SPECIALIZED FEEDING OF LAMBS FOR OPTIMIZED PERFORMANCE DURING THE FINISHING PHASE

by

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that the reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

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ABSTRACT

The aim of this study was to determine whether a lamb finishing ration that is balanced for essential amino acids (EAA), at a specific level of non-structural carbohydrates (NSC) would yield better feedlot performance when compared to a standard commercial finishing ration.

Metionine (Met) and Lysine (Lys) were identified as the first limiting amino acids for growing lambs. These two amino acids, as well as Threonine (Thr), Arginine (Arg), Leucine (Leu), Isoleucine (Ile) and Phenylalanine (Phe) were included in an optimized protein (OP) feedlot ration at optimal levels. This ration was formulated to contain 157 g/kg crude protein (CP) and 477 g/kg NSC, with Met, Lys, Arg and Thr at 2.48, 7.45, 8.51 and 8.12 g/kg on dry matter (DM) basis respectively. The second treatment, FIN, was a standard commercial lamb finishing feed without optimized amino acids and contained similar total protein and NSC to OP of 152 g/kg CP and 468 g/kg NSC but with Met, Lys, Arg and Thr at 2.08, 5.49, 7.47 and 4.80 g/kg on DM basis respectively. A third treatment, a low protein (LP) diet served as a positive control and was formulated on lower specifications (139 g/kg CP, 455 g/kg NSC) and was also not optimized for amino acids. This treatment contained Met, Lys, Arg and Thr at 1.93, 4.99, 6.66 and 4.73 g/kg on DM basis respectively. Lambs grazing kikuyu pasture served as the negative control (CON) group. These lambs also received additional supplementary feed at 500 g/day as a production lick to be comparable to a scenario where lambs are finished on grazing.

Forty cross-bred Merino x Döhne-Merino lambs with an average weaning weight of 24.35 ± 0.648 kg were finished in a feedlot for 57 days where after they were slaughtered at an average weight of 41.41 ± 1.259 kg. During the feedlot trial lamb performance was measured by monitoring daily growth rates and feed intake. Also, an *in vivo* digestibility study was carried out on the OP and FIN lambs. During the slaughter process the rumen was removed which was done to collect a sample of the rumen wall from next to the rumino-reticular fold. These rumen samples were mounted onto slides so that the development of the rumen could be examined. The

M. longissimus dorsi from both sides of the carcass between the 2nd and 3rd last thoracic vertebra and the 4th and 5th lumbar vertebra were removed.

There was no significant difference ($P > 0.05$) between any of the concentrate feed treatments with regards to the average daily gain (ADG), feed conversion ratio (FCR) or dressing percentage (DP). The CON lambs, as expected, had lower growth rates ($P < 0.05$) than the concentrate fed lamb and thus showed significant differences ($P < 0.05$) in terms of ADG. The DP of 45.96 % ± 0.711 for the CON lambs differed significantly ($P < 0.05$) from the OP (51.44 % ± 0.358), FIN (52.72 % ± 0.653) and LP (51.74 % ± 0.611) treatments. As expected the concentrate feeds were much more effective in maintaining higher growth rates when compared to the CON lambs while the optimizing of EAA in the OP diet did not lead to improved feedlot performance as the FIN and LP treatments were able to achieve similar ($P > 0.05$) growth rates.

Within the feedlot treatments there was no significant difference ($P > 0.05$) with regard to the papillae length and rumen wall thickness. There was however a numerical increase in the papillae length as the NSC levels in the feed increased. The CON lambs differed from the OP lambs ($P < 0.05$) in terms of papillae length. This illustrated the importance of having increased levels of NSC in a feedlot diet as it is this fraction that is responsible for the initialisation and maintenance of rumen morphological development.

The *in vivo* digestibility study therefore confirmed that the commercial finishing feed was just as effective as the optimized feed in terms of nitrogen retention as well as in maintaining suitable energy balance. Although the *in vivo* digestibility for Met and Lys in the OP feed was higher ($P < 0.05$) than the FIN feed, this did not lead to improved feedlot performance of the OP lambs. The increased digestibility of these amino acids is due to the fact that the OP diet was higher in levels of bypass amino acids than the FIN feed. The *in vitro* true digestibility (IVTD) of the OP feed was higher ($P < 0.05$) than that of the FIN feed.

OPSOMMING

Die doel van hierdie studie was om te bepaal of ‘n lamafrond rantsoen wat gebalanseerd is vir beperkende essiensiële aminosure (EAA) teen ‘n spesifieke vlak van nie-strukturele koolhidrate (NSK) beter voerkraal prestasie teweeg sou bring wanneer gemeet word teen ‘n standaard kommersiële afrond rantsoen.

Metionien (Met) en Lisien (Lis) is geïdentifiseer as die eerste beperkende aminosure vir groeiende lammers. Hierdie twee aminosure, asook Treonien (Tre), Leusien (Leu), Isoleusien (Ile) en Fenielalanien (Fen) is teen optimale vlakke ingesluit in ‘n geoptimeerde voerkraal rantsoen, OP. Hierdie rantsoen is geformuleer om 157 g/kg RP en 477 g/kg NSK te bevat asook Met, Lis, Arg en Tre teen 2.48, 7.45, 8.51 en 8.12 g/kg onderskeidelik. Die tweede behandeling, FIN, was ‘n kommersiële lamafrond voer waarin die aminosure nie geoptimeer is nie en het soortgelyke vlakke van proteïene en NSK bevat teen 152 g/kg RP en 468 g/kg NSK met Met, Lis, Arg en Tre teen 2.08, 5.49, 7.47, 4.80 g/kg onderskeidelik. ‘n Derde voer, LP, het gedien as ‘n positiewe kontrole en was ‘n lae proteïen voer met laer spesifikasies (139 g/kg RP, 455 g/kg NSK) waarin die aminosure ook nie geoptimeer is nie. Die LP voer het Met, Lis, Arg en Tre bevat teen 1.93, 4.99, 6.66 en 4.73 g/kg onderskeidelik. Die negatiewe kontrole behandeling, CON, is verteenwoordig deur lammers wat op kikuyu gewei het terwyl addisionele supplementêre voeding teen 500g/lam/dag voorsien is. Hierdie supplementêre voeding het gedien as ‘n produksie lek om sodoende vergelykbaar te wees met scenario waar lammers op weiding afgerond word.

Veertig kruisgeteelde Merino x Döhne-Merino lammers met ‘n gemiddelde gewig van 24.35 ± 0.648 kg is vir 57 dae in ‘n voerkraal afgerond waarna hulle, teen ‘n gemiddelde gewig van 41.41 ± 1.259 kg, geslag is. Tydens die voerkraal proef is prestasie gemonitor deur die meet van daaglikse groei en voerinname. Hiertydens is daar ook ‘n *in vivo* verteringsproef op die OP en FIN lammers gedoen. Tydens die slagproses is die rumen verwyder waarna ‘n monster van die rumenwand langs die rumino retikulêre vou geneem is. Hierdie rumenmonsters is op skyfies geplaas sodat die ontwikkeling van die rumen ondersoek kan word. Die *M. longissimus dorsi* was

aan beide kante van die karkas tussen die 2de en 3de laaste torakale werwels en die 4de en 5de lumbale werwels verwyder.

Daar was geen betekenisvolle verskille (P > 0.05) tussen enige van die konsentraat behandelings ten opsigte van gemiddelde daaglikse toename (GDT), voeromset verhouding (VOV) of uitslag persentasie nie. Die CON lammers het egter, soos verwag, beduidend (P < 0.05) stadiger gegroei en het dus verskille getoon ten opsigte van GDT. Die uitslag persentasie van 45.96 % ± 0.711 vir die CON lammers het ook betekenisvol verskil (P < 0.05) van die OP (51.44 % ± 0.358), FIN (52.72 % ± 0.653) en LP (51.74 % ± 0.611) behandelings.

Binne die voerkraal behandelings was daar geen betekenisvolle verskille (P > 0.05) ten opsigte van die papillae lengte en rumenwand dikte nie, alhoewel daar ‘n numeriese toename in papillae lengte was soos die NSK vlakke in die voer gestyg het. Die CON lammers het wel van die OP lammers verskil (P < 0.05) ten opsigte van papillae lengte. Hierdie bevinding het bevestig hoe belangrik NSK is in die inisiasie en instandhouding van die morfologiese ontwikkeling van die rumen.

Die *in vivo* verterings studie het daarop gedui dat die kommersiële afrond voer net so effektief soos die geoptimeerde voer was in terme van stikstof retensie asook die handhawing van ‘n geskikte energie balans. Alhoewel die *in vivo* verteerbaarheid van Met en Lis in die OP hoër was (P < 0.05) as in die FIN voer, het hierdie verskille nie gelei tot beter groei in die OP lammers nie. Hierdie verskil in verteerbaarheid is toegeskryf aan die feit dat die aminosure in die OP voer meer rumen-beskermd was as dié in die FIN voer. Die *in vitro* verteringstudie het daarop gedui dat die OP voer beduidend beter (P < 0.05) verteer is as die FIN voer.

DEDICATION

I dedicate this thesis to my parents Riaan and Margôt Moolman. Thank you for all the sacrifices you have made through the years which has allowed me to reach this goal. Thank you for raising me the way you did, instilling discipline in me and the belief that I can achieve anything if I put my mind to it. I will never be able to thank you enough.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADG	Average daily gain
ADS	Acid detergent solution
Ala	Alanine
Arg	Arginine
Asp	Asparagine
BFT	Backfat thickness
BUN	Blood urea nitrogen
$BW^{0.75}$	Metabolic body weight
CF2	Creep feed 2
CON	Control
CP	Crude protein
Cys	Cystine
DE	Digestible energy
DM	Dry matter
DMI	Dry matter intake
DP	Dressing percentage
EAA	Essential amino acids
EE	Ether extract
ERR	Empty reticulo-rumen
EUN	Endogenous urinary nitrogen
FBW	Final body weight
FCR	Feed conversion ratio
FIN	Standard commercial finisher feed
FRR	Full reticulo-rumen
GE	Gross energy
GLM	General linear models
Glu	Glutamine
Gly	Glycine
His	Histidine
Ile	Isoleucine

IVTD	In vitro true digestibility
Leu	Leucine
LI	Large intestine
LP	Low protein feed
Lys	Lysine
MCP	Microbial crude protein
ME	Metabolisable energy
Met	Methionine
MFN	Metabolic faecal nitrogen
NDF	Neutral detergent fibre
NSC	Non-structural carbohydrate
OM	Organic matter
OP	Optimized protein feed
Phe	Phenylalanine
Pro	Proline
RDP	Rumen degradable protein
RUP	Rumen undegradable protein
s.d.	Standard deviation
s.e.	Standard error
SAMM	South African mutton merino
SC	Structural carbohydrates
Ser	Serine
SI	Small intestine
TDN	Total dietary nutrient
Thr	Threonine
Tyr	Tyrosine
UDP	Undegradable protein
Val	Valine
VFA	Volatile fatty acids
WEB	Whole empty body
WHC	Water holding capacity

CHAPTER 1

GENERAL INTRODUCTION

1. Introduction

This project was conducted to optimize the efficiency with which the nutrients in finisher diets are utilized by lambs fattened in a feedlot. The specifications of the rations used in the study was based on work done by Nolte (2006) in which they reported the limiting amino acids for growing lambs. Based on their research, the amino acid requirements of growing lambs were calculated. It has been stated that ruminants would utilize protein for growth most efficiently when the supply of amino acids are the same as that required for tissue growth (Hussein 1991). Due to the lamb not having to digest solid food, and thus in effect being a "monogastric" in digestion capability, the diets were also optimized for non-structural carbohydrates (NSC). Extensive research has been done in dairy calf production on the use of various starches and concentrates to improve the efficiency of utilization of nutrients and to improve the development of the reticulo-rumen. In this regard, Nocek *et al*. (1984) showed that both the chemical composition of the feedstuffs fed as well as the end products of microbial digestion of these feedstuffs has the biggest influence on the development of villi in the reticulo-rumen. Heinrichs *et al*. (2005) stated that concentrates and diets containing starches and other nutrients results in rates of rumen development greater than that of forages. Furthermore, there is a well-documented optimal protein to energy balance for animal production (McDonald *et al.*, 2002) and based on these reasons the diets were also be optimized for energy, using NSC.

Until recently, the protein requirements of ruminants were expressed only in terms of crude protein (CP) levels, thus diets were formulated for rumen degradable (RDP) and rumen undegradable protein (UDP). Animals use most of the nitrogen they require for protein synthesis. Most of the nitrogen in feedstuffs is also present as protein and this is why the nitrogen requirements of the animal as well as the nitrogen content of the feed can be expressed in terms of protein (McDonald *et al*.,

2002). A limitation of the CP system however, is that it does not take into account the quality of the protein (amino acid profile) of the feed or the requirements of the animal (Nolte, 2006). Although the CP system indicates the nitrogen content of the feed, it is no indication of its actual value for the animal. Thus, even though the CP or nitrogen requirements of the animal might be met, individual amino acids could still be deficient (Nolte, 2006). The concept of an ideal protein has been defined by Chen & Orskov (1994) as a protein that would provide absorbed amino acids in the proportion that leads to optimal utilization of the protein. As it is important to take the amino acid levels at a tissue level into account (Schingoethe, 1996), various authors have set out to determine this ideal profile of absorbed amino acids needed for optimum growth and maintenance of the ruminant (NRC, 2000). There is a close correlation between the pattern of amino acids required for the accretion of protein in the body and the actual amino acid composition of the body and thus the amino acid composition of the whole empty body (WEB) can be used to determine this ideal pattern of amino acid absorption for optimal growth (Fuller, 1996). Therefore, by determining the duodenal amino acid profile and comparing that to the amino acid profile of the WEB, the limiting amino acids can be calculated and included in the diet (Ferreira, 1999).

Non-structural carbohydrates consist of mainly the starch fraction of the feed but also include compounds such as sugars, pectins, galactans and β-glucans. This fraction is a very important source of energy in the diet. The process of microbial protein synthesis in the rumen is an energy dependent process, therefore the supply of energy and protein to the ruminant goes hand-in-hand (Titgemeyer, 2003). There is a strong interaction between protein and energy metabolism in the rumen of animals, therefore a shortage or ineffective utilization of CP could lead to a decrease in the digestibility of carbohydrates. Similarly, a shortage of carbohydrates could lead to the loss of nitrogen in the form of ruminal ammonia (Nocek *et al*., 1984). In order to optimize production and growth it is therefore important that the rate of carbohydrate fermentation is balanced to that of protein synthesis (Russell, 1992). This can be achieved by matching the availability of carbohydrates and protein in the rumen (Aldrich *et al*., 1993).

While the supply of forages to ruminants is important in stimulating the muscular development of the rumen, whilst upholding the integrity of its epithelium, the digestion thereof does not produce enough volatile fatty acids (VFA) needed to stimulate papillary development (Norouzian *et al*., 2011). The supply of concentrate diets provides readily fermentable carbohydrates and it is the fermentation of these carbohydrates that produce VFA, which stimulate papillae development in the rumen (Steele *et al*., 2009).

It is postulated that lambs fed a feedlot ration formulated to meet the limiting EAA requirements of lambs, at a certain NSC level, would show improved feedlot performance when compared to lambs receiving a standard commercial feedlot diet. This would be achieved by lambs showing increased growth rates and improved feed efficiencies while producing meat of a good quality that meets consumer preferences.

Another aim of this study was to investigate whether a feedlot diet, optimized for EAA, and fed at a specific NSC level, would improve the nitrogen retention, energy balance and digestibility of feedlot lambs in an *in vivo study*. Also, to see whether the levels of EAA and NSC in a standard commercial finisher diet commonly used for feedlot lambs were adequate. An *in vitro* study was done to determine what the effect of optimizing the EAA, fed at specific NSC levels would have on the IVTD of the EAA.

A third aim of this study was to compare the morphological development of the rumen at marketing age (slaughter) to that at weaning age while also monitoring what the effect on rumen morphological development would be when lambs were fed a creep feed high in NSC after which being put on a feedlot diet with lower NSC levels.

Throughout this paper it is important to note that although the OP and FIN treatments had very similar CP levels, the difference between these two feeds was on an amino acid level, which was the main focus of this study.

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CHAPTER 2

LITERATURE REVIEW

2.1 Production efficiency of lambs in a feedlot

The intensive production of meat is the fastest growing sector of the world meat industry and produces 40% of meat consumed globally (Dickson-Hoyle, 2009). Globally there has been a gradual shift from extensive systems on a smaller scale to intensive, large scale production systems (Steinfeld *et al*., 2006). This could mainly be due to the fact that intensive systems are unaffected by changes in environmental factors such as global warming and climate change (Nardone *et al*., 2010). These changes lead to stress in animals resulting in a decrease in production (Nardone *et al*., 2009).

Sheep in South Africa are farmed under different ecological conditions with varying climates and natural vegetation. Sheep are hardy animals and are able to survive in climates that are unforgiving to other species like cattle. When these conditions however, don't allow for natural grazing to take place, sheep have to be fattened in a feedlot to guarantee commercial and economic viability (Van der Westhuizen, 2010). When lambs are raised on poor quality forages it leads to decreased growth rates, an increase in mortality rates and ultimately ewes have problems with conception in the subsequent mating season (Nolte, 2006). By weaning lambs early and finishing them in a feedlot, these problems can be prevented. When fattening lambs in a feedlot, the producer is looking to maintain a high growth rate or average daily gain (ADG) which equates to the amount of live weight gained (g) by the animal per day. Another important aspect of feedlotting is the feed conversion ratio (FCR) of the lambs which is the amount of feed (kg) the animal has to consume to gain one kg of bodyweight. Younger animals have higher growth rates and thus by marketing these lambs at an earlier age (21 weeks or 5 months) the producer makes use of the young animal's peaking growth rate (Malik *et al*., 1996). This will lead to the feedlot having a higher rate of turnover and ultimately better cost-effectiveness.

When changing the production system in order to advance animal productivity and economic viability, it is important to maintain a high quality product (meat) as well as consumer acceptance (Santos-Silva *et al.,* 2002). Santos-Silva *et al*. (2002) noted that due to livestock production systems becoming more intensive, concerns may arise among producers on how their production systems will affect the quality of their product. When purchasing a product, the consumer's decision is driven by multiple intrinsic (flavor, tenderness, visible fat, juiciness) and extrinsic (nutritional information, price, animal welfare, production system, environmental impact) factors (Napolitano *et al*., 2007). Initially the quality of the meat as established by the consumer is via a visual observation (colour, purge, fat) after which the consuming of the product (flavour, tenderness, juiciness) will ultimately confirm this (Acebron & Dipico, 2000). Martin & Rodger (2004) stated that the tenderness of meat primarily determines the quality thereof. Lambs fattened in a feedlot have fattier carcasses when compared to pasture based lambs and Priolo *et al*. (2001) showed that there is a positive correlation between the tenderness and the fatness of the meat. Furthermore, a positive correlation as also been shown to exist between the fatness of the meat and the juiciness (Priolo *et al*., 2001).

2.3 The feeding of sheep in intensive production systems

2.3.1 Protein in ruminant diets

Recently, accurately defining the ruminant's need for particular amino acids has been the area of focus for various authors. Rapidly growing ruminants have a high demand for amino acids. The growth potential of lambs cannot be met by only formulating diets that satisfy the nitrogen requirements of rumen microbes and it is therefore important to include sources of protein that are low in degradability or undegradable protein (UDP) (NRC, 1985). Until recently, the protein requirements of ruminants were expressed only in terms of crude protein (CP) levels, thus diets were formulated for rumen degradable protein (RDP) and rumen UDP. Animals use most of the nitrogen they require for protein synthesis. Most of the nitrogen in feedstuffs is also present as protein and this is why the nitrogen requirements of the animal as well as the nitrogen content of the feed can be expressed in terms of protein

(McDonald *et al*., 2002). A limitation of the CP system however, is that it does not take into account the quality of the protein (amino acid profile) of the feed or the requirements of the animal (Nolte, 2006). Although the CP system indicates the nitrogen content of the feed, it is no indication of its actual value for the animal. Thus, even though the CP or the nitrogen requirements of the animal might be met, individual amino acids could still be deficient (Nolte, 2006). Boisen *et al*. (2000) noted the profile of digestible amino acids passing to the small intestine is the most important factor influencing protein utilization by the ruminant. They further stated that in order to obtain the ideal protein at small intestine level, the amino acid profile of the microbial protein must be balanced with the dietary protein which has not been subjected to degradation by the rumen microbes (Boisen *et al*., 2000). Chen & Orskov (1994) defined the ideal protein as the supply of absorbed amino acids leading to optimized efficiency of utilization. There is a close correlation between the pattern of amino acids required for the accretion of protein in the body and the actual amino acid composition of the body and thus the amino acid composition of the whole empty body (WEB) can be used to establish this ideal pattern of amino acid absorption for optimal growth (Fuller, 1996). Therefore, by determining the duodenal amino acid profile and comparing that to the amino acid profile of the WEB, the limiting amino acids can be calculated and included in the diet (Ferreira, 1999).

2.3.1.1 The ideal protein

The ideal protein, as defined by Chen & Orskov (1994), is the supply of amino acids in proportion that would lead to maximized efficiency of utilization while Boisen *et al.* (2000) defined it as “the perfect ratio among the essential amino acids required for maintenance and production”. In an ideal protein, every individual essential amino acid (EAA) could potentially be limiting for animal performance while surplus nitrogen would be minimal. Therefore, by applying the ideal protein concept when formulating diets would lead to lower levels of nitrogen excretion without having a negative effect on animal performance (Boisen *et al*., 2000). When the profile of amino acids available for absorption in the small intestine (SI) do not lead to maximum efficiency of utilization, the amino acids must be balanced by including higher levels of UDP in the ration with the UDP including higher levels of the limiting amino acids (Nolte,

2006). By doing this, the animal's need for amino acids is met without the risk of overfeeding. However, the supplementation of amino acids to the ruminant is complicated by various factors such as a change in the animal's requirements, difficulty in predicting the quantity and quality of the amino acids that reach the small intestine, variations in protein degradability and amino acid contents of protein sources (Nolte *et al*., 2004; Merchen & Titgemeyer, 1992). Kung & Rode (1996) stated that due to the effect that the process of rumen nitrogen metabolism has on the quality and amount of amino acids that reach the SI, it is difficult to translate amino acid requirements on a tissue level to that of the diet. Also, Chen & Orskov (1994) maintained that the optimal composition of amino acids would vary depending on the type of production that is required. Therefore, the optimum amino acid composition would depend on whether the animal would require it for tissue or wool growth, maintenance or milk production (Chen & Orskov, 1994).

Fuller (1996) found a correlation between the amino acids required for the accretion of body protein and the composition of amino acids in the WEB protein. It follows then that, in terms of growth, the EAA profile of the WEB could be used as a model to indicate which amino acids are required for the accretion of protein in the body (Fuller, 1996). In corroboration with this, Chen & Orskov (1994) noted the potential similarity between those amino acids that are required for tissue maintenance and those needed for tissue growth, as tissue is the primary site of protein turnover. Therefore, the amino acid profile of the WEB protein can be used to calculate the ideal protein composition as required by the SI (Fuller, 1996).

2.3.1.2 The essential amino acids (EAA)

Proteins consist of 20 different amino acids, nine of which cannot be synthesized by most animals. The term "essential" is a reference to the animal's inability to synthesize these nine amino acids at an adequate enough rate that would meet the need of the animal and includes Lysine (Lys), Methionine (Met) and Tryptophan (Trp) (NRC, 2007). Thus, a constant supply of these nine amino acids along with adequate amounts of nitrogen for the synthesis of the other amino acids is indispensable for the processes of growth (production) and maintenance (Boisen *et al*., 2000). As

mentioned earlier, microbial protein is the major source of amino acids to reach the SI. However, growing ruminants have a high demand for amino acids and often the microbial protein is not able to meet the requirements of the animal (Merchen & Titgemeyer, 1992). Under these conditions the deficient amino acids need to be supplied in the form of UDP. When determining the amino acid requirements of the ruminant it is essential to have an understanding of the amino acids needed for maintenance, production (meat, milk, wool) and endogenous losses. Merchen & Titgemeyer (1992) noted that when amino acids are used as the basis for formulating diets, it is imperative to have an understanding of the measure of 1) microbial protein (amino acids) reaching the SI, 2) dietary UDP reaching and being absorbed from the SI and 3) the amino acids needed for production and maintenance.

Due to wool growth in sheep, the assessment of amino acid utilization is more complex than that of cattle as wool growth is characterized by a permanent loss of amino acids (Merchen & Titgemeyer, 1992). Determining the limiting amino acids for ruminants has been an area of focus for numerous researchers for many years. In growing lambs Met proved to be the most limiting amino acid, while Lys, Arginine (Arg) and Histidine (His) also proved to be limiting when microbial protein was the only source of protein (Storm & Orskov, 1984).

Wright & Loerch (1988) found that nitrogen retention and the digestibility of nitrogen, dry matter (DM) and fibre were not influenced when rumen protected Met was fed to growing lambs. In contrast to this, Mowat & Deelstra (1972) found that nitrogen digestibility responded linearly with an increase in levels of rumen protected Met.

It is evident that the profile of amino acids available for absorption is an essential factor in determining the effectiveness of protein utilization. The inadequate supply of a single amino acid will hamper the response of those that are sufficient (Cole & Van Lunen, 1994), while a surplus of amino acids in proportion to those that are limiting will be broken down and excreted with an ensuing loss of nitrogen and energy (Nolte, 2006). It is therefore vital to supply the ideal amino acid profile to the SI in order to attain maximum performance.

2.3.1.3 Estimating the limiting EAA requirements for growing lambs

Various authors have proposed methods of determining the ideal EAA requirements of animals. The ARC (1981) predicted that lean meat was representative of the ideal amino acid balance of an animal, while Hussein *et al*. (1991) hypothesized that the WEB would serve as an indication of the proportion in which animals required EAA.

As previously mentioned, the most significant aspect influencing the effectiveness of protein utilization is the EAA profile that enters the SI, which is a combination of microbial crude protein (MCP) and undegraded dietary protein (UDP) (Boisen *et al*., 2000). By using the EAA composition of the WEB, Nolte (2006) was able to determine the limiting EAA as required by growing lambs, which, together with the EAA composition of the MCP can be used to estimate the EAA profile of the UDP needed in order to meet the EAA requirements of lambs.

Limiting EAA is a function of the amount of CP necessary for a specific amount of daily growth, consequently the estimation thereof is only possible when an expected growth rate is determined (Boisen *et al*., 2000). The NRC (1996) established that at a growth rate of 250 g/day a lamb would need 168 g of CP to meet its daily need of protein for maintenance and growth. Based on the work done by Nolte (2006) the WEB amino acid concentration of Lysine is 6.74 g/100 g protein. The calculation of the amount of EAA that is required as UDP by growing lambs is found in Table 2.1 The Lys requirements can be calculated as follows:

(CP for 250 g/d gain ÷ 100) x (WEB concentration of Lys) = 11.32 g/d.

Nolte (2006) noted that the MCP amino acid profile would remain constant, regardless of the content of amino acids found in the dietary protein. Therefore, MCP is used in calculating the animal's need for EAA. The amount of MCP produced is determined by the quantity of feed the animal ingests and is calculated as 13% of the total digestible nutrient (TDN) intake (NRC, 1996). By deducting the MCP from the animal's daily requirement for CP, the amount of CP that has to be supplied by the UDP fraction of the diet is estimated. The Lys concentration in MCP is 6.69 g/100g

while 11.32 g/d of Lys is required, therefore the total required MCP is calculated as follows:

11.32 g/d ÷ (6.69 g/100g protein ÷ 100) = 169.2 g/d.

By subtracting the actual quantity of produced MCP (13% of TDN), the insufficient quantity of MCP is determined. At a DM intake of 1.26 kg/d with a TDN intake of 720 g/kg DM, the amount of MCP produced is 117.94 g/d. The required EAA is then calculated:

11.32 g/d – (117 g/d x 6.69) = 3.43 g/d

For a lamb weighing 25.0 kg and consuming 3.0% of its bodyweight, the amount of Lys that needs to be included in the feed is then calculated:

25.0 kg x (3/100) = 0.75 kg daily intake

3.43 g/d ÷ 0.75 kg = 4.57 g/kg Lys in feed.

Table 2.1 The EAA requirements of Merino lambs growing at 250 g/d when MCP is the only source of protein [Adapted from Nolte (2006); Le Roux (2011)]

EAA[1]	EAA Comp: MCP[2]	EAA Required: Merino	MCP Required: Merino	Estimated MCP deficiency: Merino	EAA required as UDP[7]: Merino
	(g/100g protein)[3]	(g/d)[4]	(g/d)[5]	(g/d)[6]	(g/d)[8]
Arg	5.54	11.90	215.96	98.03	5.43
His	1.29	4.31	335.01	217.07	2.79
Ile	4.02	5.44	135.35	17.42	0.70
Leu	5.85	13.10	234.56	116.62	6.82
Lys	6.69	11.32	169.25	51.32	3.43
Met	0.79	2.55	322.34	204.41	1.62
Phe	3.41	6.87	201.49	83.56	2.85
Thr	3.85	6.44	167.38	49.44	1.90
Trp	1.54	1.86	120.25	2.31	0.04
Val	4.89	9.21	188.34	70.41	3.44

[1]Essential amino acid
[2]Duodenal crude protein pre-dominantly derived from microbial protein
[3]From Nolte & Ferreira (2004)
[4]Calculated as Y = (X/100)*Z, where Y = EAA requirement for a growth rate of 250 g/d; X= a CP requirement of 168 g/d to allow an average daily gain of 250 g/d (NRC, 1985); Z = the WEB amino acid composition for each breed (Nolte & Ferreira, 2004)
[5]Calculated as Y = X/ (Z/100), where Y = MCP required (g/d); X = individual amino acid required(g/d); Z = concentration of individual amino acid in MCP (g/100g protein)
[6]Calculated as Y = X – Z where Y = estimated MCP deficiency; X = calculated MCP requirement and Z = estimated MCP production. Z is calculated as 13 % of the total digestible nutrient intake (NRC,1996), for an average daily voluntary feed intake of 1.26 kg DM of a diet with a TDN content of 720 g/kg (DM - basis) = 117.94 g/d
[7]UDP
[8]Calculated as Y = X – (Z/100*A), where Y = essential amino acid A required as UDP; X = essential amino acid requirement to allow a growth rate of 250 g/d; Z = estimated MCP production; A =concentration of essential amino acid A in MCP.

2.3.1.4 The relationship between energy supply and amino acid requirements

The process in which rumen microbes synthesize protein is energy-dependent; therefore it is difficult to view the physiological supply of amino acids and energy as separate entities (Merchen & Titgemeyer, 1992). Poppi & McLennan (1995) stated that an animal's gain in live weight was mainly dependent on amino acid supply as well as the supply of energy yielding substrates. They added that protein deposition is determined by how efficiently the absorbed proteins are used, which in turn, is dependent on the availability of the limiting EAA and energy yielding substrates.

For non-ruminants the study of the protein-energy relationship is less complex due to the fact that the dietary protein intake is equal to the protein reaching the SI (Chowdhury *et al*., 1997). This means that the animal's response to protein supply is dependent on the supply of energy, thus, higher energy levels will equal a greater response to protein supply.

The above mentioned principle was initially thought to be applicable to ruminants. However, uncertainty over this assumption arose when Orskov & Fraser (1973) showed that when identical diets were fed at lower levels, increased protein supply did not lead to an increase in the flow of nitrogen. This was caused by the changes in the rumen retention- and degradation times.

Chowdhury & Orskov (1996) stated that due to the ruminant's ability to mobilize its fat reserves as an energy source for the process of protein accretion, using the protein-energy ratio to determine the animal's protein requirements become invalid.

2.3.2 NSC's in ruminant diets

Non-structural carbohydrates (NSC) consist of mainly the starch fraction of the feed but also include compounds such as sugars, pectins, galactans and β-glucans. This fraction is a very important source of energy in the diet. As discussed earlier, the process of microbial protein synthesis in the rumen is an energy dependent process, therefore supply of both energy and protein to the ruminant goes hand-in-hand

(Titgemeyer, 2003). Nocek & Russel (1988) stated that when a diet is formulated to be high in rumen available protein and carbohydrates the synthesis of microbial protein will be at an optimum when compared to diets that have unbalanced carbohydrate and protein rumen availabilities or are low in rumen available carbohydrates and proteins.

The population of microbes in the rumen can be categorized into bacteria that have the ability to ferment structural carbohydrates (SC) and those that ferment NSC (Russel *et al*., 1992). The former ferments cell wall carbohydrates and can only utilize ammonia as a nitrogen source while the latter ferments the NSC (sugars, pectin, starch), can use either peptides, ammonia or amino acid as a source of nitrogen and has the ability to produce ammonia (Russel *et al*., 1992).

The growth of the microbial population is dependent on a source of carbohydrates that are readily available in the rumen and provides ATP for the biosynthesis of cell material (Nocek & Russel, 1988). Furthermore, in order to ensure optimal growth the rate at which ATP is produced (fermentation of carbohydrates) must be equivalent to the rate at which it is utilized (protein synthesis) or fermentation will become undone (Hespell & Bryant, 1979). This implies that in order to optimize microbial protein synthesis, the availability of both protein and carbohydrates need to be matched.

Stobo *et al.* (1966) stated that concentrate based diets would stimulate rumen papillae development more than roughage based diets. While forages promote a healthier rumen it is the concentrate fraction of the feed that stimulates the development of the rumen (Coverdale *et al*., 2004). The consumption of forage is important for ruminants as it stimulates both the muscular development of the rumen as well as the passage of saliva towards the rumen (Hamada, 1976). During the digestion of forage by microorganisms, volatile fatty acids (VFA) are produced. However, these VFA concentrations are not enough to stimulate the optimal development of ruminal papillae (Coverdale *et al*., 2004). The fermentation of concentrates does however produce adequate amounts of VFA, especially Butyrate, to encourage the development of papillae (Coverdale *et al*., 2004).

2.4 Digestibility

The digestibility of feed or feed constituent can be defined as the fraction of feed that is not excreted in the faeces of the animal and is subsequently, based on assumption, absorbed and utilized by the animal (McDonald *et al*., 2002). This value can be expressed in terms of a percentage or a coefficient and is calculated as:

$$\frac{\text{Nutrient consumed} - \text{Nutrient in faeces}}{\text{Nutrient consumed}}$$

There are however a few objections to these assumptions. Firstly, during the fermentation of carbohydrates methane is produced which is released via eructation and therefore not absorbed. This methane gas production (MJ/day) was estimated at 8% of the gross energy intake (McDonald *et al*., 2002). This will lead to the digestible energy content and digestible carbohydrate content being overestimated. Also, not all the faeces consists of undigested food, as it also contains enzymes and other matter secreted into the gut during digestion as well as cellular material abraded from the gut lining. Therefore, buy excreting substances into faeces that are not of dietary origin leads to the underestimation of the amount of food that is absorbed by the animal (McDonald *et al*., 2002). In terms of amino acids it is important to distinguish between those that are digestible and those that are available. Apparent amino acid digestibility is the term that refers to the percentage of consumed amino acids that is not found in the faeces or digesta and thus implies that no adjustments have been made to compensate for endogenous amino acid losses (Mosenthin *&* Rademacher, 2003). The ARC (1981) defines the bioavailability of amino acids as the fraction of dietary amino acids not linked to compounds that could impede on absorption, digestion and the utilization for tissue accretion or maintenance.

By correctly determining the nutritional value and potential digestibility of feed or feed constituent the performance of the animal (growth and milk production) can be improved due to the more accurate prediction of supplementation needed. Food intake is arguably the most important factor when determining animal performance (Ilius *et al*., 1996). The digestibility of feed determines the intake thereof, thus feeds high in digestibility are likely to be consumed in greater quantities (Ilius *et al*., 1996).

The digestibility of a feed is affected by various factors which include the composition of the feed and the ration, the method of feed preparation (e.g. grinding, chopping), supplementation of food with enzymes and as previously mentioned, the level of feeding (McDonald *et al*., 2002).

2.5 The development of the rumen

The feedlot performance of lambs during the first two to three weeks is often suppressed and can be attributed to the sudden change of diet from pre- to post weaning (Ortega-Reyes *et al*., 1991). It is due to this reason that livestock are often fed diets they will be receiving in the feedlot, just before entering the feedlot (Taylor, 1984). This effect is enhanced when lambs receive concentrate feed while still suckling their mothers (Thorhallsdottir *et al*.,1990) as well as during the period of conversion from being non-functional ruminants to ruminants (Mirza & Provenza, 1990; Thorhallsdottir *et al*., 1990). The type of diet has a big effect on early rumen development (Church, 1979) and also the stimulatory effect of concentrate diets on the development of rumen papillae is greater than that of roughage base diets (Stobo *et al*., 1966). Therefore, exposing lambs to concentrate diets during early growth and development will lead to greater responses when lambs receive similar diets in the feedlot (Ortega-Reyes *et al*., 1991).

The fermentation of concentrate feeds by rumen microbes leads to the production of volatile fatty acids (VFA) (Van Houtert, 1993) and by the intra-ruminal infusion of these VFA into milk fed animals, morphological development of the rumen epithelium is brought about (Lane & Jesse, 1997). This morphological development of the rumen entails an increase in size of the rumen, the rumen epithelium becomes keratinized and there is an increase in the length of the rumen papillae (Church, 1969).

When lambs are born, oxidization of butyrate and glucose by the epithelial cells of the rumen occur at a similar rate (Baldwin & Jesse, 1992). However, at 42 days to 56 days of age (typical weaning age) butyrate becomes the favoured oxidizing substrate

while the glucose oxidation rate is reduced (Baldwin & Jesse, 1992). The epithelial cell production of β-hydroxybutyrate (BHBA) also increases rapidly at weaning and a six-fold increase in BHBA production has been found (Baldwin & Jesse, 1992).

At birth, the rumen of the newborn makes up less than 30% of the total stomach mass, but when reaching maturity this figure will have increased dramatically to about 80% (Ward, 2008). The rumen surface is covered by papillae which serves as the main absorption site for VFA produced during the process of ruminal fermentation. The development of the rumen is initiated with the intake of solid food and the subsequent establishment of fermentation in the rumen, leading to an increase in ruminal VFA concentration (Sakata & Tamate, 1978). This rise in VFA concentration is central in activating the process of papillary development.

Lane *et al.* (2000) compared metabolic rumen development of lambs fed a liquid diet only (milk replacer for 84 days; then slaughtered) to lambs receiving a delayed solid diet (milk replacer until 48 days, solid diet until 84 days; then slaughtered.) Lambs receiving the solid diet resulted in higher concentrations of propionate, butyrate, acetate and valerate. Theoretically it is this increase in butyrate and propionate concentrations that stimulate the development of the rumen. However, the papillae lengths of the lambs fed the solid diet were found to be shorter when compared to those of lambs raised conventionally. In a conventional rearing system lambs start to receive solid feed from 14 days of age (as opposed to the 48 days in the trial of Lane *et al*., 2000). Therefore, the rumen epithelium of lambs raised in this way will be subjected to higher levels of VFA for a longer time period (Lane *et al.,* 2000). This highlights the advantage of feeding lambs concentrate feed while they are still suckling the ewe.

2.6 Pasture or feedlot finishing of lambs

2.6.1 Production systems: Extensive vs Intensive

In recent years animal production systems have made a shift and have moved from being small-scale extensive systems to more intensive, large-scale operations which

has led to an increase in the productivity and subsequently profitability (Steinfeld *et al*., 2006). This transition can be attributed to a variety of environmental factors which include global warming, change in climate and waning land (Nardone *et al.,* 2010). In a review article by Nardone *et al*. (2010) it was stated that environmental changes, especially global warming, will not only have an adverse effect on the wellbeing of the animal but will also bring about stress, leading to decreased feed intake and ultimately a decrease in productivity.

By using extensive production systems (e.g. Free ranging) producers are reliant on the natural resources to provide food and water and due to environmental changes, the sustainability of these systems are at risk (Hanekom, 2010). Contributing 40% of global meat production, feedlotting is the fastest growing meat production system worldwide (Nierenberg, 2005). Fattening lambs in a feedlot benefits the producer in several ways and includes an increase in productivity, higher yields, improved efficiency, higher turn-over rate, lower cost of production coupled with an increase in profits and less land needed for a production unit (Hanekom, 2010).

2.6.2 Consumer preferences and effect of production system on meat quality

With the transition from extensive to intensive production systems, animal welfare and other concerns have been raised by consumers. The perception of the consumer is that intensive production systems have an adverse effect on the welfare of the animal and that extensively produced meat (grazing, free-range) is preferred (Hughes, 1995). However, contradictory to this, Turner & Dwyer (2007) maintained that animals in an extensive production unit will be exposed to a variety of extreme elements (disease, water and food availability, climate) which will impact animal welfare negatively. In order to remain competitive, it is of critical importance that producers monitor and consider the perceptions of consumers and, if need be, adapt to it in order to ensure that their needs are being met (Hanekom, 2008).

Consumer behaviour is very difficult to determine as it is motivated by various factors. When it comes to the actual purchasing of the product these factors will include the colour of the meat and visible amount of fat, while during the

consumption tenderness, juiciness, aroma and the flavour of the product will be of importance (Acebron & Dopico, 2000; Napolitano *et al*., 2007) and, as already discussed, factors such as animal welfare and environmental impact are becoming increasingly important.

Various studies have focused on the qualitative differences in the carcass obtained when lambs are fattened on a concentrate diet (feedlot) and when lambs are raised on a pasture-based diet. The major differences have been found in the fatness of the carcass, the colour of the subcutaneous fat and the meat flavour (Priolo *et al*., 2001). Lambs fattened in a feedlot will have more subcutaneous fat than pasture fattened lambs while the meat of pasture fattened lambs will be darker in colour (Diaz *et al*., 2002). Lambs grazing pasture will be more active and therefore have increased energy requirements, while also consuming more forage which increases the basal metabolism, which leads to a further increase in energy requirements (Diaz *et al*., 2002). Lambs fattened on pasture accrue less fat than lambs fed a concentrate diet. Therefore, these lambs will reach an acceptable slaughter weight without excess fat on the carcass (Borton *et al.,* 2005).

The meat of feedlot fattened lambs has been found to be more juicy and tender than that from grazing lambs while there is a positive correlation between the fatness of the carcass and the tenderness of the meat (Priolo *et al*., 2002).

Dressing percentage (DP) is the weight of the carcass expressed as a percentage of the final live weight of the animal just before slaughter (after the removal of the head, feed, skin and viscera) (Schoenian, 2010) and is calculated as:

DP = carcass weight / live weight x 100.

The thin layer of subcutaneous fat coupled with their fully developed digestive system means that grazing lambs will have a lower DP when compared lambs that have been fattened in a feedlot (Borton *et al*., 2005).

2.6.3 Production systems and their economic implications

When fattening lambs in a feedlot the aim is to provide a consistent supply of a high quality product that meets market demand. Before any capital expenditure on the implementation of an intensive production system is made, it is important to first do a thorough financial analysis in order to assess the economic viability of the operation. The production efficiency of a feedlot can be defined as the income generated from selling a product per unit of feed input (Beerman *et al.,* 1986). In a commercial feedlot, feed costs can typically comprise 70-80% of the total production costs (Haddad *et al.,* 2001). When finishing lambs in a pasture-based system the profit margins for the producer will be much higher (Zervas *et al*., 1999). However, in an intensive production system (feedlot) the turnover rate will be much higher. It has also been shown that increased profits can be generated by having lambs graze pastures prior to being fattened in a feedlot (Blackburn *et al*., 1991). This is referred to as back grounding and is a practice growing in popularity.

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CHAPTER 3

GENERAL MATERIAL AND METHODS

3.1 Introduction

This chapter was included to avoid repetition whilst also maintaining the article form protocol as described by the South African Journal of Animal Sciences. This chapter will only include the materials and methods that relate to the entire study while the materials and methods that are only applicable to specific parts of the thesis will be discussed in the relevant chapters.

3.1.1 History of the lambs used in study

Ethical clearance for this study was obtained from the Stellenbosch University ethics committee (2009B03006). The design of this study is depicted in Figure 3.1. The 40 lambs used in this experiment were divided into four treatment groups while homogeneity between groups was maintained with regards to birth weight, age and litter size.

3.1.2 The formulation of diets

The amino acid requirements of the growing lambs were estimated based on calculations as suggested by Nolte (2006). Nolte (2006) determined that the first limiting amino acids for Merino and Döhne-Merino lambs are Methionine (Met), Histidine (His), Arginine (Arg), Leucine (Leu) and Phenylalanine (Phe). For this trial the first treatment, an optimized protein (OP) feed, was optimized for these amino acids at 157 g/kg crude protein (CP) and 477 g/kg non-structural carbohydrates (NSC) with Met, Lysine (Lys), Arg and Threonine (Thr) at 2.48, 7.45, 8.51 and 8.12

g/kg on DM basis respectively. The second treatments used was a standard commercial finisher (FIN) feed that contained 152 g/kg CP and 468 g/kg NSC with Met, Lys, Arg and Thr at 2.08, 5.49, 7.47 and 4.80 g/kg on DM basis respectively and served as a positive control. The amino acids in this feed were not optimized. A third treatment was a low protein (LP) feed which served as a control and was formulated to contain 139 g CP/kg and 455 g NSC/kg with Met, Lys, Arg and Thr at 1.93, 4.99, 6.66 and 4.73 g/kg (DM) respectively, without optimizing these amino acids. It is important to note that although the levels of CP for each treatment is reported, the diets were not formulated to vary in levels of CP but rather to vary in levels of amino acids. This was achieved by including different raw materials in the different treatments while also including specific synthetic amino acids when necessary. None of the feedlot diets contained synthetic amino acids as the amino acids in the raw materials used were sufficient to supply the elevated EAA specifications. The physical and chemical compositions of the experimental treatments are found in Table 3.1 and Table 3.2.

Table 3.1 The nutrient composition of feedlot concentrate diets (FIN, OP and LP) as formulated by Tanqua Feeds (Riviersonderend, Western Cape, South Africa), as well as a supplementary feed fed to lambs grazing kikuyu pasture

Physical composition (DM basis)	**FIN** 152 g CP/kg 468 g NSC/kg	**OP** 157 g CP/kg 477 g NSC/kg	**LP** 139 g CP/kg 455 g NSC/kg	**CON (Supplementary feed)** 151 g CP/kg 358 g NSC/kg
Yellow maize (8.5%) g/kg	354	324.74	273.95	216.6
Molasses syrup g/kg	40.0	69.74	69.65	40.0
Wheat bran g/kg	142.8	11.8	12.82	150.0
Cotton meal (40%) g/kg	32.70	-	-	-
Cotton meal (36%) g/kg	-	-	-	65.4
Gluten g/kg	-	-	-	110.0
Soybean meal (47%) g/kg	14.1	84.03	11.14	-
Fishmeal 60 g/kg	-	15.11	-	-
Ammonium chloride g/kg	5.0	-	-	-
Ammonium sulphate g/kg	5.0	-	6.85	15.7
Salt g/kg	2.8	5.0	4.06	10.0
Magnesium oxide g/kg	-	-	-	2.9
Urea g/kg	-	-	-	0.8
Limestone g/kg	10.7	27.66	32.5	32.3
Monocalcium phosphate g/kg	-	-	-	6.8
Acid buff g/kg	2.8	-	-	-
Apple pulp g/kg	128.6	185.97	162.51	200.0
Oat bran g/kg	86.8	23.5	26.7	145.9
Oats g/kg	-	139.47	150.90	-
Lucerne g/kg	171.4	162.72	151.37	-
Malt pellets g/kg	-	-	139.30	-
Calf premix g/kg	-	-	-	3.6
Sheep + Rumensin g/kg	2.8	3.25	3.25	-
Green colourant g/kg	0.3	-	-	-
Threonine g/kg	-	2.67	-	-

Table 3.2 The chemical composition of feedlot concentrate diets (FIN, OP and LP), as formulated by Tanqua Feeds (Riviersonderend, Western Cape, South Africa), varying in levels of CP and NSC, the supplementary feed fed to lambs grazing kikuyu as well as the kikuyu grazed by CON lambs. EAA calculated for the optimized diets are indicated in Bold text

Chemical composition (DM basis)				**CON**	
	FIN	**OP**	**LP**	**Supplementary feed**	**Kikuyu grazing**
	152 g CP/kg 468 g NSC/kg 2.08 g/kg Met 5.49 g/kg Lys 7.47 g/kg Arg 4.80 g/kg Thr	157 g CP/kg 477 g NSC/kg 2.48 g/kg Met 7.45 g/kg Lys 8.51 g/kg Arg 8.12 g/kg Thr	139 g CP/kg 455 g NSC/kg 1.93 g/kg Met 4.99 g/kg Lys 6.66 g/kg Arg 4.73 g/kg Thr	151 g CP/kg 358 g NSC/kg	
DM g/kg	864.55	860.37	861.48	874.57	183.44
ME MJ/kg	11.57	11.80	11.44	10.77	9.87
TDN g/kg	769.93	793.08	765.71	715.23	-
Degradable protein g/kg	99.18	91.29	84.52	98.15	-
Non protein nitrogen g/kg	2.74	-	1.41	4.54	-
Crude protein g/kg	152.43	157.26	139.09	150.94	-
Bypass protein g/kg	50.99	59.57	48.92	45.81	283.15
Arganine g/kg	7.47	**8.51**	6.66	7.12	-
Lysine g/kg	5.49	**7.45**	4.99	4.39	-
Methionine g/kg	2.08	**2.48**	1.93	2.12	-
Threonine g/kg	4.80	**8.12**	4.73	3.93	-
Bypass methionine g/kg	0.81	**0.99**	0.73	0.78	-
Bypass lysine g/kg	1.87	**2.62**	1.58	1.51	-
NSC g/kg	467.72	477.35	454.91	358.39	-
Fat g/kg	39.37	40.29	37.84	32.58	39.23
NDF g/kg	328.61	284.29	337.43	379.59	463.24
Fibre g/kg	149.55	125.56	129.85	129.27	-

3.1.3 Management of lambs post weaning

The lambs used in this trial were obtained from a previous experiment where lambs were fed specialized creep feed diets. Upon completion of the creep feed phase, 40 lambs were slaughtered for further analysis while the remaining 40 lambs entered a feedlot for the commencement of the trial.

For this trial, 40 crossbred (South African Mutton Merino x Döhne Merino) lambs, at an average age (± s.d.) of 69 ± 1 days and an average live weight (± s.d.) of 24.35 ± 0.64 kg were randomly allocated into four dietary treatment groups of 10 lambs per group. Three of these groups received pelleted concentrate diets containing varying levels of CP and NSC, while the fourth group served as the control group and was allowed to graze kikuyu pasture. The four treatments were as follows:

- A low protein feed (LP; 139 g CP/kg, 455 g NSC/kg; Met, 1.93 g/kg; Lys, 4.99 g/kg; Arg, 6.66 g/kg; Thr, 4.73 g/kg on DM basis) not adjusted for amino acids which also served as the control diet for the other feedlot diets.
- An optimized protein feed (OP; 157 g CP/kg, 477 g NSC/kg; Met, 2.48 g/kg; Lys, 7.45 g/kg; Arg, 8.51 g/kg; Thr, 8.12 g/kg on DM basis) adjusted for amino acids.
- A standard commercial finisher feed (FIN; 152 g CP/kg, 468 g NSC/kg; Met, 2.08 g/kg; Lys, 5.49 g/kg; Arg, 7.47 g/kg; Thr, 4.80 g/kg on DM basis).
- Control treatment (CON) where lambs grazed kikuyu pasture and received additional supplementary feed at 500 g/day.

Lambs were kept on slatted floors in 1.2 x 1.8 m individual pens in a shed on the Welgevallen experimental farm in Stellenbosch. The three concentrate-fed groups were fed pelleted diets supplied by Tanqua Feeds, Riviersonderend while the CON group grazed kikuyu pasture and also received supplemental feeding (132 g/kg DM CP; 313 g/kg DM NSC).

Lambs receiving the control treatment were let out to graze during the day (08:00 – 17:00) after which they were kept indoors at night with access to water and oat hay *ad libitum*. During the first 14 days post-weaning these lambs received supplementary feeding at 250 g/day and at day 15 this was increased to 500 g/day and maintained at this level until slaughter (Figure 3.1). Lambs were grazed on two kikuyu paddocks on a rotational basis with the supplementary feed being provided in pellet form in feeding troughs.

3.1.4 Data collected

Lambs were kept under feedlot conditions with the exception that they were kept in individual pens to allow individual and more accurate data collection. Lambs were given fresh feed every morning (07:00) after removing and weighing the refused feed from the previous day, thus allowing the calculation of the exact 24 hour intake of each lamb. Lambs were weighed once a week on the same day and time in order to determine their average daily gain (ADG) as well as their feed conversion ratio (FCR). This weighing was conducted after removal of the previous 24 hours refusal and was thus not an "empty weight". The FCR is a measure of an animal's productive efficiency and is defined as the amount of feed (in kg) an animal must consume in order to gain one kg of body weight (Ryan *et al*., 1993).

3.1.5 The slaughtering of lambs and post-slaughter protocol

The lambs were slaughtered after a 57 day feedlot period when the target weight of 40 kg was reached. Lambs were not fasted prior to slaughter. Lambs were slaughtered at an average age (±s.d.) of 131 ± 3 days and at an average weight (±s.d.) of 40.41 ± 1.26 kg according to standard commercial practice which included electrical stunning followed by exsanguinations.

3.2 Laboratory materials and methods

Feed and kikuyu samples were analyzed according to methods described by the Association of Analytical Chemists (AOAC) as described below.

3.2.1 Moisture

The moisture content of samples was determined by method 934.041 (AOAC International, 2002d). Individually marked moisture free crucibles were weighed and their weights recorded after which a 2 g sample was then weighed and placed in the crucible. The sample-containing crucibles were placed in an oven at a 100ºC for 24 hours. With the completion of the 24 hour drying period the crucibles were removed from the oven and placed in a desiccator for 30 minutes to cool off, after which their weights were then recorded. The calculation of the moisture content is:

$$\text{Moisture \%} = \frac{[\text{Moisture free crucible + sample mass (g)}] - [\text{Mass of moisture free crucible (g)}] \times 100}{\text{Sample mass (g)}}$$

The dry matter (DM) was then calculated as:

DM% = 100 – moisture %

3.2.2 Crude protein and amino acid analyses

The CP content of samples was determined by using the Dumas combustion method 992.15 (AOAC International, 2002b). The LECO® FP – 528 Nitrogen & Protein Analyzer (LECO® Corporation, St. Joseph, USA) is an apparatus which determines the nitrogen content of a sample. By multiplying this nitrogen content by a factor of 6.25 the CP content of the sample is calculated. The LECO® was first calibrated for faecal and feed samples using alfalfa with a nitrogen content (± s.d.) of 3.32 ± 0.04%, while for meat samples EDTA (LECO®) a nitrogen content (± s.d.) of 9.57 ± 0.03% was used. A 0.1 g sample was weighed into a small tin foil pouch, closed and

placed into a port on LECO®'s carousel. After this the sample goes through the process of combustion after which the nitrogen reading is given and recorded.

For the amino acid analysis, the samples were first hydrolyzed at the Department of Animal Sciences (Stellenbosch University) after which the samples were sent to the Central Analytical Facility (CAF) at the Department of Biochemistry (University of Stellenbosch). Samples were hydrolyzed (AOAC International, 1997) using 6 M HCl and sealed in a glass tube filled with nitrogen which was then kept at 110ºC for 24 hours.

At the CAF, ion-exchange chromatography of the protein was done to determine the individual amino acid content of the samples. Samples were analyzed using the EZ:Faast kit. Samples were prepared according to the method described in the EZ:Faast user guide. Homoarginine, homophenylalanine and methionine-D3 (included in the EZ:Faast kit) were used as internal standards. The amino acid composition was determined by a Waters API Quattro Micro system.

3.2.3 Energy

In order to determine the energy content samples were compressed into a small tablet form, each weighing 0,5 g. A CP 500 Bomb Calorimeter (Eltra Africa, South Africa) was used to determine the gross energy content. Each sample (tablet) was rested on a wire on a metal crucible, which in turn was connected to two electrodes. The crucible along with the electrode was then inserted into the bomb and closed up. The lid of the bomb is mounted with a valve through which oxygen was transferred into the bomb to attain a pressure of 3000 KPa. The bomb was then placed inside the calorimeter after which the contents were ignited. The resultant reading (MJ/kg) was displayed on a digital screen on the calorimeter.

3.2.4 Neutral detergent fibre

The neutral detergent fibre (NDF) content of the samples was determined according to Robertson & Van Soest (1981). This method entailed the use of a Fibretec

apparatus and included making up a neutral detergent solution (NDS) by adding 30 g of sodium laurylsulphate to a solution of 500 ml distilled water and 10 ml 2-ethoxyethanol (solution 1). In a separate solution, 6.81 g of $Na_2B_4O_7.10H_2O$ and 18.61 g of EDTA was added to 200 ml of distilled water (solution 2) which was then heated. When *solution 2* had fully dissolved it was added to *solution 1.* Then 4.56 g of Na_2HPO_4 was added to 100 ml distilled water, dissolved and added to the previous solution to complete the NDS.

A 1 g sample was weighed and added to 100 ml NDS in a glass crucible which was then placed in the Fibretec instrument and brought to boil at 100ºC. After adding 0.1 ml α-Amylase the temperature was reduced to and maintained at 65ºC for 60 minutes. The NDS in the crucible was then drained and the residue washed three times with boiling water and once with acetone. Samples were then dried in an oven for 48 hours at 100ºC after which they were allowed to cool in a desiccator and weighed. After recording the weight samples were ashed by placing them in an oven for six hours at 500ºC after which they were left in the oven to cool. Samples were then removed, placed in a desiccator and weighed.

Calculations:

$$\text{NDF\%} = \frac{[\text{sample residue after 48 hours drying (g)}] - [\text{sample residue in crucible after ashing (g)}]}{\text{Samples mass (g)} \times [\text{sample DM \%}]} \times 100$$

NDF analyses on faecal samples were done on an Ankom 200/220 (Ankom® Technology Corp., Fairport, NY, USA) Fibre Analyzer as per the manufacturer's prescribed method (ANKOM, 2011). Filter bags were first washed with acetone where after they were dried in an oven for two hours at 100ºC. Bags were then filled with 0.5 g of the sample, heat sealed and placed into the Ankom 200/220 Fibre Analyzer with a NDS with added heat stable α-amylase and sodium sulphate as prescribed by the manufacturer (Ankom® Technology Corp., Fairport, NY, USA). Filter bags, along with the enclosed sample, were kept in the fibre analyzer and washed at 100ºC for 75 minutes after which the NDS was flushed out. Upon removal, filter bags were washed once with warm water and α-amylase where after

two washes were done with warm water. The filter bags were then dabbed dry using paper towels before being placed in acetone for three minutes. After the filter bags were removed and the acetone allowed to evaporate, the bags were dried in an oven for two hours at 100ºC. The filter bags were then placed in a desiccator for 30 minutes after which they were weighed.

Calculation:

$$NDF\% = \frac{W3 - (W1 \times C1)}{W2 \times DM} \times 100$$

W1 = bag tare weight (g)
W2 = sample weight (g)
W3 = weight after extraction process (g)
C1 = blank bag correction (final oven dried weight/original blank bag weight)

3.2.5 Acid detergent fibre

Acid detergent fibre (ADF) was determined by the method described by Goering & Van Soest (1970) with the help of an ANKOM 220 Fibre Analyser (ANKOM Technologies, Fairport, NY).

Acid detergent solution (ADS) was prepared by adding 20 g N-Cetyl-N,N,N-Trimethyl Ammonium Bromide (CTAB) to 1 L of standardized H_2SO_4. The standardized H_2SO_4 was prepared by measuring 28 ml of 98% H_2SO_4 and filling to volume (1 L) with distilled water. The method for determining the ADF contents were the same as for the calculation of NDF, with the following differences:

• No sodium sulphite or heat-stable alpha amylase was used during step five and 1.9 – 2 L of ADS was added instead of NDS;
• The solution was left to boil for 60 minutes during step seven, instead of 75 minutes.

The ADF (%) was calculated as follows:

ADF (As-is basis) = [(W 3 – (W 1 x C 1)) x 100] / W 2

ADF (DM basis) = [(W 3 – (W 1 x C 1)) x 100] / (W 2 x DM)

3.2.6 Ether extract

The fat content of samples were determined through ether extraction (AOAC International, 2002c) using the Tecator Soxtec System HT 1043 Extraction unit. Included in this unit are aluminium cups which were placed an oven at 100ºC and allowed to dry overnight after which they were placed in a desiccator for 30 minutes and allowed to cool down before their weights were recorded. Cellulose extraction thimbles were placed on a scale and 2 g of the sample was then weighed into the thimble. Cotton wool was placed in the top of the thimble in order to avoid the sample spilling out. Thimbles were then fitted with a metal ring which would allow being mounted on an extraction tube on the extraction unit. The aluminium cups were filled with diethyl ether and placed on an element on the extraction unit under corresponding thimbles. The thimbles were then lowered into the diethyl ether and allowed to boil for 15 minutes. The thimbles were then rinsed for 30 minutes after which the valve was closed for 15 minutes allowing the ether to collect. After removing the aluminium cups from the extraction unit they were placed in an oven at 100ºC for two hours, enabling evaporation of the ether to take place. The aluminium cups were placed in a desiccator for 30 minutes to cool down and upon removal were weighed.

Calculation:

$$\text{Fat \%} = \frac{[\text{mass of aluminium cup and fat (g)}] - [\text{mass of aluminium cup (g)}]}{\text{Sample mass (g)}} \times 100$$

3.2.7 Ash

The ash content of the samples was determined by method 942.05 (AOAC International, 2002a). Moisture free porcelain crucibles were weighed. A 2 g sample (moisture free) was then weighed into the crucible before being placed in an oven for six hours at 500ºC. After having allowed the oven to cool down for two hours, the crucibles were removed and placed in a desiccator for 30 minutes. Each crucible was then taken out of the desiccator and weighed. The ash percentage was then calculated.

Calculation:

$$\text{Ash\%} = \frac{[\text{mass of crucible and ash (g)}] - [\text{mass of moisture free crucible (g)}]}{\text{Sample mass (g)}} \times 100$$

The percentage of organic matter (OM) was then calculated:

OM% = 100 – ash%

3.3 References

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CHAPTER 4

THE FEEDLOT PERFORMANCE AND CARCASS CHARACTERISTICS OF CROSSBRED LAMBS FED DIETS VARYING IN ESSENTIAL AMINO ACIDS (EAA) AND NON STRUCTURAL CARBOHYDRATE (NSC) LEVELS

Abstract

The cost of feed plays a very important role in determining profitability when lambs are fattened under intensive conditions. The aim of this study was to increase the efficiency of production and feed utilization by optimizing the diet fed to feedlot lambs for specific amino acids. This will hold economic advantages for both the producer and the consumer. Forty lambs (age = 69 days; weight 24.35 kg) were used to evaluate the effect of different diet spesifications on lamb growth and performance. The diets were optimized for non-structural carbohydrate (NSC) content and for essential amino acids (EAA), Methionine (Met), Lysine (Lys), Arginine (Arg) and Threonine (Thr). Lambs (n = 10 per group) were randomly assigned to four diets: Optimized protein (OP) which contained 157 g/kg crude protein (CP) on a dry matter (DM) basis, 477g/kg DM NSC and optimized amino acid levels, a commercial finisher diet (FIN) containing 152 g/kg DM CP and 468 g/kg DM NSC, low protein (LP) which had 139 g/kg DM CP, 455 g/kg DM NSC, not optimized for EAA and the negative control (CON) where the lambs were finished on pasture (kikuyu) with supplementary feed supplied at 500 g/day containing 151 g/kg DM CP and 358 g/kg DM NSC. The concentrate-fed lambs performed markedly better than the lambs finished on kikuyu pasture, with higher growth rates and slaughtering weights. However, there were no significant differences within the different concentrate-fed groups regarding total intake, average daily gain (ADG) or feed conversion ratio (FCR). The lambs finished on pasture had a lower back fat thickness and lower dressing percentage when compared to the concentrate-fed lambs. Within the concentrate-fed groups there was no difference in back fat thickness or dressing percentage. It is therefore concluded that feedlot lambs finished on a concentrate diet will perform better than lambs finished on pasture. However, lambs fattened on diets optimized for NSC and EAA did not perform significantly better than lambs

fattened on a standard commercial finisher diet.

4.1 Introduction

With intensive livestock production systems the focus is always on profitability which is mainly achieved by optimizing animal performance. By intensifying livestock production systems, producers will be able to market lamb at more competitive prices and subsequently increase consumer demand. Fattening lambs in a feedlot implies that lambs are weaned at a younger age, decreasing the amount of time until subsequent mating of ewes (Van der Westhuizen, 2010). However, when pursuing increased productivity and profitability it is of vital importance that the quality of the product as well as consumer acceptance be upheld (Santos-Silva *et al*., 2002). Consumers are becoming more aware of the impact that production systems have on animal welfare and recently this has become an important factor in determining consumer preferences (McInerney, 2004)

.

In order for a livestock production system to become economically feasible and ultimately successful, the key is optimal performance. Due to this, intensively raised animals are required to have a high efficiency of nutrient utilization, which in turn has a direct influence on converting feed to the final product. The ability of animals to maintain high nutrient utilization efficiency will ultimately determine the profitability of the production system by affecting profitability standards such as the feed conversion ratio, average daily gain and dressing percentage (Nolte, 2006).

The main factor in determining protein utilization efficiency in the ruminant is the profile of amino acids that escapes ruminal degradation to reach the small intestine (Boisen *et al*., 2000). Boisen (2000) added that in order to attain the ideal protein levels at the small intestine (SI), it is required to balance microbial protein (MP) with the undegradable protein that has escaped ruminal degradation. In cases of inadequate MP production or high amino acid requirements, the MP produced in the rumen may not meet the amino acid needs of the animal. This will lead to suboptimal production if amino acids of a non-microbial source are not supplied (Merchen &

Titgemeyer, 1992). Nolte (2006) noted that due to their high demand for nutrients, growing lambs are susceptible to amino acid deficiencies.

Non-structural carbohydrates consist mainly of the starch fraction of the feed but also include compounds such as sugars, pectins, galactans and β-glucans. This fraction is a very important source of energy in the diet. The process in which rumen microbes synthesize protein is energy dependent; therefore it is difficult to view the physiological supply of amino acids and energy as separate entities (Titgemeyer, 2003). When there is a shortage of CP supply or when CP is insufficiently utilized the carbohydrate digestibility could decrease, while a shortage of carbohydrates could lead to nitrogen being lost as ruminal NH_3 (Nocek, 1987).

Within the rumen, the NSC and protein found in feeds are broken down at different rates and to different degrees, which is significant in determining the availability of energy and nitrogen used for microbial growth (Hoover & Stokes, 1991). The process of microbial growth is dependent on ATP, produced during the fermentation of rumen-available carbohydrates, which enables the synthesis of cellular material (Nocek & Russell, 1988). In order to achieve optimal microbial growth it is essential to balance the rate at which ATP is produced (fermentation of carbohydrates) with the rate of its use (protein synthesis), which will prevent the uncoupling of fermentation. Therefore, in order to maximize MP synthesis, the availability of carbohydrates and protein within the rumen need to be matched (Aldrich *et al*., 1993).

By using the EAA composition of the whole empty body (WEB), Nolte (2006) was able to estimate the limiting EAA requirements of lambs. This EAA composition of the WEB, together with the EAA composition of the microbial crude protein (MCP) that reaches the SI, can be used to estimate the rumen undegradable amino acids or bypass amino acids needed to meet the EAA requirements of lambs (Nolte, 2006). Also, Hammond (1992) showed that the production of MP increased as the available energy to nitrogen ratio increased (Hammond, 1992). Therefore, the fermentable carbohydrates found in NSC are a significant and are an essential source of energy for the microbial population (National Research Council, 2007).

Various studies have focused on the qualitative differences in the carcass obtained when lambs are fattened on a concentrate diet (feedlot) or when lambs are raised on a pasture-based diet. The major differences have been found in the fatness of the carcass, the colour of the subcutaneous fat and the meat flavor (Priolo *et al.*, 2002). Lambs fattened in a feedlot will have more subcutaneous fat than pasture fattened lambs while the meat of pasture fattened lambs will be darker in colour (Dıaz *et al*., 2002). Lambs grazing pasture will have increased activity levels and subsequently increased energy requirements, while also consuming more forage which increases the basal metabolism, which leads to a further increase in energy requirements (Dıaz *et al*., 2002). Lambs fattened on pasture accrue less fat than lambs fed a concentrate diet. Therefore, these lambs will reach an acceptable slaughter weight without excess fat on the carcass (Borton *et al.*, 2005).

It has also been documented that the diet influences the meat quality of lambs. One of the quality attributes affected is the colour of meat. The CIELab (Commission International De l'Eclairage, 1976) colorimetric system is a widely used system for the objective measuring of meat colour (Honikel, 1998). This system uses three coordinates namely L*, a* and b*, with L* representing the lightness of the meat (0 = black; 100 = white), a* the red/green spectrum (negative = green; positive = red) and b* yellow/blue spectrum (negative = blue; positive = yellow). The chroma and hue angle values (calculated from the a* and b* values) refer to the colour intensity and dimension of the meat samples respectively. The meat of lambs fattened on pasture tend to have lower L* values as their meat is darker (Dıaz *et al*., 2002; Priolo *et al*, 2002). This is due to extensively raised lambs being more exercised and thus having higher concentrations of haemic pigments in the meat (Renerre, 1986). The colour of meat is an influential factor in determining the acceptance of a product by consumers (Dıaz *et al*., 2002).

A high muscle ultimate pH value is often an indication that animals were stressed preslaughter (Martínez-Cerezo *et al*., 2005). This is often the case with lambs fattened on pasture as they are not used to being handled (Muir *et al.*, 1998). Also, Priolo *et al.* (2002) noted that the ultimate pH from the muscles of lambs with high growth rates tend to be higher when compared to lambs with lower growth rates; diet is known to influence growth rate in lambs post weaning.

As blood urea nitrogen (BUN) is the end-product of nitrogen metabolism in ruminants, it can be used to determine the protein status of an animal. Elevated BUN levels indicate the inefficient use of nitrogen in the diet (Nousiainen *et al*., 2004). According to the Western Cape Veterinary laboratory (Department of Agriculture, Stellenbosch, South Africa) the prescribed BUN levels for adult sheep are 14.84 – 37.18 mg/100ml.

In this investigation it is postulated that lambs fed a feedlot ration formulated to meet the limiting EAA requirements of lambs, at a certain NSC level, would perform better in a feedlot when compared to lambs receiving a standard commercial feedlot diet. This would be achieved by lambs showing increased growth rates and improved feed efficiencies while producing meat of a good quality that meets consumer preferences.

4.2 Materials and methods

The lambs used in this trial were obtained from a previous experiment where lambs were fed specialized creep feed diets. Upon completion of the creep feed phase, 40 lambs were slaughtered for further analysis while the remaining 40 lambs entered a feedlot for the commencement of the trial.

For this trial, 40 crossbred (South African Mutton Merino x Döhne Merino) lambs, at an average age (± s.d.) of 70 ± 1 days and an average live weight (± s.d.) of 24.4 ± 0.64 kg were randomly allocated into four dietary treatment groups of 10 lambs per group. Three of these groups received pelleted concentrate diets containing varying levels of CP and NSC, while the fourth group served as the control group and was allowed to graze kikuyu pasture. The four treatments were as follows:

- A low protein feed (LP; 139 g CP/kg, 455 g NSC/kg; Met, 1.93 g/kg; Lys, 4.99 g/kg; Arg, 6.66 g/kg; Thr, 4.73 g/kg) not adjusted for amino acids which also served as the control diet for the other feedlot diets.
- An optimized protein feed (OP; 157 g CP/kg, 477 g NSC/kg; Met, 2.48 g/kg; Lys, 7.45 g/kg; Arg, 8.51 g/kg; Thr, 8.12 g/kg) adjusted for amino acids.

- A standard commercial finisher feed (FIN; 152 g CP/kg, 468 g NSC/kg; Met, 2.08 g/kg; Lys 5.49 g/kg; Arg, 7.47 g/kg; Thr, 4.80 g/kg).
- Control treatment (CON) where lambs grazed kikuyu pasture and received additional supplementary feed.

4.2.1 Formulation of the diets.

The diets were formulated based on calculations suggested by Nolte (2006) where the EAA needed for growth in woolled sheep were determined. In their study it was found that the amino acid profile of the MP that reaches the duodenum remains relatively constant. Therefore, by comparing the profile of amino acids from microbial origin to the essential amino acid requirements of lambs in a particular production stage, the imbalances can be rectified by the supplementation of undegradable or bypass protein. Other than the supply of protein, the process of carbohydrate fermentation is essential in supplying energy to rumen microbes and is a key factor in determining the productivity of the microbial population within the rumen (Henning *et al*., 1993). Nocek *&* Russel (1988) stated that when a diet is formulated to be high in rumen available protein and carbohydrates, the synthesis of MP will be more optimal than when compared to diets that have either unbalanced- or low rumen-availability carbohydrates or proteins (Nocek & Russell, 1988). Diets were therefore formulated to vary in levels of EAA and NSC. The detailed description of the diets is found in Chapter 3 in Table 3.1 and Table 3.2, but for convenience, the important chemical fractions are replicated here in Table 4.1.

During the creep feeding of the lambs, the first dietary treatment (OP) was optimized for the EAA and NSC at 157 g/kg CP and 477 g/kg NSC. The second treatment (CF2) was also optimized for the EAA and NSC. This however was done at higher levels to include 179 g/kg CP and 508 g/k NSC. The third treatment was a low protein feed (LP) formulated at 139 g/kg CP and 455 g/kg NSC that served as a control and was not optimized for EAA. While the CP levels are reported, it is important to note that the dietary treatments were formulated to vary in levels of EAA and not CP.

Upon weaning, lambs in the CF2 group were given a commercial finisher diet (FIN) that was not optimized for EAA and contained 152 g/kg CP and 468 g/kg NSC. This served as a positive control and to establish the effect of a feed change on subsequent animal performance. Thus, a standard phase-feeding approach was applied to these lambs by changing from a creep feed regimen pre-weaning to a commercial finishing regimen post-weaning. The other three treatment groups were kept on the same diets that they had received pre-weaning, thus avoiding possible metabolic disruptions due to adaptation.

Table 4.1 The important chemical fractions of the experimental diets fed to feedlot lambs.

DM basis	Diets				
	FIN	**OP**	**LP**	**CON**	
				Supplementary feed	**Kikuyu**
ME MJ/kg	11.57	11.80	11.44	10.77	9.97
CP g/kg	152.83	157.26	139.09	150.94	213.15
Bypass protein g/kg	50.99	59.57	48.92	45.81	-
NSC g/kg	467.72	477.35	454.91	358.39	-
Methionine g/kg	2.08	2.48	1.93	2.12	-
Bypass Methionine g/kg	0.81	0.89	0.73	0.78	-
Lysine g/kg	5.49	7.45	4.39	4.39	-
Bypass lysine g/kg	1.87	2.62	1.58	1.51	-
Arginine	7.47	8.51	6.66	7.12	-
Threonine	4.80	8.12	4.73	3.93	-

4.2.2 Management of lambs during trial

The lambs were housed in a shed on the Welgevallen experimental farm in Stellenbosch and kept on slatted floors in 1.8 m x 1.2 m individual pens. The three

concentrate fed groups (FIN, OP, LP) received pelleted diets formulated by Tanqua Feeds, Riviersonderend (South Africa). The control group (CON) was allowed to graze kikuyu while also receiving supplementary feed (151 g CP/kg, 358 g NSC/kg). The lambs in the control group were let out onto the kikuyu pasture during the day (08:00 – 17:00) while at night being kept in a shed with access to water and barley hay *ad libitum.* The control lambs received an energy lick at 250 g/day from day 1 – 14 where after this was increased to 500 g/day up unto the end of the trial.

Keeping lambs in individual pens allowed for more accurate data collection. Lambs were weighed once a week in order to determine each lamb's individual ADG, while the feed refusals were weighed back each morning. This enabled the calculation of the exact feed intake and ultimately the FCE of the lambs.

Blood samples were taken for BUN analysis on days 29 and 56 (1 day prior to slaughter) of the trial by drawing blood into a vacuumed heparin tube from the jugular vein in the neck of the lambs. Blood samples 1 and 2 were taken when lambs were 99 and 131 days of age respectively. Blood samples were sent to the Western Cape Provincial Veterinary laboratory, Department of Agriculture, Stellenbosch for BUN analysis.

4.2.3 Slaughter of the lambs

Upon completion of the feedlot trial lambs were slaughtered in order to determine carcass quality and ultimately the meat quality. Lambs were not fasted prior to slaughter. Lambs were slaughtered at an average age (±s.d.) of 131 ± 3 days and at an average weight (±s.d.) of 40.41 ± 1.26 kg.

At slaughter, lambs were electrically stunned for four seconds at 200 volts after which the jugular vein was severed and exsanguination took place. Lambs were then weighed, dressed and eviscerated as per standard South African techniques (Hoffman *et al*., 2003). Directly after exsanguination the carcass was weighed and recorded as the weight at slaughter. Then the head, skin, trotters and internal offal were removed to give the carcass weight (kg). The kidneys and the pelvic fat were

kept intact on the carcass as per South African procedures. Upon removal the following parts were weighed and recorded:

- Head
- Skin
- Trotters
- The full reticulo-rumen as well as the reticulo-rumen minus its content – The reticulo-rumen was severed at the pyloric junction and was weighed with the oesophagus still attached.
- Large intestine and small intestine which were separated at the caecum, digesta removed, rinsed with water and weighed individually.
- Liver – The liver was weighed with the gall bladder still attached.
- Heart
- Lungs – The lungs were weighed along with the trachea still attached.
- Spleen
- Omentum fat

Upon removal of organs the carcasses were moved to a cooling room where they were hung by both Achilles tendons at 4ºC for 24 hours. At 24 hours post slaughter the *M. longissimus dorsi* on both the right and left side of the carcass was removed. This was done between the 2nd and 3rd last thoracic vertebrae and the 4th and 5th lumbar vertebrae. Proximate analysis was done on the *M. longissimus dorsi* sample taken from the left side while the sample from the right side was used to determine the physical characteristics of the carcass. Upon removal of these muscle samples, a calliper was used to measure the back fat thickness of each carcass in the middle of where the *M. longissimus dorsi* muscle was removed.

4.2.4 Proximate chemical analysis of the meat

Upon removal of the *M. longissimus dorsi*, the visible fat was removed and meat was homogenized in a bowl cutter before proximate analysis was done. The moisture content (%) (100ºC, 24 hours) of the meat sample was determined according to AOAC method 934.01 (AOAC International, 2002b:). The ash content (%) (500ºC, 6 hours) of the meat sample was determined using the AOAC method 942.05 (AOAC

International, 2002a:). The fat content (%) was determined using a 1:2 chloroform to methanol mixture (Lee, 1996). After extraction of the fat, the fat free meat samples were dried and grinded in preparation for protein and amino acid analysis. The CP content (%) of the meat was determined by placing a 0.1 mg sample in a Leco® FP-528 Nitrogen/Protein Analyzer (Leco® Corporation, St Joseph, USA) and multiplying the nitrogen content (%) with a factor of 6.25 (AOAC International 2002).

For the amino acid analysis, samples were hydrolyzed using 6 M HCl after being sealed in a glass tube filled with nitrogen and maintained at 110ºC for 24 hours (AOAC, 1997). The samples were then diluted and derivatised using the EZ:Faast method as described in the user's manual. Labeled Homoarginine, homophenylalinine and Methionine-D3 were used as internal standards and comes included in the EZ:Faast kit. A Waters API Quattro Micro system was used in determining the amino acid composition.

4.2.5 Physical characteristics of the meat

The right side of the *M. longissimus dorsi* was used to determine the physical characteristics of the meat. All procedures started at 48 hours post mortem.

The pH of the meat was taken using a Testo 205 pH meter (Testo AG, Germany) after calibration using buffers supplied by the manufacturer.

The drip loss, cooking loss and colour assessment was done according to methods described by Honikel (1998). For drip loss, 1.5 cm thick meat samples were sealed in inflated plastic bags (with the meat sample not touching the bag) and suspended inside a cold room at 4ºC. After 24 hours, the samples were removed from the bags, lightly dabbed dry with paper towels and weighed. The drip loss (%) was then calculated as follows:

$$\frac{\text{Total weight lost during storage (g)}}{\text{Initial weight of meat sample (g)}} \times 100$$

To determine the cooking loss (%), 1.5 cm thick meat samples were weighed, sealed in a thin plastic bag and placed into a heated water bath at 80ºC for 60 minutes. Upon completion of the cooking period, samples were removed and allowed to cool, dabbed dry with paper towels and their final weights recorded. The cooking loss was calculated as follows:

$$\frac{\text{Weight loss during cooking (g)}}{\text{Initial weight of meat sample (g)}} \times 100$$

The shear force was determined using cooked meat samples (same samples used to determine cooking loss). Five cylindrical samples, each 1.27 cm in diameter, were removed from one cooked sample. In order to determine the amount of force (Newton) needed to shear a cooked meat sample perpendicular to the muscle fiber, an Instron Universal Testing Machine (Apollo Scientific, South Africa) fitted with a 1.2 mm thick Warner Bratzler blade was used. A 2 kN loading cell was fitted onto the Instron measuring unit. The tests were done at a crosshead speed of 100 mm/min. Each sample's shear force value was measured and recorded in Newton (N) (Honikel, 1998).

For the Lab colour measurement on the meat, a Colour-guide 45º/0º (BYK-Gardner, USA) was used. Each meat sample was cut into 1.5 cm thick slices and allowed to bloom (being exposed to the atmosphere) for 30 minutes (Honikel, 1998). Three colour measurements per sample were taken and the mean calculated for further statistical analysis. The hue angle (dimension) and chroma (colour intensity) values were calculated as follows:

$$\text{Hue angle} = \tan^{-1}(b^*/a^*)$$
$$\text{Chroma} = \sqrt{(a^{*2} + b^{*2})}$$

4.2.6 Blood urea nitrogen (BUN)

Blood samples were taken for BUN analysis on days 29 and 56 (1 day prior to slaughter) of the trial by drawing blood into a vacuumed heparin tube from the

jugular vein in the neck of the lambs. Blood samples 1 and 2 were taken when lambs were 99 and 126 days of age respectively. Blood samples were sent to the Western Cape Provincial Veterinary laboratory, Department of Agriculture, Stellenbosch for BUN analysis.

4.2.7 Gross margin of feedlot diets

The calculations of the gross margin of the feedlot diets were based on a profit calculator for sheep feedlots which was developed by the Grootfontein Agricultural Development Institute (Department of Agriculture, Forestry and Fisheries, Middelburg, South Africa).

Gross margin = [A(B-C) + D(B-E)] – L

With:

A = Weight gained in feedlot.

B = Expected net income per kg live weight.

C = Feed cost per kg bodyweight gained in feedlot

D = Initial bodyweight (when entering feedlot)

E = Purchase price per kg live body weight

L = Losses

4.3 Statistical analysis

The data was analyzed using SAS software for Windows Version 9.3. All the data were analyzed by means of One-Way Analysis of Variance using the GLM Procedure with the treatments as the main effect. The model for the experimental design is indicated by the following equation:

$Y_{ij} = \mu_i + \beta_j + \varepsilon_{ij}$

The terms are defined as the overall mean (μ_i), the effect of the treatment (β_j) and the error associated with the effects of the treatments (ε_{ij}). The LS means and Bonferroni *Post hoc* tests were used to compare levels within treatments. P-values smaller than 0.05 were considered significant ($P < 0.05$).

4.4 Results & discussion

4.4.1 Feedlot performance

The results obtained from the feedlot trial are summarized in Table 4.2. There were no differences ($P > 0.05$) within the concentrate fed groups (FIN, OP, LP) in terms of ADG, total weight gain, total feed intake or FCR. This is in agreement with Obeidat *et al.* (2008) where the feeding of rumen-protected methionine did not lead to an increase in lamb performance. According to Obeidat *et al.* (2008), this could be due to either the composition of the diet or the composition of the protein in the diet being imbalanced. Wiese *et al.* (2003) also reported a lack in response when growing lambs were fed rumen-protected Methionine. A possible explanation could be that when lambs receive a high quality diet *ad libitum*, they are closer to obtaining sufficient quantities of EAA, such as Methionine, than when fed restricted diets or diets of lower quality such as found with lower protein content (Wiese *et al*., 2003). The lack of differences among the concentrate fed groups can also be explained by the source of grain that was used. The OP treatment, for instance, contained 35% maize compared to the 32.5% and 27.4% in the FIN and LP diets respectively. The OP diet contained no oats while the FIN and LP diets contained 13.9% and 15.1% respectively. Herrera-Saldana (1990) determined the ruminal availability of the starch in oats to be 98% while that of maize was 62% (Herrera-Saldana *et al*., 1990). It could therefore be plausible that the degradation rates of the starches and proteins were not synchronized.

Even though the grazing lambs (CON) received supplementary feed, they still had a lower ($P < 0.05$) ADG and total weight gain compared to those fed concentrates. This is in agreement with literature as diets high in energy and protein allow for higher growth rates resulting in lambs reaching slaughter weight more rapidly (Borton *et al*., 2005; Fimbres *et al*., 2002; Sultan *et al*., 2010).

Table 4.2 Mean (± SEM) feedlot performance of lambs fattened on diets varied in CP and NSC levels

	FIN 152 g CP/kg 468 g NSC/kg 2.08 g/kg Met 5.49 g/kg Lys 7.47 g/kg Arg 4.80 g/kg Thr	**OP** 157 g CP/kg 477 g NSC/kg 2.48 g/kg Met 7.45 g/kg Lys 8.51 g/kg Arg 8.12 g/kg Thr	**LP** 139 g CP/kg 455 g NSC/kg 1.93 g/kg Met 4.99 g/kg Lys 6.66 g/kg Arg 4.73 g/kg Thr	**CON**
Beginning weight (kg)	25.5^{ab} ± 0.90	26.3^{ab} ± 1.40	24.5^{a} ± 1.20	21.2^{b} ± 1.22
Slaughter weight (kg)	45.0 ± 0.85	46.3 ± 1.85	43.8 ± 1.72	29.6 ± 5.11
ADG (kg)	0.34^{a} ± 0.014	0.35^{a} ± 0.016	0.34^{a} ± 0.016	0.16^{b} ± 0.012
Total weight gain (kg)	19.5^{a} ± 0.78	20.1^{a} ± 0.89	19.4^{a} ± 0.92	9.0^{b} ± 0.66
Total feed intake (kg)	85.9 ± 1.39	83.5 ± 2.47	84.4 ± 2.71	ND
FCR[3]	4.4 ± 0.15	4.2 ± 0.18	4.4 ± 0.12	ND[2]

Values within rows with different superscripts ^{a,b}differ ($P<0.05$).
[1] Lambs were slaughtered after 57 days when the last concentrate fed group reached market weight (± 40 kg).
[2] The FCR of the CON was not determined (ND) as it was not possible to record the feed intake of the lambs while they were grazing.
[3]Calculated using the 'as is' feed intake.

4.4.2 Carcass and meat characteristics

At slaughter, there were no statistical differences between the three concentrate-fed groups for their final body weights. However, the lambs in the CON treatment had significantly lower body weights at slaughter ($P < 0.05$).As discussed; this was due to

the feedlot lambs receiving a concentrate diet high in energy and protein which allowed higher growth rates (Table 4.2). Subsequently the carcass weights for the feedlot lambs were also significantly higher than the grazing lambs ($P < 0.01$) while within the concentrate fed lambs the carcass weights showed no significant differences.

While there were no significant differences ($P > 0.05$) within the concentrate fed groups (~52%) in the dressing percentage (DP), the CON group (46.0%) had a significantly ($P < 0.01$) lower yield. Fluharty *et al.* (1999) also compared the carcass characteristics of lambs fattened on concentrate to those of lambs allowed to graze lucerne while receiving a supplemental concentrate. The concentrate fed lambs had a DP of 51.2%, compared to a DP of 48.3% for the grazing lambs (Fluharty *et al*., 1999). Murphy *et al*. (1994) also showed that the DP for concentrate fed lambs was greater than for lambs grazing lucerne, when slaughtered at the same weight. The difference in DP between intensively and extensively raised lambs can be ascribed to the fully developed digestive tract of lambs reared in extensive conditions (Priolo *et al*., 2002). This can also be explained by the increased intestinal fat found in the concentrate fed lambs (Table 4.3). Borton *et al.* (2005) noted that the fully developed digestive tract along with the thin subcutaneous fat layer found in extensively produced lambs, would lead to a lower DP when compared to intensively produced lambs.

Table 4.3 The mean (± SEM) carcass characteristics of lambs fed optimized feedlot diets or produced on kikuyu grazing

	FIN	OP	LP	CON
Final body weight (FBW; kg)	45.0^{a} ± 0.85	46.3^{a} ± 1.85	43.8^{a} ± 1.72	30.2^{b} ± 1.53
Carcass weight (kg)	23.8^{a} ± 0.62	23.8^{a} ± 1.02	22.7^{a} ± 0.99	13.9^{b} ± 0.80
Dressing percentage (%)	52.7^{a} ± 0.65	51.4^{a} ± 0.36	51.7^{a} ± 0.61	46.0^{b} ± 0.71
Small intestinal weight (SI; kg)	1.0^{a} ± 0.03	1.0^{a} ± 0.06	0.8^{a} ± 0.09	0.8^{b} ± 0.03
Large intestine (LI; kg)	0.6^{a} ± 0.04	0.6^{a} ± 0.04	0.6^{a} ± 0.04	0.4^{b} ± 0.03
Full reticulo rumen (kg)	4.8 ± 0.25	5.0 ± 0.26	4.7 ± 0.24	5.2 ± 0.34
Empty reticulo rumen (kg)	1.48^{a} ± 0.06	1.5^{a} ± 0.09	1.4^{a} ± 0.06	1.1^{b} ± 0.05
Backfat thickness (mm)	11.44^{a} ± 1.71	11.7^{a} ± 0.62	11.9^{a} ± 1.55	3.81^{b} ± 0.63
Omentum fat (kg)	0.7^{a} ± 0.06	0.9^{a} ± 0.08	0.8^{a} ± 0.07	0.2^{b} ± 0.07
LI as % of FBW	1.4 ± 0.08	1.3 ± 0.09	1.3 ± 0.09	1.4 ± 0.10
SI as % of FBW	2.2^{a} ± 0.09	2.2^{a} ± 0.12	1.9^{b} ± 0.21	2.6^{a} ± 0.11

Values within rows with different superscripts ^{a,b}differ ($P < 0.05$).

There were no differences ($P > 0.05$) within the concentrate-fed groups in terms of back fat thickness (BFT) or the weight of the omentum fat while the CON lambs had thinner ($P < 0.05$) BFT and a lighter ($P < 0.05$) omentum fat depot than these lambs. Although it was expected that concentrate-fed lambs would have greater BFT measurements, the vast differences (7.63 mm to 8.14 mm more back fat than the control group) found in these results between concentrate fed lambs and the grass fed lambs are much bigger than reported in literature. Daniel *et al*. (2004) found a 2.10 mm difference in BFT when lambs were fed dehydrated grass pellets compared to lambs fed a limited concentrate diet, which was limited to achieve the same growth rate as lambs receiving grass pellets. This difference increased to 3.91 mm when lambs were fed a concentrate diet *ad libitum*. Similarly, Borton *et al.* (2005) found a 3.60 mm difference while McClure *et al.* (1995) reported a 2.00 mm difference in BFT between concentrate fed and grazing lambs. The big differences in

the present investigation may be due to the concentrate fed lambs being slaughtered at much heavier slaughter weights compared to the grazing lambs (Table 4.3). Borton *et al. (*2005) found that increasing the slaughter weight from 48 kg to 70 kg brought about a 5.00 mm increase in the BFT. Kemp *et al*. (1972) also found a 5.00 mm increase when the slaughter weight increased by 20 kg, while Lloyd *et al.* (1980) reported that a 10 kg increase in slaughter weight resulted in a 2.00 mm increase in BFT. These increased BFT measurements will have negative economic implications for the producer. Carcasses with excessive fat will receive a poorer classification while consumers also prefer meat with less visible fat (Napolitano *et al*., 2007).

As expected the omentum fat for the concentrate lambs was also significantly higher than that of the lambs grazing. The decreased fattiness of grazing lambs might be explained by several factors. Grazing lambs are a lot more active than lambs kept in a feedlot and this increased physical activity leads to the mobilization of fat reserves, which in turn is converted to muscle tissue (Dıaz *et al*., 2002). Also, grazing lambs consume less energy and there is a positive correlation between energy intake and the amount of fat in the carcass (Field *et al*., 1990). Both Borton *et al.* (2005) and Joy *et al. (*2008) noted that forage fed lambs store less energy as fat when compared to lambs on concentrate diets.

Due to the large difference in final body weight (FBW) between the feedlot lambs and the grazing lambs, the empty reticulo-rumen weight for the grazing lambs was significantly lower than that of the lambs receiving concentrate. However, when the empty reticulo-rumen weight was expressed as a percentage of the FBW, there were no differences among treatments. This is in agreement with both Fluharty *et al.* (1999) and Joy *et al.* (2005) who also found no differences in the empty reticulo-rumen weight between grazing- and concentrate-fed lambs, when lambs were slaughtered at the same FBW.

No differences ($P > 0.05$) were found in the SI weights between the FIN, LP and CON lambs. There were however differences ($P < 0.05$) between the OP and CON lambs ($P < 0.05$). No differences ($P > 0.05$) were found between the concentrate-fed lambs for the large intestine (LI) weight. There was a significant difference ($P < 0.05$) for LI weight between the CON lambs and the concentrate-fed lambs. However,

when the SI weight was expressed as a percentage of the FBW, the CON lambs tended to be numerically higher than the concentrate fed groups and differed significantly from the LP lambs. If the lambs were therefore slaughtered at the same bodyweight, the CON lambs would have significantly higher SI weights. This is in agreement with Joy *et al*., (2008) who compared the carcass characteristics of grazing lambs with those of concentrate-fed lambs, slaughtering them at the same FBW. They found that grazing lambs had significantly heavier ($P < 0.05$) SI weights. However, the LI and stomach weights were found to be similar among treatments. Fluharty *et al.* (1999) subjected lambs to two treatments with the one group grazing lucerne while receiving additional concentrate while the second group was fed an all concentrate diet. Lambs grazing lucerne had greater SI and LI weights compared to the concentrate lambs.

For grazing lambs to attain the same body weight as concentrate fed lambs, they have to consume substantially higher amounts of dry matter, which leads to greater amounts of digesta reaching the SI, therefore stimulating tissue growth (Álvarez-Rodríguez *et al*., 2010). This factor is also of economic importance, as concentrate-fed lambs will have heavier carcasses due to the proportionately lower intestinal mass when slaughtered at a constant body weight (Priolo *et al*., 2002).

The results obtained from the chemical analysis of the meat can be found in Table 4.4. The pH 24 hours post-slaughter (pH_{24}), did not differ ($P > 0.05$) between any of the concentrate fed groups or between the concentrate fed groups and the CON lambs. This is in agreement with Diaz *et al*. (2002) who found no differences between concentrate fed lambs (pH_{24} 5.65) and lambs fattened on pasture (pH_{24} 5.56). Normally, extensively reared lambs are not used to being handled, making them more susceptible to stress when in contact with humans. During stress, the glycogen reserves in the body are depleted, leading to a high ultimate pH value in the muscle post mortem (Martínez-Cerezo *et al*., 2005). This condition would have an undesirable effect on the meat as high ultimate pH values lead to dark, firm and dry (DFD) meat as well as a decrease in the shelf life of the product (Lawrie, 1998). It would therefore be expected that extensively reared lambs would have an elevated pH_{24}. During the course of the experimental period, the CON lambs were handled

daily, possibly explaining the lack of differences in pH_{24} between the extensively and intensively raised lambs.

No differences in drip loss or cooking loss were found between any of the treatments. Drip loss is the loss of water due to a decrease in the water holding capacity (WHC) of meat. There is a direct relationship between the ultimate pH and the WHC of meat. The lower the pH, the lower the WHC and therefore the higher the drip loss (Lawrie, 1998). However, for the pH to significantly affect the WHC of meat, the variations in pH (between two samples) have to be considerable (Shackelford *et al*.,1992). It is thus plausible to conclude that because the pH_{24} of the treatments did not differ, the drip loss between treatments would also not show differences. This is in agreement with Diaz *et al.* (2002) and Carrasco *et al.* (2009) who found no differences in the drip loss between lambs finished on pasture and lambs fattened on a concentrate diet in a feedlot (Carrasco *et al*., 2009; Dıaz *et al*., 2002). The factors that influence the drip loss of meat are also the same factors that influence the cooking loss of meat as both are determined by WHC. However, the moisture loss due to cooking will be higher as the high temperatures lead to protein denaturation which causes a decrease in WHC. Also, at high temperatures fat is melted which further increases the cooking loss (Lawrie, 1998). There is also a correlation between the cooking loss and the juiciness of meat. Meat that loses a large volume of moisture during cooking will also present less juicy to the palate, which is an important factor when it comes to consumer preferences (Lawrie, 1998).

No differences in shear force were found between any of the treatments. However, more importantly, there were no differences between the concentrate fed lambs and the pasture based lambs. This finding is not in agreement with literature and there is no plausible explanation for this result except that it is known that *ante mortem* stress could lead to tougher meat. However as discussed, the pasture reared lambs in this experiment were handled daily and would thus not experience higher stress than the concentrate-fed lambs. Priolo *et al.* (2002) found the meat from concentrate-fed lambs to be more tender than that of lambs fattened on pasture. They attributed this to the differences in carcass fatness with the fat causing a dilution of the collagen per surface area within the muscular tissue. Another possible explanation for this is the increased physical activity of lambs grazing pasture (French *et al*.,

2001). The higher collagen levels found in the meat of grazing lambs will also lead to more tough meat as there is a positive correlation between collagen levels and meat tenderness (Dıaz *et al*., 2002). However, observations of the pasture reared lambs in this investigation did not indicate excessive activity as the lambs were reared in small paddocks (400 m^2) in close proximity to the barn where they were housed overnight.

No differences ($P > 0.05$) in muscle colour were found between any of the treatments. Diaz *et al.* (2002) found that pasture fattened lambs had lighter meat (higher L* value) compared to concentrate fed lambs. In contrast to this, Priolo *et al.* (2002) established that grass-fed lambs had darker meat than lambs fed concentrate diets. Teixeira *et al*. (2005) also found that there is a decrease in both the lightness (L*) and yellowness (b*) with the increase of live weight. Differences in meat colour can often be ascribed to the differences in pH as meat with higher pH's tend to be darker in colour (Ledward *et al*., 1986). Exercise is also known to result in darker (more myoglobin) coloured meat (Hanekom, 2010), however, as discussed, the pasture grazing lambs in this investigation were young and did not have a heavy exercise regime. Another factor that influences meat colour (L* values) is the amount of intramuscular fat. Meat with a higher intramuscular fat content (intensive production systems) will be lighter (increased L* value) as fats possess a high scattering/reflective property (Hedrick, 1983).

Table 4.4 The mean (± SEM) physical characteristics[1] of the *M. longissimus dorsi* of lambs fed a feedlot diet varying in levels of EAA and NSC, and a pasture based diet supplemented with a concentrate

	FIN	OP	LP	CON
	152 g CP/kg 468 g NSC/kg 2.08 g/kg Met 5.49 g/kg Lys 7.47 g/kg Arg 4.80 g/kg Thr	157 g CP/kg 477 g NSC/kg 2.48 g/kg Met 7.45 g/kg Lys 8.51 g/kg Arg 8.12 g/kg Thr	139 g CP/kg 455 g NSC/kg 1.93 g/kg Met 4.99 g/kg Lys 6.66 g/kg Arg 4.73 g/kg Thr	
pH	5.37 ± 0.01	5.37 ± 0.02	5.35 ± 0.02	5.39 ± 0.04
Drip loss (%)	1.78 ± 0.19	1.59 ± 0.06	1.52 ± 0.13	1.99 ± 0.23
Cooking loss (%)	33.15 ± 1.36	31.97 ± 1.28	32.23 ± 1.04	34.38 ± 1.19
Shear force (%)	51.90 ± 3.38	53.00 ± 4.07	63.11 ± 1.94	63.33 ± 4.46
L* value	35.17 ± 1.05	35.80 ± 0.48	35.46 ± 0.73	36.59 ± 0.60
b* value	11.35 ± 0.51	10.25 ± 0.19	10.36 ± 0.23	10.15 ± 0.31
a* value	15.52 ± 0.49	13.90 ± 0.33	14.38 ± 0.33	14.69 ± 1.02

[1]Note that none of the parameters' means differed significantly between feeds (P > 0.05).

4.4.3 Blood urea nitrogen (BUN)

By using the blood urea nitrogen (BUN) levels as an indicator, the protein status of a specific group of animals can be determined. Therefore, BUN is used to indicate the efficiency of nitrogen utilization (Kohn *et al*., 2005). It can also be used to evaluate how effective a specific energy source is in promoting nitrogen balance (Preston & Pfander, 1963). The BUN results are given in Table 4.5. At 29 days no differences (P > 0.05) in BUN were found between any of the four treatments. At 56 days (one day before slaughter) no differences were found between the concentrate fed groups. However, the BUN of the OP lambs as well as the LP lambs did show differences when compared to the CON lambs. The highest BUN level recorded was that of the CON lambs at 56 days (20.09 mg/100ml) while the lowest was that of the LP lambs (12.92 mg/100ml), also at 56 days.

Table 4.5 Blood urea nitrogen levels (mg/100ml) measured in lambs fed different feedlot diets compared to lambs grazing kikuyu and receiving supplementary feed, LSMeans±s.e. Blood was collected at 29 and 56 days into the feedlot period

Blood collected at:	**FIN**	**OP**	**LP**	**CON**
	152 g CP/kg 468 g NSC/kg 2.08 g/kg Met 5.49 g/kg Lys 7.47 g/kg Arg 4.80 g/kg Thr	157 g CP/kg 477 g NSC/kg 2.48 g/kg Met 7.45 g/kg Lys 8.51 g/kg Arg 8.12 g/kg Thr	139 g CP/kg 455 g NSC/kg 1.93 g/kg Met 4.99 g/kg Lys 6.66 g/kg Arg 4.73 g/kg Thr	
BUN mg/100ml at 29 days	14.92 ± 0.7	15.00 ± 0.7	16.13 ± 0.7	17.04 ± 0.7
BUN mg/100ml at 61 days	16.44^{ab} ± 1.0	13.98^{a} ± 1.0	12.92^{a} ± 1.0	20.09^{b} ± 1.0

^{ab}LSMeans with different superscripts within a row show significant differences (P ≤ 0.05)

These BUN levels are on the lower end of what is prescribed by the Western Cape Veterinary laboratory (Department of Agriculture, Stellenbosch, South Africa) for adult sheep (14.84 – 37.18 mg/100ml), which shows that the nitrogen was effectively utilized. However, these values are in line with that of Sarwar *et al*. (2011) who found BUN values of 17.8 – 19.8 mg/100ml when growing lambs were fed diets with different protein sources. Also, Fimbres-Durazo *et al*. (2013) found BUN values of 16.8 – 23.2 mg/100ml when growing lambs were fed high grain finishing diets. Preston and Pfander (1963) found a direct correlation between the protein and BUN level in the diet, while the energy and BUN level was inversely correlated. It therefore stands to reason that the elevated BUN levels found in the CON lambs are due to a lack of sufficient energy in the diet or due to the high rumen degradability of the nitrogen in the kikuyu grazing.

4.4.4 Economic analysis

The results obtained from a gross margin above feed costs calculation can be seen in Table 4.6. These calculations were based on a profit calculator for sheep feedlots which was developed by the Grootfontein Agricultural Development Institute (Department of Agriculture, Forestry and Fisheries, Middelburg, South Africa). The feed intake and growth rate of lambs are considered the most important factors determining profitability (Montanholi *et al*., 2008). The costs incurred by optimizing the amino acids in the OP diet ultimately lead to this diet having the lowest gross margin above feed cost. The lambs on the LP diet had the highest margin above feed cost. Although the LP diet was formulated at lower protein levels, therefore decreasing its price, the feed was still able to sustain growth rates similar to that of the OP and FIN diets. Feeding the LP diet in a commercial feedlot would therefore prove to be more profitable.

Table 4.6 The calculation of the gross margin above feed cost for three feedlot diets, varying in CP and NSC levels (Mean ± SEM)

	FIN	OP	LP	CON
	Value of lambs before feedlotting			
Initial bodyweight (kg)	25.48 ± 0.897	26.26 ± 1.404	24.46 ± 1.196	21.20 ± 1.218
Buying price (R/kg liveweight before feedlotting)	17.50	17.50	17.50	17.50
	Value of animals after feedlotting			
Slaughter weight (kg)	45.0[a] ± 0.85	46.31[a] ± 1.852	43.8[a] ± 1.72	30.21[b] ± 1.527
Meat price (R/kg)	36.00	36.00	36.00	36.00
Dressing percentage (%)	52.72[a] ± 0.653	51.44[a] ± 0.358	51.74[a] ± 0.611	45.96[b] ± 0.711
Losses	0	0	0	0
	Nutrition			
Feed conversion efficiency	4.44 ± 0.147	4.22 ± 0.178	4.40 ± 0.120	ND[1]
Feed cost (R/kg)	2.20	2.31	1.80	1.47
Gross margin above feedcost (R)	R215.76	R202.84	R234.36	R49.75

[ab]LSMeans with different superscripts within the same row show significant differences (P ≤ 0.05).

[1]Feed intake was not measured but calculated as 3.50% of bodyweight. Additional feed fed at 250 g/lamb/day for first 7 days of trial, thereafter 500 g/lamb/day. Cost of kikuyu taken at R800/ton DM; cost of additional feed R2077/ton. Bodyweight calculated as (initial bodyweight + slaughter weight) ÷ 2.

4.5 Conclusion

It was postulated that lambs fed a feedlot diet balanced for limiting EAA (OP) would elicit greater growth rates and improved feed efficiency when compared to lambs fed a standard commercial finisher diet (FIN) and a low protein feed (LP). Furthermore, it was hypothesized that maintaining the lambs on the same diet (OP) pre- and post-weaning would further improve this response due to the lambs not having to adapt to a new diet.

However, this study revealed that both the FIN and LP lambs performed similar to the OP lambs with no significant differences in terms of the growth parameters or the

slaughter data. A plausible explanation for this is the underestimation of the EAA requirements of lambs. The initial formulation and EAA balancing was done for suckling lambs growing at 250 g/d while the lambs in this study achieved growth rates of more than 300 g/d. The requirements of a weaned lamb growing at 350 g/d are different to that of a suckling lamb growing at 250 g/d while the amount of feed consumed will also vary significantly between these two stages. A solution to this would be a phase feeding approach where both the creep feed and the finisher feed are balanced for limiting EAA at the specific age and growth rates.

It was hypothesized that the lambs maintained on the same diet pre- and post-weaning (OP) would have an advantage over the lambs that were phase-fed (CF2 to FIN), but this was not the case. It is possible that the shock of weaning the lambs nullified the theoretical advantage that this approach would hold.

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CHAPTER 5

THE IN VITRO AND IN VIVO DIGESTIBILITY OF LAMB FINISHER DIETS VARYING IN LEVELS OF NON STRUCTURAL CARBOHYDRATES AND ESSENTIAL AMINO ACIDS.

Abstract

The effect of varying levels of essential amino acids (EAA) and non-structural carbohydrates (NSC) in lamb feedlot diets on nitrogen retention, energy balance and digestibility was investigated. It was hypothesized that the diet optimized for NSC and EAA would improve nitrogen retention, energy balance and the digestibility of the feed. The two diets used for the *in vivo* digestibility trial was an optimized protein feed (OP) which contained 477 g/kg NSC and 157 g/kg crude protein (CP) on a dry matter (DM) basis and a commercial finisher diet (FIN) containing 468 g/kg NSC and 152 g/kg CP. For the *in vitro* study, a third treatment, a low protein diet (LP) which had 455 g/kg NSC and 139 g/k CP, was added. Results indicated that there were no differences in *in vivo* digestibility, nitrogen retention or energy balance between OP and FIN. The *in vivo* digestibility of Methionine (Met) and Lysine (Lys) was significantly higher ($P < 0.05$) for OP. The *in vitro* true digestibility (IVTD) of FIN differed significantly from that of OP and LP, while the IVTD of OP and LP did not show significant differences. The FIN diet showed the highest *in vitro* amino acid degradability ($P \leq 0.05$) for Alanine (Ala), Serine (Ser), Valine (Val), Tyrosine (Tyr), Threonine (Thr), Arginine (Arg), Histidine (His), Lys, Leucine (Leu) and Glycine (Gly). It was concluded that that the EAA balance as well as NSC level in the standard commercial finisher diet was adequate in supporting nitrogen retention as well as maintaining energy balance.

5.1 Introduction

Various authors have proposed methods of determining the ideal essential amino acid (EAA) requirements of animals. The ARC (1981) predicted that lean meat was representative of the ideal amino acid balance of an animal, while Hussein *et al*. (1991) hypothesized that the whole empty body (WEB) would serve as an indication of the proportion in which animals required EAA.

The most significant aspect influencing how protein is utilized is the EAA profile that enters the small intestine (SI), which is a combination of microbial protein (MP) and undegraded dietary protein (UDP) (Boisen, 2000). By using the EAA composition of the WEB, Nolte (2006) was able to determine the limiting EAA required by growing lambs, which, together with the EAA composition of the microbial crude protein (MCP) can be used to estimate the EAA profile of the undegradable protein (UDP) needed in order to meet the EAA requirements of lambs. When the profile of amino acids that are available for absorption in the SI do not lead to maximum efficiency of utilization, the amino acids must be balanced by including higher levels of UDP in the ration with UDP sources containing higher levels of the limiting amino acids (Nolte, 2004).

It is evident that the profile of amino acids available for absorption is an essential factor in determining the effectiveness of protein utilization. The inadequate supply of a single amino acid will hamper the response of those that are sufficient (Cole & Van Lunen, 1994), while a surplus of amino acid in proportion to the limiting amino acid will be broken down and excreted with an ensuing loss of nitrogen and energy (Nolte, 2006). It is therefore vital to supply the ideal amino acid profile to the SI in order to attain maximum performance.

The growth of the microbial population is dependent on a source of carbohydrates that are readily available in the rumen and provides ATP for the biosynthesis of cell material (Nocek & Russel, 1988). It has been found that when rapidly digestible energy and protein sources are fed, microbial protein synthesis is maximized (Herrera-Saldana *et al*., 1990). Therefore, when there is a balance between the rumen availability of the energy and protein source, the synthesis of microbial protein

will be optimized. Furthermore, in order to ensure optimal growth the rate at which ATP is produced (fermentation of carbohydrates) must be equivalent to the rate at which it is utilized (microbial protein synthesis) or fermentation will ineffective and wasteful by-products such as excess ammonia will be evident in the rumen fluid (Hespell & Bryant, 1979).

The aim of this study was to investigate whether a feedlot diet, optimized for EAA, and fed at a specific NSC level, would improve the nitrogen retention, energy balance and digestibility of feedlot lambs in an *in vivo study*. A second aim was to see whether the levels of EAA and NSC in a standard commercial finisher diet commonly used for feedlot lambs were adequate.

An *in vitro* study was done to determine what the effect of optimizing the EAA, fed at specific NSC levels would have on the IVTD of the EAA.

5.2 Materials and methods

5.2.1 In vivo digestibility trial

The *in vivo* digestibility trial, as described by McDonald *et al.* (2002), was conducted using two treatments: An optimized protein feed (OP) where the EAA in the feed were optimized at a specific NSC level (157 g/kg CP, 477 g/kg NSC; Met, 2.48 g/kg; Lys, 7.45 g/kg; Arg, 8.51 g/kg; Thr, 8.12 g/kg on DM basis); a standard commercial finisher diet (FIN) where the EAA were not optimized (152 g/kg CP, 468 g/kg NSC; Met, 2.08 g/kg; Lys 5.49 g/kg; Arg, 7.47 g/kg; Thr, 4.80 g/kg on DM basis). Although these treatments did not vary greatly in CP levels, they were formulated to differ on EAA basis. The physical and nutrient composition of the experimental diets is discussed in Chapter 3, but for convenience the dietary composition is depicted in Table 5.1. Nolte (2006) showed that the microbial protein (MP) amino acid profile that reaches the duodenum in sheep stays relatively constant, regardless of the source of protein used. Therefore, if the EAA requirements of an animal were identified, the lack and/or surplus of an individual EAA could be determined and

adjusted through the inclusion of a rumen protected amino acid supplement (Nolte, 2006).

Table 5.1 The important chemical fractions of the experimental diets fed to feedlot lambs during a seven day digestibility study

DM basis	Experimental diets	
	FIN	OP
ME MJ/kg	11.57	11.80
CP g/kg	152.83	157.26
Bypass protein g/kg	50.99	59.57
NSC g/kg	467.72	477.35
Methionine g/kg	2.08	2.48
Bypass methionine g/kg	0.81	0.89
Lysine g/kg	5.49	7.45
Bypass lysine g/kg	1.87	2.62
Arginine g/kg	7.47	8.51
Threonine g/kg	4.80	8.12

Five rams and three ewes were placed in each treatment and subjected to feedlot conditions. The lambs weighed 35.3 ± 2.36 kg and 33.2 ± 3.19 kg for the FIN and OP treatments respectively. The ram lambs were placed in 1.8 m x 1.2 m individual pens on slatted floors while the ewe lambs were placed in metabolic crates. To enable the measuring of total faecal and urine outputs faecal bags and urinary funnels were attached to the ram lambs whilst the crates allowed for separate urine and faecal matter collection on the ewe lambs.

The lambs were placed in the crates and pens four days prior to the commencement of the digestibility trial. This allowed the ram lambs to get used to the faecal collection harnesses and urine funnels and the ewe lambs were able to adapt to the metabolic crates. Animals were however already fully adapted to the diets during the preceding feedlot period. The digestibility study was done during the 57 day feedlot period. Feed intake was also determined during the four day adaptation period as

this allowed for minimal feed wastage. Feed wastage can lead to the underestimation of digestibility as this feed is not ingested but is calculated as such. Every morning at 07:00 the total amount of faeces and urine excreted was collected and weighed, while a 10% sample and a 5% sample of the faeces and urine respectively was frozen and stored for analysis at a later stage. Sampling was repeated seven times, one for each day of the digestibility trial, thus providing a composite faeces and urine sample for each lamb. The total feed intake and faecal and urine output was determined for the seven day trial period. It is essential to keep in mind that the digestibility coefficients determined are apparent digestibility coefficients rather than true digestibility coefficients. In ruminant feeds the digestible carbohydrates and digestible energy contents are often overestimated (McDonald *et al*., 2002). This is due to the fact that the methane produced during carbohydrate fermentation is lost through eructation and is therefore not absorbed. Also, not all nutrients found in faeces are undigested food residues as enzymes can also contribute to faecal material while abraded parts of the gut lining also end up in the faeces. This leads to the underestimation of the amount of food that is truly absorbed by the animal (McDonald *et al*., 2002).

Faecal samples collected during the *in vivo* digestibility trial were dried for 48 hours at 60º C in order to determine the total dry faecal output. Dried faecal samples were then milled for analysis.

The collected representative samples of the faeces and feed were analyzed for dry matter (DM), crude protein (CP), gross energy (GE), ether extract (EE), neutral detergent fibre (NDF) and acid detergent fibre (ADF). Both the feed and faecal samples were analyzed for amino acids. The methods used for the mentioned analyses are discussed in section 5.2.4 of this chapter. The methane gas production (MJ/day) was estimated at 8% of gross energy intake (McDonald *et al.*2002). The urine energy was estimated as 5% of gross energy intake (Van der Merwe & Smit, 1973). Nitrogen retention was corrected for both the metabolic faecal nitrogen (MFN) and the endogenous urinary nitrogen (EUN) (McDonald *et al.,* 2002):

MFN (g) = 5 g N/kg dry matter intake
EUN (g) = 0.18 g N/kg $BW^{0.75}$/day

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

The NSC fraction of the feed was calculated as:

NSC = 100 – (CP% + NDF% + EE% + Ash%) x (DM/100)

5.2.3 *In vitro* digestibility trial

The *in vitro* true digestibility (IVTD) of three experimental feedlot diets was determined using an ANKOM®Daisy^{II} *in vitro* fermentation system (ANKOM Technologies, Fairport, NY) according to the methods described by the manufacturer. An optimized protein (OP) diet was optimized for EAA and contained 157 g/kg DM CP and 477 g/kg DM NSC. The second treatment was a standard commercial finisher (FIN) feed that contained 152 g/kg DM CP and 468 g/kg DM NSC. The EAA in the FIN feed was not optimized. A third treatment was a low protein (LP) diet that contained 139 g/kg DM CP and 455 g/kg DM NSC.

The feed samples were first milled through a 1 mm screen, after which the sample was then sieved through a 106 µm sieve. The coarse feed sample left on the sieve was then used. By doing this, smaller particles were removed which ensures more accurate calculations of *in vitro* digestibility. Smaller particles can escape through the filter bags which would lead to the over estimation of digestibility (Cruywagen *et al*., 2003).

The F57 filter bags (ANKOM Technology Corp., Fairport, NY) used were first soaked in acetone which removed any residue from the surface of the bag which could prevent microbes from entering the bags. Bags were dried overnight at 100°C and weighed. A 0.5 ± 0.05 g (± s.d.) feed sample was weighed into every bag and each bag was sealed. Seven filter bags were used for each treatment as follows:

- Three sample filled bags (triplicate measurement) were incubated for 48 hours with the NDF process according to the manufacturer's method in order to determine the IVTD.

- Three sealed filter bags containing 0.5 ± 0.05 g (± s.d.) were incubated for 48 hours and used to determine the amino acid content.
- The last sealed bag was washed in water after which the amino acid content was determined as an estimate of the amount of material that washes out of the bag.

The buffer solution was adjusted and based on the buffer system described by Goering & Van Soest (1970).
The buffer solution was made up as follows:

Deionised water – 500 ml
Rumen buffer solution – 250 ml
Macro mineral solution – 250 ml
Resazurin (0.2 %, w/v) – 2 ml
Micromineral solution – 0.12 ml
Reducing agent – 53 ml
Trypticase – 1.25 g

For the macro mineral solution 11.4 g Na_2HPO_4, 12.4 g KH_2PO_4 and $MgSO_47H_2O$ were measured and added to a 2 L volumetric flask, entirely filled with deionised water and allowed to dissolve.

To make up the micromineral solution 50 ml of deionised water was added to a 100-ml volumetric flask, after which 13.2 g $CaCl_2.2H_2O$, 10.0 g $MnCl_4.4H_2O$, 1.0 g $CoCl_2.6H_2O$ and 8.0 g $FeCl_3.6H_2O$ were added. The flask was then filled to volume.
The cysteine sulphide reducing agent was made up by adding 312 g cysteine hydrochloride, 20 ml 1 N NaOH and 312 mg $Na_2S.9H_2O$ to 48 ml of deionised water.
Rumen inoculum was taken from cannulated sheep at the Welgevallen experimental farm (Stellenbosch University, South Africa). The rumen content was collected and squeezed through a double layered cheese cloth into a thermal flask, which was warmed at 39º C. The flask was filled to the brim and immediately closed to maintain anaerobic conditions. At the *in vitro* laboratory (Department of Animal Science, Stellenbosch University, South Africa) the inoculum was poured from the thermal flask into a heated industrial blender, whilst allowing the inoculum to purge with CO_2

in order to maintain the anaerobic conditions. The blender separated the microbes from the solid material after which 400 ml of rumen inoculum was poured into Daisy^{II} incubator jars, whilst allowing the inoculum to purge with CO_2. The reduced buffer was pre-heated to 39ºC of which 1600 ml was then added to the inoculum. The jars were kept in the Daisy^{II} incubator for 48 hours, after which the bags were removed and rinsed under cold water in order to stop the microbial activity.

The sample bags were placed in an oven for 24 hours at 70ºC allowing for the calculation of DM disappearance from the bags. The NDF procedure was then done by using the ANKOM$^{200/220}$ (ANKOM Technologies, Fairport, NY) FiberAnalyzer according to methods described by the manufacturer in order to calculate the IVTD.

The IVTD was calculated as follows:

$$\% \text{ IVTD} = 100 - [((NDF_v - (W_1 \times C_1)) \times 100) / W_2$$

With:

W_1 = Bag tare weight (g)
W_2 = Sample weight (DM) (g)
NDF_v = Final bag weight after NDF (g)
C_1 = Blank bag correction (final oven-dried weight/original blank bag weight)
After 48 hours of incubation the amino acid degradation was calculated as follows:
$D (\%) = [(A-B) / A] \times 100$

With:

A = individual amino acid before incubation (g/100 g sample)
B = individual amino acid after 48 hour incubation (g/100 g sample)

For the amino acid analysis (three bags per treatment), the samples were first hydrolyzed at the Department of Animal Sciences (Stellenbosch University) after which the samples were sent to the Central Analytical Facility (CAF) at the Department of Biochemistry (University of Stellenbosch). Samples were hydrolyzed

(AOAC International, 1997) using 6 M HCl and sealed in a glass tube filled with nitrogen which was then kept at 110ºC for 24 hours.

At the CAF, ion-exchange chromatography of the protein was done to determine the individual amino acid content of the samples. Samples were analyzed using the EZ:Faast kit. Samples were prepared according to the method described in the EZ:Faast user guide. Homoarginine, homophenylalanine and methionine-D3 (included in the EZ:Faast kit) were used as internal standards. The amino acid composition was determined by a Waters API Quattro Micro system.

5.2.4 Laboratory analyses

The methods of analyses of feed and faecal samples for moisture, ash, EE, CP, NDF, ADF and GE are discussed in Chapter 3.

5.3 Statistical analysis

The data was analyzed using SAS software for Windows Version 9.3. All the data were analyzed by means of One-Way Analysis of Variance using the GLM Procedure with the treatments as the main effect. The model for the experimental design is indicated by the following equation:

$$Y_{ij} = \mu_i + \beta_j + \varepsilon_{ij}$$

The terms are defined as the overall mean (μ_i), the effect of the treatment (β_j) and the error associated with the effects of the treatments (ε_{ij}). The LS means and Bonferroni *Post hoc* tests were used to compare levels within treatments. P-values smaller than 0.05 were considered significant ($P < 0.05$).

5.4 Results and discussion

The results of the *in vivo* digestibility trial are depicted in Table 5.1. It must be noted that all the values discussed are apparent digestibility coefficients and not true digestibility coefficients. True digestibility involves the correction for endogenous losses, while apparent digestibility does not.

There were no differences ($P > 0.05$) in total feed intake or total faecal excretion between the groups for the duration of the trial. The total dry matter intake (DMI) of feed is mainly determined by the energy density of the feed (Lu & Potchoiba, 1990). The energy density in the two treatments was very similar and could explain why the DMI was the same.

Dry matter (DM), organic matter (OM), CP, EE, NSC, NDF and ADF digestibility showed no differences ($P > 0.05$) between the treatments. This is in agreement with Nolte (2006) who found no differences in DM, OM and NDF digestibility when diets varying in amino acid levels were fed to lambs. This could suggest that the profile of amino acids that reaches the small intestine has no effect on the digestibility of these nutrients.

Table 5.2 The results[1] of a seven day *in vivo* digestibility trial on lambs fed feedlot diets with varying levels of NSC and EAA composition

	FIN	**OP**
	152 g/kg CP	157 g/kg CP
	468 g/kg NSC	477 g/kg NSC
Daily DM feed intake,(g)	1358.88 ± 40.99	1395.75 ± 44.64
Daily DM faecal excretion (g)	355.13 ± 26.88	369.38 ± 13.93
	Feedlot diets chemical composition (DM basis) g/kg	
Dry matter	888.0	889.2
Organic matter	926.3	921.6
Crude protein	154.7	155.1
Ether extract	29.5	31.9
Nonstructural carbohydrates	460.9	472.3
NDF	281.5	261.8
ADF	152.4	154.7
	Faecal chemical composition (DM basis) g/kg ±s.e.	
Dry matter	336.63 ± 18.79	338.25 ± 12.88
Organic matter	877.63 ± 4.58	852.88 ± 10.03
Crude protein	172.25 ± 5.43	157.41 ± 3.30
Ether extract	22.05 ± 0.56	24.93 ± 1.99
Nonstructural carbohydrates	85.76 ± 11.39	85.30 ± 9.34
NDF	597.57 ± 12.23	537.02 ± 40.44
ADF	361.23 ± 15.56	355.45 ± 17.89
	Digestibility, % ±s.e.	
Dry matter	73.87 ± 1.84	73.50 ± 0.73
Organic matter	75.25 ± 1.71	75.67 ± 0.72
Crude protein	70.82 ± 2.19	72.75 ± 1.12
Ether extract	82.64 ± 1.39	82.95 ± 0.95
Nonstructural carbohydrates	95.33 ± 0.48	95.17 ± 0.58
NDF	44.26 ± 4.55	41.68 ± 1.64
ADF	35.15 ± 1.12	33.81 ± 1.09

[1]Note that none of the parameters' means differed significantly between feeds ($P > 0.05$)

The amino acid composition and digestibility coefficients for the experimental diets can be found in Table 5.2. The method used for amino acid analysis, HCl hydrolysis, has some limitations. During this process, the residual oxygen could increase the

thermal breakdown of hydroxyl and sulphur containing amino acids (Thr, Ser, Tyr and Hydroxyproline) which leads to recoveries of 50% - 90% while the recovery of Met ranges from 25% - 75%. This could explain why the amino acid composition for the feedlot diets differed from that which was originally formulated.

Both Met and Lys digestibility showed significant differences ($P < 0.05$) between the two treatments with the OP diet showing higher digestibility coefficients for these two amino acids. None of the other amino acids that were specifically balanced for (Isoleucine (Ile), Thr, Leu and Phenylalanine (Phe)) showed differences in this regard. With the original formulation of the diets the OP feed was formulated to contain 2.48 g/kg Met compared to the 2.08 g/kg in the FIN diet, while the OP feed contained 7.45 g/kg Lys compared to 5.49 g/kg in the FIN feed. Both the bypass Met and Lys in the OP diet were also higher compared to the FIN feed (Table 5.1). It would appear that feeding higher levels of these amino acids improve the *in vivo* tract digestibility thereof. The digestibility of aspartic acid also showed significant differences ($P < 0.05$).

The results of the nitrogen balance study conducted during the *in vivo* digestibility trial are reported in Table 5.4. No differences ($P > 0.05$) in DMI or nitrogen intake were found between the two treatments. The similar nitrogen intake is due to the similar CP levels between the two treatments. The FIN diet was formulated to contain 152 g/kg CP while the OP diet was formulated to contain 157 g/kg CP. The actual CP levels of the two experimental diets (Table 5.1) were 154.7 g/kg CP for the FIN treatment and 155.1 g/kg CP for the OP treatment. It is important to remember however that even though these two treatments had similar CP levels, they were formulated to differ on amino acid basis, as the amino acids in the OP diet were optimized (Table 5.1).

Table 5.3 The daily DM intake and faecal excretion, dietary and feacal amino acid composition and digestibility coefficients of feedlot diets varying in levels of NSC and CP

		FIN 152 g/kg CP 468 g/kg NSC	**OP** 157 g/kg CP 477 g/kg NSC
	Daily DM feed intake,(g)	1358.88 ± 40.99	1395.75 ± 44.64
	Daily DM faecal excretion (g)	355.13 ± 26.88	369.38 ± 13.93

	Amino acid composition of feed. (g/kg DM)		Amino acid composition of faeces (g/kg DM)		Digestibility (%)	
	FIN	OP	FIN	OP	FIN	OP
Alanine	4.1	3.9	5.7 ± 0.61	5.2 ± 0.53	64.53 ± 3.64	64.25 ± 3.64
Threonine	3.4	3.5	5.3 ± 0.36	4.5 ± 0.28	58.93 ± 3.26	66.86 ± 3.26
Serine	3.7	4.2	3.9 ± 0.32	3.5 ± 0.38	72.46 ± 2.09	78.13 ± 2.09
Arginine	4.6	4.8	2.8 ± 0.25	2.7 ± 0.27	84.90 ± 1.12	85.25 ± 1.12
Glutamic acid	10.4	12.2	10.9 ± 1.05	9.9 ± 1.06	72.90 ± 2.82	78.39 ± 2.82
Valine	4.5	4.4	5.1 ± 0.29	5.3 ± 0.36	70.68 ± 2.24	68.44 ± 2.24
Histidine	2.1	2.0	2.4 ± 0.14	2.3 ± 0.17	69.96 ± 2.21	69.56 ± 2.21
Aspartic acid	8.3	10.8	10.6 ± 0.75	9.9 ± 0.57	**66.83[a] ± 3.53**	**75.63[b] ± 3.53**
Lysine	3.8	5.4	7.8 ± 0.38	6.6 ± 0.36	**46.99[a] ± 2.92**	**67.35[b] ± 2.92**
Proline	6.0	5.8	4.7 ± 0.21	4.64 ± 0.15	79.81 ± 1.72	78.73 ± 1.72
Methionine	0.5	1.2	0.7 ± 0.06	0.7 ± 0.06	**67.32[a] ± 3.31**	**84.28[b] ± 3.31**
Tyrosine	2.3	2.5	3.7 ± 0.17	3.6 ± 0.18	56.54 ± 3.44	62.35 ± 3.44
Cysteine	0.3	0.4	0.7 ± 0.04	0.7 ± 0.03	47.17 ± 4.26	56.42 ± 4.26
Isoleucine	3.0	3.3	3.6 ± 0.23	3.4 ± 0.2	68.77 ± 2.53	72.76 ± 2.53
Phenylalinine	3.7	4.1	4.0 ± 0.21	3.9 ± 0.25	71.35 ± 2.34	74.63 ± 2.34
Leucine	10.6	10.6	8.1 ± 0.43	7.9 ± 0.37	80.01 ± 1.30	80.13 ± 1.30
Glycine	3.8	4.4	4.6 ± 0.24	4.4 ± 0.25	68.38 ± 2.26	73.40 ± 2.26

[ab]LSMeans with different superscripts within the same row show significant differences (P ≤ 0.05).

Table 5.4 Nitrogen metabolism[1] of feedlot lambs fed finishing diets varying in levels of NSC and CP during a 7 day *in vivo* digestibility trial (LSMeans±s.e.)

	FIN	OP
Dry matter intake (g/day)	1358.9 ± 41.0	1395.8 ± 44.6
Nitrogen content of the diet (g/kg)	24.6	24.8
Nitrogen intake (g/day)	33.5 ± 1.0	34.6 ± 1.1
Nitrogen intake (g N/kg $BW^{0.75}$/day)	2.4 ± 0.1	2.3 ± 0.1
Faecal DM excretion (g/d)	355.1 ± 26.9	369.4 ± 13.9
Faecal N content (g/kg)	3.6 ± 0.5	3.5 ± 0.3
Faecal nitrogen (g/day)	9.7 ± 0.7	9.4 ± 0.5
Urinary nitrogen (g/day)	16.7 ± 0.5	16.2 ± 0.4
Total nitrogen excreted (g/day)	26.4 ± 0.8	25.7 ± 0.5
Faecal nitrogen (% of nitrogen intake)	29.2 ± 2.2	27.2 ± 1.1
Urinary nitrogen (% of nitrogen intake)	50.0 ± 1.7	47.4 ± 2.5
Total nitrogen excreted (% of nitrogen intake)	79.2 ± 2.9	74.7 ± 2.9
Metabolic faecal nitrogen (g/day)	6.8 ± 0.2	7.0 ± 0.2
Endogenous urinary nitrogen (g/day)	2.5 ± 0.1	2.7 ± 0.1
Nitrogen retention (g N/kg $BW^{0.75}$/day)	1.2 ± 0.1	1.2 ± 0.1
Nitrogen retention (% of nitrogen intake)	48.5 ± 2.8	53.3 ± 2.9

[1]Note that none of the parameters' means differed significantly between feeds (P > 0.05).

There were no significant differences (P > 0.05) in nitrogen retention (g N/kg $BW^{0.75}$/day) between the two treatments, which also the case when the nitrogen retention was expressed as a percentage of the nitrogen intake.

The results of the energy metabolism study conducted during the *in vivo* digestibility trial are reported in Table 5.4. No significant differences (P > 0.05) in DMI or energy intake were found between the two treatments. Also, no differences (P > 0.05) were observed for energy excreted as urinary energy, faecal energy or methane gas, between the two treatments. There were also no differences (P > 0.05) in the energy retention (MJ/day) or the energy retention expressed as a percentage of the energy intake.

Table 5.5 Energy metabolism[1] of feedlot lambs fed finishing diets varying in levels of NSC and CP during a seven day *in vivo* digestibility trial (LSMeans±s.e.)

	FIN	OP
Dry matter intake (g/day)	1358.9± 41.0	1395.8 ± 44.6
Gross energy (MJ/kg)	17.9	17.8
Energy intake (MJ/day)	24.4 ± 0.7	24.9 ± 0.8
Faecal DM excretion (g/d)	355.1 ± 26.9	369.4 ± 13.9
Faecal energy (MJ/kg)	18.6 ± 0.1	17.9 ± 0.1
Faecal energy excreted (MJ/day)	6.5 ± 0.5	6.6 ± 0.3
Urinary energy (MJ/day)[2]	1.2 ± 0.0	1.2 ± 0.0
Methane gas production (MJ/day)[3]	2.0 ± 0.1	2.0 ± 0.1
Total energy excreted (MJ/day)	9.7 ± 0.6	9.8 ± 0.3
Faecal energy (% of energy intake)	26.8 ± 2.0	26.6 ± 0.7
Urinary energy (% of energy intake)	5.0	5.0
Total energy excreted (% of energy intake)	39.8 ± 2.0	39.6 ± 0.7
Energy retention (MJ/day)	14.7 ± 0.6	15.0 ± 0.5
Energy retention (% of energy intake)	60.2 ± 1.9	60.4 ±2.1
Metabolizable energy (MJ/kg)	10.8 ± 0.4	10.8 ± 0.1

[1]Note that none of the parameters' means differed significantly between feeds (P > 0.05).
[2]Urinary energy calculated as 5% of gross energy intake (Van der Merwe & Smit, 1973).
[3]Methane gas production calculated as 8% of gross energy intake (McDonald *et al*., 2002)

The metabolizable energy (ME) obtained from the *in vivo* trial was lower than formulated. The FIN diet was formulated to contain 11.57 MJ/kg while the OP diet was formulated to contain 11.80 MJ/kg. These differences could be attributed to the feed making and pelleting process.

It has been shown that supplementing sheep diets with rumen-protected Met and Lys improves nitrogen retention (Lynch *et al*., 1991). Chong Li *et al*. (2011) found that by infusing lambs with Met, the nitrogen utilization efficiency improved. Also, the reduction in EAA availability has a negative effect on the nitrogen retention of lambs (Nolte *et al*., 2008). It would appear that, although not optimized, the balance of EAA in the commercial finisher diet (FIN) was sufficient in allowing the lambs to achieve a similar nitrogen balance when compared to lambs receiving a diet optimized for EAA.

The IVTD of the three feedlot diets are reported in Table 5.6. The results indicate that the OP and LP diets were more digestible than the FIN diet, while the digestibility of the OP diet did not differ from the LP diet. While there was no difference in the total tract digestibility (*in vivo* digestibility) between the OP and FIN diets, there was differences ($P < 0.05$) in the *in vitro* digestibility. This could possibly be explained by a higher feed intake that leads to an increase in passage rate and lower digestibility of nutrients (Campling & Freer, 1966). Although not significant, the OP lambs did show a 3% higher daily DM feed intake during the *in vivo* trial which could have resulted in the OP diet showing slightly decreased *in vivo* digestibility. In the *in vitro* study however, the OP and FIN feeds were both incubated for the same period.

Table 5.6 The in vitro true digestibility for feedlot diets with varying levels of NSC and CP; LSMeans±s.e.

	In vitro true digestibility
FIN	84.86 ± 0.35[a]
OP	86.62 ± 0.35[b]
LP	85.16 ± 0.35[ab]

[ab]LSMeans with different superscripts within the same column show significant differences ($P \leq 0.05$).

The *in vitro* degradability of the amino acids in the *in vitro* study are reported in Table 5.7. The commercial finisher diet showed the highest amino acid degradability ($P < 0.05$) for Ala, Ser, Valine (Val), Tyr, Thr, Arg, His, Lys, Leu and Gly. Therefore, the *in vivo* digestibility of the amino acids (total tract digestibility) in the OP feed was higher while the amino acids in the OP feed were less digestible *in vitro*. This means that the OP feed was higher in bypass amino acids, which is consistent with the initial formulations.

By comparing the chemical composition for amino acids of the FIN and OP diet in Table 5.2 to that of Table 5.7, it is clear that there were some variation in the results. This could be explained by the sieving process which the feed samples were

exposed to during sample preparation of the *in vitro* study. Also, as previously discussed, the HCl hydrolysis method used for analyzing amino acids, does have limitations. Residual oxygen can increase the thermal breakdown of hydroxyl and sulphur containing amino acids (Ser, Thr, Hydroxyproline, and Tyr) resulting in recoveries of between 50 % and 90%, while the Met recovery ranges between 25% to 75%. Hydrophobic amino acids (Val, Ile and Leu) may require longer hydrolysis times (72h) as their peptide bonds are difficult to break. Try and Cystein (Cys) are usually destroyed by acid hydrolysis and thus require other hydrolysis procedures for their accurate quantization. Gly yields tend to exceed 100% due to background protein contamination. Base hydrolysis is required for the accurate quantification of Tryptophan, Asparagine (Asp) and Glu.

Table 5.7 *In vitro* soluble- and undegradablefraction of three feedlot diets as well as the chemical composition of these diets

	Chemical composition (g/kg)			Soluble fraction (%)[1]			Undegradable fraction (%)[2]		
	FIN	OP	LP	FIN	OP	LP	FIN	OP	LP
Ala	4.1	4.7	5.2	29.2	41.7	47.1	35.40^{a} ± 1.12	50.52^{b} ± 0.29	49.82^{b} ± 2.96
Thr	2.6	3.3	3.3	10.2	17.0	32.9	12.86^{a} ± 1.51	23.65^{b} ± 0.45	38.95^{c} ± 3.58
Ser	3.3	4.2	4.4	5.6	15.2	39.0	23.60^{a} ± 1.32	34.63^{b} ± 0.38	53.46^{c} ± 2.74
Arg	4.9	7.4	6.3	22.0	25.1	27.5	30.31^{a} ± 1.20	67.70^{b} ± 0.19	71.16^{b}± 1.70
Glut	9.4	11.1	10.1	15.7	3.8	36.2	36.18^{a} ± 1.10	38.89^{a} ± 0.36	58.47^{b}± 2.45
Val	4.2	5	5.2	29.2	38.4	40.6	38.46^{a} ± 1.06	52.29^{b} ± 0.28	47.28^{b}± 3.11
His	2.6	3.3	4	18.3	29.5	44.6	44.77^{a} ± 0.95	61.83^{b} ±0.22	71.10^{c}± 1.70
Asp	10.7	13.8	14.3	35.8	23.6	41.8	33.49^{a} ± 1.15	35.16^{a} ± 0.38	50.34^{b} ± 2.93
Lys	4.8	7	5.8	36.6	26.7	30.6	17.56^{a} ± 1.42	36.32^{b} ± 0.37	42.48^{b} ± 3.39
Pro	6.3	7.2	7.4	32.6	33.4	40.1	52.89^{a} ± 0.81	61.87^{b} ± 0.22	58.04^{ab} ± 2.47
Met	0.6	1.2	0.6	15.1	31.5	32.9	74.47^{a} ± 0.44	78.47^{a} ± 0.13	58.71^{b} ± 2.43
Tyr	2.9	3.7	3.4	19.5	18.6	25.0	13.07^{a} ± 1.50	22.30^{b} ± 0.46	36.86^{c} ± 3.72
Iso	3.4	4	3.6	29.2	23.0	23.5	20.22^{a} ± 1.38	33.78^{b} ± 0.39	26.60^{ab} ± 4.33
Phe	3.8	5.1	4.4	12.5	18.1	10.0	19.38^{a} ± 1.39	27.80^{ab} ± 0.42	37.69^{b} ± 3.67
Leu	9.6	11.7	10.7	17.4	22.8	19.1	28.53^{a} ± 1.23	39.81^{b} ± 0.35	39.19^{b} ± 3.58
Gly	3.6	4.6	5.1	17.4	24.1	34.2	31.74^{a} ± 1.18	45.23^{b} ± 0.32	44.95^{b} ± 3.24

^{abc}LSMeans with different superscripts within the same row show significant differences (P ≤ 0.05).
[1]The soluble fraction calculated using one sample, therefore no variance recorded.
[2]Calculated as follows: 100 – Degradable fraction; LSMeans±s.e.

5.5 Conclusion

It was hypothesized that the feeding of a feedlot diet optimized for EAA and at increased NSC levels would improve both the nitrogen retention and energy balance of feedlot lambs. Furthermore, it was suggested that the increased NSC levels would improve the digestibility of the lambs as the growth of the microbial population is dependent on a source of readily digestible carbohydrates which provide ATP for the synthesis of cell wall material (Nocek & Russell, 1998).

The results obtained from this study showed no differences in the *in vivo* digestibility between the optimized (OP) diet and the standard commercial finisher feed (FIN) for DM, OM, CP, EE, NDF or ADF. There was however a significant difference in the amino acid digestibility of Met and Lys, with the OP feed showing improved digestibility for both. Nolte (2006) stated that natural protein sources that are high in bypass Met would improve the growth performance of lambs. The OP diet was optimized for EAA and was higher in bypass Met and Lys levels than the FIN diet, which lead to an increase in the *in vivo* digestibility of the amino acids. However, this did not lead to improved feedlot performance (Chapter 4). The OP feed showed increased IVTD compared to the FIN feed which, however, was not reflected in the total tract (*in vivo*) digestibility.

The balancing and optimizing of EAA in the OP diet did not lead to improved nitrogen retention or energy balance, suggesting that the balance of EAA as well as the ratio of energy to protein in the commercial finisher feed used was adequate in supporting nitrogen retention while also maintaining the same energy balance.

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CHAPTER 6

RUMEN DEVELOPMENT OF FEEDLOT LAMBS FED CONCENTRATE DIETS VARYING IN LEVELS OF NON-STRUCTURAL CARBOHYDRATES (NSC)

Abstract

The effect of varying levels of non-structural carbohydrates (NSC) in feedlot diets on the rumen development of lambs was investigated. The microbial fermentation of NSC leads to the production of volatile fatty acids (VFA) which stimulates rumen papillary growth. The improvement of rumen development in lambs will lead to the optimal absorption of nutrients, which will optimize production efficiency. Forty weaned lambs (age = 69 days; weight 24.35kg) were used to evaluate the effect of varying levels of NSC on rumen development. Lambs (n=10 per group) were randomly assigned to four diets: Optimized protein feed (OP) which contained 477 g/kg NSC on a dry matter (DM) basis, a standard commercial finisher feed (FIN) containing 468 g/kg NSC, a low protein feed (LP) which had 455 g/kg NSC, and the negative control (CON) where the lambs were finished on kikuyu pasture with supplementary feed supplied at 500 g/day containing 358 g/kg NSC. Microscopy slides of the rumen wall were prepared and measurements captured included the papillae length and width as well as the thickness of the epithelium, mucosa, collagen and rumen wall. The feedlot lambs had longer papillae when compared to the grazing lambs. Within the concentrate treatments there was also an increase in papillae length as the NSC level in the diet increased. There were no differences in the rumen wall thickness between any of the treatments as different mechanisms are responsible for the papillary and muscular development of the rumen.

6.1 Introduction

At birth, the rumen of the newborn lamb is undeveloped, non-functional, exhibits a smooth surface and only makes up about 30% of the total weight of the stomach. In contrast, the rumen of an adult sheep will comprise of about 80% of the total weight of the stomach, while displaying fields of long and dense papillae (Ward, 2008). The process of rumen development is triggered upon the ingestion of solid food by the newborn lamb and the resultant establishment of fermentation in the rumen. While forages stimulate the development of rumen muscles as well as uphold the integrity of the rumen epithelium, it does not promote the development of papillae. During the digestion of forages by microorganisms, VFA are produced. However, the concentrations of these VFA are insufficient in stimulating papillary development (Coverdale *et al*., 2004). The feeding of concentrate diets provide the carbohydrates needed for microbial fermentation which produces more VFA and of a different composition than forage based diets. Diets that are high in fibre will proportionately produce more acetic acid (typically in molar proportions of 70:20:10 for acetic acid:propionic acid:butyric acid) whereas starch-rich diets will produce higher proportions of propionic acid at the expense of acetic acid (France *et al.* 2005). There is a positive correlation between papillary growth and increased levels of propionic and butyric acid while the relationship with acetic acid is inverse (Ortega-Reyes *et al*., 1992). While both butyric and propionic acid are the main VFA in stimulating morphological development of the rumen, butyric acid has been shown to be the most important (Kauffold *et al,* 1977). Mentschel *et al.* (2001) found a four-fold increase in the length of papillae when butyric acid was additionally fed as a salt to calves, while the feeding of propionic acid led to a two-fold increase in papillae length.

The microbial population within the rumen can be grouped into microorganisms that ferment structural carbohydrates (SC) and those that ferment NSC (Russell *et al*., 1992). In order to ensure optimal microbial growth, ingested nutrients need to be readily available. Therefore, rumen development will be optimized when the diet is easily

fermentable and allows for undigested feed particles to be passed through the digestive system at a more rapid rate (Andrews, 1969). Non-structural carbohydrates mainly constitute the starch fraction of the feed but also comprises of sugars, pectins, galactans and β-glucans (McDonald *et al*., 2002). These feedstuffs are rapidly fermented (Carruthers *et al*., 1997) which will allow an increased turnover rate in the rumen. The growth of the microbial population is dependent on a source of carbohydrates that are readily available in the rumen and provides ATP for the biosynthesis of cell material (Nocek & Russell, 1988).

During the first two or three weeks, the performance of lambs in a feedlot is often suppressed, which can be ascribed to the sudden change of diet from pre- to post weaning (Ortega-Reyes *et al*., 1992). To prevent this from happening, just prior to entering the feedlot, lambs are fed the diet they will be receiving in the feedlot to allow them to adapt (Taylor, 1984). This effect is exploited by providing lambs with a concentrate diet while still suckling (Thorhallsdottir *et al*.,1990), known as creep feeding, as well as during the transition from being "monogastric" to ruminants (Mirza & Provenza, 1990). It is clear from literature that lambs fed creep feed while still being suckled adapt easier and perform better when fattened in a feedlot post-weaning. Bhatt *et al.* (2009) showed that lambs with a higher weaning weight would perform better during the post-weaning phase. Baldwin *et al.* (2004) reported that calves consuming lower amounts of solid food while receiving milk *ad libitum,* displayed poor post-weaning performance due to delayed rumen development.

The aim of this study was to compare the morphological development of the rumen at marketing age (slaughter) to that at weaning age. Another aim was to see what the effect on rumen morphological development would be when lambs were fed a creep feed high in NSC after which being put on a feedlot diet with lower NSC levels.

6.2 Materials and methods

Forty crossbred (South African Mutton Merino x Döhne Merino) lambs, at an average age (± s.d.) of 69 ± 1 days and an average live weight (± s.d.) of 24.4 ± 0.64 kg were randomly allocated into four dietary treatment groups of 10 lambs per group (5 males, 5 females). Three of these groups received pelleted concentrate diets containing varying levels of NSC, while the fourth group served as the control group and was allowed to graze kikuyu pasture. The four treatments were as follows:

- A low protein feed (LP; 139 g CP/kg, 455 g NSC/kg; Met, 1.93 g/kg; Lys, 4.99 g/kg; Arg, 6.66 g/kg; Thr, 4.73 g/kg on DM basis) not adjusted for amino acids which also served as the control diet for the other feedlot diets.
- An optimized protein feed (OP; 157 g CP/kg, 477 g NSC/kg; Met, 2.48 g/kg; Lys, 7.45 g/kg; Arg, 8.51 g/kg; Thr, 8.12 g/kg on DM basis) adjusted for amino acids.
- A standard commercial finisher feed (FIN; 152 g CP/kg, 468 g NSC/kg; Met, 2.08 g/kg; Lys 5.49 g/kg; Arg, 7.47 g/kg; Thr, 4.80 g/kg on DM basis).
- Control treatment (CON) where lambs grazed kikuyu pasture and received additional supplementary feed..

6.2.1 Formulation of the diets

The lambs used in this study were obtained from a preceding creep feed study where suckling lambs were fed specialized creep feeds. For the creep feed study, the first dietary treatment (OP) was optimized for the essential amino acids (EAA) and NSC at 157 g/kg DM CP and 477 g/kg DM NSC. The second treatment (CF2) was also optimized for the EAA and NSC. This however was done at higher levels to include 179 g/kg DM CP and 508 g/kg DM NSC. The third treatment was a low protein feed (LP) formulated at 139 g/kg DM CP and 455 g/kg DM NSC that served as a control and was not optimized for EAA. While the CP levels are reported, it is important to note that the dietary treatments were formulated to vary in levels of EAA and not CP.

After the lambs were weaned the lambs hat had received the CF2 feed during the creep phase were given a commercial finisher diet (FIN) that was not optimized for EAA and contained 152 g/kg DM CP and 468 g/kg DM NSC. This served as a positive control and to establish the effect of a feed change on rumen development. Thus, a standard phase-feeding approach was applied to these lambs by changing from a creep feed regimen pre-weaning to a commercial finishing regimen post-weaning. The other three treatment groups were kept on the same diets that they had received pre-weaning, thus avoiding possible metabolic disruptions due to adaptation and to investigate the effect on rumen morphological characteristics.

The aim of this study was to compare rumen histological development of lambs subjected to a phase-feeding regimen to that of lambs receiving the same diet pre- and post-weaning.

6.2.2 The management of the lambs in the feedlot

Lambs were kept on slatted floors in 1.2 x 1.8 m individual pens in a shed on the Welgevallen experimental farm in Stellenbosch. The three concentrate-fed groups were fed pelleted diets supplied by Tanqua Feeds, Riviersonderend while the control group (CON) grazed kikuyu pasture and also received supplementary feed (132 g CP/kg; 313 g NSC/kg).

Lambs receiving the control treatment were let out to graze during the day (07:00 – 17:00) after which they were kept indoors at night. These lambs had access to water and Oat hay in feeding troughs, *ad libitum* at night. During the first 14 days post-weaning these lambs received supplementary feed at 250 g/day and at day 15 this was increased to 500 g/day and maintained at this level until slaughter. Individual supplementary feed intake could not be measured as all the CON lambs received this feed in a feeding trough, placed in the grazing paddock.

6.2.3 The slaughter of the lambs and rumen sample collection

Upon the completion of the feedlot trial, lambs were slaughtered in order to enable the collection of rumen samples. The lambs were slaughtered at an average age (±s.d.) of 131 ± 3 days and at an average weight (±s.d.) of 40.41 ± 1.26 kg. The lambs were not fasted prior to the slaughter. The slaughtering of the lambs took place at an abattoir also situated on the Welgevallen experimental farm. During slaughter the lambs were electrically stunned for a period of four seconds at a voltage of 200. The jugular vein was severed followed by the exsanguination of the lambs.

The lambs were eviscerated to allow for the sampling of the reticulo-rumen. The reticulo-rumen, with the oesophagus attached, was removed from the rest of the digestive tract at the pyloric sphincter of the abomasum. An incision was made across the dorsal side of the atrium after which the rumen content was gently removed by rinsing it with water in order not to damage the papillae. A small rectangular sample of 2 x 3 cm was then taken from the rumen wall in the left cranial ventral sac, approximately 2.5 cm to the right of the rumino-reticular fold. Each sample was immediately placed in 100 ml of formaldehyde solution.

6.2.4 Rumen wall sample preparation

The fixing of the rumen wall samples in histological slides was done at the Department of Biomedical Sciences, Anatomy and Histology laboratory at the University of Stellenbosch. Staining was done using Harris's Haematoxylin (Merck Chemicals (Pty) Ltd.) while counterstaining was done using Eosin (0.5%). The processing of the samples was done on a Shandon Elliot Duplex Processor (Optalabor (Pty) Ltd.) which allowed the sample to go through numerous stages of alcohol dehydration. During the dehydration, the water inside the fixed sample was systematically replaced with paraffin wax. This prevents the damage of samples during sample cutting. This was done by

placing the fixed samples in steadily increasing concentrations of alcohol (70% alcohol for 90 minutes; two cycles of immersion in 96% alcohol for 90minutes each and finally two cycles each of immersion in 100% alcohol for 90 minutes).

A Leica EG 1160 Embedder (SMM Instruments, South Africa) was used for the embedding process during which the sample was positioned in a cast filled with paraffin wax. The samples were then allowed to set and harden by being placed on an ice table. Upon setting, the casted samples were ready to be cut and mounted on glass slides. This was done using a Leica RM 2125 RT microtome (SMM Instruments) which sliced the samples into 5 µm segments. The sample slice was placed on the glass slide and then incubated at 60ºC for an hour, which allowed the rumen tissue to join to the glass. Wax from the samples was then removed by placing the samples in one litre Xylene dishes, which was done twice for two minutes per dish.

Staining was done using a H&E (Haematoxylin and Eosin) Processor (Leica Auto Stainer XL, SMM Instruments, South Africa). The slides were hydrated by placing them in alcohol, first in 100% alcohol, with two cycles of immersion of one minute each. There after two cycles of immersion in 96% alcohol for one minute each and then in 70% alcohol for one minute. The alcohol was rinsed off with tap water and then the slides were stained by placing it in Harris' Haematoxylin for four minutes. The slides were then rinsed with tap water for three minutes before being stained in Eosin for 2.3 seconds. The slides were then finally rinsed in tap water for another two minutes. The slides then went through another dehydration process by being placed in a sequence of increasing alcohol concentrations. This was done by immersing slides in 70% alcohol for 0.20 seconds, two cycles of immersion in 96% alcohol for 0.15 seconds each, and finally two cycles of immersion in 100% alcohol for 0.15 seconds each. The alcohol was then removed by placing slides in Xylene for one minute. Each sample from each of the ten lambs in each treatment was prepared and mounted in duplicate.

6.2.5 Microscopic measurements of the rumen

Measurement of the rumen features was performed using an Olympus CH30 microscope with a Zeiss West Germany 47 lens at 4x magnification. To enable the measuring of papillae lengths images of the slides were taken using a Nikon DS – Fi1 Digital Sight camera. The measurements were then conducted using Nis elements imaging software (Nikon, Japan).

In order to measure the length of the papillae, a line was drawn from the base of the papillae while the width was measured by drawing a line at the widest part of the papillae at a right angle to the line measuring the length (Hill *et al*., 2005). On each slide the four longest papillae were measured and captured for statistical analysis. The method of measuring the rumen properties are depicted in Figure 6.1. The collagen and epithelium layers were measured around each investigated papillae. The rumen wall thickness was established by drawing a straight line from the edge of the rumen wall to the rumen epithelium on the luminal side. A total of four measurements were done per slide and therefore 40 measurements per treatment.

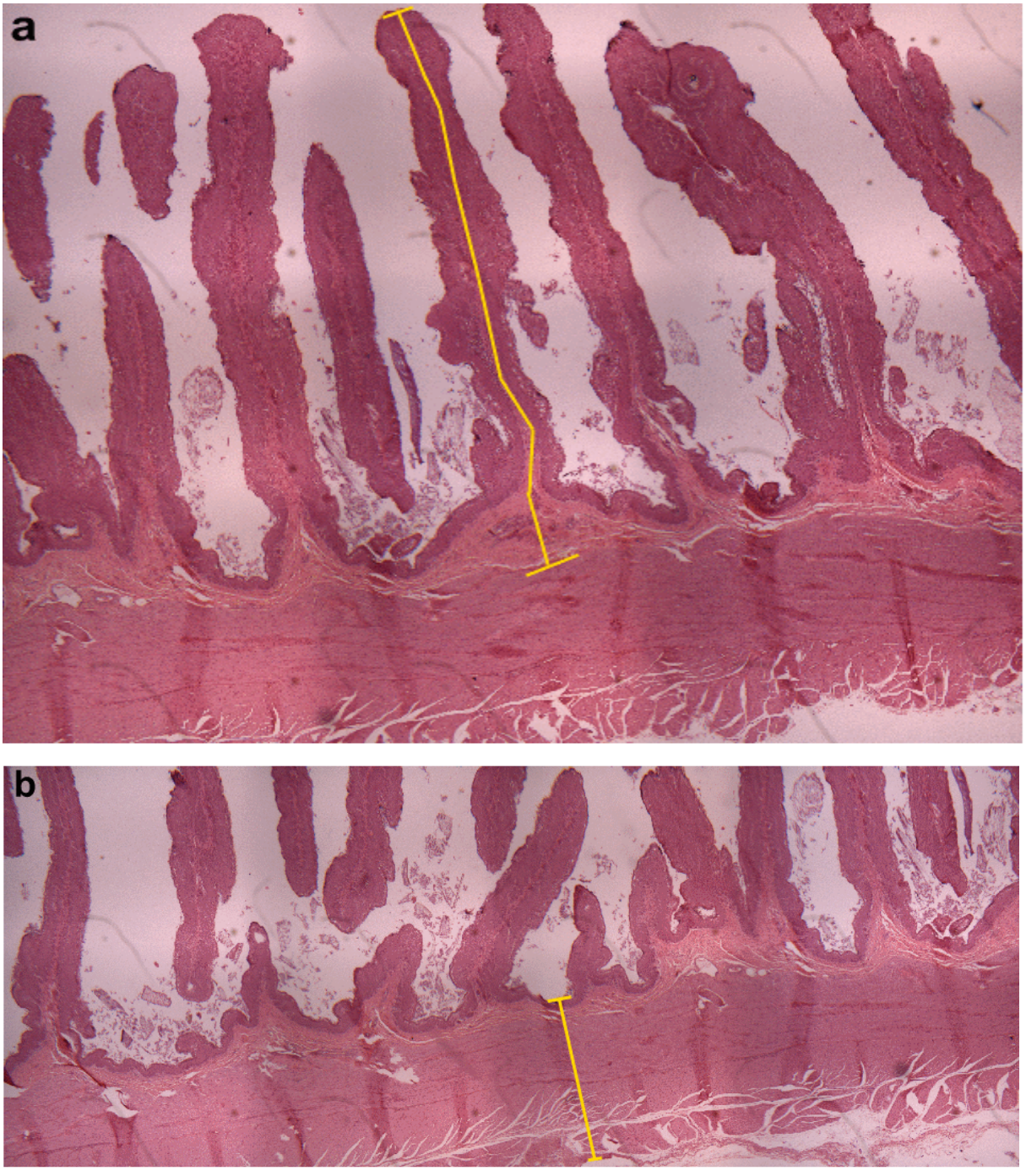

Figure 6.1 Schematic representations indicating the method of measuring papillae length (a) and rumen wall thickness (b).

6.3 Statistical analysis

The data was analyzed using SAS software for Windows Version 9.3. All the data were analyzed by means of One-Way Analysis of Variance using the GLM Procedure with the treatments as the main effect. The model for the experimental design is indicated by the following equation:

$Y_{ij} = \mu_i + \beta_j + \varepsilon_{ij}$

The terms are defined as the overall mean (μ_i), the effect of the treatment (β_j) and the error associated with the effects of the treatments (ε_{ij}). The LS means and Bonferroni *Post hoc* tests were used to compare levels within treatments. P-values smaller than 0.05 were considered significant ($P < 0.05$).

6.4 Results and discussion

The carcass and digestive tract characteristics are discussed in detail in Chapter 4 however, Table 6.1 is a summary of these results. The CON lambs had a lower ($P < 0.05$) FBW, carcass weight, small and large intestinal weight and empty reticulo rumen weight. Forage-fed lambs will have heavier SI when compared to concentrate fed lambs, due to increased digesta flow to the SI which stimulates tissue growth (Joy *et al*., 2008). The results obtained in this study did however not show this. This could be due to the big difference in slaughter weight between the concentrate fed lambs and the CON lambs. In a study by Baldwin *et al*. (2000) the gastrointestinal development of lambs on a 75% forage diet was compared to that of lambs on a 75% concentrate diet. The lambs were slaughtered at a weight of 41.7 kg and 44.9 kg for the forage and concentrate lambs respectively. When expressed as a percentage of bodyweight, the forage-fed lambs had a heavier rumen and SI. Therefore, had the CON lambs in the current study been slaughtered at the same weight as the concentrate fed lambs, it is likely that CON lambs would have a heavier digestive tract.

Table 6.1 The mean (± s.e.) carcass characteristics of lambs fed optimized feedlot diets or produced on kikuyu grazing

	FIN	OP	LP	CON
Final body weight (FBW; kg)	45.04[a] ± 0.849	46.31[a] ± 1.852	43.84[a] ± 1.715	30.21[b] ± 1.527
Carcass weight (kg)	23.76[a] ± 0.619	23.84[a] ± 1.024	22.71[a] ± 0.993	13.92[a] ± 0.798
Small intestinal weight (SI; kg)	0.97[a] ± 0.032	1.02[a] ± 0.066	0.84[a] ± 0.086	0.78[b] ± 0.033
Large intestinal weight (LI; kg)	0.61[a] ± 0.039	0.60[a] ± 0.040	0.58[a] ± 0.040	0.41[b] ± 0.025
Full reticulo rumen (FRR; kg)	4.82 ± 0.245	5.04 ± 0.257	4.74 ± 0.243	5.18 ± 0.339
Empty reticulo rumen (ERR; kg)	1.48[a] ± 0.061	1.47[a] ± 0.088	1.44[a] ± 0.060	1.06[b] ± 0.053
LI as % of FBW	1.36 ± 0.084	1.30 ± 0.086	1.34 ± 0.090	1.40 ± 0.112
SI as % of FBW	2.16[a] ± 0.086	2.21[a] ± 0.122	1.95[b] ± 0.207	2.66[a] ± 0.131
FRR as % of FBW	10.75 ± 0.593	10.87 ± 0.311	10.83 ± 0.408	17.12 ± 0.546
ERR as % of FBW	3.29 ± 0.114	3.17 ± 0.127	3.30 ± 0.109	3.53 ± 0.106

[ab]Means with different superscripts differ significantly ($P < 0.05$).

In this investigation there were no differences ($P > 0.05$) in the length of the papillae among the concentrate fed lambs (Table 6.2). However, there was a numerical increase in papillae lengths as the levels of NSC in the concentrate diets increased. The papillae length between the OP (highest NSC level) and the CON lambs differed ($P < 0.05$). The differences in the properties of these two treatments can be seen in Figure 6.2a (OP) and Figure 6.2b (CON). No differences were observed for any of the other histological measurements.

Castells *et al*. (2013) found that the rumen papillae were longer when Holstein calves were fed only concentrate compared to calves receiving concentrate with oat hay or concentrate with lucerne. As with this study, Castells *et al*. (2013) also only showed statistical differences with regard to the papillae lengths whilst other rumen measurements were similar across treatments (Castells *et al*., 2013).

Table 6.2 The histological measurements (µm) of the rumen of lambs fed varying levels of EAA and NSC, Mean ± SEM

Histological parameters		**FIN**	**OP**	**LP**	**CON**
Long papillae	Length	5018.9[a] ± 215.5	5596.1[a] ± 416.1	4505.8[a] ± 383.3	3852.7[b] ± 261.0
	Width	409.7 ± 22.8	382.0 ± 13.5	406.7 ± 23.4	400.5 ± 22.0
Epithelium		90.3 ± 5.8	88.6 ± 4.7	89.2 ± 5.6	84.4 ± 4.4
Collagen		187.6 ± 26.9	198.2 ± 26.3	166.6 ± 15.5	182.6 ± 28.3
Mucosa		278.0 ± 25.2	286.7 ± 26.8	255.9 ± 16.4	267.0 ± 27.6
Rumen wall thickness		1732.8 ± 132.1	1775.9 ± 247.8	1786.4 ± 100.2	1568.2 ± 81.7

[ab]Means with different superscripts differ significantly ($P < 0.05$).

Lane & Jesse (1997) found that papillae length increased by up to 50% when VFA were ruminally infused to meet the net energy requirements of the animal. Adding sponges to the rumen of lambs only encouraged muscular development and rumen capacity but had no effect on the development of papillae. These results illustrate that even though the bulkiness of feed is essential in ensuring rumen growth and rumen muscle development, morphological development of the rumen epithelium requires fermentation which produces VFA.

Alvarez-Rodriguez *et al*. (2012) set out to determine what effect forage supply would have on the morphological development of the rumen. Lambs were weaned early and

assigned to one of two treatments. The first group was placed in an indoor feedlot (CON) after weaning while the second group was placed on lucerne grazing (ALF), also receiving the same concentrate as the indoor lambs, *ad libitum*.

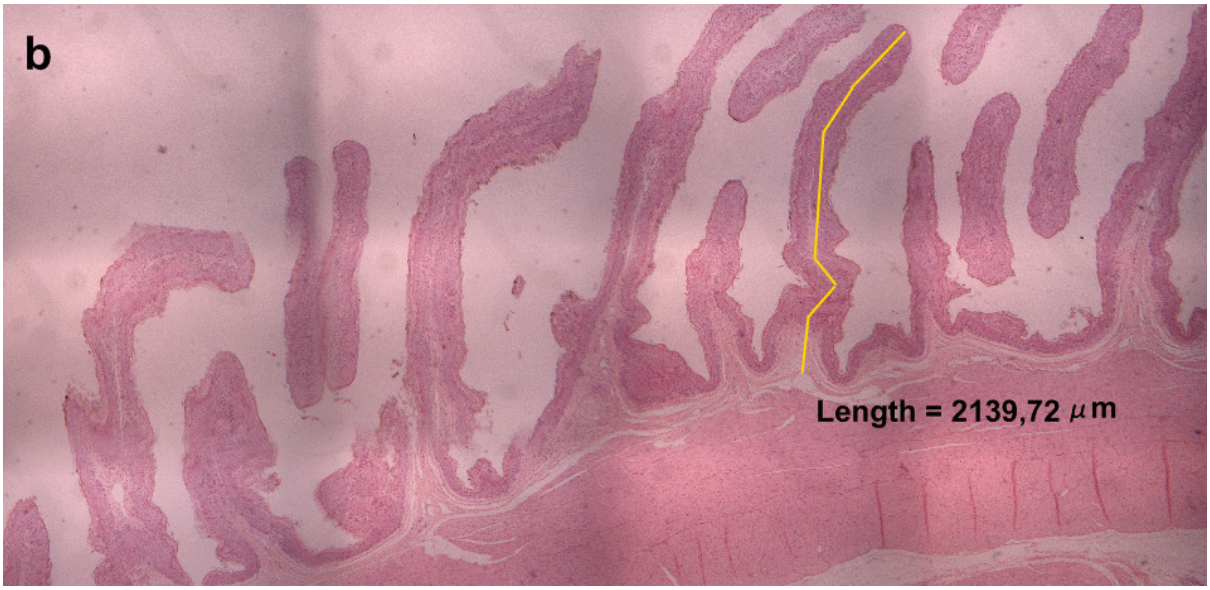

Figure 6.2 The rumen papillae of lambs receiving a concentrate diet, OP1 (a) and lambs grazed on pasture, CON (b)

The CONC lambs also had access to barley hay *ad libitum*. Upon slaughter, rumen samples were taken from both the dorsal and ventral sac in order to determine the extent of rumen development. Although rumen papillae of the CONC lambs were numerically longer than the ALF lambs in both the dorsal and ventral sac (3.05 mm vs 2.76 mm and 2.74 mm vs 2.52 mm respectively), these differences were not significant ($P > 0.05$). There were also no differences ($P > 0.05$) in the papillae width or muscular layer thickness between treatments, although the latter was numerically thicker in the ALF lambs in both the dorsal and ventral sac (1.17 vs 1.07 mm and 1.40 vs 1.05 mm respectively). Differences were however found in the surface area of papillae (mm^2) in the dorsal sac, with the CONC lambs having a larger surface area than the ALF lambs (3.61 vs 3.10 mm respectively). The surface area in the ventral sac was also larger in the CONC lambs although not statistically so (3.22 vs 3.06 mm) (Álvarez-Rodríguez *etal*., 2012).

Based on these result, the differences among treatments were more apparent in the dorsal sac than in the ventral sac. A plausible explanation for this is due to the formation of a 'rumen mat' in ruminants where coarse particles collect and float at the top of this mat, allowing increased physical stimulation in the dorsal sac (Evans *et al.*, 1973).

Gäbel *et al.* (1987) fed six fistulated sheep four consecutive diets for a period of 15 weeks each: (1) Hay only, (2) 64% concentrate, 36 % hay, (3) 90% concentrate, 10% hay and (4) only hay. The feeding of the 64% concentrate treatment increased the concentrations of butyric and propionic acid by 120% and 35% respectively while these concentrations increased to 180% and 88% when sheep received the 90% concentrate diet. Also, the rumen surface area increased by 200% when the 64% concentrate diet was fed and this doubled to 400% when the 90% concentrate was fed. Along with these results, Gäbel *et al*., (1987) showed that this increase in rumen surface area led to an increase in the absorptive capacity for magnesium, chloride and sodium. What was

interesting was when the sheep were put back on to the control (only hay) diet, the amount of cell layers in the rumen returned to the original amount. This shows that the morphological development of the rumen can regress if not maintained. Harrison *et al.* (1960) found this retrogression of rumen papillae when the diet of Ayrshire calves was changed from a concentrate back to milk for a period of 18 weeks. While they found that the rumen papillae had almost completely disappeared, the deterioration of the rumen muscles were less evident (Harrison, 1960).

The above mentioned study (Gäbel *et al*., 1987) illustrates the importance of maintaining NSC levels to ensure maximum absorptive capacity while also preventing the regression of rumen development. A comparison of the papillae length and rumen wall thickness of lambs receiving the experimental diets pre- and post-weaning can be found in Table 6.3. The results in the present study show that even if lambs receive creep feed prior to weaning, the rumen has still not finished developing, which further stresses the importance of maintained NSC levels post-weaning. The papillae length of lambs receiving the OP treatment increased by a further 34% during the feedlot phase, while the CON lambs showed an increase in papillae length of 31%. The increase in papillae length of the CON lambs can be attributed to the supplementary feed that this group received after weaning. Pre-weaning these lambs only grazed kikuyu while still suckling. The lambs that had received the CF2 (508 g/kg NSC) experimental diet during creep feeding was transferred onto a commercial finisher diet (FIN; 457 g/kg NSC), which is standard feedlotting practice. However, instead of showing further significant papillae development, as with the OP lambs, the papillae length of these lambs only increased by a further 12%.

Table 6.3 A comparison of the papillae length (µm ± SEM) and rumen wall thickness between lambs slaughtered at weaning (69 days) and lambs slaughtered after fattening in a feedlot (126 days).

	CF2/FIN	**OP**	**LP**	**CON**
	Papillae length			
Weaning age (69 days)	4483.0 ± 146.1	4165.1[a] ± 157.5	4027.3[a] ± 183.1	2943.8a ± 161.4
Marketing age (126 days)	5018.9 ± 215.5	5596.1[b] ± 416.1	4505.8[b] ± 383.3	3852.7b ± 261.0
	Rumen wall thickness			
Weaning (69 days)	1519.8[a] ± 75.9	1740.7[a] ± 79.5	1351.6[a] ± 83.9	1851.4[a] ± 83.9
Marketing (126 days)	1732.8[a] ± 132.1	1775.9[a] ± 247.8	1786.4[b] ± 100.2	1568.2[b] ± 81.7

[ab]Means with different superscripts in the same column differ significantly (P < 0.05).

With regards to the rumen wall thickness, there was no significant increase for either the OP or FIN lambs from weaning to market weight, which would suggest that the muscular development of the rumen is complete at weaning.

6.5 Conclusion

It is well established that the feeding of NSC to ruminants is crucial in initializing and establishing fermentation in order to ensure rumen morphological development. In this trial the increasing levels of NSC (455, 467 and 477 g/kg NSC respectively) in the feedlot diets did not lead to significant increases in papillae length. However, the importance of maintaining adequate levels of NSC in pre-weaning diets was clearly illustrated. By applying a phase-feeding approach, there was a decline in the

development rate of papillae when compared to papillae of lambs maintained on the same diet pre- and post-weaning. This was due to a decrease (51 g/kg) in the NSC content of the diet that the FIN lambs received in the feedlot.

Lane *et al.* (1997) stated that while VFA do stimulate various aspects of the morphological growth of the rumen, it might not be solely responsible for every facet (metabolic and morphological) of development. They concluded that other rumen fermentation end products such as branched-chain fatty acids and ammonia could possibly be key in ensuring complete development and maturation of the rumen epithelium.

Lesmeister *et al*. (2004) stated that papillae length could possibly be the most significant variable in the research of rumen development while also representing the greatest effect of different treatments on rumen development. The treatment with the highest NSC level, OP, also had the longest papillae. When compared to the standard commercial finisher treatment (FIN), the papillae of the OP lambs were longer and thinner, meaning a greater volume to surface ratio. It could therefore be expected that the OP lambs would be more efficient at absorbing nutrients.

6.6 References

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CHAPTER 7

GENERAL CONCLUSION

The aim of this study predominantly was to evaluate whether a lamb finisher diet, optimized for essential amino acids (EAA) at a certain non-structural carbohydrates (NSC) level, would lead to improved feedlot performance, when compared to a standard commercial finisher diet, while also maintaining economic viability. This would be achieved by greater feed efficiency in the feedlot and increased daily gains.

Three feedlot diets were formulated to vary in levels of EAA and NSC: A finisher diet, Optimized Protein (OP), which was formulated to contain 157 g/kg CP and 477 g/kg NSC and optimized for EAA (Met, 2.48 g/kg; Lys, 7.45 g/kg; Arg, 8.51 g/kg; Thr, 8.12 g/kg); a standard commercial finisher (FIN) diet, containing 152 g/kg CP and 468 g/kg NSC (Met, 2.08 g/kg; Lys, 5.49 g/kg; Arg, 7.47 g/kg; Thr, 4.80 g/kg); a Low Protein (LP) feed which contained 139 g/kg CP and 455 g/kg CP of which the EAA were not optimized (Met, 1.93 g/kg; Lys, 4.99 g/kg; Arg, 6.66 g/kg; Thr, 4.73 g/kg). A fourth group served as the negative control group (CON) and was rotated on kikuyu pasture paddocks while also receiving supplementary feed (151 g/kg CP; 358 g/kg NSC) at 500 g/day.

As expected, the feedlot lambs performed markedly better than the lambs grazing pasture. The feedlot performance trial showed no difference between any of the concentrate fed groups for average daily gain (ADG), feed conversion efficiency (FCE) or total feed intake. The OP lambs were kept on the same concentrate diet before and after weaning while a phase-feeding approach was applied to the FIN lambs as they had received a creep feed before weaning. It was thought that by placing the OP lambs in the feedlot, already adapted to their diet, would give them an advantage over the FIN lambs. Although the FIN lambs had to adapt to a new diet in the feedlot, no differences

in growth rates were found. This could have been due to the weaning shock nullifying the theoretical advantage of this approach.

Although the LP diet was formulated to contain less protein than the OP and FIN diets, lambs receiving this treatment was still able achieve similar growth rates to that of the higher protein diets. At slaughter there were also no differences in the final body weight (FBW) between any of the concentrate fed lambs. Due to the slower growth rate of the CON lambs, their FBW differed significantly from that of the concentrate fed lambs. Subsequently, the CON lambs also had a lower dressing percentage (DP) compared the concentrate fed lambs. By calculating the gross margin above feed costs, it was determined that the OP diet proved the least profitable of the feedlot groups. Optimizing the OP diet for EAA subsequently increased the cost thereof. The LP diet proved to be the most profitable as it was cheaper, yet allowed lambs to achieve similar growth rates. As indicated by the results, when formulating feeds, even at lower CP and EAA levels, the quality of the feed ingredients will have a marked effect on animal performance. It is hypothesized that any amino acid deficiencies were masked by the oversupply of protein, and therefore amino acids, resulting in good animal performance. It would be interesting to repeat the study and evaluate a diet formulated to high EAA specifications, but using poorer quality and cheap feed ingredients in comparison to the normal practice of feeding expensive, yet often limited in supply, high quality protein feedstuffs.

The digestive tract of the concentrate fed lambs was also markedly heavier than that of the CON lambs, which is owed to the fact that the feedlot lambs were so much heavier at slaughter. However, when the digestive tract was expressed as a percentage of the body weight, no differences were found. The rumen of the concentrate lambs was also more developed than that of the CON lambs, while within the feedlot treatments, no differences were found in the rumen parameters. This statement is made based on the long, dense mat of papillae observed in all the concentrate fed treatments as opposed to the significantly shorter papillae of the pasture fed lambs. There was however an

increase in the papillae length as the NSC level in the diet increased; suggesting that the OP lambs would be able to absorb nutrients more efficiently.

The *in vivo* digestibility trial did not show any differences in digestibility between the OP and FIN lambs. Analysis of the amino acids in the *in vivo* study indicated that the digestibility coefficients for Met and Lys were higher in the OP lambs. This meant that the optimizing of the EAA did lead to improved digestibility of Met and Lys, *in vivo* for the simple reason that better quality feedstuffs or synthetic amino acids were used to meet the requirements. Coupled with this, the degradability of the EAA were in most cases lower, resulting therefore in a substantially higher “bypass” or rumen undegradable digestible amino acid fraction. As indicated earlier, unfortunately this positive attribute of the OP diet did not culminate in improved animal performance over commercial finisher diets. The results obtained from this study also showed no differences in nitrogen retention or energy balance, suggesting that the balance of EAA as well as the ratio of energy to protein in a commercial finisher diet was adequate in supporting nitrogen retention while also maintaining energy balance.

The results obtained from the current study showed that although the optimizing of EAA did lead to the improved *in vivo* digestibility of Met and Lys, this did not lead to improved feedlot performance. It was also proved expensive to optimize the OP diet, leading to it being less profitable. While the LP diet proved to be more profitable, it would be interesting to see the effect this treatment would have had on the nitrogen retention in an *in vivo* study. In future, feedlot diets will have to be formulated to elicit maximized feedlot performance, remain profitable while having a minimum impact on the environment. This will have to be done with the ever increasing demand and short supply of good quality protein feedstuffs available to the ruminant feed industry. Therefore, continued research on EAA nutrition and feed formulation is of importance as the feed industry is increasingly having to rely on feed and food by-products such as Dried distillers grains, Brewers grains to name a few. The feedstuffs are characterized by EAA deficiencies which can easily be corrected using synthetic amino acids.