^b	4.03 ^{ab}
SD					± 0.23	± 0.25	± 0.57	± 0.13
Thr	3.3	4.02	3.8	5.8	3.88 ^a	3.95 ^a	3.59 ^b	4.01 ^a
SD					± 0.23	± 0.26	± 0.38	± 0.08
Val	5.01	5.65	5.3	6.2	5.31 ^a	5.23 ^a	5.5 ^a	5.30 ^a
SD					± 0.26	± 0.28	± 0.62	± 0.12
Trp	0.99	1.42	2.17	1.0	1.40 ^b	1.64 ^a	1.14 ^c	0.82 ^d
SD					± 0.37	± 0.18	± 0.12	± 0.23

^{a, b, c, d} Values in rows bearing different superscripts are significantly ($P < 0.05$) different.

¹⁾ Bacteria values from NRC (1996)

The diets from the present study contained some maize UDP that could have resulted in different duodenal amino acid concentrations. Meissner & Du Plessis (1992) however mentioned that the diets used in feedlots in South Africa do not contain plant escape proteins that have the potential to alter the amino acid passage to the duodenum. The concentration of the duodenal methionine and lysine varies ($P < 0.05$) in the diets, indicating that the supply of these amino acids are influenced to a greater extent than other amino acids. This was consistent with the observations of Boisen *et al.* (2000) where the contribution of lysine, methionine and threonine, which are often considered first limiting, was increased in the duodenal digesta.

The comparison of the EAA content of the WEB and that of the duodenal digesta is informative on the limiting amino acids for growth (Rohr & Lebzien, 1991; Ferreira *et al.*,

1999). The duodenal digesta of Diet 1,2 and 3 was significantly lower ($P < 0.05$) in arginine and histidine (Table 3). Diets 1 and 3 were also lower ($P < 0.05$) in methionine and lysine. In the duodenal digesta of Diet 3, threonine was also lower ($P < 0.05$) in relation to the WEB requirement. Considering all three diets, arginine (Diet 1,2 and 3), histidine (Diet 1,2 and 3), lysine (Diet 1 and 3) and methionine (Diet 1 and 3) are substandard in the duodenal digesta relative to the WEB requirements. Methionine, lysine and histidine have been cited as most often limiting amino acids in ruminant nutrition (Rulquin & Vérité, 1996). From the above comparison one is however not able to distinguish between first- and second-limiting amino acids (Ferreira *et al.*, 1999). According to Ferreira *et al.* (1999), the chemical score (Table 4) provides a better evaluation of amino acids resulting in an EAA index. The chemical score represents the proportion of an individual EAA in the duodenum relative to its concentration in the WEB resulting in the EAA index.

The chemical scores in Table 4 suggest that the first-to-third-limiting amino acids in the duodenal digesta of beef cattle receiving diets 1 to 3 are: histidine, lysine, methionine/arginine (Diet 1), histidine, arginine, lysine (Diet 2) and arginine, methionine, histidine (Diet 3). Comparisons of the amino acid compositions of carcass and digesta have generally identified arginine or histidine as first limiting, followed by lysine and methionine (Leibholz & Naylor, 1971; MacRae & Reeds, 1980; Rohr & Lebzien, 1991). According to Zinn & Shen (1998), methionine was first limiting and lysine and histidine co-limiting amino acids in steam flaked maize diets for feedlot calves. Zinn *et al.* (2000) further reported, according to the chemical scores, histidine, lysine, methionine, threonine, phenylalanine and valine limiting on steam flaked maize diets. It is clear that the order of limitation may vary within diets with several amino acids becoming closely co-limiting (Buttery & Foulds, 1988; Merchen & Titgemeyer, 1992). The difference in the order of limitation is an indication that the feed's UDP content has the ability to modify the duodenal amino acid flow. According to Newbold (1988), the importance of arginine may be overestimated when comparing tissue with duodenal digesta, as arginine tends to be only semi-essential (Boisen *et al.*, 2000) for ruminants. It however is not known if arginine is synthesised or released at rates adequate to meet arginine requirements (Zinn & Owens, 1993). Furthermore, Zinn *et al.* (2000) states that histidine requirements may be over-estimated by using tissue chemical scores. This statement is based on the

Table 4 Chemical score and essential amino acid index (mean \pm SD) of the three diet's, bacteria and duodenal digesta (n = 10)

EAA	Diet 1	Diet 2	Diet 3	Bacteria ³⁾	Duodenal digesta 1 ¹⁾	Duodenal digesta 2 ¹⁾	Duodenal digesta 3 ¹⁾
Arg	81	90	148	75	85 \pm 9	82 \pm 4	57 \pm 7
His	88	111	87	73	66 \pm 6	81 \pm 4	77 \pm 5
Ile	90	99	100	140	100 \pm 4	108 \pm 6	109 \pm 5
Leu	101	124	124	117	116 \pm 7	102 \pm 10	119 \pm 12
Lys	66	65	62	105	77 \pm 7	96 \pm 7	86 \pm 11
Met	56	57	64	128	85 \pm 6	102 \pm 7	75 \pm 17
Phe	100	114	106	125	100 \pm 6	109 \pm 6	100 \pm 14
Thr	82	100	94	144	97 \pm 6	99 \pm 6	90 \pm 9
Val	93	105	99	115	100 \pm 5	99 \pm 5	104 \pm 12
Trp	99	166	264	117	170 \pm 19	200 \pm 21	139 \pm 14
EEA Index ²⁾					95 \pm 6.	100 \pm 5	93 \pm 4

¹⁾ Chemical score represents the proportion of an individual EAA relative to its concentration in the WEB protein

²⁾ EAA index presents the proportion of all 10 EAA's relatively to that of WEB protein

³⁾ Bacteria values from NRC (1996)

fact that histidine is found in large endogenous reservoirs as non-protein dipeptides, carnosine and serine. Research based on growing swine emphasised that tissue chemical scores over-estimated histidine requirements by 25 to 50% (Izquierdo *et al.*, 1988; Chung & Baker, 1992). On the other hand, the importance of cystine, methionine and threonine may be under-estimated due to the large contribution of sulphur amino acids to endogenous N losses of digestion in the gut, hair and scurf (Boisen *et al.*, 2000). The rest of the amino acids (Table 4) tended to be well balanced, illustrating the balancing effect of rumen bacteria on duodenal digesta. A general improvement of protein quality occurred when comparing the amino acid composition of duodenal flow and rumen bacteria with dietary protein (Table 4). Ruminants therefore, have the ability to increase the biological value of dietary protein (Loëst *et al.*, 1999). According to Newbold (1988) tryptophan is frequently not measured and its importance consequently overlooked, however in the present study, the concentration of tryptophan was abundant relative to requirements.

In order to provide a direct comparison of the EAA composition between duodenal digesta and WEB (Table 5) regardless of protein, EAA's must be expressed as a percentage of lysine (Cole & Van Lunen, 1994; Loëst *et al.*, 1999).

Table 5 Diet, bacteria, duodenal (n = 10) and whole empty body (n = 8) essential amino acids expressed as a percentage of lysine (mean \pm SD)

EAA	Diet 1	Diet 2	Diet 3	Bacteria ¹⁾	Duodenal digesta 1	Duodenal digesta 2	Duodenal digesta 3	Whole Body
Arg	112	124	217	65	102 ^a	78 ^c	62 ^d	92 ^b
SD					± 16	± 3	± 8	± 5
His	49	62	51	25	31 ^b	31 ^b	33 ^b	36 ^a
SD					± 2	± 1	± 5	± 0.6
Ile	74	82	87	72	71 ^a	61 ^b	70 ^a	54 ^c
SD					± 5	± 2	± 7	± 7
Leu	149	176	185	103	142 ^a	99 ^b	133 ^a	94 ^b
SD					± 7	± 11	± 28	± 1
Lys ¹⁾	100	100	100	100	100 ^a	100 ^a	100 ^a	100 ^a
SD					± 0	± 0	± 0	± 0
Met	23	24	28	33	30 ^a	29 ^a	24 ^b	27 ^{ab}
SD					± 4	± 1	± 7	± 0.7
Phe	83	95	93	65	71 ^a	61 ^b	64 ^b	54 ^c
SD					± 2	± 2	± 13	± 1
Thr	67	82	82	73	69 ^a	56 ^b	57 ^b	54 ^b
SD					± 6	± 4	± 10	± 1
Val	102	115	114	78	94 ^a	73 ^c	87 ^b	71 ^c
SD					± 6	± 3	± 10	± 2
Trp	20	29	47	13	25 ^a	23 ^a	16 ^b	11 ^c
SD					± 7	± 3	± 2	± 3

^{a, b, c} Values in rows bearing different superscripts are significantly ($P < 0.05$) different.

¹⁾ Bacteria values from NRC (1996)

²⁾ EAA's: Lysine expresses each amino acid as a percentage of lysine (lysine = 100%)

The ideal ratio to lysine remains largely unaffected by dietary - (protein level, energy level and feed intake), environmental - (disease and heat stress) and genetic - (sex and capacity of lean vs. fat growth) factors (Baker & Han, 1994). In the light of the need for improved N economy and optimal production, Tamminga & Verstegen (1996), stated that animals require EAA's in a well-balanced and strictly defined ratio for protein deposition. Once an ideal profile of amino acids is established, the quantitative needs of the remaining nine EAA's can be estimated relative to the lysine ratio (Peisker, 1999; Mack *et al.*, 1999). Detailed information regarding the protein quality of the duodenal digesta, hence the respective diet, can be obtained from calculations of the contributions of each individual amino acid to the ideal pattern (Boisen *et al.*, 2000). The EAA to lysine ratios (Table 5) gives the impression that leucine concentrations are higher in the diet when compared to the duodenal digesta and WEB. In Diet 1,2 and 3 there are higher arginine, histidine, isoleucine, phenylalanine, threonine and valine concentrations

compared to its digesta and to the WEB requirements. There is thus a general decrease in EAA concentrations relative to lysine after passage to the duodenum, except for the methionine concentrations in Diet 1 and Diet 2. Buttery & Foulds (1988) mentioned that with a number of protein supplements, methionine was the only amino acid whose concentration was raised relative to lysine after rumen fermentation. The average lower bacterial amino acid ratios to lysine could contribute to this change, altering the diet to a more balanced diet compared to the WEB requirement. Accordingly, the latter demonstrates the assumption that ruminants are less susceptible to imbalances through extensive microbial modification. However, the generation of certain reactive metabolites from specific amino acids during rumen degradation can induce detrimental effects (D'Mello, 1994).

In Table 5, it is evident that the duodenal digesta of Diet 1 in comparison with the WEB requirement was significantly lower ($P < 0.05$) in histidine, while higher ($P < 0.05$) in the other EAA's. The duodenal digesta of Diet 2 and 3 were significantly lower ($P < 0.05$) in histidine and arginine, while higher ($P < 0.05$) in isoleucine, phenylalanine and tryptophan ratios in comparison with the WEB requirement. Diet 3 was also significantly higher ($P < 0.05$) in leucine and valine. Methionine concentrations of the three diets did not differ significantly from the whole empty body EAA ratio. Although there was a general improvement in the required amino acid ratios from feed to duodenal digesta, the EAA patterns in the duodenal digesta from the three feedlot diets remained disproportionate to the requirement for WEB growth. Ferreira *et al.* (1999) stressed that excess EAA's may result in depressed amino acid utilization, and that the latter fact is ignored when balancing diets for limiting EAA's. Based on the chemical score of the present study (Table 4), excess duodenal leucine as represented by the duodenal content of Diet 1 and Diet 3, could possibly induce a rapid fall in plasma valine and result in the depressed utilization of branched chain amino acids (isoleucine and valine) (D'Mello, 1994). The ideal protein concept is thus dependent on a perfect balance of dietary amino acids in order to maximise the efficiency of protein utilization and avoid the harmful effect of imbalances (D'Mello, 1994). With the knowledge that amino acid imbalances and toxicities occur, Loëst *et al.* (1999) stated that further research should be aimed at addressing the imbalanced profiles in the duodenum in order to enhance the efficiency of utilization of absorbed amino acids to maximise growth.

Conclusion

Results based on chemical scores and the resulting EAA index demonstrated the effect of microbial modification on the feed protein, resulting in a more balanced profile of individual amino acids and hence an increased biological value in the duodenum (Loëst *et al.*, 1999). Although the duodenal digesta were assumed constant due to the smoothing effects of microbial modification, results demonstrated the ability of feed protein to alter the duodenal amino acid composition. This was in contradiction to Meissner & Du Plessis (1992), who mentioned that diets used in feedlots in South Africa do not contain plant escape proteins that have the potential to alter the amino acid passage to the duodenum. The duodenal EAA concentrations tended to be lower in histidine while higher in isoleucine, lysine and methionine when compared to their respective diets. There was thus an increase in the concentration of lysine and methionine that were often assumed to be limiting in cattle nutrition. Although there was a general improvement in the protein quality in the duodenal digesta, amino acid concentrations in the duodenal digesta from the three feedlot diets remained disproportionate to that of WEB requirements. The results also revealed that, according to the ideal protein concept, the duodenal digesta of all three diets were limiting in basically the same amino acids for WEB growth, although not in the same order. In all three diets arginine, histidine, lysine and methionine (only Diet 1 and 3) were one of the four most limiting amino acids. These values are in accordance with previous studies based on duodenal scores where arginine or histidine was first limiting, followed by lysine and methionine (Leibhloz & Naylor, 1971; MacRae & Reeds, 1980; Rohr & Lebzien, 1991). The present study indicated that the order of limitation is dependent on the type of diet with several amino acids becoming closely co-limiting. According to Tamminga & Verstegen (1996), animals require EAA's in a well balanced and defined ratio to improve nitrogen utilization and maximise protein deposition. The excess duodenal leucine in Diet 1 and 3 can induce a rapid fall in plasma valine and result in a depressed utilization of isoleucine and valine (D'Mello, 1994). In order to maximise the utilization of the three feedlot diets, further research should not only be directed at supplying the limiting amino acids, but should also be directed at balancing the amino acids in over-supply to prevent imbalances. The ideal protein concept remains a valuable tool in diet formulation, giving the precision required to make the feeding of a given population of cattle as efficient as possible, yet allowing the flexibility to suit

different populations and varying circumstances (Fuller, 1996; Boisen *et al.*, 2000). Determination of the net requirements of limiting amino acids would form the basis for formulation rations which optimise amino acid supply and the development of rationing systems (Parker, 2001).

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CHAPTER 5

EVALUATION OF DIET AMINO ACID ADEQUACY THROUGH FACTORIAL PREDICTIONS OF REQUIREMENTS AND DUODENAL AMINO ACID SUPPLY

Abstract

Amino acid requirement and flow to the duodenum of three standard feedlot diets were simulated using the Cornell Net Carbohydrate and Protein system (CNCPS) to predict the adequacy of absorbed essential amino acids (EAA). Predictions of requirements for an ME allowable gain was limited by lysine amino acid flow in Diet 1 and 3. The use of beef cattle EAA compositions induced methionine to become additionally limiting to Diet 1 while Diet 2 became limiting in lysine. The profiles of lysine, arginine, histidine (Diet 1), lysine, arginine, histidine, methionine (Diet 2), lysine, arginine, and histidine (Diet 3) were however predicted disproportionate to requirements for optimal utilization. The predicted profiles were in accordance with observed duodenal values, except for methionine that was observed limiting in Diet 1 and 3, however the sequence and extent of limitation was different. The Rulquin and Schwab ideal protein ratios also indicated lysine and methionine as limiting for optimal utilization. Results indicate that prediction models have potential in predicting requirements; there are however still limitations for use to accurately define requirements for particular EAA's.

Key Words: essential amino acids; requirements; factorial prediction; beef steers

Introduction

The effective use of knowledge of the amino acid requirements of ruminants requires the ability to precisely determine the flow of amino acids from the rumen (Buttery & Foulds, 1988). This provides a tool in defining the ideal content of essential amino acids (EAA) in metabolizable protein (MP), resulting in a means of implementing the knowledge to select protein and amino acid supplements to optimise the balance of EAA's in MP. Different mathematical approaches (factorial and multivariate regression approaches) that estimate amino acid supply to ruminants are available (Dijkstra *et al.*, 1998; Bateman *et al.*, 2001). The Cornell Net Carbohydrate and Protein System (CNCPS) amino acid sub model (Fox *et al.*, 1992; Russell *et al.*, 1992; Sniffen *et al.*, 1992), adopted, in conjunction with the CNCPS model for Level II of the *Nutrient requirements for Beef Cattle* (NRC, 1996) was developed to predict the absolute flows of each of the EAA's. The key components of the CNCPS and the NRC (1996) protein systems, are the estimation of feed protein fractions, microbial protein synthesis in the rumen and rumen-undegraded protein (UDP), flow of protein and amino acids to the duodenum and

the absorption of protein and amino acids in the small intestines. These systems provide quantitative estimates of intestinal amino acid profiles, or flux, based on dietary composition characteristics (Rulquin & Vérité, 1993). In order to derive net requirements for beef cattle, the CNCPS and NRC (1996) also includes a model to estimate both quality and production of EAA's required by beef cattle on a specific diet due to an energy allowable gain (Fox *et al.*, 1992). Protein requirements for maintenance and growth based on requirements for absorbed EAA's can be defined using the classical factorial methods (O'Connor *et al.*, 1993) that estimate metabolizable energy, metabolizable protein and metabolizable EAA requirements and supply. The future of understanding amino acid requirements for ruminants will rely on the ability of these models to accurately predict the need for supplemental amino acids (Kung & Rode, 1996). According to Schwab (1996a), continued research and intensive field evaluation are both important to the eventual refinement of factorial models and hence amino acid requirements. The dynamics of factorial mathematical models allow the prediction of amino acid demand and supply under a wide variety of dietary, animal and environmental conditions (O'Connor *et al.*, 1993) that would aid in the diet formulation process. Zinn *et al.* (2000) lends support to the utilization of the NRC (1996) system in predicting EAA supplies to the small intestine of feedlot cattle and regards the model as a practical tool in meeting the metabolizable amino acid requirements. The ability of protein models to predict the optimum EAA requirements under different feeding situations would be a fundamental step in balancing the duodenal amino acid profile. It is therefore important to use available approaches to evaluate diets in different environments with the objective of refining present knowledge on the EAA requirements. Therefore, the aim of this study was to evaluate feedlot diets commercially used in South Africa through model simulation that would provide information on the amino acid requirements and identify limiting amino acids for growing/finishing cattle.

Materials and Methods

The complete animal management, environmental variables and dietary inputs required by the model, Level 2 of the (CNCPS v. 40) and NRC (1996), were recorded, with the objective of evaluating the diets in terms of duodenal amino acid supply.

Animal inputs

Three feedlot trials were conducted on Simmental and Hereford crosses where they received their respective diets at commercial feedlots. Steers were all implanted and received ionophores. An initial condition score of 3 was used for finishing cattle at a starting weight of 300kg. The growing/finishing period lasted approximately 100 days depending on breed. Cattle were slaughtered with an average final weight of 440 kg, which is representative of the average slaughter weights at abattoirs in South Africa. The animal weights were adapted to 288 kg shrunk body weight (SBW) and a 422 kg final shrunk body weight (FSBW). Although breeds were crossbred, it was often difficult to estimate breeding beyond the primary parental breed (i.e. Simmental, Hereford or Angus). However, the breed effects or the most likely secondary parental breeds are the same (NRC, 1996).

Environmental inputs

The average daily temperature of 26°C and average daily relative humidity of 60% were recorded into the model. Animal hide conditions were considered with an average thickness, 60mm, and some mud on the lower body. Steers did not show evidence of heat stress and night cooling did occur.

Feed inputs

Feed compositions and inclusion rates of the three diets are represented in Chapter 4. The nutrient composition of dietary ingredients remained at model default. Proximate components were obtained from NRC (1996) and Dale (1999).

Model description

Formulating diets for amino acid requirements requires accounting of the variables known to influence requirements and diet supply of energy and protein, such as provided by the CNCPS (Fox *et al.*, 1992; Russell *et al.*, 1992; Sniffen *et al.*, 1992) and NRC, (1996). Equations for predicting the requirements and supply for absorbed EAA's are described and presented by O'Connor *et al.* (1993). A summary of factorial equations to predict requirements and supply are presented in Table 1. The abbreviations of general notations used for the entities are presented in Table 2.

Table 1 Mathematical statement of the NRC (1996) and CNCPS simulating requirements and supply of essential amino acids**SUMMARY OF EQUATIONS TO PREDICT REQUIREMENTS FOR AMINO ACIDS****Maintenance Requirement**

Metabolizable requirement for i^{th} absorbed AA (g/d)	$MPAA_i = (AATISS_i \times 0.01 \times (MP_{\text{maint}} \times 0.65)) / EAAM_i$
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Metabolizable protein requirement for maintenance (g/d)	$MP_{\text{maint}} (XP) = SPA + UPA + FPN$	$MP_{\text{maint}} = 3.8 \times SBW^{0.75}$
	$UPA = 2.75 \times SBW^{0.5} / 0.67$	
	$SPA = 0.20 \times SBW^{0.6} / 0.67$	
	$FPN = 0.09 \times IDM$	

Growth Requirement

Metabolizable requirement for i^{th} absorbed AA (g/d)	$RPAA_i = (AATISS_i \times RPN) / EAAG_i$
Net protein required for gain (NPg, g/d)	$RPN = PB \times 0.01 \times EBG_g$
	$NP_g = SWG \times (268 - (29.4 \times (RE/SWG)))$
	$RE (NEg) = 0.0635 \times EQEBW^{0.75} \times EBG^{1.097}$
	$EBW = 0.891 SBW$
	$EBG = 0.956 SWG$
	$EQSBW = SBW \times (SRW) / (FSBW)$
	$EQEBW = 0.891 \times EQSBW$
	$SWG = 13.91 \times NEg^{0.9116} \times SBW^{-0.6837}$

SUMMARY OF EQUATIONS TO PREDICT SUPPLY OF AMINO ACIDS**Total Duodenal AA Supply**

Total amount of i^{th} AA in the duodenum (g/d)	$REAA_i = REBAA_i + REFAA_i$
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Bacterial AA Supply

Amount of i^{th} bacterial AA appearing in the duodenum (g/d)	$REBAA_i = \sum_{j=1}^n (AABCW_i \times 0.01 \times REBCW_j) + (AABNCW_i \times 0.01 \times REBTP_j)$
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Bacterial true protein and cell wall in duodenum (g/d)	$REBTP_j = 0.60 \times 0.625 \times BACT_j$
	$REBCW_j = 0.25 \times 0.625 \times BACT_j$

Bacterial yields fermenting SC & NSC of the j^{th} feedstuff (g/d)	$BACT_j = NFCBACT_j + FCBACT_j$
	$NFCBACT_j = (Y_{2j} \times RDCA_j) + (Y_{3j} \times RDCB1_j)$
	$FCBACT_j = Y_{1j} \times RDCB2_j$

SUMMARY OF EQUATIONS (Table 1, Continued)

Carbohydrate fractions that are ruminally digested as effected by passage rates

$$\begin{aligned} \text{RDCA}_j &= I_j \times \text{CA}_j \times (\text{Kd}_{4j}/\text{Kd}_{4j} + \text{Kp}_j) \\ \text{RDCB1}_j &= I_j \times \text{CB1}_j \times (\text{Kd}_{5j}/\text{Kd}_{5j} + \text{Kp}_j) \\ \text{RDCB2}_j &= I_j \times \text{CB2}_j \times (\text{Kd}_{6j}/\text{Kd}_{6j} + \text{Kp}_j) \end{aligned}$$

Intake carbohydrate fractions

$$\begin{aligned} \text{CA}_j (\% \text{DM}) &= \text{NFC}_j - \text{CB1}_j \\ \text{CB1}_j (\% \text{DM}) &= \text{STARCH}_j \times \text{NFC}_j / 100 \\ \text{CB2}_j (\% \text{DM}) &= \text{NDF}_j - (\text{NDFIP}_j \times 0.01 \times \text{CP}_j) - \text{CC}_j \\ \text{NFC}_j (\% \text{DM}) &= \text{CHO} - \text{CB2}_j - \text{CC}_j \\ \text{CC}_j (\% \text{DM}) &= \text{NDF}_j \times 0.01 \times \text{LIGNIN}_j \times 2.4 \\ \text{CHO}_j (\% \text{DM}) &= 100 - \text{CP}_j - \text{FAT}_j - \text{ASH}_j \end{aligned}$$

Feed AA Supply

Amount of the i^{th} AA content of UDP for the j^{th} feed stuff (g/d)

$$\text{REFAA}_i = \sum_{j=1}^n \text{AAINSP}_{ij} * 0.01 * (\text{REPB1}_j + \text{REPB2}_j + \text{REPB3}_j + \text{REPC}_j)$$

Protein fractions (B1, B2, B3, C) escaping rumen degradation of the j^{th} feedstuff (g/d)

$$\begin{aligned} \text{REPB1}_j &= I_j \times \text{PB1}_j \times (\text{Kp}_j / (\text{Kd1}_j + \text{Kp}_j)) \\ \text{REPB2}_j &= I_j \times \text{PB2}_j \times (\text{Kp}_j / (\text{Kd2}_j + \text{Kp}_j)) \\ \text{REPB3}_j &= I_j \times \text{PB3}_j \times (\text{Kp}_j / (\text{Kd3}_j + \text{Kp}_j)) \\ \text{REPC}_j &= I_j \times \text{PC}_j \end{aligned}$$

Percentage of protein fractions (B1, B2, B3, C) in the j^{th} feedstuff (%DM)

$$\begin{aligned} \text{PB1}_j (\% \text{DM}) &= \text{SOLP}_j \times \text{CP}_j \times 0.01 - \text{PA}_j \\ \text{PB2}_j (\% \text{DM}) &= \text{CP}_j - \text{PA}_j - \text{PB1}_j - \text{PB3}_j - \text{PC}_j \\ \text{PB3}_j (\% \text{DM}) &= (\text{NDFIP}_j - \text{ADFIP}_j) \times \text{CP}_j \times 0.01 \\ \text{PC}_j (\% \text{DM}) &= \text{ADFIP}_j \times \text{CP}_j \times 0.01 \end{aligned}$$

Total Metabolizable AA Supply

Total amount of the i^{th} absorbed amino acid supplied by dietary and bacterial source (g/d)

$$\text{AAAs}_i = \text{DIGBAA}_i + \text{DIGFAA}_i$$

Amount of i^{th} absorbed amino acid digested from bacteria (g/d)

$$\text{DIGBAA}_i = \sum_{j=1}^n \text{AANBCW}_i \times 0.01 \times \text{REBTP}_j$$

Amount of i^{th} absorbed amino acid digested from feed UDP (g/d)

$$\text{DIGFAA}_i = \sum_{j=1}^n \text{AAINSP}_{ij} \times 0.01 \times (\text{REPB1}_j + \text{REPB2}_j + 0.8 \times \text{REPB3}_j)$$

Table 2 Abbreviations for entities used in the model simulating requirements and supply of amino acids in the NRC (1996) and CNCPS

SYMBOL	ENTITY AND UNIT
AA	Amino acid
AAAsi	Total amount of the i^{th} absorbed amino acid supplied by dietary and bacterial sources (g/d)
AABCWi	The i^{th} amino acid content of rumen bacteria cell wall protein (g/100g)
AABNCWi	The i^{th} amino acid content of rumen bacteria non-cell wall protein (g/100g)
AAINSPij	The i^{th} amino acid content of the insoluble protein for the j^{th} feedstuff (g/100g)
AATISSi	AA composition of tissue (g/100g CP), Table 3
ADFIPj	Percentage of the j^{th} feedstuff that is acid detergent insoluble protein (%CP)
ASHj	Percentage of ash of the j^{th} feedstuff (%DM)
BACTj	Yield of bacteria from the j^{th} feedstuff (g/d)
BCP	Bacterial (Microbial) crude protein
CAj	Percentage of DM of the j^{th} feedstuff that is sugar (%DM)
CB1j	Percentage of DM of the j^{th} feedstuff that is starch (%DM)
CB2j	Percentage of DM of the j^{th} feedstuff that is available fibre (%DM)
CCj	Percentage of DM in the j^{th} feedstuff that is unavailable fibre (%DM)
CHOj	Percentage of carbohydrate of the j^{th} feedstuff (%DM)
CPj	Percentage of crude protein of the j^{th} feedstuff (%DM)
DIGBAAi	Amount of the i^{th} absorbed bacterial amino acid (g/d)
DIGFAAi	Amount of the i^{th} absorbed amino acid from dietary protein escaping rumen degradation (g/d)
DM	Dry matter
EAAGi	Efficiency of use of the i^{th} amino acid for growth (g/g), Table 3
EAAMi	Efficiency of use of the i^{th} amino acid for maintenance (g/g)
EBG	Empty body gain (kg)
EBW	Empty body weight (kg)
EBW ^{0.75}	Metabolic body weight based on empty body weight (kg)
FCBACTj	Yield of fibre carbohydrate bacteria form the j^{th} feedstuff (g/d)
FPN	Metabolic faecal protein (g/d)
EQEBW	Equivalent empty body weight (kg)
EQSBW	Equivalent shrunk body weight (kg)
FSBW	Actual final shrunk body weight at fat endpoint selected for feed lot steers
Ij	Intake of the j^{th} feedstuff (g/d)
IDM	Indigestible dry matter (g/d)
Kd _{1j}	Rumen rate of digestion of the rapidly degraded protein fraction of the j^{th} feedstuff (1/h)
Kd _{2j}	Rumen rate of digestion of the intermediately degraded protein fraction of the j^{th} feedstuff (1/h)
Kd _{3j}	Rumen rate of digestion of the slowly degraded protein fraction of the j^{th} feedstuff (1/h)
Kd _{4i}	Rumen rate of sugar digesting of the j^{th} feedstuff (1/h)
Kd _{5i}	Rumen rate of starch digestion of the j^{th} feedstuff (1/h)
Kd _{6i}	Rumen rate of available fibre digestion of the j^{th} feedstuff (1/h)
Kpj	Rate of passage form the rumen of the j^{th} feedstuff (1/h)
LIGNINj	Percentage of lignin of the j^{th} feedstuff's NDF (%NDF)

SYMBOL	ENTITY AND UNIT (Table 2, Continued)
MPAAi	Metabolizable requirement for the i^{th} absorbed AA for maintenance (g/d)
MPg	Metabolizable protein requirement (g/d)
MPmaint	Metabolizable protein requirement for maintenance (g/d)
NDFj	Percentage of the j^{th} feedstuff that is neutral detergent fibre (%DM)
NDFIPj	Percentage of neutral detergent insoluble protein in the crude protein of the j^{th} feedstuff (%CP)
NEg	Net energy for gain content of the diet (Mcal/kg)
NFCj	Percentage of the DM in the j^{th} feedstuff that is non-fibre carbohydrates (%DM)
NFCBACTj	Yield of non fibre carbohydrate bacteria from the j^{th} feedstuff (g/d)
NPg	Net protein requirement (g/d)
NSC	Non-structural carbohydrates
PAj	Percentage of crude protein in the j^{th} feedstuff that is non-protein nitrogen (%DM)
PB1j	Percentage of crude protein in the j^{th} feedstuff that is rapidly degraded protein (%DM)
PB2j	Percentage of crude protein in the j^{th} feedstuff that is intermediately degraded (%DM).
PB3j	Percentage of crude protein in the j^{th} feedstuff that is slowly degraded protein (%DM)
PCj	Percentage of crude protein in the j^{th} feedstuff that is bound protein (%DM)
PB	Protein content of empty body gain (g/100g CP)
RD	$(Kd/Kd+Kp)$ is proportion of component of feedstuff degraded in the rumen
RDCAj	The j NFC in the A (sugar) fraction to the j^{th} feedstuff ruminally degraded (g/d)
RDCB1j	The j NFC in the B1 (sugar) fraction to the j^{th} feedstuff ruminally degraded (g/d)
RDCB2j	The j NFC in the B2 (sugar) fraction to the j^{th} feedstuff ruminally degraded (g/d)
RE	Retained energy (Mcal/d)
REPB1j	Amount of ruminally escaped B1 true protein in the j^{th} feedstuff (g/d)
REPB2j	Amount of ruminally escaped B2 true protein in the j^{th} feedstuff (g/d)
REPB3j	Amount of ruminally escaped B3 true protein in the j^{th} feedstuff (g/d)
REPCj	Amount of rumen escaped bound C protein from the j^{th} feedstuff (g/d)
REAAi	Total amount of the i^{th} amino acid appearing at the duodenum (g/d)
REBAAi	Amount of the i^{th} bacterial amino acid appearing at the duodenum (g/d)
REBCWj	Bacterial cell wall protein appearing at the duodenum due to fermentation of the j^{th} feed (g/d)
REBTPj	Bacterial non-cell wall protein appearing at the duodenum to fermentation of the j^{th} feed (g/d)
RESC	$Kp / (Kd + Kp)$ is a proportion of component of feedstuff escaping ruminal degradation
RPAAi	Growth requirement for the i^{th} absorbed amino acid (g/d)
RPN	Net protein required for growth (g/d)
SBW	Shrunk body weight, 0.96 full weight (kg)
SC	Structural carbohydrates
SOLPj	Percentage of the crude protein of the j^{th} feedstuff that is soluble protein (%CP)
SPA	Scurf protein (g/d)
SRW	Standard reference weight as 478, 462,435,400 for 28%, 27,25, 22 %fat respectively
STARChj	Percentage of starch in the non-structural carbohydrate of the j^{th} feedstuff (%NFC).
SWG	Shrunk weight gain (kg)
UDP	Undegradable intake protein
UPA	Urinary protein (g/d)
Y1j	Yield efficiency of FC bacteria from the available fibre fraction of the j^{th} feedstuff
Y2j	Yield efficiency of NFC bacteria from the available starch fraction of the j^{th} feedstuff
Y3j	Yield efficiency of FC bacteria from the available fibre fraction of the j^{th} feedstuff

Results and Discussion

Predicted amino acid requirements

According to Fox *et al.* (1992), the computation of protein and amino acid requirements for growth are dependent on the energy allowable gain. The net protein required for this gain is then multiplied by the EAA concentration required for tissue production and then divided by various transfer coefficients to determine absorbable amounts (NRC, 1985). The dry matter intake (DMI) of the three diets were adapted to obtain the same energy allowable gain for all three diets, that was representative of 1.4 kg/day obtained in the three feedlots. Amino acids therefore required for tissue growth, because of a shrunk weight gain (SWG) of 1.4 kg/day, was dependent on the protein in gain (net protein retained) of 207 g/day. The net amino acid value required for growth (gAA/100g CP, or % of net protein in gain), which is a function of the whole empty body (WEB) tissue EAA concentration, is represented in Table 3.

Table 3 Amino acid composition of tissue, net amino acid requirement and the utilization of individual absorbed amino acids

Item	Arg	His	Leu	Iso	Lys	Met	Phe	Thr	Val	Trp
Amino acid composition of tissue ¹⁾	6.59	2.47	6.70	2.84	6.37	1.97	3.53	3.90	4.03	0.5
Amino acid composition of tissue ²⁾	6.81	2.69	6.96	4.02	7.43	2.01	4.03	4.01	5.30	0.8
Amino acid composition of keratin ³⁾	3.80	1.00	10.0	5.00	3.20	1.00	3.70	7.20	6.00	1.4
Net amino acid requirement of 207 g/day protein ⁴⁾	13.6	5.11	13.9	5.88	13.2	4.08	7.31	8.07	8.34	1.0
Net amino acid requirement of 207 g/day protein ⁵⁾	14.1	5.57	14.4	8.32	15.4	4.16	8.34	8.30	10.9	1.7
Efficiency of utilization for maintenance ⁶⁾	0.85	0.85	0.66	0.66	0.85	0.85	0.85	0.85	0.66	0.8

¹⁾ Amino acid composition as g/100 g of protein, average of three studies summarized by WEB values of Ainslie *et al.* (1993)

²⁾ Amino acid composition as g/100 g of protein, WEB values of beef cattle (Chapter 3)

³⁾ Amino acid composition as g/100 g of keratin protein (Block & Bolling 1984 cited by O' Connor *et al.*, 1993)

⁴⁾ Net amino acid requirement, using amino acid composition of Ainslie *et al.* (1993)

⁵⁾ Net amino acid requirement, using amino acid composition of beef cattle reported in the present study (Chapter 3)

⁶⁾ Utilization of individual absorbed amino acids for maintenance (CNCPS) according to Evans & Patterson (1985), while NRC (1996) adopted an average value of 100 % for all amino acids

The metabolizable EAA's required for growth (Table 4) were obtained by dividing the net amino acid requirement by the efficiency of use of absorbed protein for growth. Amino acid requirements for growth vary with stage of growth, as determined by Ainslie *et al.* (1993): $EAAG_i = 0.834 - (0.00114 \times EQSBW)$, where EAAG is the efficiency factor of amino acids for growth and EQSBW is the equivalent shrunk body weight as described by Fox *et al.* (1992). In the present study, the efficiency of MP that is used for growth in cattle was estimated as 49,6%. The net requirements based on the amount of product and amino acid composition of the product can be measured reliably. Variations in literature, such as the higher values of tissue EAA composition, reported by Rohr & Lebzien (1991) and results from Chapter 3, can result in a reduction of EAA allowable ADG due to the higher EAA requirements. Furthermore, the use of fixed transfer coefficients for efficiency of use of absorbed protein (Rohr & Lebzien, 1991; NRC, 1996) dictates that production responses are linear, regardless of the amount of nutrient supplied, resulting in over-prediction of production responses by factorial methods (NRC, 1996). The over-prediction of efficiency will result in the under-prediction of the metabolizable EAA requirement. Campbell *et al.* (1997) reported a low efficiency of utilization of 24 % for methionine. The efficiency with which amino acids are used for protein deposition also varies between amino acids (NRC, 1985), where estimated values were low (0.64) for tryptophan and high (0.93) for lysine in relation to an ideal protein. These different efficiencies would dictate different profiles of EAA's required for MP, than those based on tissue EAA composition of net protein. These variable efficiencies cause the factorial approach to deviate from the ideal protein concept and increase the bias in predicting MP and EAA allowable ADG.

Amino acid requirements are dependent on the relative proportions of dietary amino acids utilised for maintenance and for body gain upon the amino acid composition of protein deposited (Bikker *et al.*, 1994). Maintenance requirements for MP are for replacing amino acids irreversibly lost from tissue protein pools. The amino acid requirements for maintenance are based upon the amino acid composition of tissues and depend on the prediction of sloughed protein and net tissue turnover losses (NRC, 1985). The CNCPS computes MP requirements for maintenance as the sum of scurf protein (SPA), urinary protein (UDA) and metabolic fecal protein (FAN) (Fox *et al.*, 1992). The EAA content of the UDA and FAN were related to that of muscle tissue while SPA was estimated from keratin (Table 3). The metabolizable requirements for SPA

and UPA were 9 g/day and 10 g/day for all three diets respectively as the latter are related to SBW. However, the requirements for FAN varied in the diets (Diet 1, 187; Diet 2, 216; Diet 3, 217), as the latter are related to the indigestible dry matter (IDM) intake of the diets. In contrast, the NRC (1996) computes the requirements as a function of metabolic weight (Wilkerson *et al.*, 1993), hence obtaining the same MP requirement for maintenance value for all three diets. The values given in Table 4 reflect the average metabolizable EAA requirements for maintenance and growth (288 kg SBW to 422 kg FSBW) obtained with the NRC (1996) and CNCPS.

Table 4 Metabolizable essential amino acid requirements for maintenance and growth (g/d) estimated according to the factorial approach

Requirements (g/day)	Arg	His	Iso	Leu	Lys	Met	Phe	Thr	Val	Trp
Maintenance ¹⁾	8.8	6.6	7.4	17.8	17.0	5.3	9.3	10.4	10.6	1.6
Maintenance (Diet 1) ²⁾	9	7	10	24	18	6	10	11	15	1
Maintenance (Diet 2) ²⁾	10	8	11	27	20	6	11	12	16	2
Maintenance (Diet 3) ²⁾	10	8	12	27	20	6	11	12	16	2
Growth ³⁾	27(14)	10	12	28	27	8	15	16	17	2
Growth ⁴⁾	28	11	17	29	31	8	17	17	22	3

¹⁾ Maintenance requirement estimated as $3.8 \times BW^{0.75}$ g/d by NRC (1996)

²⁾ Maintenance requirement estimated as SPA + UPA + FAN by CNCPS

³⁾ Growth requirement estimated by NRC (1996) and CNCPS

⁴⁾ Growth requirement estimated using amino acid concentrations of beef cattle (Chapter 3)

Value in parenthesis is the decreased requirement as arginine is regarded a semi-essential amino acid

The NRC (1996) adopted the maintenance efficiency value of 100% for amino acids while the CNCPS used lower values, 66 to 85 % depending on the amino acid (Table 3), explaining the lower and variable metabolizable requirements obtained (Table 4) for maintenance according to the NRC (1996). The largest part of amino acid requirements is used for body protein accretion, which is deposited with relatively the same amino acid compositions at different rates of gain (Fuller, 1996). Maintenance requirements amount to a smaller fraction of the total amino acid needs of the non-ruminant, thus the requirements are primarily determined by the pattern of amino acids in the body protein accreted. However due to the higher fractional growth rates in ruminants compared to non-ruminants, a smaller fraction of absorbed amino acids would be used for growth relative to the amounts used for maintenance (Owens & Pettigrew, 1989). Although maintenance requirements represents a smaller proportion of the total requirements, the

pattern of amino acids required for maintenance is different to that required for body protein accretions as considerable differences exist between the optimal amino acid composition for maintenance, including endogenous losses, and that for growth (Fuller *et al.*, 1989; Fuller, 1996). Animal factors such as body weight, sex, genotype, and nutritional factors such as feeding level and dietary compositions, affect the ratio between amino acids used for maintenance and those used for growth. As a consequence, the proportion of amino acid requirement is not a fixed entity but varies with the animal's age, growth rate, capacity for lean growth and with live weight in that the maintenance requirements increases with live weight and age, while protein deposition is often static or declines at heavier weights (Tamminga & Verstegen, 1996; Mack *et al.*, 1999). This ratio between maintenance and growth may influence the ideal amino acid composition of the diet (Fuller, 1996; Bikker *et al.*, 1994; Edwards & Campbell, 1993). Considering that maintenance requirements increase with age it is essential to determine the amino acid content related to maintenance more accurately as this becomes more important with increasing age.

Predicted intestinal amino acid supply

A fundamental approach in controlling the flow of metabolizable amino acids and thus utilizing amino acids to the maximum is essentially dependent on the prediction of duodenal EAA compositions (Rulquin *et al.*, 1998). Table 5 represents the estimated total EAA's appearing in the duodenum with the absorbable fraction of amino acids. The quantity of amino acids appearing at the duodenum was calculated as the sum of bacterial amino acids and dietary protein escaping rumen degradation as estimated by O'Connor *et al.* (1993). The estimated intestinal supplies of individual amino acids were different between diets, resulting in the different values predicted for the metabolizable EAA supply using the CNCPS. The estimated proportion of bacterial and feed amino acid compositions appearing in the duodenum, with the absorbable fraction, is represented in parenthesis in Table 5. Differences in the proportions of amino acids appearing in the duodenum, and the fraction available for absorption could be related to the proportions of bacterial cell wall and non-cell wall fractions. Quantities of bacterial amino acids escaping and appearing at the duodenum were determined by multiplying the amino acid content of each bacterial protein fraction (cell wall and non-cell wall) by each bacterial fraction produced in the rumen (O'Connor *et al.*, 1993). Only the non-cell

Table 5 The total essential amino acid supply and the absorbable fraction (g/d) provided by microbial and feed amino acids as estimated by the CNCPS

	Duodenal supply (g/d)	Arg	His	Leu	Iso	Lys	Met	Phe	Thr	Val	Trp
Diet 1	Supply	56 (64)	22 (68)	71 (59)	43 (74)	54 (83)	19 (84)	44 (66)	40 (75)	49 (71)	12 (83)
	Absorbed	45 (67)	18 (61)	55 (58)	34 (74)	42 (83)	14 (79)	33 (67)	32 (75)	38 (68)	8 (88)
Diet 2	Supply	64 (66)	27 (63)	84 (58)	51 (73)	64 (80)	22 (82)	52 (65)	48 (71)	59 (68)	13 (85)
	Absorbed	52 (65)	22 (59)	67 (55)	41 (71)	50 (80)	16 (81)	40 (62)	39 (79)	46 (65)	9 (89)
Diet 3	Supply	53 (72)	24 (63)	80 (55)	45 (73)	56 (82)	20 (80)	46 (65)	43 (72)	52 (69)	12 (83)
	Absorbed	43 (72)	19 (63)	63 (52)	36 (72)	44 (82)	15 (80)	35 (66)	34 (74)	41 (66)	8 (88)

Values in parenthesis represent the proportion of amino acids from microbial origin. The difference of this fraction from 100 will provide the proportion from dietary protein

wall fraction is considered digestible, therefore the absorbable fraction represents the amino acid content of the non-cell wall that is dependent on the quantity of true protein produced as a result of NSC fermentation (O'Connor *et al.*, 1993). A change in the values for microbial amino acid composition can have a dramatic effect on the EAA allowable gain. The CNCPS level 2, uses different absorbable amino acid compositions (O'Connor *et al.*, 1993) to the reported values of Clark *et al.* (1992) for whole bacteria. Clark *et al.* (1992) further stressed that there are differences in bacterial amino acid compositions, and that large errors can result by assuming a constant composition. Factors influencing the prediction of microbial yield are considered the most critical in predicting the EAA supply. Recent evidence of Bateman *et al.* (2001) suggests that errors in predicting the total passage of CP to the duodenum and in predicting the proportion of total CP that is microbial and feed CP, could contribute to differences in the measured and predicted passages of individual amino acids to the small intestines. Bateman *et al.* (2000) further demonstrated that the CNCPS tends to over-predict the CP originating from bacteria and under-predicts that of feed undegradable protein escaping rumen degradation. Robinson *et al.* (1998) suggests that the appropriate criterion for an amino acid adequacy in the diet is its calculated quantitative intestinal delivery in relationship to its calculated intestinal requirements, rather than its intestinal

delivery relative to the other EAA's. The predicted metabolizable supply of EAA's in relation to calculated requirements according to the factorial approach of the CNCPS are recorded in Table 6. According to the factorial assessment of net EAA requirements, the

Table 6 Model predicted absorbed AA requirements, supply and balance for a 400kg feedlot steer

AA	Diet1			Diet2			Diet3		
	Req (g/d)	Supply ¹⁾ (g/d)	Req %	Req (g/d)	Supply ¹⁾ (g/d)	Req %	Req (g/d)	Supply ¹⁾ (g/d)	Req %
Arg	24 (37)	45	188 (122)	24 (38)	52	218 (137)	24 (38)	43	176 (113)
His	17 (18)	18	105 (100)	18 (19)	22	122 (116)	18 (19)	19	106 (100)
Ile	22 (27)	34	153 (126)	23 (28)	41	174 (146)	24 (29)	36	152 (124)
Leu	52 (53)	55	106 (104)	55 (56)	67	121 (120)	55 (56)	63	114 (113)
Lys	44 (49)	42	95 (86)	47 (51)	50	108 (98)	47 (51)	44	93 (86)
Met	14 (15)	14	103 (93)	14 (15)	16	113 (107)	14 (15)	15	103 (100)
Phe	25 (27)	33	136 (122)	26 (28)	40	155 (143)	26 (28)	35	136 (125)
Thr	27 (28)	32	117 (114)	28 (29)	39	136 (134)	28 (29)	34	120 (117)
Val	31 (37)	38	121 (103)	33 (38)	46	140 (121)	33 (38)	41	122 (108)
Trp	3 (4)	8	242 (200)	4 (5)	9	261 (180)	4 (5)	8	231 (160)

Values in parenthesis are estimated from tissue amino acid composition of the present study (Chapter 3)

¹⁾ Represents the total absorbable fraction, estimated as the sum of digestible microbial and feed amino acids

results indicate, that in terms of absolute absorbable individual EAA flow, Diets 1 and 3 are limiting lysine quantitatively in the duodenum. When the amino acid composition of the present study (Chapter 3) was used, Diet 1 was additionally limiting in methionine while Diet 2 became limiting in lysine. Diet 3 reported borderline values for histidine and methionine when using the EAA composition for beef cattle in the present study. If assumed that the factorial values were predicted accurately, then Diet 2 could be considered the best in terms of amino acid adequacy to obtain an energy allowable gain

of 1.4 kg/day. Evidence exists (Zinn & Shen, 1998) that the factorial estimates may be inaccurate in estimating the metabolizable EAA's due to deviations between tabular and actual coefficients from feed ingredients and (or) due to limitations in the models. The British model (NRC, 1996) and American model (CNCPS) can have limitations for use because inputs to these models are often not available in South African production settings to mechanistically predict the supply of net energy and amino acids from feeds (NRC, 1996; Bateman *et al.*, 2001). The incorrect computation of net energy of diets will affect the energy allowable gain that is used to estimate EAA requirements. Furthermore, considering that the CNCPS microbial synthesis is based on the rate of carbohydrate fermentability in the rumen, rumen carbohydrate availability, and passage rate from the rumen, errors in the model would arise in predicting values of amino acids appearing in the duodenum (Bateman *et al.*, 2001). These errors arise from the limited database of feedstuffs and their respective rates of digestion and passage of carbohydrates and protein for beef cattle (NRC, 1996), providing a possible explanation for the differences in profiles predicted and observed in Table 7. According to Bateman *et al.* (2001), the CNCPS predicted a smaller passage of leucine, phenylalanine, valine, and threonine and higher methionine and arginine than observed *in vivo*. Considering this, the value of methionine could also become limiting and this can also explain the high value of arginine predicted, compared to its observed limiting nature. The NRC (1996) predicted similar values for Diet 2 and 3, however in contrast, the CNCPS predicted histidine the most limiting amino acid in Diet 1 followed by lysine. The NRC (2000) recognises that factorial methods can over-estimate production responses due to the under-estimated net requirements. Considering that the EAA's that were limiting based on the chemical score (Chapter 4), the CNCPS over-estimated the intestinal supply of methionine, lysine, threonine and arginine. This was consistent with the observations of Zinn & Shen (1998) who showed that the same amino acids were over-estimated by 38, 12, 14, and 54% respectively by the factorial approach. These observations are however not directly comparable due to different definitions assigned to absolute flows of individual amino acids represented by the factorial approach and individual amino acid profiles represented in chemical scores. Amino acid requirements can either be expressed in daily amounts (g/d) or on the basis of profiles. The value expressed in daily amounts is a quantity approach that provides information on the amino acid sufficiency. However, due to the different proportions of individual amino acid flows over the limiting or potentially limiting amino acids, the change of a specific

amino acid disproportionate (g/d) to another can have an effect on the profiles of amino acids, and therefore utilization. Therefore, profiles express the quality aspect of amino acids in the duodenum as a result of the different quantity of individual amino acid supplied to the duodenum. Profiles are regarded more consistent as they can be determined more accurately and are also consistent with the ideal protein concept (Schwab, 1995). Table 7 represents EAA profiles relative to total EAA's that were determined in Chapter 4, in comparison with the profiles adapted from estimated flows of individual EAA's expressed as a percentage of total EAA's.

Table 7 Essential amino acid composition as a percentage of total amino acids predicted and observed appearing in the duodenum

Item	Diet 1		Diet 2		Diet 3		Requirements	
	Predicted ¹⁾	Observed ²⁾	Predicted ¹⁾	Observed ²⁾	Predicted ¹⁾	Observed ²⁾	WEB ³⁾	WEB ⁴⁾
Arg	13.7 (88)	13.8 (89)	8.8 (57)	12.8 (83)	12.3 (79)	9.5 (61)	15.5	16.9
His	5.4 (89)	4.3 (70)	3.7 (61)	5.0 (82)	5.6 (92)	5.0 (82)	6.1	6.3
Leu	17.3 (109)	19.3 (122)	11.5 (73)	16.2 (103)	18.6 (118)	20.2 (128)	15.8	17.2
Ile	10.5 (115)	9.6 (105)	7.0 (77)	10.0 (110)	10.4 (114)	10.7 (118)	9.1	7.3
Lys	13.2 (79)	13.7 (82)	8.8 (52)	16.4 (98)	13.0 (77)	15.5 (92)	16.8	16.4
Met	4.6 (100)	4.1 (89)	3.0 (65)	4.7 (102)	4.6 (100)	3.7 (80)	4.6	5.1
Phe	10.7 (118)	9.7 (107)	7.1 (78)	10.0 (110)	10.7 (118)	9.8 (108)	9.1	9.1
Thr	9.8 (108)	9.3 (102)	6.6 (73)	9.1 (100)	10.0 (110)	8.8 (97)	9.1	10.0
Val	12.0 (100)	12.8 (107)	8.1 (68)	12.0 (100)	12.1 (101)	13.4 (112)	12.0	10.4
Trp	2.9 (153)	3.4 (179)	1.8 (95)	3.8 (200)	2.8 (147)	3.4 (179)	1.9	1.3

Values in parenthesis represent the proportion of amino acids relative to the requirements estimated in Chapter 2, providing a chemical score

¹⁾ Predicted values of absolute amino acid flows based on the factorial approach of the CNCPS, altered to a profile basis by expressing values as a percentage of total EAA's

²⁾ Observed profiles of amino acids expressed as a percentage of total EAA's that appeared in the duodenum (Chapter 4)

³⁾ EAA requirements based on WEB composition reported in Chapter 3, expressed as percentage of total EAA's

⁴⁾ EAA requirements reported by Ainslie *et al.* (1993) expressed as percentage of total EAA's

EAA's expressed as a percentage of total EAA's that were predicted by the factorial approach, tended to deviate from the observed values. The profiles of leucine, lysine, valine and tryptophan were under-predicted in all three diets while the profiles of histidine, methionine, phenylalanine and threonine were over-predicted by Diet 1 and 3, and under-predicted in Diet 2. According to Bateman *et al.* (2000) the differences in the assumed microbial and feed amino acid concentrations in addition to the estimated amounts of the relevant fractions, could probably contribute to the differences in measured and predicted values. When the individual EAA requirements based on empty body tissue, were expressed as a percentage of total EAA's (Table 7), the requirements reported by Ainslie *et al.* (1993) tended to be higher in arginine, leucine, methionine, threonine and tryptophan, while lower in isoleucine and valine, than values reported in Chapter 3. The change in differences can be attributed to the lower total EAA concentration of 39% reported by Ainslie *et al.* (1993) compared to the reported value of 44% in Chapter 3. The definition of net requirements, due to different reported ideal profiles, will be dependent on the reference of the ideal protein used. With the objective to compare predicted results with Chapter 3, the tissue EAA concentration of the present study was used to determine chemical scores. The order of EAA limitation of the predicted and observed values in the duodenal digesta of Diet 1 to 3 are: Predicted; lysine, arginine, histidine and observed; histidine, lysine, arginine/methionine (Diet 1). Predicted; lysine, arginine, histidine, methionine and observed; histidine, arginine, lysine (Diet 2). Predicted; lysine, arginine, histidine and observed; arginine, methionine, histidine, lysine and threonine (Diet 3). It is clear that both the predicted and observed are limiting in similar amino acids, the sequence of limitation however tends to differ. Results from Table 7, illustrate that although there were sufficient amino acids for a daily growth rate of 1.4 kg, the profiles of certain amino acids were disproportionate and unbalanced to the requirements that could possibly lead to a decrease in utilization. Considering that the objective of ruminant research is not only directed at supplying amino acids for a given gain, but also at improving the efficiency of utilization (Hvelplund & Madsen, 1996; Schwab, 1996a), it is important that these profiles are corrected.

According to Hvelplund & Madsen (1989), the prediction of absolute amino acid flow values is more affected by variation in CP flow than by the variation in amino acid composition of protein. Bateman *et al.* (2001) stated that factorial approaches are prone to errors in estimating the passage of duodenal CP from either microbes or feed, and

that these errors would lead directly to errors in estimating passage to the total duodenal CP and amino acids. Rulquin *et al.* (1998) formulated an ideal protein prediction model based on the PDI system (INRA, 1989) that accurately estimates protein flow to the duodenum, and predicts individual amino acid methionine and lysine contents in the duodenum and not the absolute flow of individual amino acids. This approach provided a true integration of the amino acid sub-model within the protein model. In addition, and in contrast to the described factorial models in which both the structure and parameters were determined on theoretical grounds, multivariate regression approaches allow some parameters to be determined by regression. Schwab (1996b) developed regression equations to predict the contributions of lysine and methionine to the total EAA's in the duodenal digesta of cattle. Table 8 represents the amino acid ratios based on the methionine and lysine concentration in MP and on the percentage of the two latter amino acids in total EAA's. According to the Rulquin ratios, the metabolizable protein is

Table 8 **Estimated methionine and lysine ratios expressed as a percentage of metabolizable protein and as a percentage of total essential amino acids in the duodenum**

	Required	Diet 1		Diet 2		Diet 3	
		Supply	% Req	Supply	% Req	Supply	% Req
Rulquin ¹⁾							
Met	2.1	2.1	100	2.0	95	2.0	95
Lys	>6.5	6.3	97	6.3	97	6.0	92
Schwab ²⁾							
Met	5.1	4.4	86	4.3	84	4.4	86
Lys	16.3	13.2	81	13.2	82	13.0	80

¹⁾ Rulquin system expresses methionine and lysine as a percentage of metabolizable protein flow to the small intestine

²⁾ The Schwab system expresses methionine and lysine as a percentage of EAA flow to the small intestines

limiting in methionine (Diet 2 and 3) and lysine in all three diets. The regression values determined by Schwab support this concept, however the amino acids tend to be more limiting. In accordance with the predicted values of the factorial approach and observed amino acid ratios of lysine and methionine in total EAA's, Schwab also determined the latter amino acids to be limiting. The lysine and methionine concentrations observed showed a tendency to be under-predicted by both the factorial (Table 7) and Schwab ratios (Table 8) in Diet 2, while Diet 3's lysine ratio was under-predicted and methionine

over-predicted. Schwab (1996) calculated requirements for amino acids using both the factorial system and his ideal protein system, and determined that the factorial system approximated his system when there were no excesses of the other EAA's. Clearly, from this report, most diets will result in excess of one or more amino acids when fed to cattle; therefore, it is difficult to determine how sensitive these requirements are to changes in supplies of the other amino acids to cattle. The predicted Schwab and adapted factorial ratios tended to be similar between diets, however the observed ratios ranged from 13.7 to 16.4 for lysine and from 4.1 to 4.7 for methionine between diets. Erasmus (1993) mentioned that most of the variation between lysine and methionine profiles in the duodenal digesta could be attributed to the UDP contribution to diet CP and the content of lysine and methionine in diet UDP. The constant microbial amino acid composition reported by the factorial approach, that tends to be over-predicted in relation to UDP, leads to smaller variation of amino acids predicted in the duodenum. According to Zinn & Shen (1998), the NRC (1996) may over-estimate methionine supply to the small intestine, which was the case in Diet 1 and 3 in the present study (Table 7). The logic of efficient protein utilization of balanced EAA's profiles has emphasized the need to identify the EAA that is most limiting in cattle diets when fed different feed compositions (NRC, 2000). According to Erasmus (1998), methionine and lysine are limiting in diets typically used in South Africa. The present study, in addition, indicates that other EAA's may further be limiting for optimal utilization. Knowledge of the sequence of amino acid limitation as affected by diet composition, provides an opportunity to improve AA profiles with feed supplements.

Table 9 represents the dietary effects on the predicted performance of cattle observed in the present study. From the results, ADG can be limited by the first limiting (lysine) EAA in Diet 1 and 3. In Diet 2 where lysine was in excess, the ADG was limited by the ME allowable gain. When other nutrients are limiting, any approach to define amino acid requirements would be incomplete because responses can only be improved to the level allowed by these limitations. Increasing ADG by supplying the limiting EAA can hold great value, as this can represent up to 150 grams growth/day in Diet 3. The effective amino acid supply from the small intestines can influence the efficiency with which ME is used for growth (Oldham, 1996). The effective amino acid supply, defined not only by sufficiency of EAA's, but also by the proportionality of amino acids relative to each other,

Table 9 **Dietary effects on the 100-d performance of growing-finishing crossbred steers**

Item	Diet 1	Diet 2	Diet 3
DMI (kg/day) ¹⁾	7.60	8.40	8.50
NE available for growth (Mcal/day) RE ²⁾	5.79	5.74	5.79
EQSBW ³⁾	297	297	297
ME Allowable gain (kg/day) ⁴⁾	1.41	1.40	1.41
MP Allowable gain (kg/day) ⁵⁾	1.38	1.72	1.48
Lysine Allowable gain (kg/day)	1.29	1.72	1.25
Urea Cost (Mcal/day)	0.21	0.17	0.18
Excess N excreted (g/day)	42.0	32.0	45.0

¹⁾ The DMI of each feedstuff

²⁾ The Net energy that is available for growth

³⁾ Equivalent shrunk body weight

⁴⁾ Metabolizable energy allowable gain

⁵⁾ Metabolizable protein allowable gain

can influence the use of absorbed energy resulting in an increase in the ME allowable ADG relative to MP and EAA allowable gain (Fox *et al.*, 1992). The metabolizable energy value of a food will therefore vary according to whether the amino acids it supplies are retained by the animal for protein synthesis, or are delaminated and their nitrogen excreted in the urine as urea (Chen & Ørskov, 1994). Absorbed amino acids in excess of the demands and/or imbalanced profiles contribute to recycling of nitrogen to the rumen (Hvelplund & Madsen, 1996) after transformation to urea in the liver. However, 62% of urea produced by the liver is excreted in urine, resulting in a net loss of nitrogen. In addition to net nitrogen loss, ammonia imposes an increase in energy expenditure emphasizing its metabolic cost and the importance of considering the formation of this compound in the rumen when balancing a diet. According to Table 9, Diet 2 appears to have the least N excreted with the lowest urea cost that coincides with the better profile of EAA's in the duodenal digesta (Table 7) and the sufficient quantities of EAA's supplied (Table 6).

Conclusion

According to the present study, the CNCPS protein model predicted that Diet 1 and 3 were insufficient in lysine to provide an energy allowable gain of 1.4 kg/day, that related to 207g of protein retained per day. Although the EAA compositions are regarded reliable, the variable EAA compositions required for growth used in the factorial models

and the higher values reported in the present study, induced Diet 1 to become additionally limiting in methionine while Diet 2 became limiting in lysine. The use of the fixed efficiency coefficient of 49.6 % over the whole growth period, according to the factorial assessment, is assumed constant and the same for all amino acids. Factorial approaches therefore can erroneously predict the metabolizable amounts and proportions required for a given gain as efficiencies change with age, are dictated by dietary amino acids in relation to the ideal protein, and differ between amino acids. These variable efficiencies cause the factorial approach to deviate from the ideal protein concept and increase the bias in predicting MP and EAA allowable ADG. Literature reports (Zinn & Shen, 1998; Bateman *et al.*, 2001) mentioned that the CNCPS over-predicts the methionine, lysine, threonine and arginine supply, implying that these amino acids can also become limiting. The conversion of the predicted factorial EAA flow into profiles, tended to deviate from observed duodenal profiles. The factorial approach generally under-predicted the profiles of leucine, lysine valine and tryptophan, while phenylalanine, threonine, methionine and histidine were over-predicted in Diet 1 and 3 and under-predicted in Diet 2. The ability of models to predict profiles, as a result of individual amino acid flows to the duodenum, was demonstrated by estimating similar limiting EAA's based on the chemical scores of observed values. The sequence and extent of limitations tended to vary. Results from the present study pointed out that the order of EAA limitation of the predicted and observed values in the duodenal digesta of Diet 1 to 3 were: Predicted; lysine, arginine, histidine and observed; histidine, lysine, arginine/methionine (Diet 1). Predicted; lysine, arginine, histidine, methionine and observed; histidine, arginine, lysine (Diet 2). Predicted; lysine, arginine, histidine and observed; arginine, methionine, histidine, lysine and threonine (Diet 3). Differences between predicted and observed values could be due to error in estimating the proportions of microbial and feed protein, and their relevant amino acid concentrations that appear in the duodenum, due to deviations between tabular and actual coefficients from feed ingredients under South African conditions. Considering that the net protein requirement is dependent on the ME ADG, any error in estimating energy available from the feeds, will yield errors in predicting the metabolizable EAA required for a given gain. Although diets were sufficient to provide enough quantities of EAA's to the duodenum for growth, the quality was disproportionate to that required for optimal utilization. The development of more accurate feed compositions with their relevant digestion rates, and more mechanistic approaches to predict utilization of absorbed amino acids, will result in

improved predictability of dietary amino acid adequacy for cattle (Fox & Barry, 1996). The data reported in the present study can be utilized in preparing or evaluating diets based on maize for feedlot cattle. The challenge is to develop a rumen microbial protein and a ruminal bypass protein combination that presents a more desirable array of amino acids in the lower digestive tract, which is more digestible and absorbable than normally presented.

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CHAPTER 6

GENERAL DISCUSSION AND CONCLUSION

Growing cattle require dietary nitrogen and amino acids to meet the requirements of both the microbial population and the animal's metabolic requirements for amino acids, which are the building blocks of proteins. With the understanding of the biology underlying specifics of protein nutrition in ruminants, research was directed towards formulating diets on an amino acids basis because the tissues of the ruminant actually require amino acids for protein synthesis, rather than protein *per se*. The determination of amino acid requirements is based on a fundamental concept of an optimal pattern among essential amino acids that correspond to the needs of the animal. This optimum pattern is defined as the "Ideal Protein". The basic concept of amino acid balancing is interpreted by comparing the amino acid flow to the small intestine with the amino acid requirement for the synthesis of tissue. Amino acids that make up tissue protein is encoded in the genes within the animal; therefore, the amino acid composition of tissue is reasonably constant and characteristic of the types of proteins synthesized by a given tissue.

Research from the present study shows that there are differences in essential amino acids of the whole empty body values reported in literature, hence different ratios required for the ideal protein. In Chapter 3, the whole empty body essential amino acid composition was determined from three body protein pools, namely, the carcass, metabolic organs and residual fractions. As reported, each of these fractions had its own unique essential amino acid composition. If these fractions change through either growth or diet manipulation (implants), there could be an altered whole empty body essential amino acid concentration. It has also been emphasised in the present study, to define the requirements of a specific genotype and species, as not only the protein pools vary between the latter, but also certain genotypes have a greater potential for meat protein production. Considering that the tissue amino acid composition of the skeletal muscle tends to be high in most essential amino acids, it was hypothesised that the genotypes of beef breeds bred for high average daily gain, have a higher requirement for certain essential amino acids related to muscle production. The ratio of amino acids used from body composition studies in relation to lysine can only be used as estimates, as no accurate values for tissue turnover rates and maintenance requirements are known.

The ideal protein system is based on the concept that amino acids will be used for productive functions in a characteristic *pro rata*; therefore, balancing on an ideal protein basis will maximize the efficiency of nitrogen utilization. The evaluation of the three standard feedlot diets (Chapter 4) through the investigation of the duodenal essential amino acid composition, clearly indicated that there was variation between the profiles of the three diets. If assumed that microbial amino acid compositions are constant, the variation could be ascribed to the different undegradable protein essential amino acid compositions of the diets. Although there was a general increase in lysine and methionine profiles, which are often considered limiting, in the duodenal digesta after microbial fermentation of the dietary protein, the chemical scores still recorded deficient profiles of several essential amino acids (Chapter 4). The chemical scores suggested that the first-to-third-limiting amino acid in the duodenal digesta of beef cattle that received the three commercially fed feedlot diets were: histidine, lysine, methionine/arginine (Diet 1), histidine, arginine, lysine (Diet 2) and arginine, methionine, histidine (Diet 3). Clearly from this report, the sequence of limitation varies between diets with several amino acids becoming closely co-limiting.

The provision of duodenal essential amino acids in the correct amounts and proportions to ruminants determines the adequacy (sufficiency) of a specific diet as result of rumen fermentation of protein and carbohydrates. There are two principal systems used to formulate diets on an amino acid basis. One is the factorial system used in the CNCPS; the second is the ideal protein system of Rulquin and Schwab, with the same approach used in the present study. The factorial system calculates amino acid requirements using the net amount of protein synthesized for maintenance and growth, its amino acid composition, and efficiency factor for the conversion of absorbed (metabolizable) amino acids to the net amino acid requirements. As reported, each of these steps has variance associated with it, and these systems are particularly sensitive to the efficiency factors for maintenance and growth. The factorial system can be associated with bias in predicting metabolizable protein and essential amino acid allowable average daily gain and tends to deviate from the ideal protein concept because of the different efficiencies. These systems provide information on the absolute flow of individual amino acids to the small intestine. In many situations, efficiencies of one or more amino acids may limit production quantitatively. The factorial assessment on net requirements for absorbed amino acids (Chapter 5) identified lysine as limiting for an energy allowable gain of 1.4

kg/day, yet it tends to overestimate absolute flows of individual amino acids that could possibly be limiting in terms of quantity for this gain.

When profiles were estimated using the factorial approach (Chapter 5), the order of essential amino acid limitation of the predicted duodenal digesta of Diet 1 to 3 were: lysine, arginine, histidine (Diet 1), lysine, arginine, histidine, methionine (Diet 2) and lysine, arginine, histidine (Diet 3). It is clear that both the predicted (Chapter 5) and observed profiles (Chapter 3) are limiting in similar amino acids; the sequence and extent of limitation however tends to differ. The predicted profiles of essential amino acids according to Rulquin and Schwab, as well as the adapted CNCPS factorial approach in comparison with the observed profiles; all indicated lysine and methionine limiting for optimal growth in all three diets. Imbalances (observed in all three diets) or large excesses of amino acids (leucine in Diet 1 and 3) resulted in increased calculated catabolism of amino acids by the liver and excretion of urea at an energy cost of the steers. Amino acid balancing provides the incentive to meet amino acid requirements while simultaneously decreasing the crude protein content of the diet, which may have positive implications on the environment from a nitrogen excretion point of view.

The evaluation of duodenal digesta based on chemical scores is assumed to over-predict histidine, due to endogenous reservoirs, and under-predict methionine and threonine requirements due to the contribution of these amino acids to endogenous losses associated with maintenance. Arginine is also assumed to be over-predicted, as it is regarded as a semi-essential amino acid that can be synthesised from glutamine. Accordingly, the factorial approach tends to consider this by decreasing the arginine requirement. The factorial approach also includes maintenance requirements for amino acids, however the accuracy of the essential amino acids associated with the fractions of scurf, urinary and endogenous losses needs further attention. Furthermore, the chemical scores also represent the total supply of amino acid profiles and not the absorbable fraction that would be available for metabolism. The profiles of the supply and absorbable fraction may differ, as different profiles are associated with bacterial wall and non-cell fractions, which are represented by the factorial approach. The computation of bacterial amino acids are assumed constant by the factorial approach, however bacterial amino acid compositions can vary according to the composition of the diet, feeding frequency, passage rates and substrate fermented. The assumption of

constant bacterial amino acid composition by the factorial approach can lead to bias in predicting the amino acid content originating from bacteria. On the other hand, the prediction of absolute amino acid flow values is more affected by variation in CP flow than by variation in amino acid composition of protein. Therefore, profiles are regarded more consistent as it can be determined more accurately and is consistent with the ideal protein concept.

From the present study, it is clear that the diets were insufficient to meet the metabolic requirements. Therefore, the challenge is to develop a rumen microbial protein and ruminal bypass protein combination that presents a more desirable array of amino acids to the lower digestive tract that is more digestible and absorbable than normally presented. This approach provides an option that is economical viable for the feedlot operator. Chase (1996) mentioned a variety of approaches that can be used to increase amino acid flow and change the profiles to the duodenum to achieve maximum energy allowable ADG:

1. Maximize dry matter intake.
2. Increase microbial protein synthesis.
3. Altering the degradability of protein sources by chemical or heat treatments.
4. Selecting protein sources to control ruminal degradation.
5. Selecting protein sources which enhance the conversion of crude protein to absorbable protein.
6. Feeding high levels of commercially available undegradable protein sources.
7. Use of rumen protected amino acids.

Maize based diets could be supplemented with high lysine content ingredients such as fishmeal, soybean products (Erasmus, 1998) and canola (Schingoethe, 1996). The escape protein of these supplements can be utilized efficiently, suggesting that the amino acid spectrum is complementary to that of microbial protein. However considering the high price of protein sources, economic imperatives may underscore the importance of high quality protein supplementation to meet the amino acid requirements. Therefore, to realize a cost-of-gain advantage of balanced feedlot rations for rapidly growing cattle, the total economics need to be considered, not just the relative cost of the protein supplement. Supplementation with protein sources that provide limiting amino acids, may increase the feed conversion efficiency, thus lowering feed costs per unit of weight

gain or production. It is therefore essential to evaluate supplements on a cost-per-unit of protein produced, in order to achieve maximal profitability.

Although the balancing of amino acids has potential to optimise production, more research must be conducted on the whole empty body essential amino acid composition, maintenance and efficiency of utilization, before requirements for individual amino acids can be determined accurately. Further investigation into the amino acid requirements of rapidly growing steers fed maize based diets will be required before it can be expected to economically improve performance of growing cattle via manipulation of amino acid supply. Nevertheless, sufficient progress has been made to begin to improve intestinal amino acid profiles to allow for increased and more efficient production. A high research priority is to increase our ability to accurately predict both the extent, and composition of microbial growth on a particular diet. The importance to accurately predict the amino acid composition of rumen escape proteins is essential if the amino acid requirements of the animals are to be met, but not exceeded. Once factorial methods can be regarded reliable in estimating net requirements for nutrients in the commercial field, it would be regarded as a valuable step in nutrient research and therefore, animal production.

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