

# **SPECIALIZED CREEP FEEDING FOR LAMBS TO OPTIMIZE PERFORMANCE**

by

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*Pectora roborent cultus*

## **DECLARATION**

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## ABSTRACT

**Title:** Specialized creep feeding for lambs to optimize performance

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The following study is to determine if a creep feed balanced for limiting essential amino acids (EAA) at a certain non structural carbohydrate (NSC) level will elicit greater responses than lambs reared on a commercial creep feed.

Lysine, Threonine, Methionine, Isoleucine, Phenylalanine and Leucine were determined as the limiting amino acids for nursing lambs. These amino acids were incorporated into the creep feed treatments CF1 and CF2 representing 157 g CP/kg, 477 g NSC/kg and 179 g CP/kg, 508 g NSC/kg, respectively. A commercial creep feed with no optimisation for EAA is represented as CFC with 139 g CP/kg and 455 g NSC/kg. A negative control (CON) treatment represents lambs receiving no creep feed but with *ad libitum* access to suckle their dams while feeding on kikuyu pasture.

A growth trial was conducted on Merino x Döhne-Merino cross twin lambs averaging a birth weight of 4.42 kg  $\pm$  0.11 for 60 days following with a digestibility trial towards the end of the trial. Half of the lamb crop was slaughtered at an average live weight of 23.6 kg  $\pm$  0.56. The *M. longissimus dorsi* was removed on both the left and right half of the carcass between the 2<sup>nd</sup> - 3<sup>rd</sup> last thoracic vertebrae and the 4<sup>th</sup> - 5<sup>th</sup> lumbar vertebrae. A sample from the rumen wall was taken at the rumino-reticular fold to determine development characteristics.

Results indicate that the feed conversion ratio for the CFC lambs were better than CF1 (P = 0.052) but not more than CF2 (P = 0.307). The FCR was 0.88, 1.19 and 1.01 (kilogram feed required to gain 1 kg in bodyweight) for CFC, CF1 and CF2, respectively. Dressing percentage was higher for CF2 than for both CFC (P = 0.012) and CF1 (P = 0.077). Along with BUN data it was concluded that the high CP level of CF2 resulted in the higher fat deposition. The optimised creep feeds had higher nitrogen and energy balances than the commercial CFC, this implicates that the optimised creep feeds were more efficiently utilised. Optimised creep feed treatments had longer papillae than CON (P < 0.0001). Papillae of creep feed 2 was longer than CFC (P = 0.0537). Papillae width decreased as the NSC level increased thus it is surmised that higher NSC levels resulted in longer but thinner papillae. Rumen muscularization was equally developed between all the treatments and was ascribed to the lambs' access to course roughage. Meat quality in terms of physical and chemical characteristics was found to be in range with that expected for lambs at higher slaughter weights (40 kg).

In conclusion, the balancing of the limiting EAA increases the benefits of creep feeding while simultaneously being more efficiently utilised. Intensive sheep production systems may benefit from the feeding of such creep feeds provided it fits economically into their farming system.

## OPSOMMING

**Titel:** Gespesialiseerde kruipvoeding vir lammers om prestasie te optimaliseer

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Die volgende studie is om te bepaal of 'n kruipvoer wat gebalanseerd is vir beperkende essensiële aminosure (EAA) by 'n vasgestelde nie-strukturele koolhidraat (NSK) vlak verbeterde groei sal ontlok teenoor lammers wat grootgemaak word op 'n kommersiële kruipvoer.

Lisien, Treonien, Metionien, Isoleusien, Fenylalanien en Leusien is bepaal as die beperkende aminosure vir suipende lammers. Hierdie aminosure is opgeneem in die geoptimaliseerde kruipvoer behandelings CF1 en CF2 wat 157 g RP/kg, 477 g NSK/kg en 179 g RP/kg, 508 g NSK/kg onderskeidelik verteenwoordig. 'n Kommersiële kruipvoer met geen optimalisering vir EAA is verteenwoordig as CFC met 139 g RP/kg en 455 g NSK/kg. 'n Negatiewe kontrole (CON) behandeling is verteenwoordig deur lammers wat geen kruipvoer ontvang het nie, maar wat *ad libitum* toegang tot hul ooi gehad het om te soog terwyl die ooi op 'n kikoejoeveld wei.

'n Groei proef is uitgevoer met Merino x Döhne-Merino kruis tweelinglammers met 'n gemiddelde geboorte gewig van  $4,42 \pm 0,11$  kg vir 60 dae en 'n verteerbaarheids proef is na aan die einde van die studie uitgevoer. Helfte van die lam kudde is geslag by die lewende gewig van  $23,6 \pm 0,56$  kg. Die *M. longissimus dorsi* was op beide die linker-en regter helfte van die karkas tussen die 2de - 3de laaste torakale werwels en die 4de - 5de lumbale werwels verwyder. 'n Monster van die rumen wand is geneem langs die rumino retikulêre vou sodat die rumen ontwikkeling eienskappe daarmee bepaal kon word.

Resultate dui daarop aan dat die voeromsetverhouding (VOV) vir CFC lammers beter was as vir CF1 ( $P = 0,052$ ), maar nie meer as vir CF2 ( $P = 0,307$ ) nie. Die VOV was 0,88, 1,19 en 1,01 (kilogram voer wat nodig is om 1 kg liggaamsmassa aan te sit) vir CFC, CF1 en CF2 onderskeidelik. Uitslag persentasie vir CF2 was hoër as beide CFC ( $P = 0,012$ ) en CF1 ( $P = 0,077$ ). Saam met die bloed, urea en stikstofbalans data is daar tot die gevolgtrekking gekom dat die hoër RP inhoud van CF2 gelei het tot 'n hoër vetneerlegging. Die geoptimaliseerde kruipvoere het 'n hoër stikstof- en energiebalaans gehad teenoor die kommersiële CFC behandeling wat dus impliseer dat die optimale kruipvoere doeltreffender benut was. Die geoptimaliseerde kruipvoer behandelings het langer papillae gehad as CON ( $P < 0,0001$ ). Papillae lengte van die CF2 behandeling is langer as die van CFC ( $P = 0,0537$ ). Papillae breedte het dunner geraak soos wat die NSK-vlak gestyg het dus word vermoed dat hoër NSK-vlakke langer maar dunner papillae tot gevolg het. Rumen bespiering is ewe ontwikkel tussen

al die behandelings en word toegeskryf aan die lammers se toegang tot growwe ruvoer. Vleis kwaliteit in terme van fisiese- en chemiese eienskappe was inlyn met wat verwag kan word vir lammers by hoër slaggewigte (40 kg).

Ten slotte, die balansering van beperkende aminosure verhoog die voordele van kruipvoeding en word terselfdertyd doeltreffender benut. Intensiewe skaap produksie stelsels kan dus voordeel trek uit die voeding van sodanige kruipvoere mits dit ekonomies pas in die boerdery stelsel.

## **DEDICATION**

I dedicate this thesis to my parents Philip and Corrie le Roux. Thank you for your encouragement, assistance and providence through my life and academic career to help me reach one of my greatest goals and achievements. It is your love, commitment and ambition that kindled this growing ambitious attitude and love towards life.

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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
BUN	Blood urea nitrogen
BW <sup>0.75</sup>	Metabolic body weight
CF1	Creep feed 1
CF2	Creep feed 2
CFC	Creep feed control
CON	Control
Cys	Cystine
DE	Digestible energy
DM	Dry matter
DP	Dressing percentage
E.U.	Experimental unit
EAA	Essential amino acids
EE	Ether extract
ERR	Empty reticulo-rumen
EUN	Endogenous urinary nitrogen
FBW	Final body weight
FCR	Feed conversion ratio
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
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
Phe	Phenylalanine
Pro	Proline
PUN	Plasma urea nitrogen
RDP	Rumen degradable protein
RUP	Rumen undegradable protein
s.d.	Standard deviation
s.e.	Standard error
SAMM	South African mutton merino
Ser	Serine
TDN	Total dietary nutrient
Thr	Threonine
Tyr	Tyrosine
Val	Valine
VFA	Volatile fatty acids
WAS	Weight at slaughter
WEB	Whole empty body

## **CHAPTER 1**

### **GENERAL INTRODUCTION**

The main challenge in the global feed environment today is to produce more food with affordable price tags. The increase in food demand is not only related to the increase in population size but also due to the increasing amount of consumers being able to afford food from animal origin, especially in developing countries. Production and availability of raw materials on the other hand has decreased over the last few years due to droughts, recession and the increasing use of crops for the production of biofuels ([www.oup.com](http://www.oup.com); AFMA, Chairman's report 2009/2010). Be it as it may, it should be the priority of the animal feed industry to produce feeds that are efficient with low wastage that also increase animal production.

Creep feed, an intensive feed ration for suckling lambs, is such a feed that is commercially available and has the potential to elicit good performance results, especially since lambs are very efficient in feed turnover during this exponential growth phase. Such a ration should provide the correct balance of energy, protein and minerals while providing stimulus for the rumen to develop adequately (Jones *et al.*, 1996). To gain the economic edge a creep feed ration should elicit greater efficiency and production outputs while simultaneously represent the correct balance of energy, protein and minerals.

It has been shown that increasing the level of crude protein (CP) results in better feed efficiency and higher average daily gains (ADG; Craddock *et al.*, 1974; Jones *et al.* 1996). It is also known that protein quality rather than CP level *per se* results in better feed utilisation. Protein quality is typically determined by its amino acid profile based on the ideal amino acid concept. This concept basically stipulates that the absence or undersupply of a single essential amino acid will inhibit further synthesis of protein even if the other amino acids are in adequate supply (Cant *et al.*, 2003). The other amino acids in adequate supply are therefore in excess of protein synthesis and are deaminated to carbon skeletons and ammonia. Ammonia is excreted in the urine while carbon skeletons are used as energy sources for fat deposition. This process being energetically inefficient allows for valuable energy normally available to the animal being lost. Protein utilization can therefore be said to be more efficient when the limiting essential amino acids are supplied. Protein quality, in turn, therefore is measured according to its essential amino acid (EAA) profile.

Even though ruminants are able to synthesise both essential and non essential amino acids through their microbial population, the physiological demand of high producing animals require higher amounts of amino acids than what can be supplied via the microbial protein (Jones *et al.*, 1996). It has furthermore been confirmed that microbial crude protein is deficient in the EAA's Histidine, Methionine, Leucine, Arginine and Phenylalanine (Nolte, 2006). Suckling lambs however, do not have a microbial population at first as the rumen is undeveloped and they are therefore dependent on milk or feed intake to provide their amino acid requirements. Since the amino acid requirement of an animal is linked to its performance level (Titgemeyer, 2003; Nolte, 2006) it is likely that lambs that are

capable of higher performance (genetically) require more amino acids than that provided by the milk from its dam, quantitatively and qualitatively.

Nolte (2006) determined the essential amino acid requirement of growing Merino and Döhne-Merino lambs from the whole empty body amino acid composition as it was proposed to be an excellent indication of what the requirement and proportion of amino acids are (Hussein *et al.*, 1991). Nolte (2006) determined that Histidine, Methionine, Leucine, Arginine and Phenylalanine as the first limiting amino acids for Merino and Döhne-Merino lambs.

The study of Schroeder *et al.* (2006) further indicated that growing steers supplemented with energy, irrespective of its source, had an increase in nitrogen retention and thus also on the efficiency of crude protein utilization. In preceding studies it has been found that pre-ruminant calves with increasing energy intakes on both high and low protein diets had improved protein retention, which was suggested to be due to the increase efficiency in individual amino acid utilization (Donnelly & Hutton, 1976; Gerrits *et al.*, 1996). Hammond, (1992) indicated that as the ratio of available energy to nitrogen increases, the amount of microbial protein production also increases.

The non structural carbohydrate (NSC) fraction of feeds could function as the immiscible energy source required for intensive rations. This fraction is a readily fermentable carbohydrate source (Carruthers & Neil, 1997) and is a vital energy source for the microbial population (NRC, 2007). Suckling lambs or pre-ruminants further specifically require this energy source for the proliferation of a microbial population in their undeveloped rumens. This immature population typically requires that a feed is highly fermentable with a fast outflow rate of indigested feed (Cheng, 1991), which is represented by feeds with high NSC fractions.

The NSC fraction is also believed to be important in the physical development of the rumen. Grains, typically high in NSC, have been shown to be responsible for rumen papillary development, rather than roughages (Warner *et al.*, 1956). It has been indicated by Stern *et al.* (1978) that the proportion of the volatile fatty acid (VFA) butyrate increases as the NSC level increases. Butyrate, specifically, has largely been indicated as the VFA that has the highest influence on rumen papillae development (Baldwin & McLeod, 2000). This is not surprising seeing as 90% of butyrate produced during fermentation is metabolized within the rumen wall with little butyrate circulating the peripheral blood supply (Baldwin & McLeod, 2000).

It is furthermore important to remember the effect of feeds on meat quality, and not just the ability to increase production output. The consumer inevitably determines whether the carcass (and its commercial cuts) obtained from such a feed will gain entry into the market, irrespective of what the growth performances of such lambs were. Consumers in turn typically assess meat quality based on attributes such as colour (Miltenburg *et al.*, 1992), tenderness (Koohmaraie *et al.*, 1990) and juiciness (Safari *et al.*, 2001).

In light of all above mentioned, the ideal creep feed ration would therefore be one:

- Supplying the most limiting EAA for suckling lambs
- At an adequate NSC level to provide energy for efficient CP and amino acid utilisation as well as providing substrate for the microbial population which will also elicit optimum rumen development
- That has no detrimental effects on meat quality

It is therefore hypothesised that a creep feed optimised for the limiting EAA balance at certain NSC levels would be more efficient with higher production responses as compared to a commercially available counterpart.

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## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **2.1 Introduction**

Creep feed as a management tool has been around and studied for decades, not only for the benefits of increased growth rate but also for the economic gain of early weaning and less days to market weight. The nutritional aspect of creep feed has been studied in an array of good articles with different approaches with regards to nutrient composition. Any feed supplementing milk intake will elicit an increased growth response mainly because extra nutrients are available beyond the requirements of maintenance. However with increasing feed costs, environmental friendly conscience, and the global economic situation it remains that smart and efficient utilisation of feed mixtures triumphs at the end of the day. This thesis focuses on the refinement of creep feed for lambs in terms of amino acid requirements and non structural carbohydrate level. Therefore literature is focussed on that pertaining to the requirements of pre-weaning lambs as far as possible.

##### ***2.1.1 What is creep feeding***

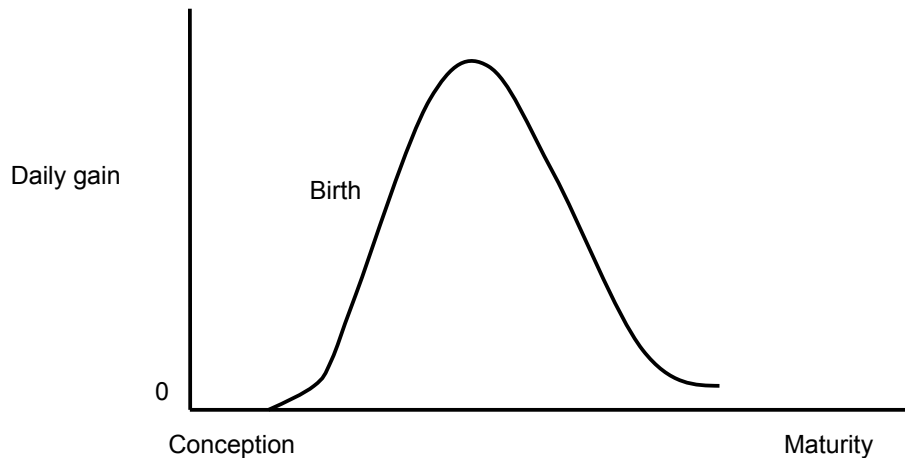
Creep feeding entails the supply of feed supplements to nursing lambs through a “creep” which excludes entrance to the ewes. The “creep” is usually an opening in a gate or fence just large enough for a lamb to use but too small to be used by ewes (Alcock, 2006). This type of system allows the lambs to have access to supplemental feed while suckling, thereby making up for the inevitable drop in milk intake as lactation progresses. Creep feeding is particularly used in intensive sheep production systems where early weaning of lambs is practised. Creep feeding can be viewed as more economical since the efficiency of feed utilization is far greater when feed can be converted to lean gain as opposed to feed converted to milk and milk to lean gain (Coetzee, 2009). However, creep feeding can also be uneconomical when animals are reared on adequate high quality green pasture (Alcock, 2006). The decision to creep feed will therefore be influenced by factors such as time of lambing (autumn and winter lambing for summer rainfall areas), multiple births, stud breeding and type of slaughter lamb production.

#### **2.2 Animal growth and development**

Animal growth can be divided into two phases, hyperplasia (increase in cell number) and hypertrophy (increase in cell size). Hyperplasia of muscle cells occurs mostly prenatally (Allen *et al.*, 1979) and only to some extent postnatally (Bergen & Merkel, 1991). Muscle hypertrophy is accompanied by protein synthesis while adipose tissue is accompanied by the accumulation of lipids (Hossner, 2005). For meat production the event of protein synthesis and lean meat deposition (muscle hypertrophy) is of utmost importance, especially since it is the meat on a carcass that gains economic benefit from meat retailers while consumers also prefer lean meat over fat meat.



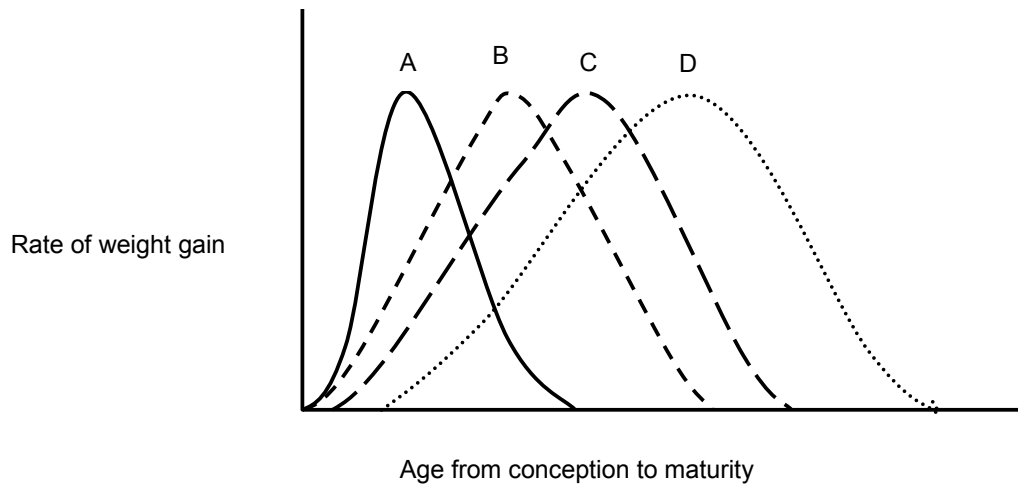
Growth has a phenomenon trend over several animal species in that it keeps the same sigmoidal pattern up to mature weight when age is plotted against gain in weight. The sigmoidal growth starts off with an exponential growth phase where the slope of the curve is at its maximum. An inflexion point occurs where the slope of the curve changes from very rapid growth to a more moderate slower growth rate. This is followed by the growth plateau where growth is slow and finally ceases. This sigmoidal growth curve is also followed for organs of animals and plant components (Figure 2., Hossner, 2005).



**Figure 2.1** Daily weight gain curve (adapted from Hossner, 2005)

As is evident from Figure 2., the different organs and tissues of the body grow in specific growth waves chronologically, i.e. some tissues start to mature before others. For example neural tissue starts growing before bone, muscle and adipose tissue. In turn the development of each tissue, depending on its location, varies between early, medium and late maturing. Fat deposition around the kidney precedes deposition at intermuscular, intramuscular and subcutaneous sites. For optimum growth rates the supply of nutrients need to be coordinated with the progression of growth (Owens *et al.*, 1993). It is therefore important to optimise growth during the exponential phases of growth (Figure 2.) in order to gain economic profit with regards to meat production.

Protein accretion (growth) is limited by the genetic capacity of the animal, i.e. it sets the maximum level to the extent of protein accretion. Factors such as live weight, age, physiological state and energy: protein ratio influences protein deposition within the limits set by genetics (Tamminga & Verstegen, 1996).



**Figure 2.2** Different growth rates of different tissues and body regions (adapted from Hossner, 2005) Where “A” represents head, brain and kidney fat; “B” represents neck, bone intermuscular fat; “C” represents thorax, muscle, subcutaneous fat; “D” represents loin, fat, intramuscular fat

### **2.2.1 Nursing behaviour of lambs**

The behaviour of suckling lambs has received some interest, as it has provided the key in understanding the nutrient requirement of lambs. This type of research has allowed scientists to manage the husbandry of pre-weaned lambs far better. It is also this natural innate behaviour that needs to be accounted for during research with such lambs.

In general, as lactation progresses, lambs suckle their ewes less frequent and for shorter suckling bouts (Munro, 1956; Ewbank, 1967; Hinch, 1989). It was observed that during the first two weeks of lactation ewes allowed their lambs to suckle at any time. After two weeks, ewes prevent their lamb(s) from suckling at certain times by simply walking away or running forward (Ewbank, 1967). Twins seem to suckle more frequently than singles (Ewebank 1967; Hinch, 1989) with an accompanied lower duration of the suckling bout (Hinch, 1989). Even further, light weight twins tended to suckle more frequently than heavy weight twins (Ewbank, 1967). Sucking bouts per hour was observed to range between 0.15 and 0.55 throughout the day and peaked early morning and late afternoon (Hinch, 1989). “Sucking duration tended to be highest in the early morning and mid-afternoon, and paralleled the peak grazing periods” (Hinch, 1989).

Table 2.1 illustrates how the duration of individual suckling bouts decrease over the weeks of lactation.

**Table 2.1** Duration of single and twin lamb suckling bouts as time progresses

Approximate age of lambs (weeks)	Duration of individual suckling bouts (sec)	
	Single lambs	Twin lambs
1	41	42
2	33	31
3	20	19
4	15	17
5	13	15
6	13	13
7	14	14
8	14	13

Adapted from Ewbank (1967)

There seems to be a positive correlation between the sucking behaviour of lambs on the milk yield of their ewes, but this effect on milk yield cannot be separated from the effect of age and litter size on milk yield (Hinch, 1989).

### **2.2.2 Lamb growth as affected by milk yield of ewe**

It is known that the growth rate of pre-weaned lambs is highly correlated to the milk production of their dams (Burriss & Baugus, 1955). Lambs from high milk producing ewes have greater growth rates compared to lambs from low milk producing ewes; this advantage was shown to be maintained throughout the suckling period (Burriss & Baugus, 1955). The positive correlation between milk production and growth rate of lambs, up to 56 days of age, was confirmed by Snowden & Glimp (1991). The same study also indicated that the growth rate of twin lambs were affected less by milk production than for single lambs. This implies that twin lambs are less dependent on milk than singles but more dependent on other sources of nutrients to grow, i.e. they become dependent on dry feed earlier than singles.

Milk yield is at its highest at four weeks postpartum with dramatic declines over the next four weeks of lactation, followed with a less rapid decline (Burriss & Baugus, 1955). Gardner & Hogue (1964) maintained that ewes with twins did not only have a higher milk yield but that fat and protein percentages also increased. The study of Snowden & Glimp (1991) however indicated that protein percentage did not differ between single or twin bearing ewes but the fat percentage was higher for twin bearing ewes.

Milk production of ewes decreases steadily over the lactation period. The first month's milk production can account for 45 – 50 percent of the total milk yield, for a 16 week lactation (Theriez, 1984). By the time the lamb is 9 to 12 weeks old the amount of gain originating from milk intake is very low, in fact the correlation between gain in weight of lamb to milk intake is already very low at seven weeks of age (Barnicoat *et al.*, 1949; 1956). Table 2.2 illustrates how milk production decreases over a 12 week period for twin and single bearing **Error! Reference source not found.**ewes.

**Table 2.2** Milk consumption of lambs over a 12 week period from birth

**Table 2.2** Milk consumption of lambs over a 12 week period from birth

Class	Average Daily Milk Consumption (grams) by Weekly periods											
	1	2	3	4	5	6	7	8	9	10	11	12
Singles	1260	1215	1215	1080	945	900	810	675	675	675	675	540
Twin	1260	1350	1260	1170	1035	855	810	810	765	675	720	540

Adapted from Burris & Baugus, 1955

Lambs grow at phenomenal rates before weaning (60 – 80 days) with high efficiency (Coetzee, 2009). Creep feeding was brought about to supplement milk intake in order to gain profit from high growth rates by providing adequate nutrients. Creep feed provides additional nutrients to lamb for growth while providing substrate for establishing a microbial population and allowing early weaning with reduced weaning stress (Ørskov, 1992).

The study of Poe *et al.* (1969) indicated that lambs supplied with creep feed either as grounded roughages or concentrates had better growth rates than lambs on milk alone even when creep intake was low. After early weaning (28 days), weight gains did not differ significantly and at 16 weeks of age rate of gain was similar between treatment lambs and control lambs (Poe *et al.*, 1969). Creep feed lambs maintain the advantage of creep feeding after weaning due to the fact that they reach market weight earlier than non creep feed lambs even if the rate of gain is similar after weaning.

## 2.3 Carcass composition

### 2.3.1 Growth rate and feeding system on carcass composition

It has been suggested that rate of gain is associated positively with lean tissue accretion and negatively with fat accretion (Whiteman *et al.*, 1966). This means that a fast growing animal will deposit less fat than a slow growing animal. Lambuth *et al.* (1970) tested and confirmed this phenomenon in depth: carcasses from slow gaining lambs had more fat trim than their fast gaining counterparts which had a higher bone proportion; the percent ether extract in the muscles of lambs from faster gaining lambs were less than from slow gaining lambs.

Rate of gain is also affected by the type of feeding system. Lambs with access to concentrates gained weight faster than lambs reared on pasture alone (Murphy *et al.*, 1994; Santos-Silva *et al.*, 2002). Concentrate feeding systems however have been shown to produce carcasses with higher amounts of fat compared to pasture feeding systems (Priolo *et al.*, 2002; Santos-Silva *et al.*, 2002; Joy *et al.*, 2008). Rate of gain should therefore be distinguished from genetic ability and as an effect from nutritional means.

In the study of Lambuth *et al.* (1970) lambs were separated into their ability to gain slow or fast while being reared on the same diets; rate of gain was therefore a sole response to genetic ability. Rate of gain in the studies of Santos-Silva *et al.* (2002) and Murphy *et al.* (1994) were responses to types of feeding, i.e. nutritional means. Murphy *et al.* (1994) argued that propionate production from concentrate diets increase insulin secretion which in turn stimulates protein and fat synthesis.

It is further argued that lambs on pasture (or low energy diets) consumed sufficient energy for bone and muscle deposition with little energy partitioned to fat deposition therefore the leaner carcasses (Murphy *et al.* 1994). As tissue maturation occurs in the order of bone, muscle and finally fat, the partitioning of energy follows the same pattern until maximum deposition is reached. Each respective tissue matures as quickly as it can with the amount of energy available. Concentrate or high energy diets therefore provide over and above the energy required by bone and muscle, thereby increasing fat deposition.

### **2.3.2 Slaughter weight and carcass composition**

As an animal matures and slaughter weight increases the composition of the carcass changes. It was previously stated (section 2.2) that the growth of tissues can be early, medium or late maturing. Subcutaneous fat which is a late maturing tissue characterise a carcass that is at its last stage of maturity.

Increase in slaughter weight is therefore typically associated with higher fat trim from carcasses, higher ether extract, higher fat around kidney and pelvic regions (Lambuth *et al.*, 1970), decrease in muscle proportion (Santos-Silva *et al.*, 2002) and higher dressing percentages (Lambuth *et al.*, 1970)

Slaughter weight however does not seem to have an effect on meat quality attributes (Mendenhall & Ercanbrack, 1979), except for its effect on L\* and b\* colour values which decrease as slaughter weight increase. Meat therefore becomes darker and less yellow with increasing slaughter weight (Santos-Silva *et al.*, 2002).

## **2.3. Compilation of feed rations for intensive sheep production systems**

Rations intended for the intensively feeding of young lambs require the correct balance of protein, energy and minerals while the composition thereof should stimulate the development of the immature rumen (Jones *et al.*, 1996). For this thesis the emphasis is placed on compiling a creep feed with an optimum protein balance at the correct energy level (manipulated via level of non structural carbohydrates) and therefore these components are discussed.

### **2.3.1 Protein in ruminant diets**

It is no new theory that ruminants are capable of synthesising protein from non-protein nitrogen (NPN) sources through their microbial population in the rumen. However, for rumen microbes to synthesise microbial crude protein (MCP) they require adequate amounts of carbon skeletons (from protein and carbohydrates), non-protein nitrogen and inorganic sulphur (Atasoglu *et al.*, 1998). *In vitro* studies indicated that maximum growth of microbes requires that intact amino acids (Maeng & Baldwin, 1976; Atasoglu *et al.*, 1998; 2001) and peptides (Cotta & Russell, 1982) are included in the diet, i.e. it is imperative to include rumen degradable protein rather than just NPN and rumen undegradable protein (UDP).

Non structural carbohydrate (sugar and starch) degrading bacteria prefer intact amino acids and peptides while ammonia is the chief nitrogen source for cellulolytic bacteria (Russell *et al.*, 1992). When amino acids and peptides are incorporated into microbial protein the energy cost for protein synthesis is much less than when amino acids need to be synthesized from ammonia (Nolan *et al.*, 1976). Microbial growth is therefore more efficient when amino acids and peptides are the sources of nitrogen for protein syntheses (Baldwin & Allison, 1983).

Ruminants are further “exempt” from the requirement of essential amino acids seeing as the microbial population produces both essential and non essential amino acids. However, the physiological demand of high producing animals requires higher amounts of amino acids than what can be supplied via the microbial protein (Jones *et al.*, 1996). Microbial protein has been shown to be deficient in the EAA's Histidine, Methionine, Leucine, Arginine and Phenylalanine (Nolte, 2006). It is therefore apparent that ruminants also require rations balanced for essential amino acids (EAA) which are supplied as UDP. Ruminants therefore require not only RDP for efficient MCP production but also UDP to provide those limiting amino acids for high producing animals.

Craddock *et al.* (1974) indicated that as protein supplementation increased it resulted in higher average daily gains (ADG) and improved feed efficiency in lambs. This study of Craddock *et al.*, (1974) was but only a reiteration of that found by workers previously, thereby illustrating the importance of CP supplementation. Jones *et al.* (1996) confirmed earlier research that when protein levels are increased an improvement in growth rate, feed intake and feed efficiency is the consequence. It was further suggested by Jones *et al.* (1996) that lambs growing at 300 g/d need a CP content of 160 – 170 g/kg.

However, the choice of protein sources to be supplemented need to be evaluated on the basis of their quality, seeing as not all protein sources are equal.

The proportion or balance of amino acids that make up a protein is what distinguishes a superior protein source from a poor protein source. This has led to the promotion of the ideal protein concept of a protein which refers to the proportion or balance of amino acids within a protein source. An ideal protein is defined as that perfect proportion between the essential amino acids needed for maintenance and production. It implies that a change in amino acid requirement, such as increased milk production in a dairy cow, will not change the proportion to which it is required, only the amount (Boisen *et al.*, 2000). However, this definition should include that non-essential amino acids influence the composition of the essential amino acids and should the requirement be expressed as a ratio of essential amino acids relative to CP or nitrogen requirement (Boisen *et al.*, 2000). Furthermore the concept of the first limiting amino acid states that protein can only be synthesised up to the first amino acid that is limiting, until that amino acid requirement is filled and the next limiting amino acid will limit protein synthesis and so forth (Cole & van Lunen, 1994; Cant *et al.*, 2003).

Lambs which can only be considered as fully functional ruminants at two months after birth are very dependent on the quality of the protein they consume. The essential and non essential amino acid

contribution via MCP production is almost negligent at first, as very little dry feed is consumed soon after birth. Since the amino acid requirement of an animal is linked to its performance level (Titgemeyer, 2003; Nolte, 2006) it is likely that lambs that are capable of higher performance (genetically) require more amino acids than that provided by the milk from its dam, quantitatively and qualitatively.

It is therefore imperative to balance the amino acid supply of a creep feed to ensure that lambs from intensive feeding systems will grow at optimum rates and efficiency.

### **2.3.2 Estimation of limiting EAA requirement for growing ruminants**

Determining the requirement for amino acids for a ruminant entails the knowledge of amino acids required for maintenance, endogenous losses and synthesis of products such as milk, meat, wool, etc. It is also necessary to know the resources from where the ruminant gets its amino acid supply. As mentioned previously, the ruminant's protein source is mainly that of microbial protein and rumen undegradable protein. An immense amount of research has been conducted on the rumen microbial population and not in vain. Protein passing to the small intestine is 60 – 85% of microbial protein in origin (Smith, 1975; ARC, 1980). The amino acid composition has also been shown to stay constant differing only in quantity and not quality of amino acids with different types of diets (Nolte, 2006; Storm & Ørskov, 1984). Microbial protein is the best metabolisable source of protein while also being the source of origin for most of the amino acids arriving at the small intestine (Storm & Ørskov, 1984). Rumen undegradable protein (UDP) makes up the rest of the protein arriving at the small intestine. It has been a common practice to feed UDP in dairy rations to provide extra protein to the lactating cow but also to make-up for the amino acid deficiency of microbial protein.

Studies for determining the limiting amino acids for domestic livestock started many decades ago while the process still continues in refinement. Schelling & Hatfield (1968) maintained that Lysine was the first limiting amino acid and not Methionine. Storm & Ørskov (1984) thereafter found that Methionine and Lysine were the first and second limiting amino acids in microbial protein while Arginine and Histidine were also limiting.

Nimrick *et al.* (1970) further determined the first three limiting amino acids for growing lambs when urea was their only nitrogen source. It was maintained that Methionine was first limiting followed by Lysine as second limiting and Threonine as the third limiting amino acid.

In all above mentioned studies the amino acids that were found to be limiting were related to the basal diet the lamb was consuming. In other words, a basal diet high in sulphur containing amino acids will indicate Lysine as first limiting while a diet low in sulphur amino acids will indicate Methionine as first limiting. There seems to be no clear indication to what the required proportions of amino acids are, since it is related to the basal diet consumed.

After the ARC (1981) proposed lean meat as representing the ideal amino acid balance of an animal, the whole empty body (WEB) was proposed to be an excellent indication of what the requirement and proportion of amino acids are (Hussein *et al.*, 1991).

Nolte (2006) attempted to study the quality and quantity of amino acids needed by growing Merino and Döhne-Merino lambs by estimating the essential amino acid distribution of whole empty body weights. The method used by Nolte (2006) to determine the limiting EAA requirement of lambs is discussed here after.

The limiting EAA requirement of a lamb (estimated from the EAA composition of the WEB) together with the EAA composition of microbial crude protein can be used to determine the rumen undegradable amino acids required by the lamb to meet all EAA requirements (Nolte, 2006).

Limiting EAA requirement can only be calculated with an expected growth rate because an essential amino acid is a function of the amount of CP required for a certain amount of growth per day (Boisen *et al.*, 2000). At a growth rate of 250 g/d a CP requirement of 168 g/d (NRC, 1996) is necessary to meet the protein deposition for growth and maintenance. According to Nolte (2006), the amino acid concentration of Arginine in the WEB is 7.12 g/100g protein. Arginine requirement can then be estimated as: (crude protein for a 250 g/d gain ÷ 100) x (concentration of arginine in the WEB) = 11.90 g/d.

MCP production contributes to the amino acid supply received by the animal in a constant amino acid profile, regardless of the type and profile of protein in feed (Nolte, 2006). Thus MCP forms part of the calculation of the limiting EAA required. Quantitative MCP production in turn is dependent on the amount of feed the animal consumes and can be estimated as 13% of the total digestible nutrient intake (NRC, 1996). Nolte (2006) used the amino acid profile of MCP to calculate the amount of individual amino acid required to meet the needs of the animal. After the MCP requirement is calculated the actual MCP produced can be subtracted equalling that which needs to be provided by UDP. If the Arginine concentration in MCP is 5.54 g/100g and 11.96 g/d of Arginine is required the total MCP required is calculated as  $11.96 \text{ g/d} \div (5.54 \text{ g/100g protein} \div 100)$  equalling 216.06 g/d of MCP required. After subtracting the real amount of MCP produced (13% of TDN) the MCP deficiency is known. MCP produced from a 1.26 kg intake with a TDN of 720 g/kg equals 117.94 g/d. Essential amino acid requirement is then calculated as the essential amino acid required for a 250 g/d namely 11.96 g/d minus  $(117.94 / 100 \times 5.54)$  equalling 5.43 g/d. Table 2.3 summarises the limiting EAA requirements for Merino lambs growing at 250 g/d as determined by Nolte (2006).



**Table 2.3** Essential amino acid requirements of Merino lambs growing at 250 g/day if microbial protein is the sole protein source (adapted from Nolte (2006))

EAA <sup>1</sup>	EAA comp: MCP <sup>2</sup> (g/100g protein) <sup>3</sup>	EAA: Required Merino (g/d) <sup>4</sup>	MCP required: Merino (g/d) <sup>5</sup>	Estimated MCP deficiency: Merino (g/d) <sup>6</sup>	EAA required as UDP <sup>7</sup> : Merino (g/d) <sup>8</sup>
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

<sup>1</sup> Essential amino acid

<sup>2</sup> Duodenal crude protein pre-dominantly derived from microbial protein

<sup>3</sup> from Nolte & Ferreira (2005)

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

<sup>5</sup> 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)

<sup>6</sup> 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

<sup>7</sup> Undegradable protein

<sup>8</sup> Calculated as  $Y = X - (Z/100 \cdot A)$ , 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

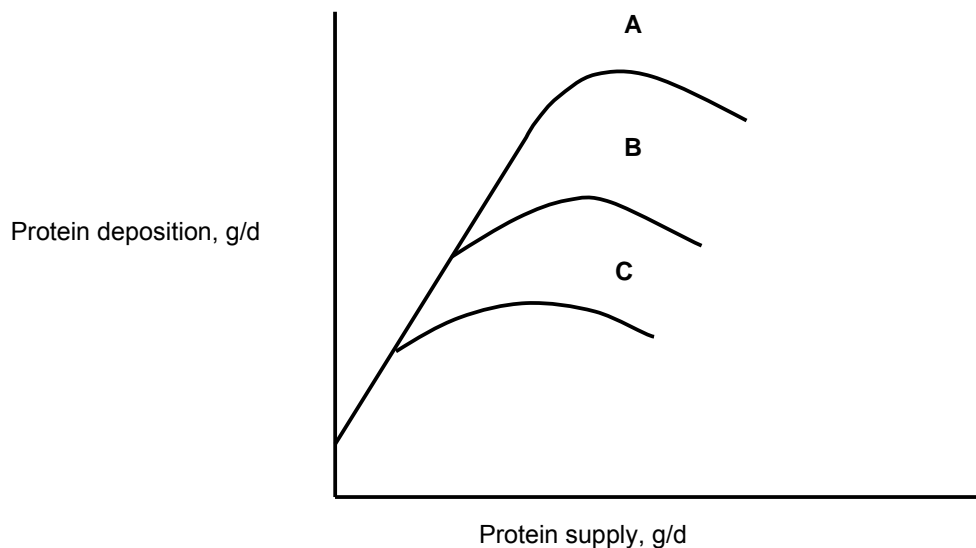
### 2.3.3 Energy: protein ratio

The concept of protein- and energy dependent phases of growth is used to describe the relationship between energy and protein supply on protein accretion (Chowdhury & Orskov, 1997; Titgemeyer 2003). This relationship between protein and energy has been used in models to predict the requirements of monogastric animals as well as that of some ruminants, however due to the complex nature of ruminant digestion the application to ruminant requirements remains doubtful. Some studies indicate that ruminants do follow this trend while other studies disagree (Schroeder & Titgemeyer, 2008).

The protein dependent phase depicts that when protein is limiting and a subsequent supplementation of protein is given, protein accretion will increase linearly until energy becomes limiting and accretion plateaus. Similarly, protein accretion can only occur up to the point where energy supply is limiting and therefore no increase in accretion even if protein supply increases (Figure 2.).

Two assumptions are implied based on the relationship between energy and protein supply. Firstly, specific amino acids are required at certain energy levels therefore amino acid requirements are

related to energy supply. Because of the linear relationship between rate of protein accretion and energy supply, requirements for amino acids can be expressed as relative to energy intake. Secondly, protein accretion is not affected when energy supply is increased while protein is limiting, therefore efficiency of amino acid utilization is not affected by energy supply (Schroeder & Titgemeyer, 2008).



**Figure 2.3** Protein- and energy dependent phases of growth (adapted from Schroeder & Titgemeyer 2008) Where “A” represents high energy supply, “B” intermediate energy supply, and “C” low energy supply

However, the study of Schroeder *et al.* (2006) indicated that growing steers supplemented with energy, irrespective of its source, had an increase in nitrogen retention and thus also on the efficiency of utilization for amino acids. In preceding studies it has been found that pre-ruminant calves with increasing energy intakes on both high and low protein diets had improved protein retention, which was suggested to be due to the increased efficiency in individual amino acid utilization (Donnelly & Hutton, 1976; Gerrits *et al.*, 1996). Therefore the assumption that efficiency of amino acid utilization is not affected by energy supply in the case of ruminants is not true, and warrants further investigation (Schroeder & Titgemeyer, 2008).

#### **2.3.4 Non structural carbohydrates in ruminant diets**

Non structural carbohydrates are considered as the starch and sugars of raw feed constituents but also include pigments, fructans, organic acids and pectins. Animal food sources are divided into the fractions moisture, crude protein, ether extract, crude fibre, ash and nitrogen-free extractives according to the proximate analysis of food. The fractions crude fibre and nitrogen-free extractives represents the carbohydrates of food. The nitrogen-free extractives can be estimated by subtracting the sum of the other fractions moisture, crude protein, ether extract, ash and crude fibre (expressed in g/kg) from a 1000. The proximate analysis procedure has been criticised with regards to the nitrogen-

free extractives and therefore were revised. The starches and sugars are now collectively called the non structural carbohydrates (NSC). Similar to the calculation of the NFE, NSC is calculated by subtracting the other fractions, moisture, crude protein, ether extract and crude fibre and ash from a 1000 (McDonald *et al.*, 2002).

As the ratio of available energy to nitrogen increases, the amount of MCP production increases (Hammond, 1992). Hence, readily fermentable carbohydrates such as that found in the non structural carbohydrate (NSC) fraction of feeds (Carruthers & Neil, 1997) are the most vital energy sources for the microbial population (NRC, 2007).

Pre-ruminants specifically require this energy source for the proliferation of a microbial population in their undeveloped rumens. The immature microbial population requires feed that is highly fermentable with a fast outflow rate of indigested feed (Cheng, 1991).

The nature of the carbohydrate is crucial to the type of fermentation that takes place. By-products of carbohydrate fermentation include the volatile fatty acids (VFA) of which acetate, propionate and butyrate are the most abundant. Diets high in structural carbohydrates such as forage-type diets give rise to a microbial population better adapted to a higher pH with a higher acetate proportion. Higher propionate proportions accompany a population that's more adapted to a low pH, usually brought about when concentrates are fermented. Soluble carbohydrates (mainly sugars and starch) are largely contained in concentrates. The fate of acetate once absorbed through the rumen or omasum wall is to be transported to the liver where it is mainly used for the *de novo* synthesis of cholesterol and long chain fatty acids (Van Houtert, 1993). Some acetate can also be found in the peripheral blood supply where it can be absorbed by the mammary gland, adipose tissue, and muscle for the synthesis of fatty acids as mentioned above. Propionate on the other hand is extensively metabolised by the liver for gluconeogenesis, therefore propionate circulating in peripheral blood supply is very low in forage type diets. Butyrate which is produced in smaller quantities than both propionate and acetate is metabolized to acetoacetate and beta-hydroxy-butyrate in the rumen wall where after it is oxidized or used for fatty acid synthesis in peripheral circulation.

Non structural carbohydrate has been shown to improve the utilization of rumen  $\text{NH}_3\text{-N}$  (Stern *et al.*, 1978). The extent to which ruminal ammonia is utilized for microbial growth is highly affected by carbohydrate availability (Stern *et al.*, 1978), i.e. the propionate production from the fermentation of non structural carbohydrates is directly related to the amount of microbial protein synthesis (Dove & Milne, 1994; Trevaskis *et al.*, 2001). Since the optimum ratio of NSC to nitrogen for microbial growth has not yet been determined, Stern *et al.* (1978) studied the effects of different NSC levels on microbial protein synthesis. Non structural carbohydrate levels were low, medium and high representing 490 g NSC/kg, 326 g NSC/kg en 196 g NSC/kg, respectively. The study indicated that butyrate proportions increased as the level of NSC increased while the molar percentages of acetate and propionate did not change significantly between the different levels of NSC, however as the NSC levels increased the amount of ammonia losses decreased. This same result was reported by Chalmers *et al.* (1954), Phillipson *et al.* (1962) and Robertson & Hawke (1965) for *in vitro* and *in vivo*

studies where starch more than that other carbohydrates decreased the ammonia levels. Stern *et al.* (1978) however concluded that “these results indicate that source of carbohydrate and its rate of availability to rumen microbes play an important role in nitrogen utilization and warrants further study to test these effects *in vivo*. In light of this present work, studies should be conducted to evaluate the formulation of ruminant rations in which the non structural carbohydrate content is controlled, as well as the energy or TDN level”

## **2.4 Digestibility, and the factors affecting it**

The digestibility of a feed/ feed stuff is typically defined as that proportion which is not excreted. It is assumed that that which is not excreted is absorbed by the animal. There are however two discrepancies to this assumption. Firstly, methane production from fermentation in the rumen is lost by eructation and not absorbed. Energy contained in this fraction is lost and not available to the animal at all, thus digestible energy and digestible carbohydrate content is overestimated (McDonald *et al.* 2002). Secondly, not all of the material in faecal matter is of dietary origin. Nitrogen originating from cellular material abraded from the lining of the gut and enzymes that were not absorbed contribute to the nitrogen content of faeces. This fraction is known as metabolic faecal nitrogen. Other substances of metabolic origin include ether extractable substances and excreted minerals (represented as the ash component in proximate analyses). Digestion therefore seems to be underestimated. It is for this reason that values obtained from digestibility trials are called *apparent* digestibility coefficients as opposed to true digestibility coefficients (McDonald *et al.*, 2002). “True digestibility coefficients are difficult to determine, because the fractions of the faeces attributable to the food and to animal are in most cases indistinguishable from one another” (McDonald *et al.*, 2002).

### **2.4.1 Factors affecting digestibility:**

As the level of feeding (quantity) increases the digestibility of the feed decreases. High levels of feeding cause a reduction in the time feed is exposed to digestion enzymes (Schneider & Flatt, 1975; McDonald *et al.*, 2002). Likewise, high feeding levels increase the rate of passage through the rumen which lowers the digestion of slowly digestible feed particles. It is therefore important that the feeding level, of a certain feed, during a digestibility trial is the same as that in practice.

The chemical composition of feed/feed stuff is the most important factor affecting digestibility (Schneider & Flatt, 1975; McDonald *et al.*, 2002). Schneider & Flatt (1975) maintained that a digestion coefficient of a feed/feed stuff should never be reported without also reporting the chemical composition thereof. This will be especially applicable when the digestion coefficient of a feedstuff is compared to the same feed stuff from a different source, i.e. using reported digestion coefficients as a standard to one's own feed stuff. Changes in one chemical component could also lead to a different digestion coefficient in another chemical component. Level of protein is an example of such a component. An increase in protein has been shown to increase the digestibility of the crude fiber component of feeds. This change may not just be because of the change in metabolic nitrogen but

purely because of the effect of protein on the growth and activity of microorganisms in the rumen (Schneider & Flatt, 1975).

Associative effects (ration composition): The digestibility of a feed stuff is not only influenced by its own chemical composition but also by the composition of other feed stuffs with which it is fed, e.g. a ration (McDonald *et al.*, 2002). Easily digestible carbohydrates typically decrease the digestion of celluloses and hemicelluloses. This is explained by the fact that different types of microbial populations are responsible for the fermentation of different carbohydrates (non structural versus structural carbohydrates). Fermentation of easily fermentable carbohydrates, such as NSC, lowers the rumen pH due to propionic acid production. Cellulolytic microbes which digest the resistant (structured) carbohydrates do not function optimally at the lower rumen pH, hence a reduced fibre digestion (McDonald *et al.*, 2002). Apart from the effect on the rumen pH, there seems to be a direct effect on cellulolysis when easily fermentable carbohydrates are fermented (McDonald *et al.*, 2002). Schneider & Flatt (1975) stated that the microbes utilize these easily fermentable carbohydrates rather than attacking the structured carbohydrates.

Feed preparation or processing changes the physical form of the feed which influences the digestibility thereof (Schneider & Flatt, 1975). Chopping, chaffing, grinding or cooking are some of the commonest processes of preparation and help to gain maximum digestibility (McDonald *et al.*, 2002)). Grounding of roughages lowers digestibility because the small particles pass through the rumen faster preventing proper fermentation.

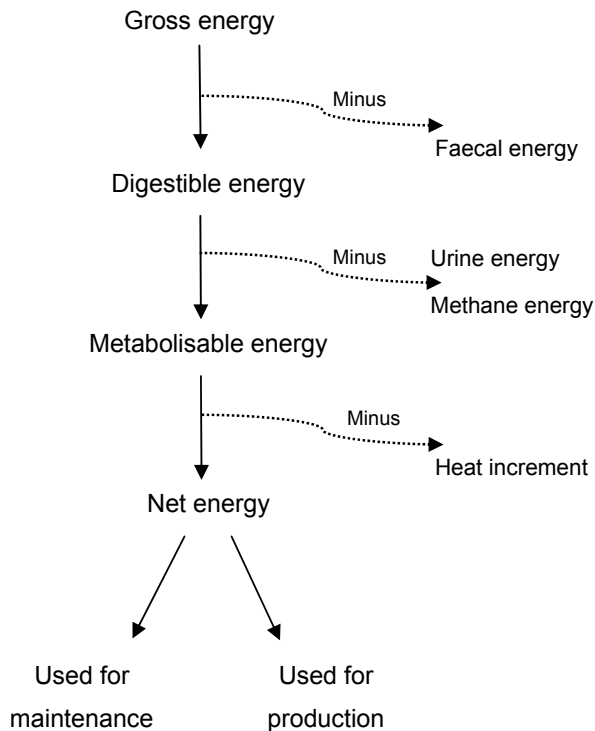
Species is the most important animal factor affecting the digestibility of a feed/feed stuff. Monogastric animals differ in their ability to digest from ruminants. High fibre (structured carbohydrates) diets are poorly digested by monogastric animals such as poultry. Pigs usually have higher apparent digestibility coefficients, because their excretion of metabolic faecal nitrogen is smaller than for ruminants. Sheep tend to digest highly digestible feeds, such as cereals, better than cattle which in turn digest high fibrous feeds better than sheep (McDonald *et al.*, 2002).

#### **2.4.2 Partitioning of dietary energy within the animal**

It is known that the organic nutrients of feeds are used by animals for the construction of body tissues and synthesis of products such as milk and meat. Energy obtained from these nutrients is in turn used to fuel the “work” that needs to be done by the animal (McDonald *et al.*, 2002). “A unifying feature of these diverse functions is that they all involve a transfer of energy, and this applies both when chemical energy is converted into mechanical or heat energy, as when nutrients are oxidised, and when chemical energy is converted from one to another, as for example when body fat is synthesised from food carbohydrate” (McDonald *et al.*, 2002). The nutritive value of a food therefore lies in its ability to supply energy. It has become a common practice to use energy values to express the nutritive value of feed stuffs and to describe how an animal responds to it (Schneider & Flatt, 1975).

The gross energy (heat of combustion) of a feed does not represent the full amount of energy useful and available to the animal for maintenance or production. Energy can be lost via solid, liquid and

gaseous excretions and also from the production of heat (McDonald *et al.*, 2002). The partitioning of food energy is depicted in Figure 2.4. That amount of energy remaining after the energy losses from faecal excretion, urinary excretion, methane production and heat increment are subtracted is the energy available for maintenance and production.



**Figure 2.4** Partition of food energy in the animal, adapted from McDonald *et al.* (2002)

Even though Net energy is the best energy to scrutinise a feed on its ability to provide energy for maintenance and production, it is the Metabolisable energy (ME) that is most commonly used for this purpose. The following section is therefore dedicated to ME and the factors affecting it.

### **2.4.3 Metabolisable energy and the factors affecting it**

Metabolisable energy represents the energy that remained after the losses of energy contained in faeces, urine and gasses (mostly methane) were deducted. Importantly, the ME of a feed will vary according to the species of animal consuming it or, put differently, the type of digestion it is subjected to (McDonald *et al.*, 2002). Metabolisable energy is therefore very much an expression of a feeds nutritive value/quality as well as an animal's ability to digest it.

Of all the energy losses, the energy lost via faeces is by far the most important. Undigested feed particles excreted in the faeces contribute more energy loss than urine or methane. Those factors

influencing digestibility is therefore the most important factors affecting ME (McDonald *et al.*, 2002). Factors affecting digestibility were discussed in section 2.4.1.

Urine energy is contained in the nitrogen containing substances such as urea, hippuric acid, creatinine, allantoin and the non nitrogenous compounds such as glucuronates and citric acid. The amino acid profile of a feed will therefore have an effect on ME because amino acids in excess of the limiting amino acid, will be deaminated and excreted in the urine as urea. Higher excretion of nitrogen compounds such as urea will increase energy excretion and decrease ME (McDonald *et al.*, 2002).

Methane is the most prominent combustible gas that is lost via the rumen and its production is closely related to feed intake. High feed intakes increase rate of passage in the rumen (as mentioned previously) and lowers the amount of methane produced. In fact, any factor affecting fermentation time in the rumen either increases or decreases methane production. Increased rate of passage due to the feeding of concentrates, pelleted rations, finely grounded roughages, etc. decrease the amount of methane production. The reduction in methane production from these factors however, is offset by the increase in undigested faecal material output (McDonald *et al.*, 2002).

In digestibility trials where methane production cannot be measured directly, it can be estimated as 8% of the gross energy intake. It is also possible to determine the ME by assuming that urine and methane loss is 20% of digestible energy (McDonald *et al.*, 2002).

## **2.5 Rumen development of suckling lambs**

A young ruminant who is essentially a monogastric at birth has to go through a transition from a suckling monogastric to a grazing ruminant. The rumen which at first constitutes a very small proportion of a young ruminant's stomach grows and develops to become the largest compartment of the stomach compared to the reticulum, omasum and abomasum (Sisson & Grossman, 1938). Growth and development not only occurs with respect to size but also in papillary growth, muscularization and vascularisation of the rumen wall (Warner *et al.*, 1956). A functional rumen allows the animal to consume and utilize dry feed and roughages.

Jones *et al.* (1996) elaborated that rumen development or dry feed intake is a better guide to determine if a lamb is ready for weaning as opposed to live weight. "Lambs suckling ewes milking heavily would meet live weight criteria but might have a less developed rumen owing to minimal consumption of dry feed" (Jones *et al.*, 1996 ).

The rate at which the rumen develops rather than rumen development *per se* is important when rearing domesticated animals. The rate at which the rumen develops influences the time at which the animal can become dependent on rumen microorganisms as their source of protein. This rate of development can be manipulated via a nutritional means (Ørskov, 1992).

### **2.5.1. Histological appearance of the rumen wall**

The rumen can be described as a fermentation vat where microorganisms populate and ferment the feed that enters the rumen. The type of microorganisms, their establishment, substrate of preference and end products were discussed in detail in the NSC section 2.4. Dobson *et al.* (1955) described the histological appearance of the rumen wall in sheep as is described here.

The rumen is lined with tongue shaped papillae varying in size and shape between its different compartments. Papillae could be up to 6 mm long and 2 mm wide. The core of a papilla consists out of collagen fibers which are surrounded by a stratified epithelium layer. "The deeper layers are irregular, being arranged around papillary processes of the central core: these extend into the epithelium towards the cavity of the rumen. The larger blood and lymphatic vessels are found in the central core, and from the former small vessels, accompanied by connective tissue, penetrate into the papillary bodies. No muscle fibres have been found in the papillae and elastic fibres penetrate only a short way up the central core" (Dobson *et al.*, 1955).

A keratin layer is present as the last layer on papillae nearest to the rumen cavity. This layer is in a continuous process of sloughing into the rumen cavity and therefore can be missing at times. Networks of fine capillaries are found in abundance ca. 50 µm from the rumen cavity, confirming the immense absorptive capacity of the rumen. Numerous mitochondria are present within the rumen wall and are perpendicular to the papillae. The different tissue layers within the rumen wall vary considerable (Dobson *et al.*, 1955).

### **2.5.2. Factors affecting rumen development**

It is important for the transition from a monogastric to a ruminant to be a smooth one with minimal loss in growth. A smooth transition entails that the reticulo-rumen is of adequate size and optimally developed to utilized dry and forage based diets (Heinrichs, 2005). It is for this reason that so much research has gone into establishing what the factors are that influence rumen development.

Firstly for rumen development to commence the presence of dry feed in the rumen is of at most importance. Cheng (1991) reported that immediately after birth a bacterial population proliferates in the fluid phase of the rumen; by 38 hours the digestive tract is colonized. Fungi and protozoa responsible for mostly cellulose digestion start to colonise in the fluid phase at 8 to 10 and 12 to 20 days respectively (Cheng, 1991). Bacteria in the rumen come from the environment as young ruminants suckle, play and nibble on anything that enquires their interest. Young ruminants start nibbling at whatever their dams are consuming out of curiosity, as early as 3 days after birth, and inevitably consume some feed which becomes available to the bacterial population. This bacterial population is therefore dependent on feed entering the rumen to survive.

Furthermore, feed that allows for rapid fermentation and removal of indigestible feed particles will give rise to greater rumen development (Andrews *et al.*, 1969). Feed needs to enter the rumen and be available to the microbes before their generation time is over, i.e. nutrients from feed needs to



become available as quickly and frequently as the microbes need it to grow and populate or they will die off. Therefore a young lamb's immature population requires feed that is highly fermentable with a fast outflow rate of indigestible feed so that the population can proliferate without shortcomings.

It has been reported that rumen papillae and rumen wall muscularization occurs independently from one another (Brownlee, 1956; Harrison *et al.*, 1960), i.e. dietary factors attributing to papillae development does not attribute to rumen wall muscularization.

Cereal grains rather than roughages are responsible for rumen papillary development (Warner *et al.* 1956). Baldwin & McLeod (2000) attributed this increased papillary development to the higher proportions of VFA (propionate and butyrate) as response to the fermentation of cereal grains. When rumens were infused with sodium butyrate and sodium propionate it was found that butyrate has the largest effect on rumen papillary development (Baldwin & McLeod, 2000). This is not surprising seeing as 90% of butyrate produced during fermentation is metabolized within the rumen wall with little butyrate circulating in the peripheral blood supply. It has also been indicated that a decreasing rumen pH and increasing butyrate production increases the metabolism of butyrate in the rumen wall (Baldwin & McLeod, 2000).

Particle size furthermore plays a role in preventing a condition called parakeratosis whereby the squamous epithelial cells becomes hardened due to the diet's inability to remove the degenerating epithelial cells (Hinders & Owen, 1965). This condition restricts the absorption of substances, such as VFA, through the rumen wall by creating a physical barrier. In severe cases papillae degeneration and sloughing can occur (Beharka *et al.*, 1998). Parakeratosis is typically the result when finely ground concentrates or roughages are fed which decreases rumen buffering capacity and thereby lowering the rumen pH (Heinrichs, 2005). An example of parakeratosis is found in the study of Suárez *et al.* (2006) who reported focal and multifocal patches of coalescing and adhering papillae with a sticky mass of feed hair or cell debris in the rumen of lambs reared on pelleted diets.

Heinrichs (2005) suggested that the ideal feed for rumen development would be coarsely ground concentrates that will stimulate rumen papillae development, wall muscularization and limited incidences of parakeratosis.

### **2.5.3. Methods for measuring rumen development**

Due to high within rumen variation, Lesmeister *et al.* (2004) decided to study which area within the rumen is best suited for rumen sampling as well as the amount of samples required and which measurement indicates treatment differences more correctly. Lesmeister *et al.* (2004) however, used calf rumen tissue to establish the above mentioned. Even though lamb rumen tissue were scrutinised for this thesis, the finding of Lesmeister *et al.* (2004) will also be applicable here.

Lesmeister *et al.* (2004) found that samples taken from one area of the rumen are just as good as samples taken from all over the rumen in detecting dietary treatment differences. Even samples taken from the ventral portion of the caudal ventral blind sac will be able to show dietary treatment

differences. Papillae in this area are typically smaller than those found elsewhere in the rumen and were therefore not normally used for sampling, in literature. This illustrates the sensitivity of papillae to dietary treatments.

It was recommended that tissue samples should be obtained from four areas in the cranial and caudal sacs of the ventral and dorsal rumen, with three random tissue samples from each area and two measurements per sample for papillae length, papillae width and rumen wall thickness (Lesmeister *et al.*, 2004). However, it was also stated that if only one sampling area within the rumen was possible it should be ensured that the same site be used across animals and treatments (Lesmeister *et al.*, 2004).

Measurements normally taken on rumen samples include papillae length, papillae width, rumen wall thickness and papillae density. Papillae length and width were named the primary and secondary most important and strongest variables, respectively, for rumen development research. Papillae width reported in literature as a rumen development response variable is very limited (Lesmeister *et al.*, 2004). Seeing as papillae are tongue shaped; they consist out of a flat and narrow end. The predicament therefore lies in which side is used to estimate papillae width. Some researchers measure with millimetre rules (Ortega-Reyes *et al.*, 1992) and therefore inevitable end up measuring the flat side of the papillae while others use a more histological technique on microscopic levels. The problem therefore resides in the fact that it is difficult to compare one study to that of another study because it is often not noted which side of the papillae was measured for papillae width.

Hill *et al.* (2005) published a technique which is stated to be more efficient and accurate; the technique makes use of histological sectioning with scanning electron microscopy coupled with computer software. In this technique all papillae variables are measured from a histology slide with computer software. A series of straight lines were traced from the base of the papillae to the top of the papillae to determine papillae length. Width was determined by tracing a straight line at the widest part of the papillae at a right angle with the lines determining length (Hill *et al.*, 2005). This study of Hill *et al.* (2005) however does not define exactly what constitutes as the base of the papillae. In good sectioned slides it is possible to distinguish the epithelial layer from the connective collagen core of the papillae. The question therefore remains as to what presents the base of the rumen papillae, whether it be at the epithelium layer or the base of the connective collagen core?

## **2.6 Meat characteristics**

At the end of day all animal products are subjected to the preference of consumers. It is therefore crucial that any production system be measured against the favourability of it with regards to the preference of consumers. It is for this reason that the measurement of meat characteristics has become so vast and imperative to any meat production system.

Physical meat characteristics include colour, pH and water holding capacity and shear force. With regards to lean meat deposition, chemical characteristics such as moisture, fat percentage and protein percentage are important. All characteristics are discussed below.

Meat colour is the single most important characteristic to consumers seeing as it gives the consumer their very first impression with regards to freshness and ability to satisfy (Mancini & Hunt, 2005). Colour is appropriately determined by the CIELab-system which generates three colour values namely L\*, a\* and b\*. The lightness of the meat is represented by L\*; where L\*= 0 is black and L\*= 100 is white. A red-green range is represented by a\* while b\* represents a yellow-blue range (Poulson *et al.*, 2004). Hue and chroma is calculated as:

$$\text{Hue angle } (^{\circ}) = \tan^{-1} (b^*/a^*)$$

$$\text{Chroma value} = [(a^*)^2 + (b^*)^2]^{1/2}$$

Chroma value indicates the brightness of the colour; the higher the chroma value the richer and fuller the colour is. The hue angle, which is expressed in degrees (270°, 180°, 90°, 0°), gives an indication to how much a colour corresponds to blue, green, yellow and red (respectively).

Water holding capacity for this thesis is measured in drip and cooking loss. During pre-rigor pH fall, the volume in myofibrils change due to the myosin heads which attaches to the actin filaments at rigor. The breakdown of proteins during rapid pre-rigor pH fall can increase water loss (Honikel, 1998). The fluid accumulates between the fibre bundles, when a muscle is then cut this fluid drains from the surface under gravity. This fluid loss is known as the drip loss of meat.

It is no new theory that proteins denature at high temperatures, similarly meat protein denatures at varying temperatures (37-75°C). The structural changes occurring at higher temperatures due to the denaturation of meat protein, with accompanied expulsion of water, results in what is known as cooking loss.

The measurement of pH post mortem is a way of assessing glycolysis. As glycogen levels in muscle decrease and post lactic acid accumulation increase, the pH of the muscle steadily decreases (Immonen *et al.*, 2000). The rate at which these processes occur have desirable and undesirable results on meat quality. Devine *et al.* (1993) maintained that an ultimate pH greater than 5.8 can be considered as undesirable because the higher pH is associated with tougher meat and a lower shelf-life. Stressful handling of animals before slaughter typically increases the ultimate pH (Martínez-Cerezo *et al.*, 2005). According to Safari *et al.* (2001) the normal mutton ultimate pH range is between 5.40 and 5.86.

## **2.7 Breed characteristics of Döhne-Merino and South African Mutton Merino**

The Döhne-Merino dual purpose breed can be described as a hardy and versatile breed which is known for its adaptability in both feed lot and pasture feeding systems. This breed is further characterised by resistance to fleece rot and fly strike, very fertile with low birthing problems, high milk production and good mothering abilities ([www.Dohne.com](http://www.Dohne.com)).

Lambing percentages of 150% and more is possible while lambs can grow up to 350 g/d until weaning. Lambs can reach market weight (40 kg) at 4 to 6 months of age. Ewes are mature at a body weight between 55 – 75 kg and can produce three lamb drops in two years as they are not limited to a breeding season ([www.Dohne.com](http://www.Dohne.com)).

The South African Mutton Merino (SAMM) is known for its good growth rate, adaptation to all climatic conditions and renowned mothering ability. This breed is popular for use in feedlots and is efficient feed converters. The breed is also capable of utilising low quality roughage; in fact one breeding goal is to obtain optimum production at low cost (South African Mutton Merino Breeders' Society, 2001).

Lambing percentage also averages 150% and more, lambs can grow at 350 g/d and 560 g/d for ram lambs in intensive production systems. Ewes are mature at 77 kg body weight (South African Mutton Merino Breeders' Society, 2001).

The study of Cloete *et al.* (1999) showed that SAMM produced more triplet and quadruple litters, higher yearling weights and higher average lamb production than Döhne-Merino. Döhne-Merino lambs however produced more clean wool than the SAMM lambs.

In the following chapters the effect of specialised creep feeds on the performance of Döhne-Merino x Mutton merino cross lambs will be investigated. These chapters will cover growth performance, carcass composition, nitrogen- and energy balances, rumen development and the meat characteristics of lambs reared on specialised creep feeds. The compilation of such specialised creep feeds will also be discussed.

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## **CHAPTER 3**

### **GENERAL MATERIALS AND METHODS**

#### **3.1 Introduction**

*Note: To maintain the various chapters of this thesis in article form as described by the South African Journal of Animal Science with unnecessary repetition and ease of understanding, a general Materials and Methods section was incorporated. This section will function in giving all required information on the research protocols, which pertained to the entire study, in detail thus avoiding unnecessary repetition. Research chapters will therefore refer to this chapter in their relevant Materials and Methods sections.*

*It is important to note that the material and methods which are characteristic to only one study and not to the entire thesis will be discussed in its relative section and not in this General Materials and Methods section.*

Seeing as the creep feed diets were the focal point of all research in this thesis, the compilation of the different creep feed diets are discussed firstly, with a detailed discussion of the determination of a lamb's essential amino acid requirement. Though ewes were not actively part of the research done, their roles in the management and nutrition of lambs is immiscible and therefore discussed secondly. Thereafter, lamb nutrition and management is discussed followed by the slaughter protocol. Lastly the laboratory methodologies employed for the entire study are discussed.

Ethical approval was obtained from the *Stellenbosch University Animal Care and Use Committee*, reference number 2009B03006.

The study was conducted at the Stellenbosch University's experimental farm, Welgevallen.

#### **3.1.1 Estimation of lamb amino acid requirement and compilation of the creep feed diets**

Similar to Nolte's (2006) calculations (discussed in chapter 2.3.2), the essential amino acid requirement for a creep feed for pre-weaning lambs can be calculated. Pre-weaning lambs, however, obtain crude protein from milk firstly and only later from MCP as the rumen develops and becomes colonized. Milk's EAA contribution therefore needs to be taken into consideration in the same way as MCP is taken into consideration for estimating the EAA requirement of a creep feed. Milk intake however declines steadily as the lamb matures and becomes more dependent on sources other than milk. At first, the amino acid supply in milk should be efficient in EAA's for the lamb, but it decreases in quantity as the lamb grows (ewe milk production declines) and the requirements of EAA increase.

It was decided that the growth rate of pre-weaning lambs to be used in the current study would be in the range of 150 g/d. At the time of the EAA estimation the information on the type and breed of lambs to be used in the growth trial were not known. Therefore, the NRC (2007) CP

recommendations were based on the requirements of a lamb at 20 kg live weight and a daily intake of 3.91% of live weight. Protein was assumed to be 60% UDP based on the fact that lambs' fermentation capabilities are not fully functional, if even developed, inevitably leading to undegraded protein arriving at the abomasum. It was assumed that an average intake of 200 g of creep feed per day would be consumed per lamb in addition to the milk consumed. As mentioned, milk's contribution towards EAA supply is crucial to the EAA requirement calculations and therefore milk intake was assumed at 800 g (3.91% of body weight) per day per lamb over several suckling periods (NRC, 2007). Amino acid analysis from a freeze dried ovine milk sample was used to estimate the EAA contribution of milk to that required by pre-weaning lambs.

Table 3.1 represents the calculations of the EAA composition required by a creep feed ration to meet the requirements of the suckling lambs. In conclusion, it was estimated that Lysine, Threonine, Methionine, Isoleucine, Phenylalanine and Leucine needed to be included in the creep feed diets at levels of 7.4, 8.1, 1.7, 0.3, 4.3, and 3.5 g/kg respectively for suckling lambs.

NSC was included in the Creep feed rations as carbohydrate is the most vital energy source for the microbial population (NRC, 2007) and that microbial protein synthesis is energy dependent (Titgemeyer, 2003). Furthermore the extent of utilization of ruminal ammonia for microbial growth is highly affected by carbohydrate availability (Stern *et al.*, 1978).

Tanqua Feeds (Riviersonderend, Western Cape, South Africa) formulated and provided the creep feed rations to be used in the trial. A commercial creep feed with no adjustment for amino acids was formulated to function as a control for Creep feed 1 and Creep feed 2. Table 3.2 presents the physical and chemical composition of the three different creep feeds as formulated by Tanqua Feeds (Riviersonderend, Western Cape, South Africa).

**Table 3.1** Essential amino acid (EAA) requirements of suckling Döhne-Merino lambs and subsequent creep feed EAA composition to meet deficiency

EAA <sup>1</sup>	EAA requirement for a growth rate of 150g/d	Milk EAA composition (As is)	Milk EAA supply (As is) at 800g milk consumption	EAA deficiency if no other nutrient intake or MCP production	EAA composition needed in Creep feed	Suggested EAA composition for CF1 at 150g/kg CP	Suggested EAA composition for CF2 at 170g/kg CP
	(g/d) <sup>2</sup>	(g/100g) <sup>3</sup>	(g) <sup>4</sup>	(g/d) <sup>5</sup>	(g/kg) <sup>6</sup>	(g/kg)	(g/kg) <sup>7</sup>
Arg	6.58	0.22	1.83	4.74	23.72	23.7	26.9
His	2.54	0.23	1.85	0.68	3.42	3.4	3.9
Ile	3.18	0.38	3.11	0.06	0.31	0.3	0.4
Leu	7.72	0.87	7.01	0.70	3.51	3.5	4.0
Lys	6.69	0.65	5.20	1.48	7.44	7.4	8.4
Met	1.56	0.15	1.20	0.35	1.77	1.8	2.0
Phe	3.88	0.37	3.00	0.87	4.39	4.4	5.0
Thr	3.99	0.29	2.37	1.61	8.09	8.1	9.2

<sup>1</sup> EAA = Essential amino acids

<sup>2</sup> Calculated as  $Y = (X/100)*Z$ , where Y = EAA requirement for a growth rate of 150 g/d; X = a crude protein requirement of 95 g/d to allow an average daily gain of 150 g/d (NRC, 2007); Z = the whole empty body amino acid composition of a 36 kg Dohne Merino lamb (Nolte & Ferreira, 2004)

<sup>3</sup> Determined in Laboratory

<sup>4</sup> Calculated as  $Y = X*8$ , where Y = Milk EAA supply at 800 g milk consumption; X = Milk EAA composition (g/100g)

<sup>5</sup> Calculated as  $Y = X - Z$ , where Y = EAA deficiency if no other nutrient intake or MCP production, X = EAA requirement at growth rate of 150 g/d; Z = Milk EAA supply at 800g milk consumption

<sup>6</sup> Calculated as  $Y = X / (Z/1000)$ , where Y = EAA composition needed in Creep feed; X = EAA deficiency if no other nutrient intake or MCP production; Z = Creep feed consumption of 200 g/d

<sup>7</sup> Calculated as  $Y = X/Z*W$ , where Y = Suggested EAA composition for creep feed ration 2 at higher CP level of 17%; X = Suggested EAA composition for creep feed ration one at 15% CP, Z = 15%, W = 17%

**Table 3.2** Nutrient composition of two optimised creep feeds (CF1 and CF2) for amino acid composition and non structural carbohydrates and a commercial creep feed (CFC) as formulated by Tanqua Feeds (Riviersonderend, Western Cape, South Africa)

	<b>CFC</b> 139 g CP/kg; 455 g NSC/kg	<b>CF1</b> 157 g CP/kg; 477 g NSC/kg	<b>CF2</b> 179 g CP/kg; 508 g NSC/kg
	<b>Physical composition, DM (g/kg)</b>		
Soybean meal (47%)	11.14	84.03	125.44
Wheat bran	12.82	11.80	5.17
Yellow maize (8.5%)	273.95	324.74	414.75
Lucerne (grade 1)	151.37	162.72	246.96
Limestone	32.50	27.66	25.73
Salt	4.06	5.00	4.96
Apple pulp	162.51	185.97	93.48
Sheep+rumensin <sup>1</sup>	3.25	3.25	3.23
Molasses syrup	69.65	69.74	69.24
Fishmeal 60	-	15.11	-
Oat bran	26.70	23.25	-
Oats	150.90	139.47	-
Malt pellets	139.30	-	115.40
Threonine	-	2.67	2.19
MetaSmart <sup>2</sup>	-	-	0.46
Green colourant	-	-	0.35
Ammonium sulphate	6.85	-	-
	<b>Chemical Composition, DM</b>		
DM g/kg	861.48	860.37	866.54
ME MJ/kg	11.44	11.80	11.95
TDN g/kg	765.71	793.08	794.06
Degradable protein g/kg	84.52	91.29	110.06
Non protein nitrogen g/kg	1.41	-	-
CP g/kg	139.09	157.26	179.23
Bypass protein g/kg	48.92	59.57	66.14
Arginine g/kg	6.66	8.51	9.99
Lysine g/kg	4.99	7.45	8.43
Methionine g/kg	1.93	2.48	2.77
Threonine g/kg	4.73	8.12	9.11
Bypass lysine g/kg	1.58	2.62	2.80
Bypass methionine g/kg	0.73	0.99	1.09
Non Structural carbohydrates g/kg	454.91	477.35	508.60
Fat g/kg	37.84	40.29	38.44
NDF g/kg	337.43	284.29	266.70
Fibre g/kg	129.85	125.56	127.17

<sup>1</sup>Ionophore<sup>2</sup>Methionine

### **3.1.2 Management of ewes, pre and post lambing**

Forty-five twin bearing ewes (scanned to ensure that they were bearing twins) were brought to the University of Stellenbosch's experimental farm, Welgevallen, from the Heidelberg area in the Western Cape two months prior to lambing in the month of September 2009.

Ewes were housed indoors from 17h00 to 7h00 in a semi open building with slatted wooden floors. Within the building ewes were not confined into individual pens and had access to fresh drinking water from troughs.

Ewes were allowed to graze a kikuyu paddock of average quality under irrigation from 7h00 to 17h00 and were given access to supplement Wenlek (Tanqua feeds, Riviersonderend, South Africa) at 250 g/ewe/day (281 g CP/kg, 27.9 g CF/kg, 235.0 g NDF/kg, 127.8 g ADF/kg DM basis) after an adaptation period of two weeks. One month prior to parturition, lucerne mixed with molasses syrup (not more than 8% molasses syrup) was supplemented with Wenlek after the day's grazing to prevent Ca deficiency from the kikuyu grazing.

Two weeks before parturition ewes were adapted to a total mixed ration termed Lactating ewe pellets. As the ewes were to be completely housed indoors after parturition, a total mixed ration was necessary to provide all the nutrient requirements of the ewes. Lactating ewe pellets were formulated at the nutrient level set by the NRC (2007) for late lactating ewes with a body weight of 60 kg. Late lactating nutrient requirement levels were chosen so as to meet ewe requirements without stimulating high milk production in order to encourage lambs to consume creep feed. The rate of creep feed consumption is inversely related to the amount of milk intake (Ørskov, 1992) and therefore it was aimed to restrain the milk production of ewes. Calcium and phosphorus levels however were set at the early lactating level to prevent metabolic disorders from a deficiency such as milk fever. Ewes were allowed a daily lactating pellet intake of 1.9 kg/ewe/day as recommended by the NRC (2007). Table 3.4 indicates the physical and chemical composition of the lactating ewe pellets.

Up to peak lactation, Wenlek was supplied on top of the lactating ewe pellets. Peak lactation, which is a nutritionally high demanding phase, is also accompanied by lower appetite and thus lower feed intake. Wenlek was therefore supplied to supplement the lactating ewe pellets while increasing concentrate intake at peak lactation. Additional roughage was supplemented along with the lactating ewe pellets to increase rumination and also to compensate for lack of grazing behaviour thereby reducing stress by keeping ewes occupied. Lucerne and oat hay were used in a 1:2 ratio to fulfil the ewes' requirement.

At six weeks prior to lambing the ewes were dosed with an Ivomec injection (1 ml/50kg body weight) and vaccinated with Multivax P subcutaneously, to manage internal parasites and clostridia infections, respectively.

After birth ewes and their lambs were housed in individual 1.2 x 1.8 m pens within a semi open shed on wood slatted floors. Straw bedding was provided for up to three days after parturition. Ewes which



required intervention during birth, such as assistance in moving lambs from the birth canal, were given an antibiotic pessary as prescribed by a veterinarian. A pessary was necessary to prevent a fatal infection.

**Table 3.4** Physical and chemical composition of the lactating ewe pellets as fed after parturition at 1.9 kg/ewe/day

<b>Physical composition (DM %)</b>	
Wheat bran	8.72
Brewer's pellets	10.43
Yellow maize (8.5%)	7.06
Oat bran	26.07
Lucerne (grade 1)	12.73
Limestone	0.29
Salt	0.39
Monocalcium phosphate	0.17
Ammonium sulphate	0.50
Apple pomace	15.32
Sheep premix	0.17
Molasses syrup	3.48
Sunflower meal (%)	1.56
<b>Chemical composition from formulation (laboratory analyses, DM)</b>	
DM g/kg	869.79
ME MJ/kg	10.05
Crude protein g/kg	128.12
By pass protein g/kg	24.17
Isoleucine g/kg	5.68
Lysine g/kg	4.99
Methionine g/kg	2.15
Bypass lysine g/kg	1.66
Bypass methionine g/kg	0.78
Non structural carbohydrates g/kg	276.32
Fibre g/kg	223.92
Calcium g/kg	5.42
Total phosphate g/kg	3.49

Milk samples were randomly taken from ewes at various stages of lactation, so as to have milk samples representing early, medium and late lactation. Most ewes had become familiar and tame to the researcher and it was possible to milk the ewes by hand without an oxytocin hormone injection for milk let down. Milk let down, however, were enhanced when lambs' suckling behaviour at the udder was mimicked, i.e. stimulation of udder in the same manner as done by lambs. Milk samples were only intended to give an estimation of lambs' nutrient intake and were therefore pooled within each lactation phase. The pooled samples were frozen until the chemical analysis was done. Before analysis, the milk was defrosted and preserved with a dichromate preserver as used by the ARC Animal improvement scheme. Milk was analysed for total solids, protein, fat and lactose content. Milk was also analysed for amino acid content in the same way as with faeces and feed samples. Milk

however had to undergo freeze drying prior to hydrolysis seeing as the high amount of water content influences hydrolysis and subsequent amino acid results.

Milk consumption of lambs were estimated by weighing random lambs before and after suckling where the difference equals milk consumed.

### 3.1.3 Creep feed trial and lamb management

Ewes lambled over a three week period in November 2009. As lambs were born their birth weights were recorded within 24 hours (Santra & Karim, 1999) and their naval cords sprayed with Woundsep preventing possible fatal infection through the naval cord. After birth, ewes and their lambs were housed in a semi open shed within pens of 1.2 x 1.8 m on wood slatted floors. Ewes and their lambs were allowed bonding with their dam in individual pens until seven days after which commencement of the trial took place. Between three and seven days of age lambs' tails were docked and ear tags fitted while male lambs were kept intact.

Because lambs were born over a three week period they were divided into two groups to allow for the start of the trial to commence at an average lamb age of seven days. Once a group of lambs reached the average age of seven days they entered the creep feed trial period. Two groups were formed where group 1 entered the trial ten days prior to group 2. Commencement of trial represented Day 0 and lasted 60 days.

Each group was divided into four treatments, Creep feed 1 (CF1), Creep feed 2 (CF2), Creep feed control (CFC) and control (CON). Lambs were divided into their treatments in such a way as to maintain homogeneity within and between groups with regards to age, litter size and birth weight. Table 3.5 depicts the distribution of lambs in the four different treatments.

**Table 3.5** Assignment of lamb crop into their four homogenous treatments, Control, Creep control, creep feed 1 and creep feed 2

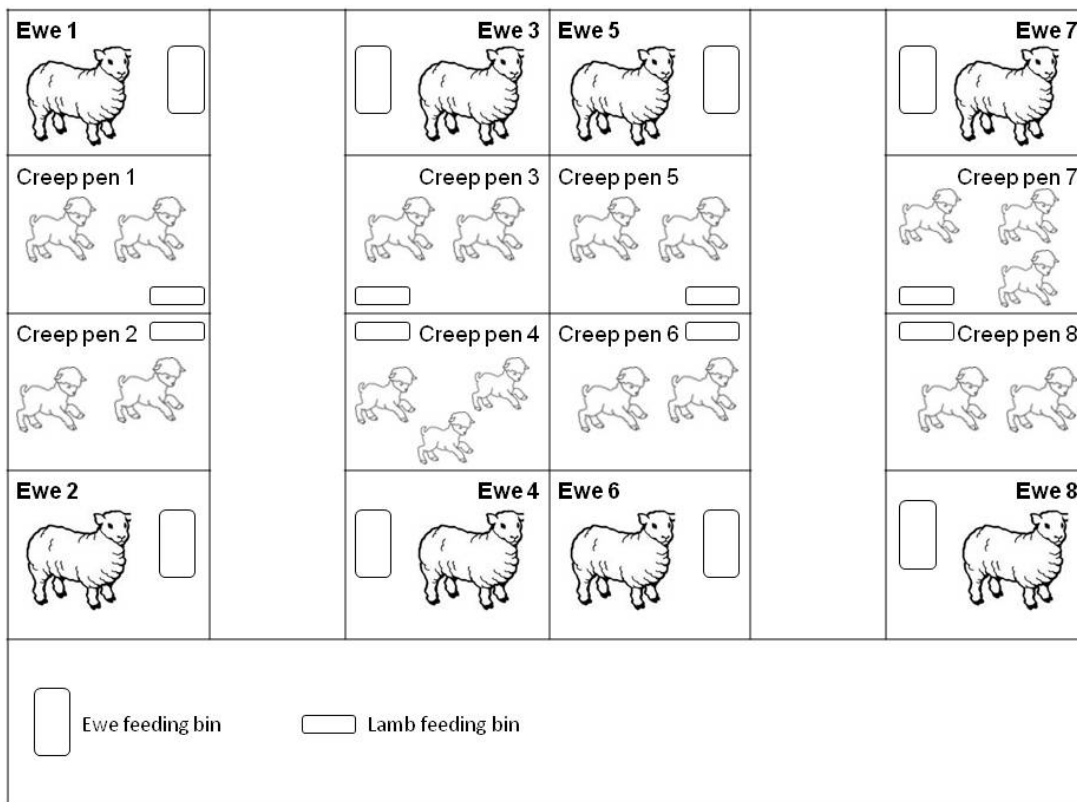
	CON (Pasture)	CFC 139 g CP/kg; 455 g NSC/kg	CF1 157 g CP/kg; 477 g NSC/kg	CF2 179 g CP/kg; 508 g NSC/kg
Average birth weights*	4.33 ± 0.23	4.53 ± 0.20	4.79 ± 0.25	4.18 ± 0.25
Number of single born lambs	1	1	1	1
Number twin borne lambs	6	7	8	7
Number triplet borne lambs	2	2	1	2
Total number of lambs	19	20	20	20
Total ram lambs	14	13	12	6
Total ewe lambs	5	7	8	14

\*Values represent mean ± s.e.

Control treatment lambs were allowed to graze for eight hours each day with their dams on a kikuyu pasture. Care was taken to ensure that CON lambs did not get access to any of the feed pellets used during the trial. This was accomplished by managing access to feed bags and also by removing the

pellets that spilled during handling of feed, when lambs were moved from the shed to the grazing paddock and *vice versa*. No restriction was placed on the amount and frequency of suckling for the CON lambs. After a day's grazing lambs and ewes were brought into the shed where fresh water and a lucerne- and oat hay mixture was supplied.

To encourage and determine lamb creep feed consumption, lambs were separated from their dams into an adjacent pen where visual and physical contact remained through the wired fence separating the individual pens, which in turn reduced stress at separation (Figure 3.1). Effort was made to arrange creep pens in such a manner that not only was contact maintained with the dam but also with lambs from other litters, which allowed for normal circadian behaviour (Figure 3.1).



**Figure 3.1** Arrangement of ewes and their lambs during separation to minimise stress while optimising creep feed intake

At day 0 of the creep feed trial (i.e. at seven days of age) lambs were separated from their dams for two hours two times a day (8h00 – 10h00 and 13h00 – 16h00) to initiate creep feed intake. From day 7 to 21 lambs were separated from their dams in the morning (7h00) and allowed a two hour suckling time at mid day and separated again until approximately 17h00 in the evening where the lambs were united again with their dams until the next morning. From day 21 to weaning, lambs were separated from dams during the day and united in the evenings from 17h00 until ca. 8h00 the next morning. Poe *et al.* (1969) also separated lambs from their dams to encourage adequate creep feed intake in a

similar way as the present study. Ørskov (1992) maintained that solid feed consumption is inversely related to the amount of milk intake and therefore above mentioned steps were taken to restrain milk intake in order to increase creep feed consumption.

Care was taken to feed ewes when lambs were separated in adjacent pens and not able to consume pellets intended for ewes. Lambs did however have access to dam's roughage which aided later in rumination and buffering of rumen pH.

Due to the fact that the separation of lambs from the same litter (for determination of individual feed intake and FCR) was not feasible, it was decided to treat a litter of lambs as if it was one animal, i.e. one experimental unit (E.U.).

Lambs were individually weighed weekly, on the same day at the same time. Control lambs were weighed first and let out to the grazing paddock as quickly as possible to ensure that the time spent on grazing is similar to the other non weighing days.

Both groups were slaughtered at the average age of 69 days for the analysis of meat characteristics and rumen development.

Blood samples were taken twice over the growth trial period, from the jugular vein into a heparin tube, to estimate blood urea nitrogen (BUN). Lamb age at blood collection period one averaged 43 and 34 days for group 1 and group 2, respectively; lamb age at second blood collection averaged 67 and 58 days for group 1 and 2, respectively. The BUN levels were analysed by the Western Cape Provincial Veterinary laboratory, Department of Agriculture, Stellenbosch. Blood urea nitrogen was measured to be compared to the nitrogen retention measurements from an *in vivo* digestibility trial as an indication of the efficient use of the dietary amino acids. Blood was taken from one lamb within an experimental unit, with preference to male lambs where possible. Male lambs were preferred to neutralise any possible gender effect. Due to the age difference between the two groups, one collection of blood on a day represents BUN levels at two different ages, i.e. the two blood collection periods represent BUN levels at four different ages namely 34, 43, 58 and 67 days.

At approximately six weeks of age lambs were dosed with Panacur (0.5 ml/10kg body weight) and vaccinated with Covexin. Panacur, an anthelmintic, provided protection against the internal parasites: roundworm, lungworm and milk tapeworm while Covexin immunised lambs against diseases from *clostridia* organisms.

### **3.1.4 Slaughter of lambs, and collection of slaughter data**

At weaning, 40 lambs were randomly chosen for slaughter, representing 10 lambs per treatment. Care was taken to select at least one lamb from every E.U. in order to have 10 lambs per treatment.

Lambs were slaughtered at an average age ( $\pm$  s.d.) of  $69 \pm 4$  days with an average weight of  $23.6 \pm 0.59$  kg and were not fasted before slaughter. Lambs selected for slaughter were taken from their experimental units and transported in groups of 10 to the abattoir less than 500 m from their holding

area. One lamb at a time was offloaded and slaughtered before the next lamb was taken out. At slaughter lambs were electrically stunned (4 s at 200 V) where after the jugular veins were severed and exsanguination took place. After exsanguination the lambs were weighed, dressed and eviscerated according to standard South African techniques. Kidneys and pelvic fat were left intact.

Immediately after exsanguination, lambs were weighed and recorded as body weight at slaughter. Carcass weight represents the weight after removal of head, trotters, skin and internal oval with the exception of the kidneys and kidney- and pelvic fat and recorded as carcass weight (kg). Head, trotters, skin and organs were individually weighed and recorded. Dressing percentage was calculated as follow: carcass weight ÷ body weight at slaughter multiplied by a 100.

The carcasses were suspended by both the Achilles tendons in a cooler at 4°C for 24 hours. The *M. longissimus dorsi* was removed on both the left and right half of the carcass between the 2<sup>nd</sup> - 3<sup>rd</sup> last thoracic vertebrae and the 4<sup>th</sup> - 5<sup>th</sup> lumbar vertebrae. The left and right *M. longissimus dorsi* samples were used for proximate chemical and physical analyses respectively. Back fat thickness was determined on the carcass where the samples were removed with a digital calliper.

The liver was weighed with gall bladder intact. Lungs were weighed with trachea intact. Omental fat was removed around the gastrointestinal tract and weighed. The small and large intestines were separated from each other at the caecum and weighed separately with contents remaining. Reticulo-rumen weights were recorded as full reticulo-rumen weight and empty reticulo-rumen weight. Reticulo-rumen represents the reticulo-rumen with oesophagus intact, severed at the pyloric junction of the small intestine. Full reticulo-rumen was weighed with contents. For rumen histological analysis a rumen wall sample had to be taken after the contents was removed but before proper washing could take place in order for papillae to sustain minimum damage, therefore empty reticulo-rumen weight represents weight of empty reticulo-rumen after a 2 x 3 cm sample was taken.

## 3.2 Laboratory methodologies

### 3.2.1 Moisture

Moisture was determined as prescribed by the AOAC (2002a) method 934.041. Moisture free porcelain crucibles were weighed and recorded. A 2 g sample was weighed into a crucible where after it was placed for 24 hours in a 100°C drying oven. After 24 hours the crucibles were placed into a desiccator to cool down to room temperature for 30 minutes where after it was weighed and recorded. Calculation:

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

The dry matter (DM) was calculated as follows: DM % = 100 - % moisture

### **3.2.2 Gross energy**

Gross energy was determined using a CP 500 Bomb Calorimeter instrument (Eltra Africa, Springs, South Africa).

Samples were pressed into pills of ( $\pm$  s.d.)  $0.5 \pm 0.1$  g. Each sample was placed on a melting wire (attached to two electrode points) suspended above a crucible that forms part of the electrode. The electrode was placed inside a bomb and filled with oxygen until a pressure of 3000 kPa was reached. The bomb was placed into the bomb calorimeter where after the sample was ignited; results were given in MJ/kg as is.

### **3.2.3 Crude protein and amino acid analyses**

Crude Protein (CP) was determined by the Dumas combustion method 992.15 (AOAC, 2002c) using the Leco® FP – 528 Nitrogen & Protein Analyzer (Leco® Corporation, St. Joseph, USA) which analysis the nitrogen percentage of a sample which in turn is multiplied with a factor of 6.25 (for feed, faecal, urine and meat samples) to gain the CP percentage. Alfalfa (Leco®) with a nitrogen percentage ( $\pm$  s.d.) of  $3.32 \pm 0.04\%$  was used as the standard to calibrate the Leco® for feed and faecal samples; EDTA (Leco®) with a nitrogen percentage ( $\pm$  s.d.) of  $9.57 \pm 0.03\%$  was used as the standard for meat samples. Samples were weighed into a tin foil cup closed and twisted and the weight recorded. Samples were then placed into the rotating platform of the Leco® where after it goes through the combustion process.

The amino acid hydrolyses were done at the Department of Animal Sciences (Stellenbosch University) where after amino acid analyses were done at the Central Analytical Facility in the Department Biochemistry (Stellenbosch University).

Amino acids were determined by ion-exchange chromatography of the acid-hydrolyzed protein. Samples were hydrolyzed (AOAC, 1997) with 6 M HCl in a sealed tube under nitrogen for 24 hours at  $110^{\circ}\text{C}$ . Thereafter samples were diluted and derivatised using the EZ:Faast LC method (with EZ:Faast column) as described in the user's guide. Labelled Homoarginine, Methionine-D3 and homophenylalanine which is part of the EZ:Faast kit was included as internal standards. A Waters API Quattro Micro system was used for the determination of the amino acid composition.

### **3.2.4 Neutral detergent fibre**

Feed neutral detergent fibre (NDF) was determined using the Fibertec instrument as described by Robertson and Van Soest (1981). A one litre neutral detergent solution (NDS) was made up of 30 g Sodium laurylsuphate in 500 ml of distilled water with 10 ml of 2-ethoxyethanol added to it. 18.61 g of EDTA and 6.81 g of  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$  was added to 200 ml distilled  $\text{H}_2\text{O}$  and heated to dissolve. After dissolving, it was added to the sodium laurylsulphate. 4.56 g of  $\text{Na}_2\text{HPO}_4$  was dissolved in 100 ml of distilled water and added to the other solutions. A 1 g sample with 100 ml cold NDS was brought to boil at  $100^{\circ}\text{C}$  where after 0.1 ml  $\alpha$ -Amylase (Sigma # A3306) was added and temperature decreased to  $65^{\circ}\text{C}$ . After an hour's boiling at  $65^{\circ}\text{C}$ , the sample was drained of NDS and washed three times with

boiling water and once with a small amount of acetone. Samples were placed in 100°C oven for 48 hours where after it was cooled to room temperature in a desiccator and weighed. After the weight was recorded the sample was ashed in a 500°C oven for 6 hours. Samples were cooled for two hours within the oven to about 200°C and to room temperature in a desiccator and weighed.

Calculation:

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

Faecal NDF analyses were done with the ANKOM<sup>200/220</sup> (Ankom® Technology Corp., Fairport, NY, USA) Fiber Analyzer according to the manufacturer's method ([www.ankom.com](http://www.ankom.com)). After filter bags were washed with acetone and dried in 100°C oven for two hours, a 0.5 ± 0.05 g sample was weighed into a filter bag and heat sealed. Sample weight was corrected for moisture content. Samples were placed within the ANKOM<sup>200/220</sup> (Ankom® Technology Corp., Fairport, NY, USA) with a neutral detergent solution (NDS) as prescribed by ANKOM (Ankom® Technology Corp., Fairport, NY, USA) with added sodium sulphite and heat stable alpha-amylase. Samples were washed for 75 minutes at 100°C. After the NDS solution was exhausted from the ANKOM<sup>200/220</sup> (Ankom® Technology Corp., Fairport, NY, USA) the samples were rinsed once with hot water and alpha amylase and thereafter another two times with hot water. Excess water was pressed out of the sample bags which were then soaked in acetone for three minutes. After the sample bags were allowed to air dry, thereby allowing the acetone to evaporate, the bags were dried in a 100°C oven for at least two hours. After drying sample bags were weighed out of a desiccator.

Calculation:

$$\text{NDF\%} = \frac{W_3 - (W_1 \times C_1)}{W_2 \times \text{DM}}$$

W<sub>1</sub> = bag tare weight (g)

W<sub>2</sub> = sample weight (g)

W<sub>3</sub> = weight after extraction process (g)

C<sub>1</sub> = blank bag correction (final oven dried weight/original blank bag weight)

### **3.2.5 Acid detergent fibre**

Feed ADF was determined by the method of Goering & Van Soest, (1970) as follows on the Fibertec instrument. One litre of acid detergent solution (ADS) was made up by adding 20 g N-Cetyl-N,N,N-trimethyl Ammonium Bromide (CTAB) to one litre standardised H<sub>2</sub>SO<sub>4</sub>. The H<sub>2</sub>SO<sub>4</sub> was made up of 28 ml 98% H<sub>2</sub>SO<sub>4</sub> in one litre distilled H<sub>2</sub>O. A 1 g sample with 50 ml cold ADS was brought to the boil at 100°C where after the temperature was decreased to 65°C. After an hour's boiling at 65°C, the sample was drained of ADS and washed three times with boiling water and once with ca. 2ml of acetone. Sample was placed in 100°C oven for 24 hours where after it was cooled to room temperature in a desiccator and weighed. After weight was recorded the sample was ashed in a

500°C oven for six hours. Samples were cooled for two hours within oven to about 200°C and to room temperature in a desiccator and weighed.

Calculation:

$$\text{ADF \%} = \frac{[\text{sample residue after 24hour drying (g)}] - [\text{sample residue after ashing (g)}]}{\text{Sample mass (g)} \times [\text{sample DM \%}]} \times 100$$

Faecal ADF analyses were done with the ANKOM<sup>200/220</sup> (Ankom® Technology Corp., Fairport, NY, USA) Fiber Analyzer according to the manufacturer's method number five ([www.ankom.com](http://www.ankom.com)). After filter bags were washed with acetone and dried in a 100°C oven for two hours, a 0.5 ± 0.05 g sample was weighed into a filter bag and heat sealed. Sample weight was corrected for moisture content. Samples were placed within the ANKOM<sup>200/220</sup> with an acid detergent solution (ADS) as prescribed by ANKOM and similar to the solution as used for feed ADF analysis. Samples were washed for 60 minutes at 100°C. After the ADS solution was exhausted from the ANKOM<sup>200/220</sup> the samples were rinsed three times with hot water for five minutes. Sample bags coming out of the ANKOM<sup>200/220</sup> were pressed to relieve of excess water and soaked in acetone for three minutes. After sample bags were allowed to air dry for acetone to evaporate it was dried in a 100°C dry oven for at least two hours. After drying sample bags were weighed out of a desiccator in order for bags to remain moisture free.

Calculation:

$$\text{NDF\%} = \frac{W_3 - (W_1 \times C_1)}{W_2 \times \text{DM}} \times 100$$

W<sub>1</sub> = bag tare weight (g)

W<sub>2</sub> = sample weight (g)

W<sub>3</sub> = weight after extraction process (g)

C<sub>1</sub> = blank bag correction (final oven dried weight/original blank bag weight)

### **3.2.6 Ether extract**

An ether extract (EE) method was used to measure the percentage of fat in the samples (method 920.39; AOAC, 2002d). The Tecator Soxtec System HT 1043 Extraction unit instrument was used with diethyl ether using the following method:

- Clean aluminium soxtec-cups were placed over night in a 100°C oven
- Aluminium soxtec-cups were cooled to room temperature in a dessicator for 30 minutes
- Weight of the aluminium soxtec-cups were recorded
- A 2 g sample was weighed into cellulose extraction thimbles (Whatman®, 30 mm x 80 mm)
- A small piece of cotton was placed in the top of each extraction thimble to prevent sample spillage during the rinse cycle
- Aluminium soxtec-cups were filled with 100 ml diethyl ether
- Thimbles were placed in the extraction tubes of the Tecator Soxtec instrument



- Aluminium soxtec-cups were placed on the Tecator Soxtec's element alongside the corresponding thimble in the extraction tube
- Thimbles were lowered into the ether and allowed to boil for 15 minutes
- After boiling thimbles were rinsed for 30 minutes
- The valve was closed to allow the ether to collect, for 15 minutes
- Aluminium soxtec-cups were placed into a dry oven for 2 hours to allow ether to evaporate (100°C oven)
- Aluminium soxtec-cups were placed in a desiccator for 30 minutes to cool down to room temperature
- Aluminium soxtec-cups were weighed and recorded out of a dessicator

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

### **3.2.7 Ash**

Ash was determined as prescribed by the AOAC (2002b) method 942.05. Moisture free porcelain crucibles were weighed before a 2 g moisture free sample was weighed in each porcelain crucible. The crucibles were placed in a furnace set at 500°C for 6 hours. After 6 hours the furnace was allowed to cool down for about 2 hours before the crucibles were taken out and placed into a desiccator to cool down to room temperature and weighed.

Calculation: Ash % =  $\frac{[\text{Mass of crucible and ash (g)}] - [\text{empty dry crucible (g)}]}{\text{Sample weight (g)}} \times 100$

The Organic matter was calculated as follows: Organic matter (%) = 100 – Ash%

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## **CHAPTER 4**

### **LAMB GROWTH FROM BIRTH TO WEANING AS A RESPONSE TO OPTIMISED CREEP FEED DIETS**

#### **Abstract**

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Specialised creep feeds optimised for the limiting essential amino acids (EAA) and non structural carbohydrate (NSC) levels were tested for its effect on Döhne-Merino x Mutton Merino lamb performance. The aim of this study was to determine the EAA required by suckling lambs and its effect with NSC's in a creep feed on lamb growth performance with specific reference to its effect on feed efficiency, average daily gain, and carcass composition. The limiting EAA for suckling lambs were determined and used to formulate two creep feeds at crude protein levels of 157 g/kg and 179 g/kg, respectively with NSC levels at 477 g/kg and 508 g/kg. A commercial creep feed with no optimisation for EAA was also formulated, representing a CP level of 139 g/kg and a NSC level of 455 g/kg. These creep feeds represent the treatments CF1 (CP 157 g/kg; NSC 477 g/kg), CFC2 (CP 179 g/kg; NSC 508 g/kg) and CFC (CP 139 g/kg; NSC 455 g/kg); a negative control (CON) treatment was included to represent lambs with no creep feed access but *ad lib.* suckling with dams on kikuyu pasture. The trial was conducted on twin lambs averaging a birth weight of 4.42 kg  $\pm$  0.11 for 60 days. After the growth trial half of the lamb crop was slaughtered at the averaged live weight of 23.6 kg  $\pm$  0.56. Results indicated that the feed conversion ratio for CFC lambs were more efficient than CF1 but not more than CF2. Carcass weight did differ significantly between CON and the creep feed treatments, however no difference was found between creep feed treatments. Dressing percentage was higher for CF2 than both CFC and CF1. It was surmised that the initial estimation of the EAA required was underestimated; therefore the similar performance between creep feeds. It was further concluded that the CP level of CF2 was set too high as lambs from this treatment had a lowered growth response as compared to CF1 indicating that energy was probably spent deaminating excess amino acids.

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Keywords: nursing lambs pre-ruminant, pre-weaning lambs, starch and sugar,

#### **4.1 Introduction**

The benefits of creep feeding in intensive sheep production systems are well known and to such an extent that many commercial creep feeds are available on the market today. The animal feed industry is a very competitive market; producers, in turn, are looking for ways to reduce their feed costs by way of increasing feed efficiency and output production.

Demands for animal protein such as meat, milk and eggs have increased worldwide, especially in developing countries, owing to the fact that more consumers are able to afford it ([www.oup.com](http://www.oup.com); AFMA, Chairman's report 2009/2010). Raw material production and availability for the feed industry in turn has decreased in the last few years ([www.oup.com](http://www.oup.com); AFMA, Chairman's report 2009/2010); this slowdown in production was expected and attributed to high grain prices and to the effect of the global recession on animal protein consumption (AFMA, Chairman's report 2009/2010). In light of these factors it could be inferred that a good profitable creep feed ration should allow for optimum utilisation and feed efficiency while minimising feed wastage. This will allow for higher lamb production while lowering feed intake and total cost on farm level.

Such creep feed rations for lambs require the correct balance of energy, protein and minerals while the composition of it must stimulate the development of the immature rumen (Jones *et al.*, 1996).

In terms of protein, an increased growth as well as improved feed efficiency was found in lambs when the protein levels of the diet increased (Craddock *et al.*, 1974, Jones *et al.*, 1996). Protein quality in turn is determined by its amino acid profile. The ideal amino acid concept stipulates that the absence or undersupply of a single essential amino acid will inhibit further synthesis of protein even if the other amino acids are in adequate supply (Cole & van Lunen, 1994; Cant *et al.*, 2003). A good quality protein therefore would be a protein supplying those essential amino acids required by an animal allowing for optimum protein deposition.

Although ruminants' microbial population are able to synthesise essential and non essential amino acids the physiological demand of high producing animals require higher amounts of amino acids than what can be supplied via the microbial protein (Jones *et al.*, 1996). Microbial protein has been confirmed to be deficient in the EAA's Histidine, Methionine, Leucine, Arginine and Phenylalanine by Nolte (2006). It is therefore apparent that ruminants also require rations balanced for essential amino acids (EAA) which are supplied as undegradable rumen protein (UDP).

However, the following study is relative to suckling lambs which are only considered as fully functional ruminants at two months after birth. As microbial crude protein (MCP) production is calculated at 13% of the TDN intake (NRC, 1985) the protein contribution from microbial protein is almost negligible at first as very little dry feed is consumed soon after birth. Since the amino acid requirement of an animal is linked to its performance level (Titgemeyer, 2003; Nolte, 2006) it is likely that lambs that are capable of higher performance (genetically) require more amino acids than that provided by the milk from its dam, quantitatively and qualitatively.

In terms of energy balance it has been indicated that as the ratio of available energy to nitrogen increases, the amount of microbial protein production also increases (Hammond, 1992). Hence that the readily fermentable carbohydrates, such as that found in the non structural carbohydrate (NSC) fraction of feeds (Carruthers & Neil, 1997), are the most vital energy sources for the microbial population (NRC, 2007). Pre-ruminants further specifically require this energy source for the proliferation of a microbial population in their undeveloped rumens. This immature population requires

feed that is highly fermentable with a fast outflow rate of indigested feed (Cheng, 1991). Higher proportion of butyrate and propionate production from the fermentation of NSC (Stern *et al.*, 1978) in turn has been shown to stimulate rumen papillae development (Baldwin & McLeod, 2000).

It was therefore hypothesised that a creep feed ration formulated to meet the limiting EAA requirement of pre-weaning lambs at a certain NSC level would prove to be superior over a commercially available creep feed. The optimised creep feed would therefore prove to elicit a higher growth rate and improved feed efficiency in lambs as compared to a commercial creep feed.

## **4.2 Materials and methods**

A detail description of the creep feeds' compilation was given in the previous chapter and is therefore discussed here in short relevant detail while ewe management (Chapter 3, section 3.1.2) is omitted from this section as it only plays an indirect part of the study and to avoid unnecessary repetition.

The study was conducted at the University of Stellenbosch's experimental farm, Welgevallen.

### **4.2.1 Compilation of creep feeds**

Amino acid requirement for lambs were estimated based on the work by Nolte (2006) and used to compile a creep feed meeting the amino acid requirement of lambs. The creep feeds were compiled based on the assumption that lambs would grow at 150 g/day while consuming 800 g/day of milk and 200 g/day of creep feed. Detailed description of how the amino acid requirements for suckling lambs were estimated and the subsequent feed compilation is found in chapter 3.

Nolte (2006) determined Histidine, Methionine, Leucine, Arginine and Phenylalanine as the first limiting amino acids for Merino and Döhne-Merino lambs. In chapter 3 it was estimated that Lysine, Threonine, Methionine, Isoleucine, Phenylalanine and Leucine needed to be included in a creep feed diet at levels of 7.45, 8.10, 1.78, 0.32, 4.39, and 3.51 g/kg respectively for suckling lambs.

Creep feed 1 (CF1) was optimised for these amino acids at 157 g CP/kg and 477 g NSC/kg feed. A second creep feed (CF2) was optimised for these amino acids at higher CP and NSC levels namely 179 g CP/kg and 508 g NSC/kg feed respectively. As control, a commercial creep feed (CFC) was formulated at 139 g CP/kg and 455 g NSC/kg feed without limiting EAA optimisation.

Tanqua Feeds (Riviersonderend, Western Cape, South Africa) mixed all the creep feeds; Table 4.1 indicates the physical and chemical composition of the three creep feed mixtures as formulated by the company.

**Table 4.1** Nutrient composition of two optimised creep feeds (CF1 and CF2) for amino acid composition and non structural carbohydrates and a commercial creep feed (CFC) as formulated by Tanqua Feeds (Rivieronderend, Western Cape, South Africa)

	<b>CFC</b> 139 g CP/kg; 455 g NSC/kg	<b>CF1</b> 157 g CP/kg; 477 g NSC/kg	<b>CF2</b> 179 g CP/kg; 508 g NSC/kg
	<b>Physical composition, DM (g/kg)</b>		
Soybean meal (47%)	11.14	84.03	125.44
Wheat bran	12.82	11.80	5.17
Yellow maize (8.5%)	273.95	324.74	414.75
Lucerne (grade 1)	151.37	162.72	246.96
Limestone	32.50	27.66	25.73
Salt	4.06	5.00	4.96
Apple pulp	162.51	185.97	93.48
Sheep+rumensin <sup>1</sup>	3.25	3.25	3.23
Molasses syrup	69.65	69.74	69.24
Fishmeal 60	-	15.11	-
Oat bran	26.70	23.25	-
Oats	150.90	139.47	-
Malt pellets	139.30	-	115.40
Threonine	-	2.67	2.19
MetaSmart <sup>2</sup>	-	-	0.46
Green colourant	-	-	0.35
Ammonium sulphate	6.85	-	-
	<b>Chemical Composition, DM</b>		
DM g/kg	861.48	860.37	866.54
ME MJ/kg	11.44	11.80	11.95
TDN g/kg	765.71	793.08	794.06
Degradable protein g/kg	84.52	91.29	110.06
Non protein nitrogen g/kg	1.41	-	-
CP g/kg	139.09	157.26	179.23
Bypass protein g/kg	48.92	59.57	66.14
Arginine g/kg	6.66	8.51	9.99
Lysine g/kg	4.99	7.45	8.43
Methionine g/kg	1.93	2.48	2.77
Threonine g/kg	4.73	8.12	9.11
Bypass lysine g/kg	1.58	2.62	2.80
Bypass methionine g/kg	0.73	0.99	1.09
Non Structural carbohydrates g/kg	454.91	477.35	508.60
Fat g/kg	37.84	40.29	38.44
NDF g/kg	337.43	284.29	266.70
Fibre g/kg	129.85	125.56	127.17

<sup>1</sup>Ionophore<sup>2</sup>Methionine

#### 4.2.2 Creep feed trial and lamb management

Ewes (Döhne-Merino x Mutton Merino) lambed over a three week period from 28 October to 20 November 2009. As lambs were born their birth weights were recorded within 24 hours (Santra &

Karim, 1999) and their naval cords sprayed with Woundsep preventing possible fatal infection through the naval cord. After birth ewes and their lambs were subsequently housed in a semi open shed within pens of 1.2 x 1.8 m with slatted floors. Ewes and their lambs were allowed bonding with their dam in individual pens until seven days after which commencement of trial took place. Between three and seven days of age lambs' tails were docked and ear tags fitted while male lambs were kept intact.

Lambs had to enter the creep feed trial at seven days of age and therefore the lamb crop was subdivided into two groups to maintain a seven day average age between lambs. Once a group of lambs reached an average age of seven days they entered the growth trial. Two groups were formed where Group 1 entered the trial ten days prior to Group 2. Commencement of trial represents Day 0 and lasted 60 days.

Each group was divided into four treatments, CF1, CF2, CFC and a negative control on pasture (CON). Similar to the study of Joy *et al.* (2008) the lambs were divided into their treatments in such a manner as to maintain high homogeneity within and between treatments. Care was taken to ensure a balanced distribution of litter sizes, and age between the treatments.

The CON treatment was allowed to graze for eight hours each day with their dams on a kikuyu pasture. Care was taken to ensure that CON lambs did not get access to any of the feed pellets used during the trial. This was accomplished by managing access to feed bags and also by removing the pellets that spilled during handling of feed when lambs were moved from the shed to the grazing paddock and *vice versa*. No restriction was placed on the amount and frequency of suckling for the CON treatment. After a day's grazing lambs and ewes were brought indoors where fresh water and a lucerne- and oat hay mixture was supplied.

Lambs in creep feed treatments were housed indoors on slatted floors. To determine lamb creep feed intake the lambs were separated from their dams into an adjacent pen where visual and physical contact remained through the wired fence separating the individual pens, which in turn reduced stress at separation (Figure 3.1). Effort was made to have lambs from different dams in adjacent pens at time of separation to aid in learning behaviour from one another.

At day 0 of the creep feed trial (i.e. at seven days of age) lambs were separated from their dams for two hours two times a day (8h00 – 10h00 and 13h00 – 16h00) to initiate creep feed intake. From day 7 to 21 the lambs were separated from their dams in the morning (7h00) and allowed a two hour suckling time at mid day and separated again until 17h00 in the evening where the lambs were united again with their dams until the next morning. From day 21 to weaning, lambs were separated from dams for the whole day and united only in the evenings from 17h00 until ca. 8h00 the next morning.

Care was taken to feed ewes (with lambs in the creep feed treatments) when lambs were separated in the adjacent pen and not able to consume pellets intended for ewes. Ewes, however consumed their pelleted feed at different rates which resulted in some left over pellets being consumed by creep feed treatment lambs. Lambs furthermore had access to dam's roughage after reunion.

A litter of lambs were regarded as one experimental unit (E.U.) and because separation of siblings was not feasible, feed intake of lambs were measured as if for one animal. Daily feed intake was measured from the average intake over a week divided by seven. Lambs did however receive fresh water and feed daily.

Lambs were weighed weekly, on the same day at the same time. Lambs in the two different age groups were weighed on their particular weighing day. This method also ensured that weighing commenced faster and lambs were allowed to consume feed sooner. CON lambs were weighed first before they were taken out to graze. On weighing days an attempt was made to keep CON lambs on pasture as long as they would have been on pasture if they were not weighed, i.e. they were kept on pasture for eight hours.

To estimate milk intake of lambs, the lamb suckling weight differential technique (Doney *et al.*, 1979) was used. Lambs were weighed before and after suckling their dams over a two hour period. The difference in weight was recorded as the amount of milk consumed. In a similar method to Ewbank (1967) the suckling rate (frequency of suckling) of lambs for the three hours of daylight in which they were united with their dams were calculated. Ewbank (1967) divided the number of times a lamb was seen to suckle by the day time watch time and then multiplied it by 12 to get the frequency of suckling expressed on 12 hours.

Both groups were slaughtered at an average age of  $69 \pm 4$  days. Lambs in Group 1 were slaughtered five days after the recording of their last live weight and Group 2 was slaughtered three days from their last weighing day. These weights represent final body weight (FBW). Weight recorded after exsanguination represents weight at slaughter (WAS).

#### **4.2.3 Slaughter of lambs, and collection of slaughter data**

Fourty lambs were randomly allocated to be slaughtered. Effort was however made to include at least one lamb per experimental unit, where it was possible, to allow for 10 lambs per treatment.

As mentioned previously, lambs were born over three weeks and were therefore divided into two groups to maintain homogeneity with regards to age difference between treatments. Group 1 was slaughtered eight days before Group 2. Lambs were not fasted before slaughter. Lambs selected for slaughter were taken from their experimental units and transported in groups of 10 to the abattoir less than 500 m from their holding area. One lamb at a time was offloaded and slaughtered before the next lamb was taken out. At slaughter lambs were electrically stunned (4 s at 200 V) where after the jugular veins were severed and exsanguination took place. After exsanguination the lambs were weighed, dressed and eviscerated. The carcasses were suspended by both the Achilles tendons in a cooler at 4°C for 24 hours.

Carcass weight represents the weight after removal of head, trotters, skin and internal oval with the exception of the kidneys and the pelvic fat depots and recorded as carcass weight (kg). Head,



trotters, skin and organs were individually weighed and recorded. Dressing percentage was calculated as follows:

$$DP = (\text{carcass/body weight at slaughter}) * 100$$

Reticulo-rumen represents the reticulo-rumen with oesophagus intact, severed at the pyloric junction with small intestine. Full reticulo-rumen was weighed with contents. For a subsequent study (Chapter 6) a rumen wall sample had to be taken after the contents was removed but before proper washing could take place in order for papillae to sustain minimum damage, therefore empty reticulo-rumen weight represents weight of the empty-reticulo rumen after a 2 x 3 cm sample was taken.

The liver was weighed with gall bladder intact. Lungs were weighed with trachea intact. Omental fat was removed around the gastrointestinal tract and weighed. The small and large intestines were separated at the caecum and weighed separately with contents remaining.

#### **4.2.4 Laboratory analyses**

The five different feeds used during this study were subjected to proximate chemical analyses in the laboratory of the Animal Sciences Department, University Stellenbosch. Moisture, CP, ether extract (EE) and ash were analysed according to the Association of official analytical chemists Inc. (AOAC, 2002). NDF was determined as according to Robertson & van Soest (1981) while ADF was determined as according to Goering & van Soest (1970). Detailed description of these analyses was given in Chapter 3.

### **4.3 Statistical analyses**

Statistical analyses were done with the aid of SAS Enterprise guide 4.0, version 9.1.3 (2002), using Proc GLM. Least square means were estimated and significance declared at the  $P \leq 0.05$ .

A complete randomised design was used.

Linear regressions were fitted to the weekly weights and weekly cumulative creep feed consumptions to obtain the ADG and daily feed intake estimates from the slopes of the linear regression. The slopes in turn were analysed for treatment differences. The intercept of the slope was used as a covariate.

Birth weight had an effect ( $P < 0.0001$ ) on all the slaughter data; similarly final body weight (FBW) also had an effect ( $P < 0.0001$ ) on the slaughter data. Birth weight and FBW therefore needed to be included in the model as covariates. It was found however, that birth weight was significantly ( $P < 0.0001$ ) correlated to FBW ( $r = 0.59$ ) and couldn't be included in the same model.

Based on the  $R^2$  value of the respective parameters, the best covariate was used on each slaughter characteristic. Weight at slaughter, carcass weight, dressing percentage and back fat thickness had birth weight as a covariate while the rest of the slaughter data had Final body weight as covariate. Gender had no significant effect on any of the growth parameters and was therefore not included as a main effect in the model.

## 4.4 Results and discussion

A total of 83 lambs were available for the growth trial, of which 79 lambs were available for the statistical analysis of the growth trial while only 40 lambs were slaughtered to obtain carcass composition data. Three lambs were fostered on cow's milk and therefore not used for statistical analyses because the feeding and nutrition differed from that of the other lambs. One lamb died from an unknown cause towards the end of the trial. Table 4.2 indicates the distribution of the lamb crop between the various treatments where lamb birth weight represents the average for individual lambs.

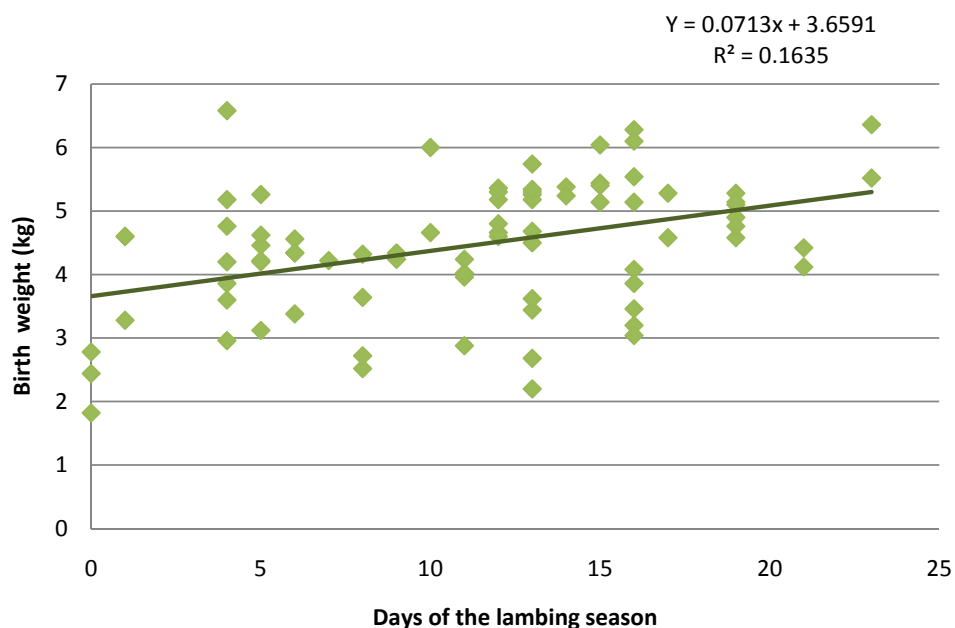
As mentioned, the lamb crop was divided into two trial groups, to ensure that lambs enter the trial at seven days of age, reducing variance between lambs within treatments. The term *Group* refers to the separation of the lamb crop entering the trial at different times, similarly the term *treatment* refers to the different dietary treatments, CON, CFC, CF1 and CF2.

**Table 4.2** Assignment of lamb crop into four homogenous dietary treatments, control (CON), creep control (CFC), creep feed 1 (CF1) and creep feed 2 (CF2)

	<b>CON</b> (Pasture)	<b>CFC</b> 139 g CP/kg; 455 g NSC/kg	<b>CF1</b> 157 g CP/kg; 477 g NSC/kg	<b>CF2</b> 179 g CP/kg; 508 g NSC/kg
Mean birth weights* (kg)	4.33 ± 0.23	4.53 ± 0.20	4.79 ± 0.25	4.18 ± 0.25
Number of single born lambs	1	1	1	1
Number twin borne lambs	6	7	8	7
Number triplet borne lambs	2	2	1	2
Total number of lambs	19	20	20	20
Total ram lambs	14	13	12	6
Total ewe lambs	5	7	8	14

\* Values represent LSMean and SE

Group 2, consisting of lambs born later in the lambing season, had significantly heavier birth weights than lambs in Group 1; this increase in birth weight as the lambing season progresses coincides with the studies of Shelton (1964) and Juma & Faraj (1966). Figure 4.1 illustrates how the birth weight of lambs from this study increased as the lambing season progressed. According to Figure 4.1 the average birth weight was 3.65 kg and increased by 70 g per day as the season progressed. The R<sup>2</sup> value for birth weight influenced by lambing day/season was very low for this fitting and if interpreted it means that only 16% of the variation was due to the effect of the lambing season; this effect was however still significant.



**Figure 4.1** Lamb birth weight as affected by the progress of the lambing season, 28 October – 20 November 2009

When the effect of diet on the growth parameters such as ADG and FBW were analysed, it became evident that the potential effect that siblings can have on the milk and/or feed intake had to be taken into account as well. Siblings in a given litter were maintained together in indoor pens, and therefore the interaction and competition for udder and feed trough space was more frequent than it would have been if they were maintained outdoors. It can thus be postulated that an individual lamb's milk/feed intake is affected by its sibling's milk/feed intake. The dominant lamb would potentially consume more milk or feed, which would potentially have the effect that the dominant lamb would outperform its less dominant sibling in terms of ADG and FBW. Competition between siblings can therefore influence the potential effect that diet can have on the ADG and FBW of lambs, resulting in a greater degree of variation within treatment groups. This was indeed observed in the current study when each lamb was treated as an experimental unit. However, when siblings were treated as an experimental unit, less variation and significant differences were found in terms of ADG and FBW.

It is for this reason, and the fact that individual feed intake could not be determined, that growth parameter data were analysed per experimental unit (E.U.), i.e. growth data were analysed for statistical differences for each litter and indicated as such. However, individual lamb performance will also be referred to in some of the text below to function as a reference for lamb growth in general and also to use as comparison with that found in literature.

Furthermore the effect of triplets and single lambs contributed to variation within treatments due to litter effects, as discussed above, and were therefore omitted in statistical analysis because the numbers of triplet and single litters were too few to analyse growth parameters upon litter basis.

#### 4.4.1 Creep feed growth parameter results

Table 4.3 represents the growth parameter data per E.U. Average E.U. birth weight showed no significant difference between treatments. Therefore it could be inferred that treatments were equally balanced for birth weight and that there were no biased treatments at commencement of trial.

Individual lamb birth weight was lower compared to the South African Mutton Merino (SAMM) and Döhne-Merino twin lambs (4.93 kg and 4.60 kg, respectively) from the study of Cloete *et al.* (2003). Individual lamb weight from the current study averaged ( $\pm$  s.d.)  $4.43 \pm 1.0$  kg. Lamb birth weight is affected by a number of factors such as litter size, year of birth, maternal birth weight, maternal nutrition, sex of lamb and the maternal body composition at mating (Gardner *et al.*, 2007). Not only were lambs from the current study a different breed than those from the study of Cloete *et al.* (2003), but the year of birth, maternal nutrition, etc. was also different, therefore the difference in birth weight.

**Table 4.3** Average growth parameters per Experimental Unit for pre-weaned lambs reared on three different creep feeds (CFC, CF1, and CF2) and a control group (CON) raised on pasture, (LSMeans  $\pm$  s.e.)

	CON (Pasture)	CFC 139 g CP/kg; 455 g NSC/kg	CF1 157 g CP/kg; 477 g NSC/kg	CF2 179 g CP/kg; 508 g NSC/kg
<b>Per experimental unit:</b>				
Birth weight (kg)	9.51 $\pm$ 0.5	9.03 $\pm$ 0.43	9.86 $\pm$ 0.43	9.31 $\pm$ 0.46
Final Body Weight (kg)	42.66 <sup>b</sup> $\pm$ 2.45	49.48 <sup>ab</sup> $\pm$ 2.14	51.83 <sup>a</sup> $\pm$ 2.14	48.19 <sup>ab</sup> $\pm$ 2.27
Total Weight gain (kg)	33.15	40.45	41.97	38.88
Average Daily Gain <sup>1</sup> (g/d)	450.64 <sup>a</sup> $\pm$ 36.66	634.76 <sup>b</sup> $\pm$ 31.60	670.92 <sup>b</sup> $\pm$ 31.83	616.64 <sup>b</sup> $\pm$ 32.74
Cumulative feed intake <sup>2</sup> (g/d)	N/D	608.65 <sup>b</sup> $\pm$ 19.56	690.03 <sup>a</sup> $\pm$ 20.18	628.81 <sup>ab</sup> $\pm$ 20.57
FCR (kg feed intake/ kg gain)	N/D	0.88 <sup>a</sup> $\pm$ 0.08	1.19 <sup>b</sup> $\pm$ 0.09	1.01 <sup>ab</sup> $\pm$ 0.08
Intake @ 4 weeks (kg/d)	N/D	0.112 $\pm$ 0.04	0.175 $\pm$ 0.04	0.171 $\pm$ 0.04
Intake @ 9 weeks (kg/d)	N/D	1.518 $\pm$ 0.12	1.34 $\pm$ 0.13	1.322 $\pm$ 0.13

<sup>abc</sup>LSMeans with a different superscript within a row are significantly different ( $P \leq 0.05$ )

Experimental unit FBW represents the body weight recorded at last weighing opportunity and not the weight at slaughter. Lambs averaged  $65.2 \pm 3.3$  days of age ( $\pm$  s.d.) at final body weight. Lambs on CF1 weighed  $51.83 \pm 2.14$  kg while the lambs on CFC weighed  $49.48 \pm 2.14$  kg. There were significant differences in FBW between lambs from CON and CF1 however no differences were found between lambs from CON, CF2 and CFC.

The E.U. ADG linear regressions slopes indicated that there were no significant differences between the creep feed treatments. Control lambs, however, differed significantly from the creep feed treatments. Experimental unit ADG for the creep feed treatments were  $634.76 \pm 31.60$  g/d,  $670.92 \pm 31.60$  g/d and  $616.64 \pm 32.74$  g/d for CFC, CF1 and CF2 respectively while CON showed an ADG of  $450.64 \pm 36.66$  g/d.

The individual mean ADGs were  $308.1 \pm 11.4$  g/d,  $316.6 \pm 14.8$  g/d,  $293.4 \pm 10.6$  g/d and  $236.7 \pm 14.1$  g/d for CFC, CF1, CF2 and CON respectively. The creep feed treatments were notably better

than CON. These ADGs for the creep feed treatments were higher than those reported by Joy *et al.* (2008) where lambs (birth to 53 days of age) were raised indoors on concentrates where the CP level at first was 182 g/kg for the first month and 167 g/kg thereafter. The difference could be attributed to different type of breeds used. Döhne-Merino and SAMM lambs were reported to be able to grow up to 350 g/d; lambs from the current study seemed to grow in relative close agreement with this, albeit a bit lower.

Craddock *et al.* (1974) found that when protein levels were raised the ADG increased with improved feed efficiency. The same effect was found in numerous other studies (Jones & Hogue, 1960; Hinds *et al.*, 1964; Huston & Shelton, 1968; Andrews & Ørskov, 1970). The absolute E.U. least square mean value for ADG in the current study did not follow this trend. Lambs on the CF2 creep feed, with the highest CP level (179 g/kg), did not have the highest ADG (616 g/d) but had similar feed efficiency at nine weeks of age with lambs on CFC (2.06 kg feed for 1 kg gain in weight).

A regression analysis was fitted for E.U. cumulative feed intake to estimate average feed intake per day (g/d) over the entire trial period. Creep feed 1 had the highest E.U. feed intake (690.03 g/d) and differed significantly from CFC, which had the lowest E.U. feed intake (608.65 g/d). Creep feed 2 did not significantly differ between CF1 or CFC. Based purely on this, CFC seems to be superior as less feed is required to reach relatively the same FBW. However CF1 did average a numerically higher FBW and ADG than CFC justifying CF1's higher feed intake.

Feed intake should rather be estimated at intervals as opposed to a linear regression, seeing as creep feed intake increases dramatically as the suckling lamb matures and become more independent of milk. Creep feed intake at four weeks of age typically is negligible when compared to an intake at nine weeks of age. Cumulative feed intake is therefore more appropriate for estimating the feed conversion ratio (FCR) over the entire trial period as supposed to real time feed intake at different time intervals.

Craddock *et al.* (1974) indicated that lambs on 80:20 concentrate to roughage ratio diet consumed less feed than lambs on a 50:50 concentrate to roughage ratio diet. Craddock *et al.* (1974) also indicated that lambs on a lower CP diet (105 g/kg) consumed significantly more feed than lambs on a higher CP diet (135 g/kg). At the age of nine weeks, the E.U. creep feed intake followed this trend where CFC with the lowest CP level and unbalanced EAA supply had the highest feed consumption (1.52 kg). However, the same trend was not followed when comparing the regressed cumulative feed intakes.

Experimental unit FCR (kg feed for every kg weight increase) for CFC, CF1 and CF2 was  $0.88 \pm 0.08$ ,  $1.19 \pm 0.09$  and  $1.01 \pm 0.08$  respectively.

These FCR values are very efficient but it is important to note that the contribution of milk towards growth was not quantified therefore these values could be somewhat underestimated. Coetzee (2009) indicated that the FCR for lambs from birth to 15 kg live weight is 1.0:1 and at 15 - 20 kg live weight it is 3.0:1.

The FCR of the CFC lambs did not differ from those of CF2 lambs, while the FCR of CF1 lambs differed from CFC lambs but not from CF2 lambs. Upon contrasting the FCR of lambs in CF1 and CF2 against lambs in CFC, the p-value was 0.046 implicating that the optimised creep feeds were not as efficiently utilised as the CFC seeing as FCR for the former lambs were higher than for CFC lambs.

At nine weeks of age milk consumption for lambs in this trial could almost be negligible seeing as lambs were separated from ewes the entire day and at reunion ewes started to self wean their lambs. Therefore a FCR at nine weeks (end of trial) will give the best indication to how efficient the feed was utilized. At nine weeks of age lambs on CFC and CF2 (2.0 kg feed for 1kg gain in weight) utilised feed more efficient than lambs on CF1 (2.4 kg feed for 1kg gain in weight).

In retrospect the assumptions made upon creep feed formulation (Chapter 3.1.1) seem to have been underestimated in comparison to the actual growth performance of the lambs. Actual lamb growth rate ranged between 236.7 g/d and 316.6 g/d while it was assumed that lambs would grow at 150 g/d. Amino acid requirements were expressed as a function of the crude protein needed at a certain growth rate (Chapter 3.1.1). This implies that the EAA requirement and therefore supply in creep feed was underestimated. However, lambs also consumed higher amounts of feed (E.U. cumulative intake ranged from 608.65 g/d to 690.03 g/d) as compared to the 200 g/d of feed intake estimated at first, satisfying the higher EAA requirement.

The essential amino acid requirement could also have been overestimated seeing as the contribution of essential amino acids from the microbial protein was not taken into consideration during the calculation of the EAA requirements. Microbial protein production, however, is calculated as 13% of the total digestible nutrient intake which implies that MCP supply had a very small contribution to EAA supply at four weeks of age where feed intake was ca. 100 g/day/lamb.

Milk consumption was estimated to be 800 g/day/lamb when the EAA requirement was estimated. Ewbank (1967) established that the suckling rate (i.e. number of suckling bouts in a 12 hour period) of twin lambs were 20.5 in the first week and decreased to 8 at six weeks of age. In a similar method to Ewbank (1967), the suckling rate of lambs for the three hours of daylight in which they were united with their dams was calculated. Therefore at three weeks of age the suckling rate was calculated to be 2.6 and 1.9 at six weeks of age.

It was further estimated that lambs consumed between 200 and 250 g of milk during a suckling session, as determined via the *Lamb suckling weight differential technique* (Doney *et al.*, 1979). Total daylight milk consumption is therefore estimated to be 520 g at three weeks of age and 380 g at nine weeks of age. These amounts are clearly lower than the original estimated 800 g/day, which furthermore suggests an underestimation of EAA requirement. However this does not take into account the amount of milk consumed during sunset. The lambs further adapted their behaviour as they became more familiar with the normal day to day routine and managed suckling bouts as soon as they heard movement imitating the sound of lambs being separated from their dams. This behaviour makes it difficult to assume suckling bouts such as those found by Ewbank (1967). Milk

intake can therefore not be accurately quantified for this study; however it would seem that the initial estimation is close to the real amount suckled during lamb-dam reunion.

Furthermore it was suggested that the efficiency of utilization for amino acids rarely exceeds 70% (INRA, 1989) which could exacerbate a possible undersupply of certain amino acids.

The similar growth performance of lambs between the optimised creep feeds (CF1 and CF2) and CFC could be ascribed to the fact that the amino acid requirement of lambs were underestimated at four weeks of age but overestimated at nine weeks of age.

#### **4.4.2 Slaughter data results**

Forty lambs from the total lamb crop were selected for slaughter at the end of the growth trial representing trial day 60 for Group 1 and trial day 59 for Group 2 where lambs averaged ( $\pm$  s.d.)  $69.5 \pm 4.6$  and  $69.3 \pm 2.9$  days of age respectively for Group 1 and 2.

Table 4.3 summarises the slaughter data with treatment differences. Unlike the growth parameter data, the slaughter data were analysed per lamb and not per EU and is reported as such.

There were no differences for weight at slaughter between any of the treatments. Carcass weight, on the other hand, was heavier ( $P = 0.023$ ) for CF2 ( $12.71 \pm 0.63$  kg) than for CON ( $10.08 \pm 0.69$  kg). Back fat thickness did not differ between CF1 and CON ( $P = 0.081$ ). Creep feed 2 and CFC were significantly higher than CON, but not significantly higher than CF1. In general the creep feed treatments had more back fat than CON, and it would appear that CF1 had the lowest back fat thickness and thus also the leanest carcass between the creep feed treatments. The data concurs with the studies of Priolo *et al.* (2002), Santos-Silva *et al.* (2002) and Joy *et al.* (2008) where stall fed lambs have heavier carcasses but grass fed lambs have leaner carcasses.

The dressing percentage of CON ( $47.33 \pm 0.83$ ) was significantly lower ( $P < 0.005$ ) than CF1 ( $52.89 \pm 0.79$ ), CF2 ( $54.91 \pm 0.76$ ) and CFC ( $51.96 \pm 0.79$  kg). Joy *et al.* (2008) also reported lower dressing percentages for lambs reared on pasture compared to lambs reared indoors on concentrates. The study of Joy *et al.* (2008) ascribed the difference between pasture reared lambs and concentrated reared lambs to the effect of the gastro intestinal tract size accompanied by a lower external fat cover of pasture reared lambs. No differences were found for empty reticulo-rumen weight between any of the treatments; it could therefore be argued that the higher dressing percentages of the creep feed treatments are due to higher fat deposition.

Díaz *et al.* (2006) indicated that as slaughter weight increased the amount of subcutaneous fat increases. This is not surprising as lambs at higher slaughter weights have reached a higher proportion of their mature weight and their growth curve plateaus while more fat than lean deposition occurs (Whiteman *et al.* 1966). It could be suggested that creep feed lambs with higher slaughter weights have reached a higher proportion of their mature body weight and therefore a higher amount of subcutaneous fat over the CON carcasses.

**Table 4.3** Slaughter data obtained from pre-weaned lambs reared on creep feeds (CFC, CF1, and CF2) or pasture (CON) and slaughtered at 69 days of age, LSmeans  $\pm$  s.e.

Per individual:	CON	CFC	CF1	CF2
	(Pasture)	139 g CP/kg; 455 g NSC/kg	157 g CP/kg; 477 g NSC/kg	179 g CP/kg; 508 g NSC/kg
Weight at slaughter <sup>1</sup> (kg)	20.98 $\pm$ 1.13	24.67 $\pm$ 1.1	24.33 $\pm$ 1.1	23.08 $\pm$ 1.03
Carcass weight (kg)	10.08 <sup>b</sup> $\pm$ 0.69	12.87 <sup>a</sup> $\pm$ 0.66	12.71 <sup>a</sup> $\pm$ 0.63	12.93 <sup>a</sup> $\pm$ 0.66
Dressing percentage (%)	47.33 <sup>b</sup> $\pm$ 0.83	51.96 <sup>a</sup> $\pm$ 0.79	52.89 <sup>a</sup> $\pm$ 0.79	54.91 <sup>a</sup> $\pm$ 0.64
Back fat thickness (mm)	1.30 <sup>b</sup> $\pm$ 0.51	3.65 <sup>b</sup> $\pm$ 0.51	3.14 <sup>ab</sup> $\pm$ 0.48	3.61 <sup>a</sup> $\pm$ 0.47
Omental* fat (%)	0.48 <sup>b</sup> $\pm$ 0.16	0.82 <sup>ab</sup> $\pm$ 0.15	1.36 <sup>a</sup> $\pm$ 0.15	1.2 <sup>a</sup> $\pm$ 0.14
Empty reticulo-rumen (kg)	0.76 $\pm$ 0.04	0.76 $\pm$ 0.03	0.75 $\pm$ 0.03	0.69 $\pm$ 0.03
Small intestine (kg)	1.14 <sup>a</sup> $\pm$ 0.08	1.02 <sup>a</sup> $\pm$ 0.07	1.00 <sup>a</sup> $\pm$ 0.07	0.98 <sup>a</sup> $\pm$ 0.06
Large intestine (kg)	0.82 <sup>a</sup> $\pm$ 0.06	0.74 <sup>a</sup> $\pm$ 0.05	0.80 <sup>a</sup> $\pm$ 0.05	0.66 <sup>a</sup> $\pm$ 0.06
Head* (%)	5.98 <sup>ab</sup> $\pm$ 0.26	6.45 <sup>ab</sup> $\pm$ 0.24	6.96 <sup>a</sup> $\pm$ 0.24	6.02 <sup>b</sup> $\pm$ 0.22
Trotters* (%)	6.51 <sup>b</sup> $\pm$ 0.16	6.3 <sup>b</sup> $\pm$ 0.14	6.09 <sup>ab</sup> $\pm$ 0.15	5.73 <sup>a</sup> $\pm$ 0.14
Skin* (%)	3.7 $\pm$ 0.33	3.89 $\pm$ 0.30	3.98 $\pm$ 0.30	3.92 $\pm$ 0.30
Lung* (%)	2.41 $\pm$ 0.18	1.93 $\pm$ 0.17	2.18 $\pm$ 0.17	2.35 $\pm$ 0.16
Liver* (%)	1.48 <sup>b</sup> $\pm$ 0.07	1.98 <sup>a</sup> $\pm$ 0.07	1.94 <sup>a</sup> $\pm$ 0.07	1.96 <sup>a</sup> $\pm$ 0.06
Heart* (%)	0.71 $\pm$ 0.05	0.70 $\pm$ 0.05	0.72 $\pm$ 0.05	0.70 $\pm$ 0.04

<sup>abc</sup>LSMeans with different superscripts within a row are significantly different ( $P \leq 0.05$ )

<sup>1</sup>Weight at slaughter represents that weight after exsanguination.

\* Expressed as a percentage of Weight at slaughter

Dressing percentage furthermore for CF2 was higher ( $P = 0.075$ ) than CFC. Since there is no difference in gastro intestinal tract size and no difference in the amount of skin weight, it is maintained that the higher dressing percentage of CF2 is due to higher fat deposition. The renal and pelvic fat, which was not measured separately but included in carcass weight, contributed a fair amount of fat deposition. Dressing percentage is typically associated with the amount of fat on the carcass. It is important to note that the amount of pelvic and kidney fats were not measured in this study. Deposition of fat in the pelvic and kidney area precedes deposition at intermuscular, intramuscular and subcutaneous sites (Owens *et al.*, 1993).

As far as dressing percentages (DP) reported in literature: Santos-Silva *et al.* (2002) reported DP's of 51.5, 53.2, and 52.2 for lambs reared on pasture, pasture with concentrate supplementation and concentrates only, while Sañudo *et al.* (1997) reported DP's of one month old Catellana (medium wool rustic type sheep breed, 55 kg live weight) and Manchega (medium wool double purpose sheep breed 70 kg live weight) breeds to be 53.81 and 52.44%.

Skin, trotters and the other organs showed no significant difference between treatments. Heads as percentage of WAS for CF1 was higher than CF2 ( $P = 0.04$ ) and CON ( $P = 0.06$ ) but did not differ from CFC ( $P = 0.7$ ).



Liver weights differed significantly between CON and the creep feeds ( $P < 0.0001$ ). The increased supply of volatile fatty acids to the liver, produced from the fermentation of NSC's and increased fermentation ability of early developed rumen, could have led to increased liver weight seen in the creep feed treatments. Joy *et al.* (2008) similarly found that liver weight was higher in concentrate fed lambs than lambs reared on pasture. It has furthermore been found that an increase in amino acid load results in an increase in amino acid extraction and urea production by the liver (Titgemeyer, 2003) and as these are related to liver metabolism it could be surmised that liver size increases as liver metabolism increases.

#### 4.5 Conclusion

It was postulated that a creep feed balanced for limiting EAA's would prove to show higher growth rates, better feed efficiency and better feed consumption over a commercial creep feed. The optimised creep feeds were formulated to meet the limiting EAA requirements of lambs growing at 150 g/d consuming 200 g of creep feed while no microbial production was anticipated as lambs' rumens were still undeveloped.

It was expected that CF1, formulated at 157 g/kg CP with a balanced limiting EAA profile, would elicit greater growth responses from lambs compared to the lambs on a commercial creep feed (CFC), at a CP level of 139 g/kg. However, the current study revealed that lambs from CFC performed similar to the lambs from CF1 with very little significant differences between growth parameters and slaughter data. It could be suggested that the similar response between these two treatments is due to the fact that the initial limiting EAA estimation was underestimated because the lambs were growing faster with higher feed consumptions than the initial assumptions of 150 g/d (ADG) and 200 g intake. Essential amino acid requirement is dependent on the performance of a lamb, i.e. a lamb growing at 150 g/d has a different EAA requirement than a lamb growing at 300 g/d. Although the initial EAA requirements for lambs were underestimated the lambs compensated for it by consuming more creep feed. The contribution of MCP amino acids also further compensated for the lower supply of EAA. Future calculations to determine the limiting EAA requirement of suckling lambs should endeavour to include the amino acid contributions from the MCP, however, it is difficult to quantify from which age the lamb's rumen is producing MCP. It is important to also realise that as the lamb grows its feed intake also increases and also the amino acids supplied via the MCP, which could result in the oversupply of certain amino acids. Phase feeding would solve this problem; however it is not feasible to formulate and supply 3 to 4 different feed rations on producer level for one lamb crop until weaning.

As MCP is deficient in certain amino acids, it is expected that certain amino acids will still be deficient albeit the compensation of higher feed consumption and MCP amino acid contribution.

It is furthermore surmised that the CP level of CF2 was set too high as lambs from this treatment had a lowered growth response as compared to CF1 and in some cases CFC, indicating that energy was probably spent deaminating excess amino acids instead of lean deposition. The DP of CF2 was also

higher than the other creep feed treatments implicating higher fat deposition from excess amino acid supply.

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## CHAPTER 5

### **THE EFFECT OF A BALANCED AMINO ACID PROFILE AT A CERTAIN NON STRUCTURAL CARBOHYDRATE LEVEL ON *IN VIVO* AND *IN VITRO* DIGESTIBILITY OF CREEP FEEDS**

#### **Abstract**

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Specialised creep feeds optimised for the limiting essential amino acids (EAA) of suckling lambs at certain non structural carbohydrate (NSC) levels were evaluated in *in vivo* and *in vitro* digestibility trials. It was hypothesised that specialised creep feeds result in better responses in nitrogen balance, BUN parameters and overall digestibility than a commercial creep feed. Two creep feeds with balanced EAA for suckling lambs were formulated and represent the treatments CF1 (CP 157 g/kg; NSC 477 g/kg) and CF2 (CP 179 g/kg; NSC 508 g/kg). A further commercial control treatment (CFC, CP 139 g/kg; NSC 455 g/kg) was formulated without the balancing of EAA. Results indicated that CF1 had higher *in vivo* DM digestibility than CFC while CF2 had higher *in vivo* DM digestibility than CFC. Nitrogen retention was higher for CF1 and CF2 than for CFC. Similarly, CF1 and CF2 had a higher ME than CFC. At 34 days of age CF2 had the highest BUN (22.55 mg/100ml) and CON the lowest (7.28 mg/100ml) while CFC and CF1 showed no significant difference between each other. At 58 days of age CF2 maintained the higher BUN level at 20.64 mg/100ml. The IVTD results indicate that DM digestibility was significantly higher for CF2 than CF1 and CFC, while the latter showed no significant differences between each other. Creep feed 2 furthermore showed to have the highest amino acid rumen degradability for Glycine, Leucine, Phenylalanine, Isoleucine, Cystine, Histidine, Valine, Threonine and Alanine. It was concluded that the specialised creep feeds did elicit higher nitrogen balances from the lambs and showed higher digestibility's than the commercial creep feed. It is argued that the NSC levels contributed to rumen development which in turn is responsible for the higher apparent absorption of nutrients. It is further suggested that an NSC level of 508 g NSC/kg will elicit greater responses than at the 477 g NSC/kg level but that a CP level of 179 g CP/kg is too high for suckling lambs.

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Keywords: digestibility, pre-weaning lambs, starch and sugar

#### **5.1 Introduction**

The ideal amino acid concept came about through von Liebig's 'Law of Minimums'. From this it has been suggested that protein utilization is dependent on the balance of amino acids. This concept basically stipulates that the absence or undersupply of a single essential amino acid will inhibit further synthesis of protein even if the other amino acids are in adequate supply (Cole & van Lunen, 1994;

Cant *et al.*, 2003). The other amino acids in adequate supply are therefore in excess of protein synthesis and are deaminated to carbon skeletons and ammonia. Ammonia is excreted in the urine while carbon skeletons are used as energy sources for fat deposition. This process being energetically inefficient allows for valuable energy normally available to the animal being lost. Protein utilization can therefore be said to be more efficient when the limiting essential amino acids are supplied. The amino acid profile of a feed is therefore an indicator of the quality of a protein source.

Nitrogen retention, also an indicator of protein quality is used as a tool to determine whether or not the amino acid balance and crude protein (CP) levels of a feed were adequate, sub-adequate or undersupplied for the animal. Many ruminant studies dealing with protein quality and the level of protein supplementation employ the use of nitrogen balance studies. When protein quality is increased by either the infusion of the limiting amino acids (Schelling *et al.*, 1973; Titgemeyer & Merchen, 1990; Nolte *et al.*, 2008) or the supplementation of a better quality protein (Ørskov *et al.*, 1970; Urbaniak, 1995), higher nitrogen retentions were recorded. Nitrogen retention in turn has also been shown to increase when energy is supplemented (Schroeder *et al.*, 2006).

Preston *et al.* (1965) further maintained that the measurement of protein adequacy based on specific nitrogen components (such as Blood urea nitrogen (BUN)) rather than production factors is more accurate. Production factors are responses to many influences and are themselves an integration of general physiological responses (Preston *et al.*, 1965). In contrast, MacRae *et al.* (1993) indicated that nitrogen balance techniques overestimate net protein accretion. It could therefore be argued that a diet formulated on a protein basis requires not only results from production responses but also from nitrogen balance techniques and BUN analyses.

It is for this reason that the creep feed rations used in the lamb growth trial (Chapter 4) had to be tested further for its effect on nitrogen balance and BUN components to gather information on its protein quality and adequacy.

It is hypothesised that a creep feed optimised for its limiting EAA balance at a certain non structural carbohydrate (NSC) level will elicit higher nitrogen retentions than from a commercial creep feed. It is also postulated that the increasing levels of NSC will increase digestibility. The latter is based on the finding of Stern *et al.* (1978) that showed an increase in the microbial population as the NSC level rises.

## **5.2 Materials and methods**

In order to determine the viability of above mentioned hypothesis an *in vivo* and *in vitro* digestibility trial was conducted with creep feeds differing in essential amino acid (EAA) optimisation and NSC levels. These trials were conducted on lambs already partaking in a growth trial on the same creep feeds (Chapter 4).

Creep feed formulation, lamb management and feeding protocol were thoroughly described in Chapter 3 and only relevant detail will be dealt with here. However, as some information is critical to the understanding of this study some detail is repeated.

### **5.2.1 Compilation of the three creep feed diets**

Amino acid requirement for lambs were estimated based on the work by Nolte (2006) and used to compile a creep feed meeting the amino acid requirement of lambs. The creep feeds were compiled based on the assumption that lambs would grow at 150 g/day while consuming 800 g/day of milk and 200 g/day of creep feed (Chapter 3).

Nolte (2006) determined Histidine, Methionine, Leucine, Arginine and Phenylalanine as the first limiting amino acids for Merino and Döhne-Merino lambs. In Chapter 3 it was estimated that Lysine, Threonine, Methionine, Isoleucine, Phenylalanine and Leucine needed to be included in a creep feed diet at levels of 7.45, 8.10, 1.78, 0.32, 4.39, and 3.51 g/kg respectively for suckling lambs.

Creep feed 1 (CF1) was optimised for these amino acids at 157 g CP/kg and 477 g NSC/kg feed. A second creep feed (CF2) was optimised for these amino acids at higher CP and NSC levels namely 179 g CP/kg and 508 g NSC/kg respectively. As control, a commercial creep feed (CFC) was formulated at 139 g CP/kg and 455 g NSC/kg without limiting EAA optimisation.

Tanqua Feeds (Riviersonderend, Western Cape, South Africa) mixed all the creep feeds; Table 5.1 indicates the physical and chemical composition of the three creep feed mixtures as formulated by the company.



**Table 5.1** Nutrient composition of two optimised creep feeds (CF1 and CF2) for amino acid composition and non structural carbohydrates and a commercial creep feed (CFC) as formulated by Tanqua Feeds (Riviersonderend, Western Cape, South Africa)

	<b>CFC</b> 139 g CP/kg; 455 g NSC/kg	<b>CF1</b> 157 g CP/kg; 477 g NSC/kg	<b>CF2</b> 179 g CP/kg; 508 g NSC/kg
	<b>Physical composition, DM (g/kg)</b>		
Soybean meal (47%)	11.14	84.03	125.44
Wheat bran	12.82	11.80	5.17
Yellow maize (8.5%)	273.95	324.74	414.75
Lucerne (grade 1)	151.37	162.72	246.96
Limestone	32.50	27.66	25.73
Salt	4.06	5.00	4.96
Apple pulp	162.51	185.97	93.48
Sheep+rumensin <sup>1</sup>	3.25	3.25	3.23
Molasses syrup	69.65	69.74	69.24
Fishmeal 60	-	15.11	-
Oat bran	26.70	23.25	-
Oats	150.90	139.47	-
Malt pellets	139.30	-	115.40
Threonine	-	2.67	2.19
MetaSmart <sup>2</sup>	-	-	0.46
Green colourant	-	-	0.35
Ammonium sulphate	6.85	-	-
	<b>Chemical Composition, DM</b>		
DM g/kg	861.48	860.37	866.54
ME MJ/kg	11.44	11.80	11.95
TDN g/kg	765.71	793.08	794.06
Degradable protein g/kg	84.52	91.29	110.06
Non protein nitrogen g/kg	1.41	-	-
CP g/kg	139.09	157.26	179.23
Bypass protein g/kg	48.92	59.57	66.14
Arginine g/kg	6.66	8.51	9.99
Lysine g/kg	4.99	7.45	8.43
Methionine g/kg	1.93	2.48	2.77
Threonine g/kg	4.73	8.12	9.11
Bypass lysine g/kg	1.58	2.62	2.80
Bypass methionine g/kg	0.73	0.99	1.09
Non Structural carbohydrates g/kg	454.91	477.35	508.60
Fat g/kg	37.84	40.29	38.44
NDF g/kg	337.43	284.29	266.70
Fibre g/kg	129.85	125.56	127.17

<sup>1</sup>Ionophore<sup>2</sup>Methionine

### **5.2.2 Lamb management during the creep feed growth trial**

Twin bearing ewes (Döhne-Merino x Mutton Merino cross) lambed over a three week period (28 October to 20 November 2009) and were therefore subdivided into two groups to maintain an approximate seven day average age between lambs and to enable the start of the trial at ca. seven days of age. Once a group of lambs reached an average age of seven days they entered the growth trial (Chapter 4). Group 1 entered the trial ten days prior to Group 2.

It is due to this that the *in vivo* digestibility trial was done twice. Group 1 had lambs entering the digestibility trial ca. ten days prior to Group 2's lambs entering the digestibility trial. Therefore reference to the term *Group* will refer to the lamb crop division as mentioned above. Likewise the term *treatment* will therefore refer to the creep feed treatments CF1, CF2 and CFC.

Each group was divided into the above mentioned creep feed treatments CF1, CF2 and CFC. Similar to the study of Joy *et al.* (2008) the lambs were divided into their treatments in such a manner as to maintain homogeneity within and between treatments with regards to age, litter size and birth weight. The lamb growth trial lasted 60 days with lambs averaging 69 days of age at the end of the trial.

Blood samples were taken twice over the creep feed growth trial period, from the jugular vein into a heparinised tube, to estimate BUN. The two collection periods were 17 December 2009 and 10 January 2010. Group 1 was approximately 43 days and Group 2 approximately 34 days old for the first collection; at the second collection the lambs were approximately 67 and 58 days old for Group 1 and 2 respectively. This allowed for a subsample at four different ages, namely 34, 43, 58 and 67 days.

Blood was taken from one lamb within a litter of lambs, with preference to male lambs where possible. The heparin tubes filled with the blood samples were placed on ice immediately after collection and remained on ice until delivered to the Western Cape Provincial Veterinary laboratory, Department of Agriculture, Stellenbosch (Private Bag x 5020, Stellenbosch, 7599, Republic of South Africa) where the BUN level was determined.

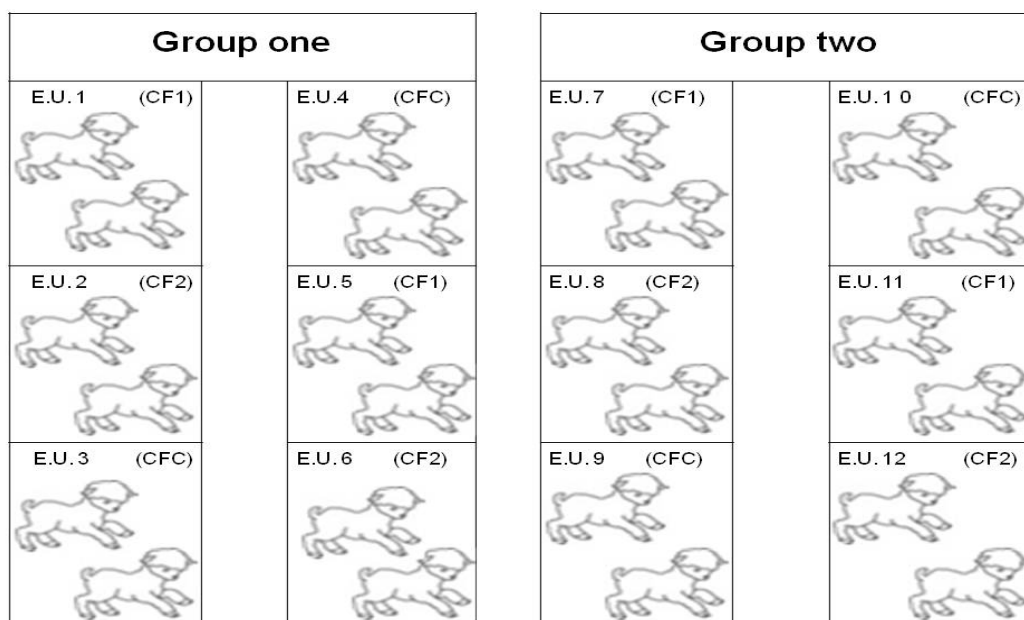
Milk samples were randomly taken as described in Chapter 3.1.2 to estimate the lambs' nutrient intake from milk. Milk samples were frozen immediately after collection. The milk samples were thawed and preserved with a dichromate preservative from the Dairy Improvement Scheme, Department of Agriculture in order for it to be analysed by the ARC – Elsenburg Analytical Services (Muldersvlei Road, PO Box 65, Elsenburg 7607, Western Cape) laboratory for its chemical composition (total solids, protein, fat and lactose content).

### **5.2.3 *In vivo* digestibility trial**

Upon selecting lambs to partake in the *in vivo* trial it was decided to only use lambs with a creep feed consumption of at least 200 g/day. The *in vivo* trial was therefore conducted towards the end of the growth trial.

Similar to the creep feed growth trial (Chapter 4) lambs in the same litter were regarded as one experimental unit. Feed intake, for example, was measured per experimental unit and not per individual lamb as it was not feasible to separate lambs. The current study employed the same protocol and did not separate lambs of the same litter. Only twin lambs were used for the *in vivo* trial.

As the lamb crop was divided into two groups the *in vivo* trial was also conducted in two groups allowing lambs to be of relative similar age. Two experimental units (two twin pairs) were selected per treatment for a specific group, e.g. *group 1* had two twin pair lambs in *treatment* CF1, CF2 and CFC. A total of 12 experimental units were therefore used for the *in vivo* digestibility trial (six experimental units per group and two per treatment), i.e. four replicates per treatment. Figure 5.1 illustrates this division. The available number of metabolic crates limited the amount of experimental units to be used to 12.



**Figure 5.1** The division of lambs into metabolic crates for the digestibility trial where E.U. represents the respective experimental unit (a twin pair) and CF1, CF2 and CFC the three different creep treatments;

Metabolic crates instead of faecal bags and urinary funnels were used for the measurement of urine and faecal output as lambs were too small to be fitted with these devices. Due to the structure of the metabolic crates, contamination of urine with faecal matter was inevitable although measures were taken to limit this to a minimum. Cotton wool was placed in a funnel which was placed in a closed urine collecting container to limit whole faeces falling into the urine container. However, urine still had to pass through faecal matter in the metabolic crate which resulted in urine contaminated with solubilised faecal matter.

Lambs were placed in metabolic crates four days prior to the start of the seven day digestibility trial to allow adaptation to their new environment and the experimental conditions (Santra & Karim, 1999). No feed adaptation period was necessary as lambs were already consuming the creep feeds at approximately 200 g/lamb/day.

Lambs were still suckling at this stage and were to return to the creep feed trial after participation in the digestibility trial and therefore were given access to their dam for suckling twice a day, morning (9h00) and afternoon (15h00), preventing weaning. Lambs were united with their dam, allowed to suckle and then placed immediately back into their metabolic crate. As an indication of milk consumption, lambs from Group 2 were used as a subsample and weighed immediately before and after a suckling period. The difference in weight gain represents milk consumed (Munro, 1956, Doney *et al.*, 1979). A suckling period represents the time it took for a lamb to suckle.

On a daily basis urine and uncontaminated faecal samples were collected to allow for enough dry material for laboratory proximate analysis. The samples were frozen until laboratory analysis could be done. Total urine and faecal output was determined over the seven day period. Feed intake was also determined for the seven day collection period, with fresh water and feed provided every morning at the same time ca. 9h00.

Subsamples of faeces and feed were pooled and analysed in the laboratory for moisture, GE, CP, NDF, ADF and EE. Amino acid analysis was also conducted on the feed and faeces. Urine was analysed for nitrogen and GE content. Analytical methods pertaining to above mentioned analyses are discussed in the section Laboratory Analysis (section 5.2.5). The digestibility of the three different creep feeds were calculated as follow (McDonald *et al.*, 2002):

Nutrient apparently digested = nutrient consumed – nutrient excreted

Apperant digestibility (%) = (Nutrient digested ÷ nutrient consumed) x 100

Methane gas production (MJ/day) was calculated as 8% of the gross energy intake (McDonald *et al.*, 2002). Nitrogen retention was corrected both for endogenous urinary N (EUN) and metabolic faecal N (MFN) according to McDonald *et al.* (2002) as follows:

$$\text{EUN (g)} = 0.18 \text{ g N/kg BW}^{0.75}/\text{day}$$

$$\text{MFN (g)} = 5 \text{ g N/kg dry matter intake}$$

$$\text{N retention (gN/kg BW}^{0.75}/\text{day)} = [\text{N}_{\text{intake}} - (\text{N}_{\text{faeces}} - \text{MFN}) - (\text{N}_{\text{urine}} - \text{EUN})] \div \text{BW}^{0.75}/\text{days}$$

The non-structural carbohydrate fraction of the feeds were determined as

$$\text{NSC} = 100 - (\text{CP}\% + \text{EE}\% + \text{NDF}\% + \text{Ash}\%) * (\text{DM}/100)$$

#### **5.2.4 *In vitro* digestibility trial**

*In vitro* true digestibility of the creep feeds was determined using the DAISY<sup>II</sup> Incubator from ANKOM Technology Corp. (Fairport, NY) using ANKOM'S Technology method 3. ANKOM's buffer solution was however replaced by the buffer solution of Goering & Van Soest (1970) as routinely used in the laboratory.

Table 5.2 represents the components of the buffer solution as described by Goering & Van Soest (1970).

Creep Feed samples were milled through a 1 mm screen and subsequently sieved through a 106 µm screen. Sample remaining on the top of the sieve was used. This procedure allows for dust and small particles to be removed out of the sample used for the *in vitro* digestibility as small particles can escape out of the filter bag during incubation, resulting in the over estimation of results (Cruywagen *et al.*, 2003).

The F57 filter bags were soaked in acetone to remove the surfactant on the bags which could inhibit microbes from entering to digest the sample. After bags were dried at 100°C overnight their empty weights were recorded. A  $0.5 \pm 0.05$  g sample ( $\pm$  s.d.) was weighed into every bag for incubation at 48 hours. The dry matter of feeds was determined prior to the incubation (AOAC, 2002a).

For each treatment seven filter bags were filled with the test sample. These sample filled bags were used as follow:

- Three sample filled bags (triplicate measurement) were subjected to the 48 hour incubation and the NDF procedure according to the manufacturer's method for *in vitro* true digestibility (IVTD) determination
- Three sample filled bags were subjected to the 48 hour incubation and pooled to analyse the amino acid content
- The last sample filled bag was washed in water where after the amino acid content was determined.

Sample filled and blank bags were heat sealed with an impulse heat sealer. Blank bags containing no sample were included to serve as blank bag correction factors.

**Table 5.2** Buffer solution for the *in vitro* true digestibility determination of the three different creep feeds (Goering & van Soest, 1970)

Reagent	Per litre
<b>Complete buffer solution</b>	
Deionized 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
Trypticase	1.25 g
Reducing agent	53 ml
<b>Rumen buffer solution</b>	
Deionized water	2.0 L
NH <sub>4</sub> HCO <sub>3</sub>	8.0 g
NaHCO <sub>3</sub>	70.0 g
<b>Macromineral solution</b>	
Deionized water	2.0 L
Na <sub>2</sub> HPO <sub>4</sub>	11.4 g
KH <sub>2</sub> PO <sub>4</sub>	12.4 g
MgSO <sub>4</sub> 7H <sub>2</sub> O	1.17 g
<b>Micromineral solution</b>	
Deionized water	100 ml
CaCl <sub>2</sub> 2H <sub>2</sub> O	13.2 g
MnCl <sub>2</sub> 4H <sub>2</sub> O	10.0 g
CoCl <sub>2</sub> 6H <sub>2</sub> O	1.0 g
FeCl <sub>3</sub> 6H <sub>2</sub> O	8.0 g
<b>Cysteine sulphide reducing agent</b>	
Deionize water	48 ml
Cysteine hydrochloride	312 mg
1 N NaOH	20 ml
Na <sub>2</sub> S 9H <sub>2</sub> O	312 mg

Rumen inoculum was taken from cannulated sheep at the Welgevallen experimental farm (Stellenbosch University). Thermo flasks were warmed at 39°C before the rumen inoculum was poured in. The sheep's rumen content was squeezed through two layers of cheese cloth to obtain ca. 2000 ml of rumen inoculum. Thermo flasks were filled to the brim, preventing aeration before being capped. At the laboratory, an industrial blender was heated by rinsing with hot tap water before the inoculum was poured in from the thermo flasks. As the inoculum was poured into the industrial blender the surface of the inoculum was purged with CO<sub>2</sub>. The industrial blender functions in

separating microbes from rumen material. Once again, as the inoculum was poured out of the blender into containers the inoculum surface was purged with CO<sub>2</sub>.

Jars previously placed into the DAISY<sup>II</sup> incubator (24 hours before) were taken out and filled with 1600 ml medium, warmed to 39°C prior to the addition of 400 ml rumen inoculum. The top layer of the rumen inoculum and medium was purged with CO<sub>2</sub> for ca. 30 seconds after the bags containing samples had been spread equally alongside the divider in the DAISY<sup>II</sup> jars, whereafter the lid was secured. Jars were placed in the DAISY<sup>II</sup> incubator for 48 hours, after which the bags were removed and washed in cold water to stop microbial activity and to remove any microbial debris. Mechanical agitation during rinsing in cold water was kept to the minimum, preventing damage to the bags.

Sample bags were placed in a 70°C oven for 24 hours to determine the DM disappearance from the bags. Thereafter, the NDF procedure was done using the ANKOM<sup>200/220</sup> (Ankom® Technology Corp., Fairport, NY, USA) Fiber Analyzer as described by ANKOM to allow for the calculation of *in vitro* true digestibility (IVTD)

IVTD was calculated as:

$$\% \text{ IVTD}_{\text{DM}} = \frac{100 - (W_3 - (W_1 \times C_1))}{(W_2 \times \text{DM})} \times 100$$

Where: W<sub>1</sub> = Bag tare weight (g)

W<sub>2</sub> = Sample weight (g)

W<sub>3</sub> = Final bag weight after *in vitro* and sequential neutral detergent treatment (g)

C<sub>1</sub> = Blank bag correction (final oven-dried weight/original blank bag weight)

The amino acid degradation after 48 hour incubation was calculated similar to the digestibility calculation.

Degradation is calculated as follow:

Degradation:

$$D (\%) = [(A - B) \div A] \times 100$$

Where:

A = individual amino acid before incubation (g/100g sample)

B = individual amino acid after incubation (g/100g sample)

### **5.2.5 Laboratory analyses**

Analysis of aliquot faecal and feed samples, pertaining to moisture, ash, CP, EE, NDF, ADF, GE and amino acid analysis are presented in the following section.

Faecal samples were dried at 60°C for 48 hours to determine dry faecal output, where after the sample was equilibrated in the atmosphere and milled for laboratory analysis. Dry matter, ash and EE were determined according to the AOAC (2002) methods.

Crude protein (CP) was determined by the Dumas combustion method 992.15(AOAC, 2002c) using the Leco® FP – 528 Nitrogen & Protein Analyzer (Leco® Corporation, St. Joseph, USA) which analysis the nitrogen percentage of a sample which in turn is multiplied with a factor of 6.25 (for feed, faecal, and urine samples) to gain the CP percentage. Alfalfa (Leco®) with a nitrogen percentage ( $\pm$  s.d.) of  $3.32 \pm 0.04$  was used as the standard to calibrate the Leco® for feed, faecal and urine samples.

Gross energy was determined using the CP 500 Bomb Calorimeter instrument (Eltra Africa, Springs, South Africa).

The amino acid hydrolyses were done at the Department of Animal Sciences (Stellenbosch University) where after amino acid analyses were done at the Central Analytical Facility in the Department Biochemistry (Stellenbosch University). The EZ:Faast LC method was used as described by its manual with an EZ:Faast column on the Waters API Quattro Micro instrument.

Amino acid analyses similar to above mentioned was conducted on the freeze-dried milk.

### **5.3 Statistical analyses**

Statistical analyses were done via SAS Enterprise guide 4.0, version 9.1.3 (2002), using Proc GLM. Least square means were estimated and significance declared at the  $P \leq 0.05$ .

A complete randomized design was followed.

No significant effect was found for group and therefore the two *in vivo* digestibility trials were pooled and analysed together.

Contrasts were used to compare the creep feed treatments between each other. The contrasts were:

- CF1 and CF2 against CFC
- CF1 against CF2
- CF1 against CFC
- CF2 against CFC.

### **5.4 Results and discussion**

#### **5.4.2 Milk consumption of lambs**

Lambs were allowed to suckle twice a day to prevent weaning during the *in vivo* digestibility trial; milk intake contributes to nutrients consumed but were not used for the calculation of creep feed



digestibility as it could not be accurately quantified, therefore creep feed digestibility results could be said to be underestimated.

Lamb milk consumption ( $\pm$  s.d.) per suckling session during the digestibility trial averaged  $253.33 \pm 87.52$  g. To limit possible loss of urine and faeces while lambs were united with their dams the suckling session ended right after the lambs had suckled. With two suckling sessions a day the total average milk intake can be calculated as the milk intake of a suckling session multiplied by two, which equals to 507 g of milk consumed per day per lamb. The chemical and amino acid composition of the milk is depicted in Table 5.3 as well as the daily nutrient intake from the total milk consumption.

**Table 5.3** Milk composition and the daily nutrient intake of individual 7 week old nursing lambs when subjected to only two suckling sessions per day

	Milk composition (g/kg)	Daily nutrient intake (g/d)
Estimated milk consumption (g/d)	507.0	-
Fat	51.7	26.2
Protein	40.0	20.3
Lactose	50.7	25.7
Total Solids	149.6	75
<b>Amino acids</b>	<b>g/kg sample (As is)</b>	<b>g/d</b>
Threonine	2.0	1.0
Lysine	3.4	1.7
Methionine	0.7	0.34
Isoleucine	2.0	1.0
Phenylalanine	2.4	1.2
Leucine	5.1	2.6
Histidine	0.8	0.4
Arginine	0.8	0.4

Santra & Karim (1999) reported milk consumption for lambs during a similar *in vivo* digestibility trial to be 410 g per day which is lower than the 507 g of milk per day for lambs from the current study. Lambs from the study of Santra & Karim (1999) were a smaller breed therefore the difference in milk consumption.

The chemical composition of milk from the current study deviates somewhat from that reported by Park *et al.* (2007) as the norm for sheep milk. Park *et al.* (2007) indicated the average lactose, protein and fat composition of sheep milk to be 49, 69 and 79 g/kg respectively. Milk composition from the current study measured 51, 40 and 52 g/kg for lactose, protein and fat respectively. These values are however in close agreement with that found by Santra & Karim (1999) for Merino/Rambouillet x Malpura cross ewes where lactose, protein and fat measured 51, 54 and 45 g/kg milk respectively. These differences could be attributed to a number of factors such as breed, diet type, stage of lactation, parity, management, etc. (Park *et al.*, 2007).

The lower fat composition could also be ascribed to the fact that the ewes received a pelleted total mixed ration where the effective physical fibre was low, thus resulting in lower milk fat production.

However supplementary hay provided to the ewes (Chapter 3.1.2) was a source of physical effective fibre and it was expected that this would cause the drop in milk fat to be lower than measured.

#### **5.4.1 *In vivo* digestibility trial, nitrogen retention and metabolizable energy**

Lambs' average age ( $\pm$  s.d.) was  $45.5 \pm 3.2$  and  $52.0 \pm 1.6$  days for the two different groups respectively when entering the *in vivo* trial. Upon data analysis, no differences ( $P > 0.05$ ) were found and the two groups were therefore pooled for further analyses.

Table 5.4 quantifies results from the *in vivo* digestibility trial, where the chemical composition of feed and faeces is specified as well as the apparent digestibility of the three different creep feeds. All digestible values are *apparent* digestibility coefficients and not *true* digestibility coefficients and reference to digestibility in the text here-on further refers to *apparent* digestibility.

There were no differences ( $P > 0.05$ ) between the three treatments for total creep feed intake. The marked variations for intake could possibly describe the reason for the lack of significance between treatments. Similarly, no difference was found between treatments for faecal output.

Dry matter digestibility showed a difference ( $P < 0.05$ ) between CFC and the optimised creep feeds, CF1 and CF2. Organic matter digestibility showed no difference ( $P > 0.05$ ) between CF1 and CFC but a difference ( $P < 0.05$ ) between CF2 and CFC.

Dry matter digestibility for suckling lambs at a similar age to those from the current study ranged from 62.2 to 63% for creep feeds at CP levels from 181 to 269 g/kg (Santra & Karim, 1999). The current study not only had lower CP levels but also showed better and worse DM digestibility where the highest was 73% (CF2) and lowest 59% (CFC).

Crude protein, CF, and NSC digestibility showed no differences ( $P > 0.05$ ) between the creep feed treatments. This could be ascribed to the marked variation within treatments between experimental units which was exacerbated by using only four E.U.'s per treatment. However, CF2 did tend to have a higher CP digestibility than CFC ( $P = 0.07$ ).

In agreement with Santra & Karim (1999), the CP digestibility increases as the CP level rises. Numerically, CP digestibility in the current study followed this trend.

**Table 5.4** Results of *in vivo* digestibility trial for three different creep feeds on different CP and NSC levels

	<b>CFC</b> 139 g CP/kg; 455 g NSC/kg	<b>CF1</b> 157 g CP/kg; 477 g NSC/kg	<b>CF2</b> 179 g CP/kg; 508 g NSC/kg
Total Creep feed DM intake per E.U. <sup>1</sup> (g)	6387.0 ± 484.2	8019.3 ± 1273.7	6749.3 ± 1000.0
Total DM faecal excretion per E.U. <sup>1</sup> (g)	2593.3 ± 178.6	2616.1 ± 384.2	1745.0 ± 183.3
<b>Creep feed chemical composition (DM basis) g/kg</b>			
Dry matter	896.8	894.1	894.2
Organic matter	911.7	908.5	903.8
Crude protein	152.6	164.6	183.8
Ether extract	312.6	294.4	284.1
Non structural carbohydrate	371.1	374.0	424.5
NDF	314.1	295.9	216.9
ADF	153.6	165.7	11.10
<b>Faecal chemical composition (DM basis) g/kg ± s.e.</b>			
Dry matter	545.5 ± 62.1	406.6 ± 30.0	413.2 ± 86.1
Organic matter	871.6 ± 4.5	858.6 ± 2.0	841.1 ± 8.5
Crude protein	150.3 ± 1.4	174.8 ± 3.6	217.3 ± 12.2
Ether extract	20.9 ± 0.3	27.9 ± 1.8	26.9 ± 18.7
Non structural carbohydrate	17.9 ± 14.2	25.8 ± 17.4	13.1 ± 11.2
NDF	692.9 ± 20.9	636.9 ± 20.7	627.3 ± 42.0
ADF	388.2 ± 6.6	379.1 ± 8.4	345.8 ± 20.1
<b>Digestibility, % ± s.e.</b>			
Dry matter	59.02 <sup>b</sup> ± 2.8	66.95 <sup>a</sup> ± 1.7	73.03 <sup>a</sup> ± 2.9
Organic matter	58.17 <sup>b</sup> ± 3.2	66.24 <sup>ab</sup> ± 2.1	71.78 <sup>a</sup> ± 2.9
Crude protein	59.71 ± 2.3	64.8 ± 2.4	68.1 ± 3.8
Crude fat	72.6 ± 2.1	69.1 ± 1.1	75.6 ± 1.5
Non structural carbohydrate	98.9 ± 2.2	98.34 ± 2.1	101.40 ± 2.6
Nitrogen free extract	93.1 ± 1.4	94.6 ± 1.4	93.8 ± 1.4
NDF	5.1 ± 5.5	23.3 ± 5.5	19.3 ± 5.5
ADF	1.9 ± 1.8	18.4 ± 4.4	16.7 ± 4.4

<sup>abc</sup>LSMeans with a different superscript within a row are significantly different ( $P \leq 0.05$ )

<sup>1</sup>E.U. = Experimental unit; Each E.U. consisted of twins

Dry matter intake and excretion was quantified over a seven day period

Stern *et al.* (1978) determined that rumen  $\text{NH}_3\text{-N}$  utilisation increased as the level of NSC increased and that microbial growth was higher at high NSC levels. As a result to this, nutrient digestion in the rumen increases as the NSC level increases, because not only are microbes more efficient in  $\text{NH}_3\text{-N}$  utilisation but their higher proliferation leads to more available microbes to digest nutrients. The *in vivo*

digestibility data supports the finding of Stern *et al.* (1978) where CF2 with the highest NSC level concomitantly also had the overall numerical highest digestibility.

As expected NDF and ADF digestibility was very low and can be explained by the fact that pre-ruminant lambs have a limited ability to digest roughage, as the rumen is relatively undeveloped at birth and develops only as dry matter is consumed. It could be argued that the decrease of NDF and ADF from CFC to CF2 is responsible for the increase in digestibility for CF2 since NDF and ADF is strongly associated with the digestibility of a feed (McDonald *et al.*, 2002).

Amino acid composition of the different creep feeds as fed on a DM basis is reported in Table 5.5 along with the digestibility coefficients thereof. The underlined values represent those amino acids that were specifically included in the optimised creep feeds CF1 and CF2 (i.e. the limiting EAA for suckling lambs) and functions in maintaining focus.

**Table 5.5** Amino acid composition for three different creep feeds (CFC, CF1 and CF2) and their respective digestibility coefficients and contents, LSmean  $\pm$  s.e.

	Chemical composition (DM g/kg)			Digestibility (%)			Digestible content (g/kg)		
	CFC	CF1	CF2	CFC	CF1	CF2	CFC	CF1	CF2
Ala	5.8	4.9	8.7	49.7 <sup>ab</sup> $\pm$ 5.3	58.2 <sup>a</sup> $\pm$ 5.3	73.7 <sup>a</sup> $\pm$ 5.3	2.9 $\pm$ 0.1	2.8 $\pm$ 0.3	6.5 $\pm$ 0.4
Thr	<u>2.7</u>	<u>5.9</u>	<u>5.6</u>	<u>19.6<sup>a</sup> <math>\pm</math> 4.9</u>	<u>67.5<sup>b</sup> <math>\pm</math> 4.9</u>	<u>70.7<sup>b</sup> <math>\pm</math> 4.9</u>	<u>0.5 <math>\pm</math> 0.1</u>	<u>4.0 <math>\pm</math> 0.1</u>	<u>3.9 <math>\pm</math> 0.3</u>
Ser	4.2	5.8	7.8	41.2 <sup>a</sup> $\pm$ 4.4	68.3 <sup>b</sup> $\pm$ 4.4	77.3 <sup>b</sup> $\pm$ 4.4	1.7 $\pm$ 0.1	4.0 $\pm$ 0.2	6.0 $\pm$ 0.4
Arg	5	4.8	9.3	70.3 <sup>b</sup> $\pm$ 2.7	72.1 <sup>b</sup> $\pm$ 2.7	88.6 <sup>a</sup> $\pm$ 2.7	3.5 $\pm$ 0.04	3.5 $\pm$ 0.2	8.3 $\pm$ 0.1
Glu	12.4	18.7	19	57.9 <sup>b</sup> $\pm$ 3.1	75.2 <sup>a</sup> $\pm$ 3.1	79.8 <sup>a</sup> $\pm$ 3.2	7.2 $\pm$ 0.1	14.1 $\pm$ 0.6	15.1 $\pm$ 0.7
Val	5.1	3.7	7.1	55.2 $\pm$ 5.1	55.5 $\pm$ 5.1	75.8 $\pm$ 5.1	2.8 $\pm$ 0.1	2.0 $\pm$ 0.2	5.4 $\pm$ 0.3
His	1.8	2.03	2.8	65.9 <sup>b</sup> $\pm$ 3.4	72.6 <sup>ab</sup> $\pm$ 3.4	80.9 <sup>a</sup> $\pm$ 3.4	1.2 $\pm$ 0.03	1.4 $\pm$ 0.07	2.3 $\pm$ 0.1
Asp	14.2	17.9	20.3	54.0 <sup>b</sup> $\pm$ 3.9	68.4 <sup>ab</sup> $\pm$ 3.9	75.5 <sup>a</sup> $\pm$ 3.9	7.6 $\pm$ 0.2	12.2 $\pm$ 0.6	15.3 $\pm$ 1.0
Lys	<u>5.7</u>	<u>6.8</u>	<u>9.5</u>	<u>49.9<sup>b</sup> <math>\pm</math> 5.2</u>	<u>62.8<sup>ab</sup> <math>\pm</math> 5.2</u>	<u>74.7<sup>a</sup> <math>\pm</math> 5.2</u>	<u>2.8 <math>\pm</math> 0.05</u>	<u>4.3 <math>\pm</math> 0.4</u>	<u>7.1 <math>\pm</math> 0.5</u>
Pro	6.7	5.8	9.6	68.3 <sup>b</sup> $\pm$ 2.9	71.6 <sup>ab</sup> $\pm$ 2.9	83.6 <sup>a</sup> $\pm$ 2.9	4.6 $\pm$ 0.1	4.1 $\pm$ 0.1	8.1 $\pm$ 0.3
Met	<u>0.5</u>	<u>0.5</u>	<u>0.8</u>	<u>43.1 <math>\pm</math> 9.1</u>	<u>53.6 <math>\pm</math> 9.1</u>	<u>60.9 <math>\pm</math> 9.2</u>	<u>0.2 <math>\pm</math> 0.04</u>	<u>0.3 <math>\pm</math> 0.02</u>	<u>0.5 <math>\pm</math> 0.01</u>
Tyr	3.9	4.8	5.5	52.5 $\pm$ 5.3	64.1 $\pm$ 5.3	71.0 $\pm$ 5.4	2.0 $\pm$ 0.1	3.0 $\pm$ 0.2	3.9 $\pm$ 0.3
Cys	0.9	0.7	1	51.0 $\pm$ 9.8	49.2 $\pm$ 9.8	64.8 $\pm$ 9.9	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1	0.6 $\pm$ 0.1
Ile	<u>3.7</u>	<u>3.7</u>	<u>5.4</u>	<u>49.5 <math>\pm</math> 5.4</u>	<u>56.4 <math>\pm</math> 5.4</u>	<u>71.2 <math>\pm</math> 5.4</u>	<u>1.8 <math>\pm</math> 0.1</u>	<u>2.1 <math>\pm</math> 0.2</u>	<u>3.8 <math>\pm</math> 0.3</u>
Phe	<u>2.7</u>	<u>4</u>	<u>4.1</u>	<u>59.0 <math>\pm</math> 5.0</u>	<u>58.0 <math>\pm</math> 5.0</u>	<u>73.0 <math>\pm</math> 5.0</u>	<u>1.6 <math>\pm</math> 0.1</u>	<u>2.3 <math>\pm</math> 0.1</u>	<u>3.0 <math>\pm</math> 0.2</u>
Leu	<u>8.9</u>	<u>10.7</u>	<u>13</u>	<u>62.0 <math>\pm</math> 3.9</u>	<u>67.3 <math>\pm</math> 3.9</u>	<u>77.0 <math>\pm</math> 3.9</u>	<u>5.5 <math>\pm</math> 0.2</u>	<u>7.2 <math>\pm</math> 0.3</u>	<u>10.0 <math>\pm</math> 0.5</u>
Gly	2.3	3.5	3.6	17.1 <sup>b</sup> $\pm$ 6.7	54.9 <sup>a</sup> $\pm$ 6.7	61.8 <sup>a</sup> $\pm$ 6.7	0.3 $\pm$ 0.1	1.9 $\pm$ 0.1	2.2 $\pm$ 0.2

<sup>abc</sup>LSMeans with a different superscript within a row are significantly different ( $P \leq 0.05$ )

Underline values represent those amino acids that were specifically balanced for the creep feeds CF1 and CF2

CFC = 139 g CP/kg, 455 g NSC/kg;

CF1 = 157 g CP/kg, 477 g NSC/kg;

CF2 = 179 g CP/kg, 508 g NSC/kg

Amongst the balanced amino acids (Threonine, Lysine, Methionine, Isoleucine, Phenylalanine, and Leucine), only Threonine and Lysine showed significant digestibility differences between treatments. Creep feed 1 and 2 had a higher ( $P < 0.05$ ) Threonine digestibility (67.5 and 70.7%, respectively) than CFC (19.6%). Creep feed control had a lower ( $P = 0.0168$ ) Lysine digestibility than CF2 with no difference between CFC and CF1.

According to the amino acid HCl hydrolysis procedure the ion-exchange chromatography recovery rate of methionine is 25 - 75% and that of the other sulphur containing amino acids are 50 - 90% due to the increased thermal breakdown from residual oxygen. These low recovery rates explain why the creep feed chemical composition shows lower methionine and threonine content compared to the original formulation (Table 5.5 and Table 5.1). It is also possible that the recovery rate of the creep feed material can be higher or lower than the recovery rate of faecal material thereby contributing to the varying results. Creep feed chemical composition (as analysed) for Methionine was in the range of 23 - 30% of what was originally formulated (e.g. 0.59 g/kg vs. 2.47 g/kg for CF1). Similarly, the Threonine chemical composition was measured as 57 to 73% of what was originally formulated for. Lysine on the other hand measured higher than the original formulation ranging at 93 to 115% of original formulation.

The nitrogen balance obtained from the *in vivo* trial is reported in Table 5.6. There were no significant difference between treatments for dry matter intake and faecal excretion, as similarly reported in Table 5.4. Although it would seem that CF1 tended to have the highest creep feed intake the statistical analyses and contrasts show no differences ( $P > 0.05$ ). However, if the sample size of each treatment was increased the variation within treatments should decrease; resulting in a lower error and significant differences at the 5% level should start being manifested.

No differences ( $P > 0.05$ ) were found between treatments for nitrogen retention. However, a large numerical difference can be noted between CF2 and CF1. With statistical contrasting it was found that CF2 had a significantly higher ( $P = 0.0277$ ) nitrogen retention than CFC but CF1 and CFC did not differ ( $P = 0.1152$ ). When CF1 and CF2 was contrasted against CFC, the P-value was 0.0587 indicating that the amino acid balance of CF1 and CF2 together tended to result in lambs having a higher nitrogen retention. This data therefore implies that the CP level of CF1 and CF2 resulted in better nitrogen retention.

However, for growing steers it has been shown that energy supplementation increases nitrogen retention thereby implicating better amino acid utilisation (Schroeder *et al.*, 2006). Similar results were found in sheep (Asplund *et al.*, 1985) and pigs (Reeds *et al.*, 1981). It could therefore be inferred that the NSC level (energy supplementation) rather than the CP level *per se* of CF2 resulted in the better nitrogen retention over CFC and CF1. This was inferred in the light of the fact that the NSC levels between CFC and CF1 are relatively similar but notably different from CF2 which is also reflected in the data.

**Table 5.6** Nitrogen balance per experimental unit for pre-weaned lambs reared on three different creep feeds (CFC, CF1 and CF2) over a seven day balance trial (LSmeans  $\pm$  s.e.)

	<b>CFC</b> 139 g CP/kg; 455 g NSC/kg	<b>CF1</b> 157 g CP/kg; 477 g NSC/kg	<b>CF2</b> 179 g CP/kg; 508 g NSC/kg
Total DM intake (kg)	6.4 $\pm$ 0.5	8.0 $\pm$ 1.3	6.7 $\pm$ 1.0
Total faecal excretion (kg)	2.6 $\pm$ 0.2	2.6 $\pm$ 0.4	1.7 $\pm$ 0.2
Total urinary excretion (L)	4.3 $\pm$ 1.8	9.4 $\pm$ 1.8	6.2 $\pm$ 1.8
Total N intake (g)	119.4 $\pm$ 21.3	159.9 $\pm$ 21.3	169.3 $\pm$ 21.3
Total N intake (gN/kg BW <sup>0.75</sup> /day)	0.9 $\pm$ 0.14	1.2 $\pm$ 0.14	1.5 $\pm$ 0.14
Total Faecal N(g)	57.4 $\pm$ 9.9	63.2 $\pm$ 9.9	52.3 $\pm$ 9.9
Total Urinary N (g)	25.1 $\pm$ 7.8	34.6 $\pm$ 7.8	28.4 $\pm$ 7.8
TOTAL N excreted (g)	82.5 $\pm$ 14.4	97.8 14.4	80.8 14.4
Faecal N (% of N intake)	47.2 $\pm$ 5.0	40.1 $\pm$ 5.0	31.8 $\pm$ 5.0
Urinary N (% of N intake)	21.3 $\pm$ 4.2	21.6 $\pm$ 4.2	16.7 $\pm$ 4.2
TOTAL N excreted (% of N intake)	68.5 $\pm$ 5.4	61.7 $\pm$ 5.4	48.5 $\pm$ 5.4
BW <sup>0.75</sup>	17.3 $\pm$ 1.02	18.7 $\pm$ 1.02	16.6 $\pm$ 1.02
MFN <sup>1</sup> (g) (MFN)	0.8 $\pm$ 0.13	0.7 $\pm$ 0.13	0.8 $\pm$ 0.13
EUN <sup>2</sup> (g) (EUN)	0.0015	0.0014	0.0016
Nitrogen retention (g N/kg BW <sup>0.75</sup> /day)	0.31 $\pm$ 0.1	0.48 $\pm$ 0.1	0.78 $\pm$ 0.1
Nitrogen retention (% of N intake)	32.17 $\pm$ 6.2	38.85 $\pm$ 3.2	52.08 $\pm$ 6.1

<sup>abc</sup>LSmeans with a different superscript within a row are significantly different ( $P \leq 0.05$ )

<sup>1</sup>MFN= metabolic faecal nitrogen

<sup>2</sup>EUN= endogenous urinary nitrogen

Dry matter intake and excretion was quantified over a seven day period

Table 5.7 indicates the calculation of the energy balance of the pre-weaning lambs to finally give the metabolizable energy value of the creep feeds.

The metabolizable energy values of 7.3, 8.6 and 9.7 MJ/kg for CFC, CF1 and CF2, respectively, was lower than the original formulation of 11.4, 11.8, 11.9 MJ/kg, respectively (Table 5.1). A number of factors can influence the ME; these factors include those affecting digestibility and those effecting energy loss via urine and methane (McDonald *et al.*, 2002). Factors such as feed composition, feed preparation, and animal factors can be eliminated as the creep feeds were prepared similarly from the same raw materials and lambs were from the same origin with similar ages. Animal factors in terms of stage of development could however influence ME, as stage of development in suckling lambs have an effect on the fermentation ability of the rumen, resulting in lower digestibility. The rumens of young suckling lambs are immature at birth and only start to develop once dry matter is consumed. It is further postulated that the increased NSC level of CF2 also attributed to better digestion by the developing microbial population as increasing NSC levels have a favourable effect on the proliferation of the microbial population (Stern *et al.*, 1978).

**Table 5.7** Energy balance of pre-weaned lambs reared on three different creep feeds (CFC, CF1 and CF2) over a seven day balance trial (LSmeans  $\pm$  s.e.)

	<b>CFC</b> 139 g CP/kg; 455 g NSC/kg	<b>CF1</b> 157 g CP/kg; 477 g NSC/kg	<b>CF2</b> 179 g CP/kg; 508 g NSC/kg
Total DM intake (kg/seven days)	6.4 $\pm$ 0.5	8.0 $\pm$ 1.3	6.7 $\pm$ 1.0
Total faecal excretion (kg/seven days)	2.6 $\pm$ 0.2	2.6 $\pm$ 0.4	1.7 $\pm$ 0.2
Total urinary excretion (L)	4.3 $\pm$ 1.8	9.4 $\pm$ 1.8	6.2 $\pm$ 1.8
Feed GE (MJ/kg)	15.8	16.0	15.8
Faecal GE (MJ/kg)	16.7	16.9	16.6
Urine GE (MJ/kg)	0.66	0.49	0.43
Total energy intake (MJ/seven days)	100.9 $\pm$ 15.6	128.7 $\pm$ 15.6	106.9 $\pm$ 15.6
Faecal energy output (MJ/ seven days)	43.3 $\pm$ 4.6	44.3 $\pm$ 4.6	29.1 $\pm$ 4.6
Digestible energy (MJ/kg)	8.9 <sup>b</sup> $\pm$ 0.4	10.5 <sup>ab</sup> $\pm$ 0.4	11.4 <sup>a</sup> $\pm$ 0.4
Urinary energy excreted (MJ/seven days)	2.1 <sup>b</sup> $\pm$ 0.9	5.7 <sup>a</sup> $\pm$ 0.9	2.5 <sup>ab</sup> $\pm$ 0.9
Methane gas production* (MJ/seven days)	8.1 $\pm$ 1.2	10.3 $\pm$ 1.2	8.5 $\pm$ 1.2
Total energy excreted (MJ/seven days)	53.5 $\pm$ 5.8	60.2 $\pm$ 5.8	40.1 $\pm$ 5.8
Total energy excreted (% of energy intake)	53.5 <sup>b</sup> $\pm$ 2.8	47.6 <sup>ab</sup> $\pm$ 2.8	38.7 <sup>a</sup> $\pm$ 2.8
Energy retention (MJ/seven days)	47.4 $\pm$ 10.6	68.4 $\pm$ 10.6	66.7 $\pm$ 10.6
Energy retention (% of energy intake)	46.5 <sup>b</sup> $\pm$ 2.8	52.4 <sup>ab</sup> $\pm$ 2.8	61.3 <sup>a</sup> $\pm$ 2.8
Metabolizable energy (MJ/kg)	7.3 <sup>b</sup> $\pm$ 0.4	8.4 <sup>ab</sup> $\pm$ 0.4	9.7 <sup>a</sup> $\pm$ 0.4

<sup>abc</sup>LSMeans with a different superscript within a row are significantly different ( $P \leq 0.05$ )

\*Calculated as 8% of Gross energy intake (McDonald *et al.*, 2002)

Dry matter intake and excretion was quantified over a seven day period

The lower ME values obtained from the *in vivo* trial against that which was formulated could partly be attributed to the effect of feed preparation such as pelleting. Pelleting and concomitantly the level of feeding increases the amount of energy lost in faeces; however, this loss can also be offset by a lowered methane production (McDonald *et al.* 2002). The stage of rumen development could further have contributed to the lower ME values as the lambs were immature and had not yet reached their full rumen fermentation ability. Also, the energy contributed via milk consumed has not been calculated resulting in more energy loss than the energy accounted for therefore reducing the ME value.

Metabolizable energy for CF2 was higher than CFC ( $P = 0.01$ ) while no difference was found between CF1 and CF2. However, upon contrasting CF1 and CF2 against CFC it was found that the optimised creep feeds had higher ME values ( $P = 0.0051$ ); even when the optimised creep feeds were individually contrasted with CFC, significant differences were found ( $P = 0.037$  and  $P = 0.0035$ , respectively).

These results could be attributed to the fact that CP and NSC level increased from CFC to CF2, making the diet more concentrated and more digestible.

It is known and stated previously that unutilized amino acids are deaminated and excreted via the urine as urea; this increases the amount of energy wasted / excreted via the urine and is responsible for lowering the ME as described by McDonald *et al.* (2002). Table 5.7, however, points out that energy lost via urine was higher for CF1 and CF2. By implication the argument arises that the ME values of the current study did not increase due to better utilization of amino acids but purely because of an increase in digestibility and CP level from CFC to CF2.

Creep feed CF1 and CF2 had the same EAA balance but at different CP levels, the difference in ME between CF1 and CF2 therefore cannot be ascribed to the effect of amino acid balance on ME, especially since no difference was found between CF1 and CF2. Feed intake did not differ significantly nor tended to differ between CF1 and CF2 thereby eliminating the effect of feed intake on ME. It has been shown however, that as CP level increases the digestibility increases and concomitantly the feed efficiency (Craddock *et al.*, 1974, Jones *et al.* 1996, Santra & Karim, 1999). However, no significant differences were found between CF1 and CF2 for CP digestibility (Table 5.3). These observations therefore further agree with the previous mentioned suggestion that the NSC level contributed to higher nitrogen and concomitantly ME balance for CF2, rather than the CP level *per se*.

It is therefore surmised that not only CP but also the NSC levels had an effect on the increased nitrogen- and energy balances of the lambs. It is postulated that rumen ammonia utilisation by the microbes increased with increasing levels of NSC (Stern *et al.*, 1978) which in turn resulted in better nitrogen utilisation, thereby increasing nitrogen retention and ME.

It has been illustrated that BUN is highly correlated to the quantity of protein in a diet (Preston *et al.*, 1965; Torell *et al.*, 1974; Pfander *et al.*, 1975) and it can be used to “fine tune” diets (Kohn *et al.*, 2005). Ruminant ammonia concentrations increase when nitrogen is in excess of available energy and spill over into the bloodstream where after it is converted to urea by the liver (Hammond, 1992); this increases the BUN concentration in the blood.

The BUN results are given in Table 5.7; it is important to note that the BUN units are given as mg/100ml and not in the SI format of mmol/L. Published work on BUN are mostly reported in mg/100ml and therefore it was decided to use this format for comparison with other studies. Values in mmol/l need to be multiplied by 2.8 to be converted to mg/100ml; each molecule of urea has two nitrogens, of which each has a molar mass of 14 g/mol.



**Table 5.8** Blood urea nitrogen measured for each Group on two collection periods for lambs reared on three different creep feeds (CFC, CF1 and CF2) compared to lambs receiving no creep feed (mg/100ml), LSmeans  $\pm$  s.e

		CON (Pasture)	CFC 139 g CP/kg; 455 g NSC/kg	CF1 157 g CP/kg; 477 g NSC/kg	CF2 179 g CP/kg; 508 g NSC/kg
		<b>Normal Range*:</b> 14.84 – 37.18 mg/100ml			
<b>Group 1</b>	43 days	11.40 $\pm$ 1.2	11.00 $\pm$ 1.2	11.51 $\pm$ 1.2	14.05 $\pm$ 1.2
	67 days	7.27 <sup>c</sup> $\pm$ 1.3	16.12 <sup>b</sup> $\pm$ 1.3	15.32 <sup>b</sup> $\pm$ 1.3	22.53 <sup>a</sup> $\pm$ 1.3
<b>Group 2</b>	34 days	9.20 $\pm$ 0.9	8.53 $\pm$ 0.8	10.46 $\pm$ 0.8	11.31 $\pm$ 0.8
	58 days	9.83 <sup>b</sup> $\pm$ 1.9	9.93 <sup>b</sup> $\pm$ 1.8	14.77 <sup>ab</sup> $\pm$ 1.8	20.64 <sup>a</sup> $\pm$ 1.8

<sup>abc</sup>LSmeans with a different superscript within a row are significantly different ( $P \leq 0.05$ )

\*As suggested by the Western Cape Provincial Veterinary laboratory, Department of Agriculture, Stellenbosch for adult sheep

For both groups no differences ( $P > 0.05$ ) were found for BUN at 34 and 43 days of age. Torell *et al.* (1974) reported that age and sampling time has no effect on BUN measurements. It can be argued however, that at the ages of 34 and 43 days the lambs had little rumen function and therefore BUN is less affected by the spill over effect of ammonia from the rumen. At 58 days there were no difference between CON and CFC and between CF1 and CF2. Creep feed 2 maintained the higher BUN level at 20.64 mg/100ml. At 67 days CF2 had the highest BUN (22.53 mg/100ml) and CON the lowest (7.27 mg/100ml) while CFC and CF1 showed no significant difference between each other.

Preston *et al.* (1965) maintains that a BUN concentration in excess of 10 mg/100ml for lambs is indicative of protein wastage. The study of Pfander *et al.* (1975) however indicated that superior performance can be achieved if the plasma urea nitrogen (PUN) concentration can be maintained at 15 mg/100ml. The PUN concentration was found to be highly correlated with that of BUN concentrations (Hammond, 1992). Accordingly it can be surmised that the BUN concentration of CF2 at 20.64 mg/100ml is higher than what is necessary for growing lambs.

It has previously been stated that BUN can be used as a tool to verify the energy: protein ratio fed to ruminants and the correct CP level. The higher BUN concentration could therefore be ascribed to either the CP level that is too high or to the NSC level which should be lifted at this CP level (i.e. increasing energy: protein ratio). It could be argued that the BUN concentration at 34 days represents the effect of the level of CP and not the energy: protein ratio on rumen ammonia production as rumen development is fairly low at this age.

#### **5.4.3 In vitro digestibility trial**

The term *digestibility* here is used interchangeable with *degradability* as *in vitro* digestibility for ruminants represent rumen degradability.

Samples for the *in vitro* digestibility trial were sieved through a 106  $\mu\text{m}$  screen and it can be argued that sample loss after washing (0 hour incubation) the non-incubated bags, is the soluble feed fraction.

The *in vitro* true digestibility (IVTD) results for dry matter disappearance between the three different creep feeds are depicted in Table 5.9. Table 5.10 represents the amino acid digestibility after a 48 hour *in vitro* incubation period in buffered rumen fluid. The table includes the amino acid content before it was incubated and the percentage bypass amino acids. The underlined values represents those amino acids that were specifically included in the optimised creep feeds CF1 and CF2 (i.e. the limiting EAA for suckling lambs) and functions in maintaining focus.

**Table 5.9** *In vitro* true digestibility for three different creep feeds (CFC, CF1 and CF2) formulated for limiting EAA's and NSC

	<i>In vitro</i> True digestibility (%)	s.e.
<b>CFC</b> 139 g CP/kg; 455 g NSC/kg	82.63 <sup>b</sup>	0.59
<b>CF1</b> 157 g CP/kg; 477 g NSC/kg	82.73 <sup>b</sup>	0.84
<b>CF2</b> 179 g CP/kg; 508 g NSC/kg	91.91 <sup>a</sup>	0.17

<sup>ab</sup> Means with different superscript within a column are significantly different ( $P \leq 0.05$ )

The IVTD results (Table 5.9) indicate that CF2 was more ( $P < 0.05$ ) digestible than CF1 and CFC while the latter showed no significant differences between each other.

Similarly to the *in vivo* results it could be argued that the decrease of NDF and ADF from CFC to CF2 is responsible for the increase in digestibility for CF2 since NDF and ADF is strongly associated with the digestibility of a feed (McDonald *et al.*, 2002).

Creep feed 2 had the highest ( $P \leq 0.05$ ) amino acid rumen degradability (Table 5.10) for Glycine, Leucine, Phenylalanine, Isoleucine, Cystine, Histidine, Valine, Threonine and Alanine. The amino acid chemical composition depicted in Table 5.10 represents those results gained from amino acid analyses of feed sieved through a screen while the amino acid chemical composition depicted in Table 5.5 represents the same feed that was not sieved. It can be noted that there is some variation between results from Table 5.10 and Table 5.5 for the individual amino acids, which can be attributed to the sieving process as well as the fact that these analyses were done in different batches. Williams (1986) associated preparation and storage of samples and the analytical stage of amino acid analyses as some of the problems contributing to the variation in amino acid analyses.

**Table 5.10** *In vitro* undegradable- and soluble-fraction of the three different lamb creep feeds (CFC, CF1 and CF2), as well as the chemical composition of the creep feeds.

	Chemical composition (g/kg) <sup>1</sup>			Soluble fraction <sup>2</sup> (%)			Undegradable fraction <sup>4</sup> (%)		
	CFC	CF1	CF2	CFC	CF1	CF2	CFC	CF1	CF2
Ala	4.3	2.4	3.6	20.9	15.7	23.0	37.1 <sup>b</sup> ± 2.4	46.5 <sup>c</sup> ± 1.5	18.9 <sup>a</sup> ± 1.8
Thr	<u>4.6</u>	<u>5.1</u>	<u>5.0</u>	<u>29.0</u>	<u>30.4</u>	<u>33.4</u>	<u>37.2<sup>a</sup> ± 2.4</u>	<u>33.5<sup>ab</sup> ± 1.1</u>	<u>26.1<sup>a</sup> ± 2.5</u>
Ser	5.6	5.8	5.8	28.5	13.9	21.7	35.0 ± 2.3	35.5 ± 1.2	27.7 ± 2.6
Arg	5.6	11.7	8.8	20.7	29.5	39.3	44.3 <sup>b</sup> ± 2.9	15.4 <sup>a</sup> ± 0.5	13.3 <sup>a</sup> ± 1.3
Glut	11.1	14	11.2	27.2	31.6	0	24.6 ± 1.6	24.5 ± 0.8	27.5 ± 2.6
Val	4.8	3.6	4.9	20.7	18.9	32.0	31.5 <sup>a</sup> ± 2.1	35.2 <sup>a</sup> ± 1.2	17.1 <sup>b</sup> ± 1.6
His	2.3	2.4	3.2	36.1	21.0	54.3	26.3 <sup>b</sup> ± 1.3	22.4 <sup>ab</sup> ± 0.7	16.3 <sup>b</sup> ± 1.5
Asp	12.1	14.0	16.0	44.6	37.4	43.5	24.5 <sup>ab</sup> ± 1.6	26.9 <sup>b</sup> ± 0.9	19.0 <sup>a</sup> ± 1.8
<u>Lys</u>	<u>6.9</u>	<u>7.3</u>	<u>8.2</u>	<u>49.5</u>	<u>28.9</u>	<u>47.9</u>	<u>24.1<sup>ab</sup> ± 1.6</u>	<u>26.7<sup>b</sup> ± 0.9</u>	<u>19.5<sup>a</sup> ± 1.9</u>
Pro	7.0	5.3	7.1	24.3	15.5	31.4	31.7 <sup>b</sup> ± 2.1	37.0 <sup>b</sup> ± 1.2	21.4 <sup>a</sup> ± 2.0
<u>Met</u>	<u>1.8</u>	<u>2.1</u>	<u>1.8</u>	<u>25.1</u>	<u>28.5</u>	<u>30.6</u>	<u>56.0<sup>b</sup> ± 3.7</u>	<u>33.1<sup>a</sup> ± 1.1</u>	<u>28.1<sup>a</sup> ± 2.7</u>
Tyr	3.4	4.8	5.2	14.4	28.8	32.1	41.7 <sup>b</sup> ± 2.7	29.4 <sup>a</sup> ± 1.0	26.7 <sup>a</sup> ± 2.5
Cys	0.5	0.4	0.6	38.2	33.5	41.4	21.6 <sup>b</sup> ± 1.4	23.3 <sup>b</sup> ± 0.8	15.1 <sup>a</sup> ± 1.4
<u>Ile</u>	<u>3.0</u>	<u>3.3</u>	<u>4.1</u>	<u>13.8</u>	<u>17.1</u>	<u>33.6</u>	<u>47.1<sup>b</sup> ± 3.1</u>	<u>38.3<sup>b</sup> ± 1.3</u>	<u>25.1<sup>a</sup> ± 2.4</u>
<u>Phe</u>	<u>5.0</u>	<u>6.0</u>	<u>7.3</u>	<u>32.6</u>	<u>26.7</u>	<u>44.3</u>	<u>36.0<sup>b</sup> ± 2.4</u>	<u>31.0<sup>b</sup> ± 1.0</u>	<u>20.6<sup>a</sup> ± 1.9</u>
<u>Leu</u>	<u>10.3</u>	<u>10.0</u>	<u>12.8</u>	<u>25.5</u>	<u>18.8</u>	<u>35.0</u>	<u>38.8<sup>b</sup> ± 2.5</u>	<u>38.1<sup>b</sup> ± 1.3</u>	<u>23.3<sup>a</sup> ± 2.2</u>
Gly	4.8	4.5	4.6	28.5	29.3	32.6	33.4 <sup>b</sup> ± 2.2	31.5 <sup>b</sup> ± 1.0	18.4 <sup>a</sup> ± 1.8

<sup>abc</sup>LSMeans with a different superscript within a row are significantly different ( $P \leq 0.05$ )

<sup>1</sup> Results from amino acid hydrolyses

<sup>2</sup> Soluble fraction obtained from one sample therefore no variance is recorded

<sup>3</sup> Least square means ± standard error (statistical analysis on these values)

<sup>4</sup> Calculated as 100 – Degradable fraction = Undegradable fraction; a 100% digestion is assumed

Underline values represent those amino acids that were specifically balanced for the creep feeds CF1 and CF2

CFC = 139 g CP/kg, 455 g NSC/kg;

CF1 = 157 g CP/kg, 477 g NSC/kg;

CF2 = 179 g CP/kg, 508 g NSC/kg

From Tables 5.10 and 5.1 the actual chemical composition versus the original formulation can be compared. The Lysine content of the feed measured 6.9, 7.3 and 8.2 g/kg for CFC, CF1 and CF2, respectively; the original formulation indicated that the content was 4.9, 7.4 and 8.4 g/kg. Threonine measured 4.6, 5.1 and 5.0 g/kg for CFC, CF1 and CF2 respectively while the original formulation indicated a content of 4.7, 8.1 and 9.1 g/kg respectively. Methionine measured 1.8, 2.1 and 1.8 g/kg for CFC, CF1 and CF2 respectively while the original formulation indicated a content of 1.9, 2.4 and 2.7 g/kg respectively. Some of these differences could be accounted for by the fact that the recovery rate of amino acids for the HCl hydrolyses procedure (as discussed in section 5.4.1) differs for the different amino acids.

Bypass Lysine content was estimated (Table 5.1) to be 1.5, 2.6 and 2.8 g/kg for CFC, CF1 and CF2 respectively. The *in vitro* results suggests the bypass Lysine content to be 1.7, 1.8 and 1.4 g/kg (s.e. =  $\pm 0.1$ ) for CFC, CF1 and CF2 respectively. Bypass Methionine content was estimated (Table 5.1) to be 0.7, 0.9 and 1.0 g/kg for CFC, CF1 and CF2 respectively. The *in vitro* results suggests the bypass Methionine content to be  $1.0 \pm 0.1$ ,  $0.6 \pm 0.02$  and  $0.4 \pm 0.04$  g/kg for CFC, CF1 and CF2 respectively. In both amino acids the bypass content for CF2 was much lower than that estimated by the original formulation. Differences between formulated and determined values did not exceed more than  $\pm 0.7$  and it can be argued that the formulated values were fairly accurate.

## 5.5 Conclusion

It was hypothesised that a creep feed optimised for the limiting EAA of suckling lambs will elicit greater nitrogen and energy balances compared to a non optimised commercial creep feed. It was also postulated that NSC within the creep feed rations will further increase nitrogen retention and simultaneously increase feed digestibility.

The current study did illustrate that the optimised creep feeds elicited higher nitrogen retentions. It was also suggested that NSC level rather than CP level *per se* increased nitrogen retention over and above the increase gained from limiting EAA supply. This coincides with the study of Schroeder *et al.* (2006) that energy supplementation increases amino acid utilization.

From the digestibility results it was speculated that a higher NSC level caused an increase in the microbial population which resulted in better digestion and therefore the results indicated higher DM disappearance for the *in vivo* trial. It is furthermore postulated that the higher levels of NSC could have resulted in better rumen development which in turn could explain the higher DM disappearance for the *in vivo* digestibility trials. This effect of NSC level on rumen development *per se* however remains to be illustrated (Chapter 6).

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## **CHAPTER 6**

### **LAMB RUMEN DEVELOPMENT: THE EFFECT OF NON STRUCTURAL CARBOHYDRATES IN CREEP FEED DIETS**

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The effect of nonstructural carbohydrate (NSC) level in creep feed rations on the rumen development of suckling lambs were investigated. Fermentation of NSC gives rise to higher butyrate and propionate which in turn stimulates rumen development. The inclusion level of NSC in creep feed diets for lambs however has not yet been determined. The current study investigated the effect of NSC level on the histological development of the rumen in suckling lambs. Three creep feed rations were formulated at NSC levels of 455, 477 and 508 g/kg to represent treatment CFC, CF1 and CF2 respectively. Another treatment (CON) was included to represent lambs with no creep feed access but access to grazing. Twin lambs averaging a birth weight of  $4.42 \pm 0.11$  kg entered the trial at an average age of seven days and were slaughtered at the average age of 69 days at a live weight of  $23.6 \pm 0.56$  kg. The rumen development characteristics which include papillae length, width, mucosa thickness and rumen wall thickness were measured from microscopic slides in micrometers. Creep feed treatments had longer papillae than CON lambs. Papillae width decreased as the NSC level increased. It seems that papillae length responds to increasing level of NSC and that the longer a papilla grows the thinner it becomes. Absorptive area is therefore increased as the surface to volume ratio increases. The creep feed diets alongside the course roughage (consumed from the dam's trough) were sufficient to prevent parakeratosis and developed those rumen characteristics (papillae and wall muscularization) that are influenced by different dietary factors simultaneously.

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Keywords: pre-weaning lambs, papillae, rumen development, starch and sugar,

#### **6.1 Introduction**

The necessity of rumen development in young ruminants has been known for many decades. A young ruminant essentially functions as a monogastric animal at birth and has to go through a transition from a suckling monogastric to a grazing ruminant. The rumen which at first constitutes a third of the total stomach capacity grows and develops to become the largest compartment, ca. 85% of the total stomach (Sisson & Grossman, 1938). Growth and development not only occurs with respect to size but also in papillary growth, muscularization and vascularisation of the rumen wall (Warner *et al.*, 1956). A functional rumen allows the animal to consume and utilize dry feed and roughages.

The production of ruminant animals within intensive rearing systems entails the responsibility of producers to see to it that ample stimulus of rumen growth and development occurs. Intensive farming systems do not always allow for the natural behaviour of domesticated animals that would inevitably have lead to a fully developed rumen. It has therefore become the responsibility of the producer to



oversee the stimuli that is needed for adequate and optimum rumen development to sustain maximum production and efficiency; at the same time this also allows for better welfare practises. The rearing of young heifers in intensive dairy production systems is a good example of increasing rumen development to sustain better efficiency later in life (Heinrichs & Lesmeister, 2005).

Effect of diet type on rumen development in lambs and calves have been the focus of most research in rumen development. It has been found that cereal grains rather than roughages are responsible for rumen papillary development (Warner *et al.*, 1956). Baldwin & McLeod (2000) attributed this increased papillary development to the higher proportions of VFA (propionate and butyrate) as a response to the fermentation of cereal grains. When rumens were infused with sodium butyrate and sodium propionate it was found that butyrate had the largest effect on rumen papillary development (Baldwin & McLeod, 2000). This is not surprising seeing as 90% of butyrate produced during fermentation is metabolized within the rumen wall with little butyrate circulating in the peripheral blood supply (Baldwin & McLeod, 2000).

It has frequently been reported that rumen papillae and rumen wall muscularization occurs independently from one another (Brownlee, 1956; Harrison *et al.*, 1960), i.e. dietary factors attributing to papillae development do not attribute to rumen wall muscularization. Roughage as opposed to cereal grains typically stimulates muscularization (Žitnan *et al.*, 1998) due to its bulkiness (Warner *et al.*, 1956; Vazquez-Anon *et al.*, 1993; Žitnan *et al.*, 1998) which in turn promotes rumen motility (McGillard *et al.*, 1965).

Feed that enters the rumen must be easily available to the establishing microbes before their generation time is over, i.e. nutrients from feed need to become available as quickly and frequently as the microbes need it to grow and populate or they will die off. Feed that allows for rapid fermentation and removal of indigestible feed particles will give rise to greater rumen development (Andrews *et al.*, 1969). Non structural carbohydrates (NSC) consisting mainly out of sugars and starches (McDonald *et al.*, 2002) function as a feedstuff easily fermentable (Carruthers & Neil, 1997) with a high outflow rate. It is therefore postulated that NSC is a vital factor for the establishment of a microbial population.

Stern *et al.* (1978) studied the effects of different NSC levels on microbial protein synthesis. NSC levels were high, medium and low representing NSC concentrations of 490, 326 and 196 g NSC/kg respectively. The study indicated that butyrate proportions increased as the level of NSC increased while the molar percentages of acetate and propionate did not change significantly between the different levels of NSC.

Extensive research on rumen development has been done in calves and to a lesser extent, in lambs. Papillary development characteristics such as papillae length, width, papillae per square centimetre, rumen wall thickness and mucosa layer thickness in calves are well documented (Heinrichs, 2005) but not as well in lambs. Even more so the rumen development characteristics of lambs as influenced by the level of NSC within creep feed rations is yet to be reported.

This study therefore determines the effect of NSC levels in three different creep feed rations on rumen development characteristics of lambs.

## **6.2 Materials and methods**

### **6.2.1 Lamb management**

Forty five twin bearing ewes were brought to the University of Stellenbosch's experimental farm Welgevallen, ca. 2 months prior to lambing. Eighty lambs were born over a three week lambing season. Detailed description of ewe and lamb management is described in the start of Chapter 3.

Lambs were born over a three week period and were therefore subdivided into two groups to maintain a seven day average age between lambs. This allowed a group of lambs to enter the creep feed trial at an average age of seven days. Two groups were formed where group one entered the trial ten days prior to group two. Commencement of trial represented Day 0 and lasted 60 days.

Each group was divided into four treatments namely: Creep feed 1, Creep feed 2, Creep feed Control and Control. The creep mixtures Creep feed 1 (CF1) and Creep feed 2 (CF2) were formulated based on the amino acid requirement for pre-weaning lambs at two different CP and NSC levels (Chapter 3). Although CF2 was formulated for a higher CP and NSC level, the balance or profile of required amino acids remained the same. Creep feed Control (CFC) was a commercial feed and not optimized for amino acid or NSC and was used as a control for the optimised creep feeds CF1 and CF2.

Table 3.2 presents the physical and chemical composition of the three different creep feeds as formulated by Tanqua Feeds (Riviersonderend, Western Cape, South Africa).

Lambs in creep feed treatments were housed in a semi open shed with 1.2 x 1.8 m pens on slatted floors. To determine lamb creep feed intake the lambs were separated from their dams into an adjacent pen where visual and physical contact remained through the wired fence separating the individual pens, which in turn reduced stress at separation (Figure 3.1). Effort was made to have lambs from different dams to be in adjacent pens at time of separation to aid in learning behaviour from one another.

At day 0 of the creep feed trial (i.e. at seven days of age) lambs were separated from their dams for two hours two times a day (8h00 – 10h00 and 13h00 – 16h00) to initiate creep feed intake. From day 7 to 21 the lambs were separated from their dams in the morning (7h00) and allowed a two hour suckling time at mid day (11h00 – 13h00) and separated again until 17h00 in the evening where the lambs were united again with their dams until the next morning. From day 21 to weaning, lambs were separated from dams for the whole day and united only in the evenings from 17h00 until ca. 8h00 the next morning.

Care was taken to feed ewes (with lambs in the creep feed treatments) when lambs were separate in adjacent pen and not able to consume pellets intended for ewes. Ewes, however consumed their

pelleted feed at different rates which resulted in some left over pellets being consumed by creep feed treatment lambs. Lambs furthermore had access to dam's roughage after reunion.

Control lambs were allowed to graze for eight hours each day with their dams on a kikuyu pasture. Care was taken to ensure that CON lambs did not get access to any of the feed pellets used during the trial. This was accomplished by managing access to feed bags and also by removing the pellets that spilled during handling of feed when lambs were moved from the shed to the grazing paddock and *vice versa*. No restriction was placed on the amount and frequency of suckling for the control treatment. After a day's grazing lambs and ewes were brought indoors where fresh water and a lucerne- and oat hay mixture was supplied.

### **6.2.2 Slaughter of lambs, and collection of rumen samples**

At weaning, 40 lambs weighing ( $\pm$  s.d.)  $23.32 \pm 4.33$  kg were slaughtered for rumen wall sampling. Lambs were not fasted before slaughter. Lambs selected for slaughter were taken from their experimental units and transported in groups of 10 to the abattoir less than 500 m from their holding area. One lamb at a time was offloaded and slaughtered before the next lamb was taken out. At slaughter lambs were electrically stunned (4 s at 200 V) where after the jugular veins were severed and exsanguination took place. After exsanguination the lambs were weighed, dressed and eviscerated. The carcasses were suspended by both the Achilles tendons in a cooler at 4°C for 24 hours.

The reticulo-rumen was separated from the rest of the gastrointestinal tract at the pyloric sphincter of the abomasum. The oesophagus was left intact. The separated reticulo-rumen was weighed and recorded as "full reticulo rumen" weight (FRR). After weighing the FRR was placed with the oesophagus in its dorsal position with the reticulum, omasum and abomasum on the left side. An incision was made from the oesophagus caudally across the dorsal side of the atrium. The rumen content in the ventral part of the atrium was gently removed from within the rumen and a 2 x 3 cm sample from the rumen wall was taken ca. 2.5 cm right of the rumino-reticular fold (in the left cranial ventral sac). Each rumen sample was fixed in 100 ml formaldehyde (4%) solution (i.e. 100 ml formaldehyde (40%) in 900ml saline (0.09%) solution). After sampling, the reticulo-rumen was washed thoroughly of all contents and weighed, representing the empty-reticulo rumen weight (ERR). Due to papillae fragility, rumen wall samples had to be taken before thorough washing in order to maintain papillae integrity for histological analyses. Figure 6.1 indicates where the rumen sample was taken.



**Figure 6.1** The area in the left cranial ventral sac of the rumen where rumen samples of 2 x 3 cm were taken for later histological measurements; a – area of sampling, b - reticulum

The small intestine was severed from the large intestine at the caecum and was weighed with contents. Similarly the large intestine was weighed with contents remaining; the rectum and anus remained attached to the large intestine.

Liver weight was recorded with the gall bladder remaining intact.

### **6.2.3 Sample preparation for microscopic slides**

Histological slide preparation of rumen wall samples was done at the University of Stellenbosch's Department of Biomedical Sciences, Anatomy & Histology laboratory, Tygerberg Campus. Harris's Haematoxylin (Merck Chemicals (Pty) Ltd; SAAR2822001LC) was used for staining and Eosin (0.5%) for counterstaining.

Rumen wall samples were processed on a Shandon Elliott Duplex Processor (Optolabor (Pty)). This processor allows the sample to go through several alcohol dehydrations where after the sample can be embedded in paraffin wax (Papa Plast, Kimix). Processing is done up to the point where the samples are immersed in Xylene for one hour.

The dehydrating process allows for the water inside the fixed sample to be replaced by a hardening medium such as paraffin wax. A fixed sample filled with water is disrupted and damaged when it is cut into sections; therefore the replacement of water with paraffin prevents this damage (Humason, 1962). Dehydration was done by placing the fixed sample in one litre dishes for 1.5 hours in a series of increasing concentrations of alcohol. The tissue samples were placed in 70% alcohol for 1.5 hours, then in two consecutive dishes containing a 96% alcohol for 1.5 hours each and finally also in two consecutive dishes with 100% alcohol for 1.5 hours each.

Alcohol remaining on the samples interferes with the infiltration process of the paraffin wax and therefore samples went through a clearing process before the paraffin infiltration process (Humason,

1962). For the clearing process, the sample is firstly placed in Xylene for 1.5 hours and thereafter in a fresh dish with Xylene for one hour.

The embedding process was done by a Leica EG 1160 Embedder (SMM Instruments). After processing, the sample was placed in a mould which was filled with paraffin wax (kept at 60°C by the Embedder) until the sample was totally submerged under paraffin wax. Samples were then placed onto the ice table provided by the Embedder to allow for the paraffin wax to set and harden. After setting of the paraffin wax the samples within their moulds were ready to be cut and placed on glass slides.

Samples were sectioned with a thickness of 5 µm with the use of a Leica RM 2125 RT microtome (SMM Instruments). After sectioning of the sample and placement thereof on a microscope-slide, the slide was incubated at 60°C in an M53c Incubator for an hour. This step allows for the tissue to stick to the glass slide and for the wax to melt which allows for easy removal of wax in the subsequent procedure. After incubation the slides were placed into one litre Xylene dishes, two times at 2 minutes per dish, in order to dewax the sample. Dewaxing is a critical step as the Haematoxylin and Eosin (H&E) staining procedure cannot occur before this has been done. Thereafter the sample was placed in an M53c Incubator for half an hour before staining commenced.

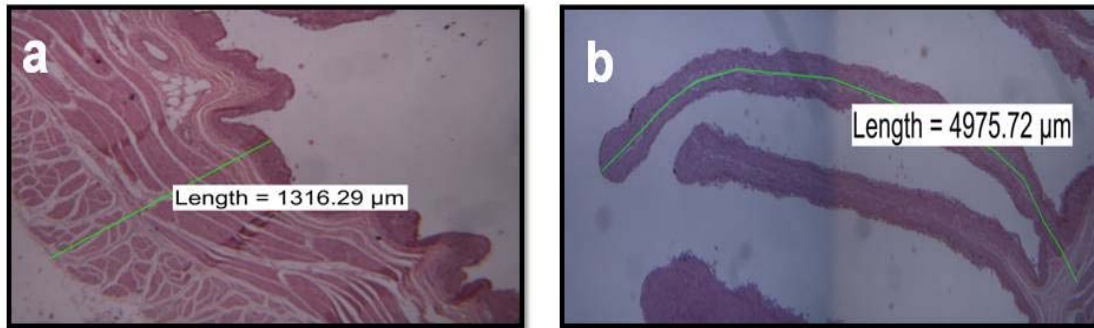
The H&E Processor (Leica Auto Stainer XL, (SMM Instruments)) was used for staining the samples. Slides were hydrated through a series of alcohols decreasing in concentration (100%, 96% and 70%). Slides were immersed in 100% alcohol two times for 1 minute each time. Thereafter in 96% alcohol two times for 1 minute each time and finally in 70% alcohol for 1 minute. After rinsing samples with tap water, staining with Harris's Haematoxylin was done by placing the slides in the haematoxylin for 4 minutes. The slides were then rinsed in tap water for 3 minutes and thereafter stained in Eosin for 2.3 seconds. The slides were again rinsed in tap water for 2 minutes.

Before mounting the slides, they need to go through a dehydration process by means of immersing the slides in a series of alcohols with increasing concentration. Slides were immersed in 70% alcohol for 0.20 seconds, then twice in 96% alcohol for 0.15 seconds and finally twice in 100% alcohol for 0.15 seconds. To remove the alcohol, the slides were immersed in Xylene for 1 minute. Using a cover slide and DPX mounting medium, the slides were finally mounted. Each rumen sample was prepared in duplicate.

#### **6.2.4 Microscopic measurements**

Microscopic measurements were done in duplicate using an Olympus CH30 microscope with a Zeiss West Germany 47 lens at a magnification of 4x (i.e. 0.99 µm/pixel at 2560 by 1920 pixels). For measuring papillae parameters the microscopic image was taken via a Nikon DS – Fi1 Digital Sight camera while image measurements were taken with the imaging analysis program: Nis elements imaging software Nikon (BR 2).

Within each slide, two long papillae and two short papillae (relative to long papillae) were measured for width and length. Length of papillae (Figure 6.2) was determined by tracing a series of straight lines across the length of each papillae (Hill *et al.*, 2005) starting from the base of the collagen fibres and ending at the tip of the papillae including the keratin layer (Dobson *et al.*, 1955). The width of each papilla was determined at the widest part of the papillae with a line perpendicular to the line determining papillae length (Hill *et al.*, 2005).



**Figure 6.2** Method of measuring (a) rumen wall thickness and (b) rumen papillae length

The stratified epithelium and collagen layers were measured around each investigated papillae. The epithelium layer was measured with a straight line on both sides of the measured papillae. The collagen layer was measured at the same place where the epithelium layer was measured in a straight line on both sides of the measured papillae.

The rumen wall thickness was determined by tracing a straight line from the edge of the rumen epithelium (lumen's side) to the outer edge of the rumen wall as demonstrated in Figure 6.2. The serosa layer on the outer side of the wall was included in the measurement. A total of four measurements were done per slide.

## 6.4 Statistical analysis

Statistical analyses were done via SAS Enterprise guide 4.0, version 9.1.3 (2002), using Proc GLM. Least square means were estimated and significance declared at the  $P \leq 0.05$ .

A complete randomized design was used.

Final body weight was used as a covariate with the slaughter data.

Contrasts were used to compare the creep feed treatments between each other. The contrasts were:

- CF1 and CF2 against CFC
- CF1 against CF2
- CF1 against CFC
- CF2 against CFC

## 6.5 Results and discussion

Full-reticulo rumen (FRR) weights were the highest for the CON lambs at  $3.85 \pm 0.21$  kg and lowest for the CF2 lambs at  $2.47 \pm 0.19$  kg (Table 6.1). FRR is indicative to some degree of the amount and type of feed consumed. Bulky feeds with high moisture content such as kikuyu grazing will lead to higher FRR weights as evident in the CON lambs. More concentrated feeds with lower water content will lead to lower FRR weights as evident from the CF2 lambs on the most concentrated diet. Similarly the FRR weight for the lambs receiving the CF1 diet, which is a more concentrated diet compared to the CFC diet, is lower than FRR from the CFC diet.

**Table 6.1** Average growth and slaughter data, per individual lamb, reared on different creep feeds (CFC, CF1 and CF2) as compared to a control group reared on pasture (CON), LSMMeans  $\pm$  s.e.

	CON (Pasture)	CFC 139 g CP/kg; 455 g NSC/kg	CF1 157 g CP/kg; 477 g NSC/kg	CF2 179 g CP/kg; 508 g NSC/kg
Weight at slaughter (kg)	23.78 $\pm$ 0.26	23.44 $\pm$ 0.24	23.14 $\pm$ 0.24	22.99 $\pm$ 0.22
Carcass weight (kg)	11.79 <sup>b</sup> $\pm$ 0.22	12.13 <sup>ab</sup> $\pm$ 0.20	12.21 <sup>ab</sup> $\pm$ 0.65	12.64 <sup>a</sup> $\pm$ 0.63
Full reticulo rumen (kg)	4.13 <sup>b</sup> $\pm$ 0.19	3.39 <sup>a</sup> $\pm$ 0.17	2.99 <sup>a</sup> <sup>c</sup> $\pm$ 0.17	2.48 <sup>c</sup> $\pm$ 0.16
Empty reticulo rumen (kg)	0.75 $\pm$ 0.04	0.76 $\pm$ 0.03	0.75 $\pm$ 0.03	0.69 $\pm$ 0.03
Small intestine (kg)	1.14 $\pm$ 0.08	1.02 $\pm$ 0.07	1.00 $\pm$ 0.07	0.98 $\pm$ 0.06
Large intestine (kg)	0.82 $\pm$ 0.05	0.74 $\pm$ 0.05	0.80 $\pm$ 0.05	0.66 $\pm$ 0.05
Liver (kg)	0.36 <sup>b</sup> $\pm$ 0.02	0.46 <sup>a</sup> $\pm$ 0.02	0.45 <sup>a</sup> $\pm$ 0.02	0.45 <sup>a</sup> $\pm$ 0.02
FRR <sup>1</sup> as % of WAS <sup>3</sup>	18.35 $\pm$ 0.91	14.73 $\pm$ 0.83	13.13 $\pm$ 0.83	10.65 $\pm$ 0.77
ERR <sup>2</sup> as % of WAS <sup>3</sup>	3.22 $\pm$ 0.13	3.24 $\pm$ 0.12	3.23 $\pm$ 0.12	3.01 $\pm$ 0.11

<sup>abc</sup> Means within the same row with different letters are significantly different at  $P \leq 0.05$

<sup>1</sup>FRR = Full reticulo rumen weight

<sup>2</sup>ERR = Empty reticulo rumen weight

<sup>3</sup>WAS = Weight at slaughter

Higher ERR weights could be expected for CON lambs on grazing compared to the creep feed treatments as forage is the primary factor influencing muscularization and volume capacity of the reticulo rumen (Zitnan *et al.*, 1998). This was not the trend however, seeing as there were no significant difference between creep feed treatments. Even if ERR is expressed as a percentage of the weight at slaughter (WAS) no numerical difference was found confirming that ERR did not differ between treatments. Creep feed treatment lambs on the other hand also had access to dry roughage and therefore perhaps show the same amount of muscularization. Rumen volume displacement would be a better indicator of rumen development in terms of total capacity rather than empty weight, seeing as empty weight also represents epithelium growth which is not indicative of increased rumen capacity.

Furthermore, as expected, diet had no influence on either the small or large intestine weights (Table 6.1).

In accordance with the study of Joy *et al.* (2008), the liver weights of the current study showed a significant increase in liver weight between CON and the creep feed treatments. The increased liver weight corresponds to the higher plane of nutrition received by the creep feed treatments (Wester *et al.*, 1995; Fluharty & McClure, 1997).

### 6.5.1 Histological parameters on rumen development

Upon rumen sampling the effect of creep feed on rumen development was clearly visible. Rumen colour for lambs on creep feed had a healthy dark gray colour and the papillae were visibly longer compared to the lambs receiving no creep feed or pellets. Rumen colour for CON lambs were pink/cream light with visibly smaller papillae, resembling that of milk fed lambs as indicated by Warner *et al.* (1956) and Heinrichs & Lesmeister (2005). Unlike the study of Suárez *et al.* (2006), no focal or multifocal patches of coalescing and adhering papillae with a sticky mass of feed hair or cell debris was seen during rumen sampling. The possible reason for this observation can be attributed to the fact that lambs in the current study had access to coarsely ground roughage as opposed to Suárez *et al.* (2006) who fed pelleted diets with no additional course roughage. The coarseness or physical effectiveness contributed by roughage prevents parakeratosis, a condition that occurs when the keratin layer around the squamous epithelium cells hardens due to the diet's inability to remove degenerating keratin cells (Hinders & Owen, 1965). The consequences of parakeratosis include reduced absorptive area, reduced epithelial blood flow, reduced rumen motility and in severe cases, lead to papillae degeneration and sloughing (Beharka *et al.*, 1998). The clumping and branching of papillae are initial signs of parakeratosis (Anderson *et al.*, 1987; Zitnan *et al.*, 1998) as was the case found in Suárez *et al.*'s (2006) study.

**Table 6.2** shows the descriptive statistics for all the papillae characteristics.

**Table 6.2** Descriptive statistics for papillae parameters measured from weaned Döhne-Merino x Merino cross breed lambs

Parameter	Mean	Std Dev	Minimum	Maximum
Length ( $\mu\text{m}$ )	3107.53	1255.38	625.87	7572.83
Width ( $\mu\text{m}$ )	432.86	136.54	88.50	272.72
Epithelium layer ( $\mu\text{m}$ )	97.90	28.44	49.16	247.74
Collagen layer ( $\mu\text{m}$ )	171.53	85.11	54.93	668.18
Mucosa layer <sup>1</sup> ( $\mu\text{m}$ )	269.41	93.87	111.59	800.86
Rumen wall thickness ( $\mu\text{m}$ )	1558.44	521.13	739.86	3356.88

<sup>1</sup>Mucosa layer equals the sum of the epithelium and collagen layers

According to Dobson *et al.* (1955), rumen papillae can be up to 6 mm long and 2 mm wide. Papillae in this study ranged beyond this with the maximum length at 7.5 mm and width at 2.7 mm. Considering the minimums and maximums of the papillae parameters, the amount of variation within parameters is clear where maximum values are threefold and more of the minimum value measured. Dobson *et al.* (1955) emphasized that there is considerable variation in the first four layers within the rumen wall,



which is confirmed in this study by the variation in the papillae parameters for length, width, epithelium- and collagen layers.

The rumen wall development characteristics are depicted in Table 6.3. Length and width of papillae are grouped for short and long papillae.

**Table 6.3** Rumen development characteristics for lambs at the age of two months reared on three different creep feeds (CFC, CF1 and CF2) and pasture (CON), LSmeans  $\pm$  s.e.

Parameter ( $\mu\text{m}$ )	CON (Pasture)	CFC 139 g CP/kg; 455 g NSC/kg	CF1 157 g CP/kg; 477 g NSC/kg	CF2 179 g CP/kg; 508 g NSC/kg	
<b>Short</b> <sup>1</sup>	Width	432.3 <sup>ab</sup> $\pm$ 22.6	502.81 <sup>a</sup> $\pm$ 22.9	461.4 <sup>ab</sup> $\pm$ 21.1	384.3 <sup>b</sup> $\pm$ 20.1
	Length	1942.0 <sup>b</sup> $\pm$ 126.5	2510.5 <sup>a</sup> $\pm$ 128.3	2553.9 <sup>a</sup> $\pm$ 120.1	2299.1 <sup>ab</sup> $\pm$ 114.5
<b>Long</b> <sup>1</sup>	Width	490.7 <sup>a</sup> $\pm$ 21.1	444.8 <sup>ab</sup> $\pm$ 24.0	398.78 <sup>b</sup> $\pm$ 20.6	376.3 <sup>b</sup> $\pm$ 19.1
	Length	2943.9 <sup>b</sup> $\pm$ 161.5	4027.3 <sup>a</sup> $\pm$ 183.1	4165.1 <sup>a</sup> $\pm$ 157.2	4483.0 <sup>a</sup> $\pm$ 146.1
Epithelium layer	91.5 <sup>b</sup> $\pm$ 2.8	98.5 <sup>ab</sup> $\pm$ 3.0	109.0 <sup>a</sup> $\pm$ 2.7	92.8 <sup>b</sup> $\pm$ 2.6	
Collagen layer	217.3 <sup>b</sup> $\pm$ 7.7	163.7 <sup>a</sup> $\pm$ 8.3	158.9 <sup>a</sup> $\pm$ 7.4	151.9 <sup>a</sup> $\pm$ 7.4	
Mucosa <sup>2</sup>	308.7 <sup>b</sup> $\pm$ 8.7	262.9 <sup>a</sup> $\pm$ 9.4	267.0 <sup>a</sup> $\pm$ 8.3	244.7 <sup>a</sup> $\pm$ 7.9	
Rumen wall thickness	1851.4 <sup>a</sup> $\pm$ 83.9	1351.6 <sup>b</sup> $\pm$ 83.9	1740.7 <sup>a</sup> $\pm$ 79.6	1519.8 <sup>ab</sup> $\pm$ 75.9	

<sup>abc</sup> LSmeans in the same row with different letters are significantly different at the  $P \leq 0.05$

<sup>1</sup>Representing measurements on short and long papillae

<sup>2</sup>Mucosa layer is the sum of the epithelium and collagen layers seeing as mucosa consists out of these layers

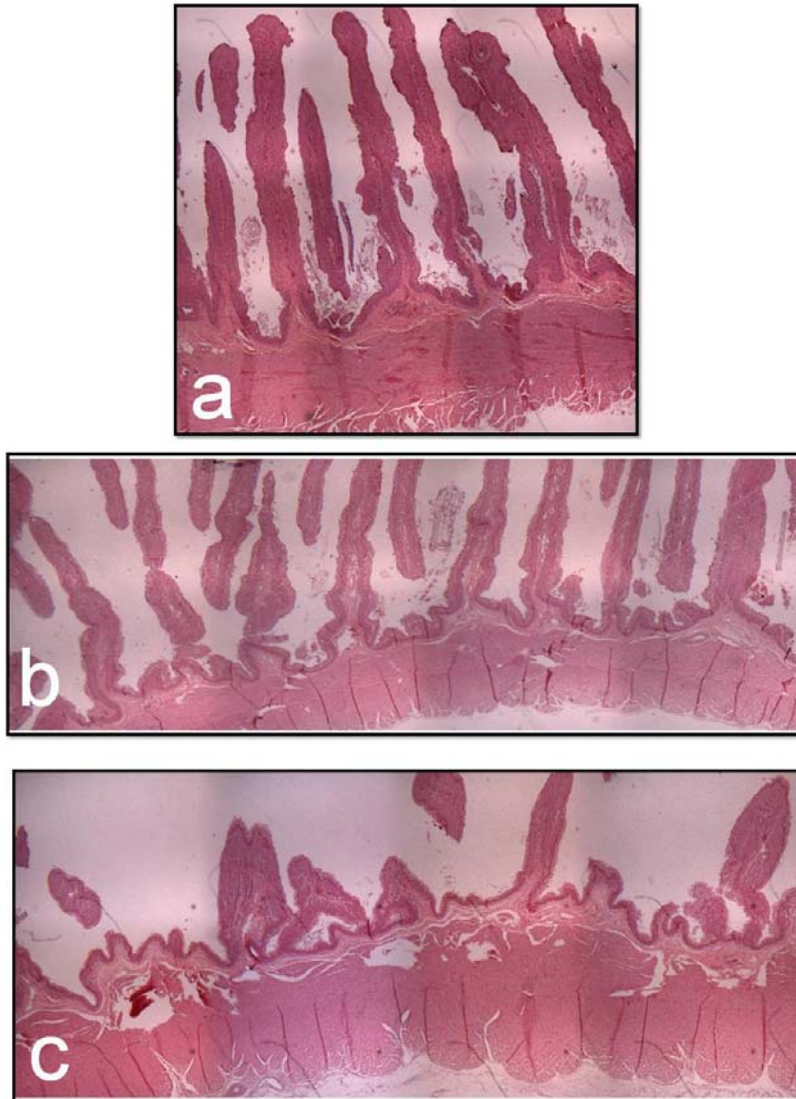
<sup>3</sup>E= Epithelium layer

<sup>4</sup>C= Collagen layer

Papillae length in both short and long papillae showed differences ( $P < 0.05$ ) between CON and the creep feed treatments while no significant differences for length materialized between creep feed treatments. Figure 6.3 depicts a histological slide section of the rumen papillae and the notable difference between lambs reared on pasture and on concentrates, respectively.

Lesmeister *et al.* (2004) indicated that a high amount of variation can be found for papillae length but that the measurement is none the less the best for detecting treatment differences, especially when dietary treatments are evaluated.

Ward (2008) indicated papillae length in male sheep to be 0.44 mm at birth and 4.47 mm in the adult. Two treatments were used in Ward's (2008) study, a control treatment with *ad lib* suckling with no access to creep feed and lambs with access to a creep feed consisting out of 191 g CP/kg and 672 g NFE/kg with *ad lib* suckling. These lambs were weaned at 12 weeks of age and had papillae lengths of 1.15 mm and 2.24 mm for the control and creep feed treatment respectively. Ward's (2008) observations correspond with that measured on the short papillae between the CON and creep feed treatment lambs of this study. In fact, measurements obtained from short papillae were longer than those found in the study of Ward (2008).



**Figure 6.3** Rumen papillae of two month old lambs reared on (a) the CFC creep feed ration (455 g NSC/kg), (b) the CF1 creep feed ration (477 g NSC/kg) and (c) pasture

Ortega-Reyes *et al.* (1992) noted that papillae length were shorter on the bottom of the ventral rumen sac as compared to papillae found in the atrium ruminis. Ward's (2008) papillae measurements were taken in the ventral rumen sac while papillae measurements in the current study were taken from the atrium. Differences in length between Ward's (2008) study and the current investigation may therefore be attributed to the area of sampling.

Ward (2008) compared papillae lengths of weaned lambs (12 weeks of age) to an adult sheep, whose age and type of treatment was not described; the length of papillae in the adult sheep corresponds extremely well with that measured on the long papillae of this study. Adult sheep in the study of Ward (2008) had papillae lengths averaging 4.47 mm while two month old lambs in this study averaged between 4.03 to 4.48 mm.

With regards to long papillae measurements, CF2 numerically had the highest least square mean ( $4483.00 \pm 146.06 \mu\text{m}$ ), followed by CF1 ( $4165.13 \pm 157.17 \mu\text{m}$ ) and then CFC ( $4027.27 \pm 183.10 \mu\text{m}$ ). It has been shown that butyrate rather than propionic acid has the highest effect on rumen papillae development (Baldwin & McLeod, 2000). Butyrate proportions increased as the level of NSC increased in the study of Stern *et al.* (1978). Diet CF2 with the highest NSC level, by inference, would also have a higher butyrate proportion resulting in longer papillae.

Width measured from the long papillae indicate that CF2 was thinner than CON ( $P = 0.0006$ ) but similar to CFC ( $P = 0.1632$ ) and CF1 ( $P = 1.000$ ). It does however seem that an increase in NSC level results in thinner papillae. Only the long papillae data supports this, which is not surprising seeing as the short papillae represent papillae with lower length within a slide which concomitantly can also be the thickest papillae; no clear conclusion can therefore be made with regards to width on short papillae.

Few researchers have used papillae width as a characteristic for measuring rumen development. The study of Ortega-Reyes *et al.* (1992) indicated papillae widths of lambs at six weeks of age to be 1.6- and 1.4 mm for lambs exposed to whole barley and a protein-mineral pellet diet and a control group that was not exposed at six weeks of age, respectively. In contrast the present study measured papillae widths (averaging 0.436 mm) thinner than Ortega-Reyes *et al.* (1992) for long and short papillae. Ortega-Reyes *et al.* (1992) used a millimetre rule to measure widths of papillae. The current study employed microscopic slide measurements on a  $5 \mu\text{m}$  sliced sample. Papillae are tongue shaped structures with a flat and narrow side. Ortega-Reyes *et al.* (1992) most probably measured the flat side. In contrast, the current study measured papillae width regardless of the papillae side. It would seem that the method employed in embedding and sectioning of the rumen samples allowed for mostly the smallest side of the papillae to be measured.

Lesmeister *et al.* (2004) indicated papillae width as the second most important parameter for estimating rumen development. However it also seems that width is better for indicating the effect of age on development rather than diet type (Lesmeister *et al.*, 2004).

Epithelium and collagen layer measurements were taken for each measured papillae. These layers vary within each slide confirming the observation of Dobson *et al.* (1955) that these layers are prone to high variation. The epithelium layer for CF1 was thicker than CON ( $P < 0.0001$ ), CFC ( $P = 0.06$ ) and CF2 ( $P < 0.0001$ ). The collagen layer on the other hand was thickest for CON ( $P < 0.0001$ ). The epithelium and collagen layers are collectively called the mucosa layer. Similar to the collagen layer, the mucosa layer was thickest for CON ( $P < 0.0001$ ).

Suárez *et al.* (2006) found that the rumen of calves reared on concentrate diets high in NDF had thin mucosa layers with thick muscular layers. The current data are in contrast to this finding of Suárez *et al.* (2006), especially with regards to the mucosa layer.

Rumen wall thickness was higher for CON and CF1 than for CF2 and CFC. It has been found that roughage stimulates the muscularization of the rumen (Zitnan *et al.* 1998) due to its bulkiness

(Warner *et al.*, 1956; Vazquez-Anon *et al.*, 1993; Zitnan *et al.*, 1998). A greater difference between CON and the creep feed treatments were therefore expected. The reason for the small difference could be attributed to the fact that the creep feed lambs also had access to their dam's roughage. The above mentioned study of Suárez *et al.* (2006) found that the NDF pelleted diet increased muscle thickness in veal calves. However it has to be emphasised that it is the physical effective fibre which promotes higher rumen motility which in turn increases muscularization rather than roughage *per se*. The kikuyu grazing which constitutes a bulkier diet compared to the dried hay and concentrated feed of the creep feed treatments attributed to the higher muscularization, albeit only numerical against CF1, found in the CON lambs.

It could be argued that healthy rumen muscularization occurred through all the treatments. More so it could be argued that the development of the rumen for the creep feed treatments was well rounded considering that the feeding regime developed the independent characteristics simultaneously.

## 6.6 Conclusion

The current study investigated the effects of different NSC levels on the development of rumen papillae parameters in pre-weaning lambs. Lambs were divided into four treatments CF1, CF2, CFC and CON. The optimised creep feeds, CF1 and CF2, represented NSC levels of 477 g NSC/kg and 508 g NSC/kg respectively. A commercial creep feed (CFC) was used as a positive control with a NSC level of 455g NSC/kg. A treatment on pasture alone with *ad lib* milk consumption functioned as a negative control (CON) to the creep feed treatments.

In corroboration with literature the creep feed diets stimulated higher rumen papillae development while CON lambs showed numerical higher muscularization of the rumen wall. As the creep feed lambs in this study had access to coarse roughage the adverse affects on rumen papillae health (parakeratosis) as reported by Suárez *et al.* (2006) was circumvented.

Literature maintains that the epithelium- and muscle- layers develop independently from one another and are stimulated by different dietary factors. The epithelium representing the mucosa layer and papillae are stimulated by grain feeding or alternatively by the products of grain fermentation (the VFA namely butyrate and propionate). The muscle layer in turn is stimulated by roughage feeding (NDF) and the physical effectiveness of roughage in promoting rumen motility. It can be surmised that the creep feed diets (which included NDF) with the additional course roughage consumed was adequate to stimulate these independent characteristics simultaneously.

The creep feed treatment with the highest NSC level, CF2, also had the longest papillae. Papillae width however decreased with increasing NSC levels. It therefore appears that the longer a papilla, the thinner it becomes. Longer and thinner papillae have a larger volume to surface ratio and could it be expected that these types of papillae would absorb nutrients better.

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## **CHAPTER 7**

### **MEAT QUALITY CHARACTERISTICS OF NURSING LAMBS REARED ON OPTIMISED CREEP FEEDS**

#### **Abstract**

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The effect of optimised amino acid and non structural carbohydrate creep feed diets on the meat quality of lambs were investigated. Three creep feed treatments were used where treatment CF1 represents 157 g CP/kg and 477 g NSC/kg, treatment CF2 represents 179 g CP/kg and 508 g NSC/kg and finally creep feed treatment CFC represents 139 g CP/kg and 455 g NSC/kg; a negative control (CON) treatment was included to represent lambs receiving no creep feed but with *ad lib.* access to suckle their dams while on a kikuyu pasture. Lambs were slaughtered at an average age ( $\pm$  s.d.) of  $69 \pm 4$  days with an average live weight of  $23.6 \pm 0.56$  kg and were not fasted before slaughter. The *M. longissimus dorsi* was removed on both the left and right half of the carcass between the 2<sup>nd</sup> - 3<sup>rd</sup> last thoracic vertebrae and the 4<sup>th</sup> - 5<sup>th</sup> lumbar vertebrae. The left and right *M. longissimus dorsi* samples were used for proximate chemical and physical analyses, respectively. No differences were found between treatments for the chemical proximate analyses. With the exception of cooking loss and chroma, there were no differences between treatments for the physical meat characteristics. Samples from the CON treatment had the highest cooking loss (31.9%) and differed significantly from the creep feed treatments while creep feed treatments tended to have a richer fuller colour (chroma value) than CON. Meat colour averaged L\* at 44.2, a\* at 7.84 and b\* at 11.21. No differences were found for muscle individual essential amino acids between treatments. Carcass pH<sub>24</sub> averaged ( $\pm$  s.d.)  $5.2 \pm 0.1$ ; the reason for the low pH<sub>24</sub> measured in this investigation is unclear and requires further investigation. It is concluded that meat quality is not affected by a creep feed ration optimised for EAA and NSC.

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Keywords: meat characteristics, non structural carbohydrate, suckling lambs

#### **7.1 Introduction**

A creep feed for lambs optimised for essential amino acids (EAA) and non structural carbohydrate (NSC) level showed to be superior to commercially available creep feeds in terms of production efficiency, nitrogen retention and rumen development as discussed in chapters 4, 5, and 6. These specialised creep feeds elicited better performance because the feed is more efficiently utilised since less energy is wasted in the deamination of excess amino acids and due to the higher absorption ability of lambs with better rumen development. As a result these specialised feeds will lower the wastage and pressure on feedstuffs such as grains for the animal feed industry, while potentially increasing meat production. This is in corroboration with current world trends where companies need

to strive to make rations more efficient while increasing production output because of the higher demands for animal protein in developing countries which is exacerbated by a decrease in grain production over the last few years ([www.oup.com](http://www.oup.com), 2010; AFMA, Chairman's report 2009/2010).

Although these specialised creep feeds have been shown to elicit good responses in suckling lambs the effect thereof on meat quality remains to be investigated. It is the consumer who inevitably determines whether the carcass (and its commercial cuts) obtained from such a feed will gain entry into the market, irrespective of what the growth performance of such lambs were. Consumers typically assess meat on quality attributes such as colour (Miltenburg *et al.*, 1992), tenderness (Koochmarai, *et al.*, 1990) and juiciness (Safari *et al.*, 2001).

Carcasses from the above mentioned study (slaughtered at ( $\pm$  s.d.)  $23.32 \pm 4.33$  kg live weight) cannot exactly be assessed on its possible attractiveness to consumers because lambs were slaughtered at weaning and weighed less than the preferred 40 kg for South African markets. However it could be that the meat quality, as it is, on these small carcasses could be indicative of what the meat quality would be at 40 kg. Mendenhall & Ercanbrack (1979) indicated that increase in slaughter weight did not result in significant differences in meat quality attributes. However, Santos-Silva *et al.* (2002) indicated that as slaughter weight increases the L\* and b\* colour values decrease, i.e. meat becomes darker and less yellow as slaughter weight increases.

The aim of the following study was therefore to determine if the meat quality of lamb is affected by the already proved successful creep feed rations optimised for EAA and NSC.

## **7.2 Materials and methods**

Physical and chemical meat quality characteristics were determined on the carcasses of the lambs slaughtered from the creep feed growth trial (Chapter 4).

Ethical approval was obtained from the *Stellenbosch University Animal Care and Use Committee*, reference number 2009B03006.

Creep feed formulation, lamb management and feeding protocol were thoroughly described in Chapter 3 and only relevant detail will be dealt with here. However, as some information is critical to the understanding of this study some detail is repeated here in brief.

### **7.2.1 Compilation of the three creep feeds**

Amino acid requirement for lambs were estimated based on the work by Nolte (2006) and used to compile a creep feed meeting the amino acid requirement of lambs. The creep feeds were compiled based on the assumption that lambs will grow at 150 g/day while consuming 800 g/day of milk and 200 g/day of creep feed (Chapter 3).

Nolte (2006) determined Histidine, Methionine, Leucine, Arginine and Phenylalanine as the first limiting amino acids for Merino and Döhne Merino lambs. In Chapter 3 it was estimated that Lysine,



Threonine, Methionine, Isoleucine, Phenylalanine and Leucine needed to be included in a creep feed at levels of 7.45, 8.10, 1.78, 0.32, 4.39, and 3.51 g/kg, respectively, for suckling lambs.

Creep feed 1 (CF1) was optimised for these amino acids at 157 g CP/kg and 477 g NSC/kg feed. A second creep feed (CF2) was optimised for these amino acids at higher CP and NSC levels namely 179 g CP/kg and 508 g NSC/kg feed respectively. As control, a commercial creep feed (CFC) was formulated at 139 g CP/kg and 455 g NSC/kg feed without EAA optimisation.

Tanqua Feeds (Riviersonderend, Western Cape, South Africa) compiled the creep feed mixtures; Table 3.2 indicates the physical and chemical composition of the three creep feed mixtures as formulated by the company.

### **7.2.2 Lamb management during the creep feed growth trial**

Twin bearing ewes (Döhne-Merino x Mutton Merino cross) lambed over a three week period (28 October to 20 November 2009) and were subdivided into two groups to maintain an approximate seven day average age between lambs when they enter the trial. Once a group of lambs reached an average age of seven days they entered the growth trial (Chapter 4). Group 1 entered the trial ten days prior to Group 2.

It is mainly due to this that the slaughter procedure also occurred twice for the two different groups. Lambs in Group 1 were slaughtered eight days prior to Group 2's lambs being slaughtered. Therefore reference to the term *Group* will hereon refer to the lamb crop division as mentioned above. Likewise the term *treatment* will therefore refer to the creep feed treatments CF1, CF2, CFC and CON.

For the creep feed growth trial each group was divided into four treatments, CF1, CF2, CFC and a negative control on pasture (CON). Similar to the study of Joy *et al.* (2008) the lambs were divided into their treatments in such a manner as to maintain high homogeneity within and between treatments. Care was taken to ensure a balanced distribution of litter sizes, and age between the treatments.

Control lambs were allowed to graze for eight hours each day with their dams on a kikuyu pasture. Care was taken to ensure that CON lambs did not get access to any of the feed pellets used during the trial. This was accomplished by managing access to feed bags and also by removing the pellets that spilled during handling of feed when lambs were moved from the shed to the grazing paddock and *vice versa*. No restriction was placed on the amount and frequency of suckling for the control treatment. After a day's grazing lambs and ewes were brought indoors where fresh water and a lucerne- and oat hay mixture was supplied.

Lambs in creep feed treatments were housed indoors on slatted floors. To determine lamb creep intakes the lambs were separated from their dams into an adjacent pen where visual and physical contact remained through the wired fence separating the individual pens, which in turn reduced stress at separation (Figure 3.1). Effort was made to have lambs from different dams in adjacent pens at time of separation to aid in learning behaviour from one another.

Lambs were slaughtered at an average age ( $\pm$  s.d.) of  $69 \pm 4$  days with an average weight ( $\pm$  s.d.) of  $23.32 \pm 4.33$  kg and were not fasted before slaughter. Lambs selected for slaughter were taken from their experimental units and transported in groups of 10 to the abattoir less than 500 m from their holding area. One lamb at a time was offloaded and slaughtered before the next lamb was taken out. At slaughter lambs were electrically stunned (4 s at 200 V) where after the jugular veins were severed and exsanguination took place. After exsanguination the lambs were weighed, dressed and eviscerated according to standard South African techniques. Kidneys with pelvic fat were left intact on the carcass.

The carcasses were suspended by both the Achilles tendons in a cooler at  $4^{\circ}\text{C}$  for 24 hours. The *M. longissimus dorsi* was removed on both the left and right half of the carcass between the 2<sup>nd</sup> - 3<sup>rd</sup> last thoracic vertebrae and the 4<sup>th</sup> - 5<sup>th</sup> lumbar vertebrae. The left and right *M. longissimus dorsi* samples were used for proximate chemical and physical analyses respectively.

The  $\text{pH}_{24}$  was measured 24 hours *post mortem* on the remaining right hand side *M. longissimus dorsi* approximately 2 cm cranially to the 2<sup>nd</sup> - 3<sup>rd</sup> last thoracic vertebrae with a Testo 205 pH probe (Testo AG, Germany) which automatically adjusts for temperature. The pH metre was calibrated after every three readings with standard buffers (pH 4.0 and pH 7.0) provided by the manufacturer. The pH metre was inserted in the same direction as the orientation of the muscle fibres.

### **7.2.3 Proximate chemical and amino acid analyses**

The left side sample of the *M. longissimus dorsi* was used for the proximate chemical and amino acid analyses. Each sample's subcutaneous fat was removed before it was homogenised in a grinder and frozen until analysed. Samples were defrosted before moisture, protein, fat, ash and amino acid analyses were done. All proximate analyses were according to the AOAC methods (AOAC, 2002).

The moisture percentage ( $100^{\circ}\text{C}$ , 24 hours) of 2.5 g homogenised meat sample was determined according to the official AOAC method 934.01 (AOAC, 2002a) and the ash percentage ( $500^{\circ}\text{C}$ , 5 hours) of the moisture free sample was determined according to the official AOAC method 942.05 (AOAC, 2002b). Fat content was determined by the chloroform: methanol extraction process as described by Lee *et al.* (1996). A 1:2 ratio of chloroform: methanol was used, seeing as the fat percentage for samples did not exceed 5%. The fat free meat samples were dried, grinded into a powder form and used for protein and amino acid analyses.

Protein was determined by the Dumas combustion method 992.15 (AOAC, 2002c) using the Leco® FP-528 Nitrogen & Protein Analyzer (Leco® Corporation, St. Joseph, USA) which analysis the nitrogen percentage of a sample which in turn is multiplied with a factor of 6.25.

Amino acids were determined on dried, defatted samples of meat by ion-exchange chromatography of the acid-hydrolyzed protein. Samples were hydrolyzed (AOAC, 1997) with 6 M HCl in a sealed tube under nitrogen for 24 hours at  $110^{\circ}\text{C}$ . After hydrolyzation samples were handed over to the Central Analytical Facility in the Department Biochemistry (Stellenbosch University) for the amino acid

analysis. There the samples were diluted and derivatised using the EZ:Faast LC method (with EZ:Faast column) as described in the user's guide of the EZ:Faast kit. Labelled Homoarginine, Methionine-D3 and homophenylalanine which is part of the EZ:Faast kit was included as internal standards. A Waters API Quattro Micro system was used for the determination of amino acid composition.

#### **7.2.4 Physical analyses**

The *M. longissimus dorsi* samples taken from the right side of the carcasses were used for physical analyses. Drip loss, cooking loss and colour analyses were determined as described by Honikel (1998). The *M. longissimus dorsi* was cut into 1.5 cm thick samples, perpendicular to the longitudinal axis of the muscle fibres. Samples used for colour determination were also used to estimate cooking loss.

For drip loss determination samples were weighed and suspended in an inflated plastic bag. Care was taken to ensure that the sample did not come into contact with the plastic bag. Samples were suspended in a cooler at 4°C for 24 hours where after the samples were blotted dry with absorbent towel paper and weighed. Drip loss was calculated as the weight loss expressed as the percentage of the initial weight (Honikel, 1998).

Samples for the determination of cooking loss were weighed before placement in thin-walled plastic bags. Samples were then placed into a water bath preheated to 80°C for one hour. Thereafter the samples were removed and placed into a container with ice slurry. After the samples had cooled down, they were blotted dry with absorbent towel paper and weighed. Cooking loss was calculated as the weight loss expressed as the percentage of the initial weight (Honikel, 1998).

Shear force was determined from the samples used to determine cooking loss, i.e. it was determined on cooked samples. Five cores of 1.27 cm in diameter were removed per sample. An Instron Universal Testing Machine (Model 4444, Apollo Scientific, South Africa) fitted with a Warner Bratzler blade, 1.2 mm thick with a triangular opening (13 mm at the widest point and 15 mm high), was used to determine the force (Newton) needed to shear the cooked cylindrical sample core perpendicular to the muscle fiber direction. The Instron Universal testing machine was fitted with a 2 kN load cell and the shear test was conducted at a crosshead speed of 100 mm/min. Shear force values for each sample was recorded in Newton (N) and a high value is indicative of a tougher sample (Honikel, 1998). The average shear force from the five core samples were recorded for analyses.

For colour assessment samples were allowed to bloom for one hour. A Colour-guide 45°/0° colorimeter (Cat no: 6805; BYK-Gardner, USA) was used to measure the coordinates L\*, a\* and b\* of the CIELab colour space (Commission Internationale de l'Eclairage, 1976). The lightness of the meat is represented by L\*; where L\* = 0 is black and L\* = 100 is white. A red-green range is represented by a\* while b\* represents a yellow-blue range (Poulson *et al.*, 2004). Hue and chroma were calculated as:

$$\text{Hue angle } (^{\circ}) = \tan^{-1} (b^*/a^*)$$

$$\text{Chroma value} = [(a^*)^2 + (b^*)^2]^{1/2}$$

### 7.3 Statistical analysis

Statistical analyses were done via SAS Enterprise guide 4.0, version 9.1.3 (2002), using Proc GLM. Least square means were estimated and significance declared at the  $P \leq 0.05$ . A complete randomized design was used.

Ten lambs per treatment were randomly selected for slaughter. The effect of *Group* on the slaughter data was not significant; slaughter data was therefore pooled for statistical analyses.

### 7.4 Results and discussion

Results pertaining to protein, fat and amino acid content of meat samples are expressed on “As is” basis as opposed to “dry matter” basis seeing as it is more informative and comprehensible to consumers (Abu-Tarboush & Dawood, 1993). The chemical proximate results are depicted in Table 7.1.

No differences ( $P > 0.05$ ) were found between treatments for the chemical proximate analyses of the meat. Creep feed 2 with numerically the highest CP level in the creep feed mixture (179 g CP/kg) is characterised by having the highest CP deposition in the meat samples (20.42 g CP/100g). Joy *et al.* (2008) found similar results from the meat samples of lambs reared on either grazing or concentrates. The protein content of the *M longissimus thoracis*, in Joy *et al.*'s (2008) study, measured 19.1 and 19.7 g CP/100g sample for lambs reared on pasture and concentrates respectively.

The *M. longissimus dorsi* CP content (Table 7.1) concurred with that found for South African Mutton Merino (SAMM) and Döhne-Merino lambs slaughtered at 40 kg in the study of Hoffman *et al.* (2003); the SAMM and Döhne-Merino lambs measured 19.79 and 19.27 g CP/100g sample, respectively.

The moisture content (75.1 g/100g sample) of the *M. longissimus dorsi* of the pre-weaned lambs were much higher than that of samples gained from Döhne-Merino and SAMM lambs (69.3 g/100g sample) slaughtered at 40 kg (Hoffman *et al.*, 2003). This is in corroboration with the fact that as an animal matures more fat is deposited which in turn reduces the moisture content as fat is associated with a lower moisture content.

Murphy *et al.* (1994) showed that the intramuscular fat depot is the least influenced by dietary or feeding management treatments. Intramuscular fat of the *M. longissimus dorsi* was not affected by the different treatments and no significant differences were found between treatments. This is expected as the lambs were all at the same relative physiological age and were not exposed long enough to treatments to find significant difference for intramuscular fat content. Joy *et al.* (2008) also noted no differences in intramuscular fat where lambs of similar age were raised on grazing or similar

concentrate diets. Significant differences were found within the other fat depots such as omental and subcutaneous fat and were discussed in chapter 4.4.2.

**Table 7.1** Proximate chemical composition of the *M. longissimus dorsi* of pre-weaned lambs reared on three different creep mixtures; g/100g meat samples

	<b>CON</b> (Pasture)	<b>CFC</b> 139 g CP/kg; 455 g NSC/kg	<b>CF1</b> 157 g CP/kg; 477 g NSC/kg	<b>CF2</b> 179 g CP/kg; 508 g NSC/kg	<b>SEM*</b>
Moisture	75.80	75.43	74.94	74.30	0.51
CP	19.17	19.78	19.98	20.42	0.36
Fat	3.63	3.73	3.99	3.96	0.20
Ash	1.13	1.10	1.08	1.16	0.04

\* Standard error of means

Carcass pH<sub>24</sub> averaged ( $\pm$  s.d.)  $5.2 \pm 0.1$ . Devine *et al.* (1993) maintained that an ultimate pH greater than 5.8 can be considered as undesirable because the higher pH is associated with tougher meat and a lower shelf-life. Stressful handling of animals before slaughter typically increases the ultimate pH (Martínez-Cerezo *et al.*, 2005). According to Safari *et al.* (2001) the normal mutton pH range is between 5.40 and 5.86. Studies on suckling lamb carcasses from lambs slaughtered at a younger age than this study also showed higher pH's, ranging from 5.5 to 5.8 (Sañudo *et al.*, 1996, 1997). The reason for the low pH<sub>24</sub> measured in this investigation could be because lambs had higher glycogen levels in their muscles (Lister, 1989; Tarrant, 1989) which lowered the ultimate pH. Furthermore it could be argued that the frequent physical handling of lambs during the whole experimental period allowed lambs to be less stressed when handled at slaughter.

Table 7.2 depicts the physical characteristics of the *M. longissimus dorsi* for the different treatments which includes drip- and cooking loss, colour assessment and the Warner Bratzler shear force tenderness.

With the exception of cooking loss and chroma, there were no differences ( $P > 0.05$ ) between treatments for the physical meat characteristics (Table 7.2). Cooking loss for CON was significantly higher (31.9%) from the creep feed treatments while the creep feed treatments did not differ significantly between each other.

According to Safari *et al.* (2001) an inverse correlation exists between cooking loss and juiciness; as the cooking loss increases the juiciness decreases. Tenderness is furthermore correlated with cooking loss and juiciness as assessed by a sensory panel and the Warner-Bratzler shear force method (Silva *et al.*, 1999). No significant differences were found between treatments for Warner-Bratzler shear force tenderness.

**Table 7.2** Physical characteristics of the *M. longissimus dorsi* from pre-weaning lambs raised on three different creep feed mixtures, LSMeans  $\pm$  s.e.

	<b>CON</b> (Pasture)	<b>CFC</b> 139 g CP/kg; 455 g NSC/kg	<b>CF1</b> 157 g CP/kg; 477 g NSC/kg	<b>CF2</b> 179 g CP/kg; 508 g NSC/kg
Cooking loss (%)	31.9 <sup>a</sup> $\pm$ 1.06	29.0 <sup>ab</sup> $\pm$ 1.01	27.2 <sup>b</sup> $\pm$ 1.01	26.3 <sup>b</sup> $\pm$ 0.96
Drip loss (%)	2.2 $\pm$ 0.75	2.3 $\pm$ 0.71	2.9 $\pm$ 0.71	2.3 $\pm$ 0.68
Shear Force (%)	29.6 $\pm$ 1.92	28.0 $\pm$ 1.82	27.3 $\pm$ 1.82	25.0 $\pm$ 1.74
L* value	43.9 $\pm$ 0.83	45.1 $\pm$ 0.79	43.9 $\pm$ 0.79	44.2 $\pm$ 0.75
b* value	10.5 $\pm$ 0.38	11.6 $\pm$ 0.36	10.9 $\pm$ 0.36	11.7 $\pm$ 0.34
a* value	7.5 $\pm$ 0.28	7.6 $\pm$ 0.26	8.3 $\pm$ 0.26	7.9 $\pm$ 0.25
Hue angle (°)	35.6 $\pm$ 1.47	33.0 $\pm$ 1.39	37.4 $\pm$ 1.39	34.5 $\pm$ 1.33
Chroma	12.9 <sup>a</sup> $\pm$ 0.31	13.9 <sup>ab</sup> $\pm$ 0.29	13.7 <sup>ab</sup> $\pm$ 0.29	14.2 <sup>b</sup> $\pm$ 0.28

<sup>abc</sup> LSMeans within a row with different superscripts differ significantly ( $P \leq 0.05$ )

The observed L\* values correspond to values obtained by Sañudo *et al.* (1995, 1997) for suckling lambs but are higher than those obtained by Cloete *et al.* (2008) for Döhne-Merino lambs slaughtered at 40 kg. The L\* values closer to zero indicate darker meat as L\* = 0 is black while values closer to 100 indicate lighter meat (L\* = 100 is white). The overall average for breeds commonly used in South-Africa (Merino, Döhne-Merino, SAMM, Dormer and Suffolk) slaughtered at 40 kg for L\* was 37.1, for a\* it was 12.1 and for b\* 9.0 (Cloete *et al.*, 2008). Lambs from this study averaged L\* at 44.2, a\* at 7.8 and b\* at 11.2. The younger lambs in this study therefore had lighter meat that is less red and more yellow than lambs at an increased slaughter weight and age (Cloete *et al.*, 2008). Santos-Silva *et al.* (2002) found that as slaughter weight increases the L\* and b\* values decrease. It is therefore expected that the meat colour of lambs in this study would become darker and less yellow if allowed to grow up to a slaughter weight of 40 kg.

For this study the a\* values which represents redness were lower than those found by Sañudo *et al.* (1995, 1997) for suckling lambs and those for lambs slaughtered at 40 kg in the study of Cloete *et al.* (2008).

Hue angle and chroma values correspond to that found in the study of Cloete *et al.* (2008) being only slightly lower. Hue angle for Döhne-Merinos averaged 37.6  $\pm$  0.8 and 35.1  $\pm$  0.7 for lambs from the current study. The chroma value for Döhne-Merinos averaged 15.0  $\pm$  0.2 and 13.7  $\pm$  0.2 for lambs from the current study.

According to the interpretation of the chroma value, suckling lambs from this study have a more dull and greyish colour compared to lambs at 40 kg seeing as high chroma values indicate a richer and fuller colour. It should also be remembered that the older 40 kg lambs were free ranging and it is well documented that animals that exercise have richer and darker coloured muscles. Creep feed 2 tended to have the highest chroma value between creep feed treatments.

Results obtained for the amino acid composition between treatments in the *M. longissimus dorsi* are depicted in Table 7.3.

**Table 7.3** Amino acid composition of the *M. longissimus dorsi* from pre-weaning lambs raised on three different creep feed mixtures (g/100g meat)

	<b>CON</b> (Pasture)	<b>CFC</b> 139 g CP/kg; 455 g NSC/kg	<b>CF1</b> 157 g CP/kg; 477 g NSC/kg	<b>CF2</b> 179 g CP/kg; 508 g NSC/kg	<b>SEM</b>
Alanine	0.86	0.80	0.81	0.81	0.04
Threonine	0.71	0.69	0.67	0.68	0.03
Serine	0.80	0.81	0.72	0.78	0.05
Arginine	0.97	1.03	0.88	0.91	0.11
Glutamate	1.86	1.81	1.76	1.96	0.16
Valine	0.68	0.63	0.65	0.67	0.03
Histidine	0.37	0.35	0.35	0.40	0.05
Aspartate	1.61	1.62	1.58	1.64	0.11
Lysine	1.35	1.13	1.18	1.24	0.07
Proline	0.71	0.66	0.65	0.66	0.03
Methionine	0.43	0.42	0.42	0.45	0.01
Tyrosine	0.67	0.61	0.63	0.71	0.03
Cysteine	0.10	0.09	0.09	0.11	0.01
Isoleucine	0.71	0.66	0.67	0.70	0.03
Phenylalanine	0.70	0.68	0.72	0.72	0.03
Leucine	1.71	1.60	1.62	1.67	0.06
Glycine	0.62	0.56	0.58	0.55	0.03

No differences ( $P > 0.05$ ) were found for amino acids between treatments. Even though differences in amino acid content have been found between different breeds (Ferreira *et al.*, 1999; Nolte, 2006) and even within the same breed at different geographical locations (Smith, 2004), the finding of this study is not surprising. Lambs used in this study, although a crossbreed, are genetically similar, i.e. the genetic control over muscle synthesis is the same. The first limiting amino acid concept states that protein synthesis will only occur up to the point where the first amino acid becomes limiting until that amino acid is supplied and the second limiting amino acid is supplied and so forth (Cant *et al.*, 2003). It implies that more protein will be deposited and not certain individual amino acids. The fact that amino acid composition is not altered by dietary treatments illustrates the efficacy of using the whole empty body (WEB) amino acid composition as the starting point for determining the amino acids required by animals.

Table 7.4 compares the amino acid composition (defatted basis) of the *M. longissimus dorsi* of the pre-weaned lambs from the current study to that found in the studies of Schweigert *et al.* (1949) and Gilka *et al.* (1989). The former study determined the amino acid composition of an unknown meat cut while the latter also used the *M. longissimus dorsi* to determine the amino acid composition.

**Table 7.4** Comparison of the amino acid composition (g/100g sample) between lamb meat from the (C) current study and from the studies of (A) Schweigert *et al.* (1949) and (B) Gilka *et al.* (1989).

	A*	B**	C**
Arginine	6.74	5.08	4.51
Histidine	2.56	4.09	1.76
Isoleucine	4.57	4.04	3.24
Leucine	7.06	5.43	7.82
Lysine	7.65	8.7	5.87
Methionine	2.43	1.85	2.04
Phenylalanine	3.82	2.89	3.33
Threonine	4.77	3.85	3.26
Valine	4.97	4.12	3.14

\* Amino acid composition was determined on an unknown meat sample cut

\*\* Amino acid composition was determined from the *M. longissimus dorsi*

The amino acid composition of the *M. longissimus dorsi* determined by Gilka *et al.* (1989) nearly matches that of the current study. Histidine, Leucine and Lysine are the only amino acids which differ by more than 1.0 unit between the current study and that of Gilka *et al.* (1989). It is not clear what the breed of lamb was from which the *M. longissimus dorsi* was obtained by Gilka *et al.* (1989). The study of Schweigert *et al.* (1949) has five amino acids that differ by more than 1.0 unit from the current study (Arginine, Isoleucine, Lysine, Threonine and Valine). Nolte (2006) emphasised that the amino acid composition between body components and even between breeds are not similar. Differences between the current study and that of Schweigert *et al.* (1949) and Gilka *et al.* (1989) can therefore be attributed to difference in breeds and or body components.

## 7.5 Conclusion

The aim of this study was to determine if the meat quality of pre-weaned lambs were affected by creep feed rations optimised for EAA and NSC.

It was found that the chemical and physical meat characteristics were not affected by the optimised creep feed mixtures. The meat quality of the weaned lambs does not deviate much from that expected of lambs slaughtered at a 40kg liveweight.

As amino acid composition was not affected by dietary treatments, with correct amino acid balances, it was concluded that whole empty body (WEB) amino acid composition is a good starting point for determining the limiting EAA's required by a certain animal.

In conclusion lamb producers may benefit economically from feeding optimised creep feed mixtures without any deterioration in carcass and meat quality characteristics.



## 7.6 References

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## **CHAPTER 8**

### **GENERAL CONCLUSION and**

### **FUTURE PROSPECTS**

It was the aim of this study to prove that a creep feed ration for lambs optimised for limiting essential amino acid (EAA) composition at certain non structural carbohydrate (NSC) levels will be superior compared to a commercially available creep feed. It is suggested that such a feed will be more attractive due to its better feed efficiency accompanied by higher levels of production (growth).

The EAA requirement of suckling lambs was calculated based on the assumptions that a 20 kg lamb growing at an initial average of 150 g/d will require 95 g CP/d with a creep feed consumption of 200 g/d and a milk consumption of 800 g/d. It was determined that Lysine, Threonine, Methionine, Isoleucine, Phenylalanine and Leucine needed to be included in the creep feed diets at levels of 7.4, 8.1, 1.7, 0.3, 4.3, and 3.5 g/kg. These amino acids were incorporated into two specialised creep feeds at the different CP levels of 157 g/kg and 179 g/kg with respective NSC levels of 477 g/kg and 508 g/kg. A commercial creep feed with no optimisation for EAA was also formulated, representing a CP level of 139 g/kg and a NSC level of 455 g/kg. These creep feeds represented the treatments CF1 (CP 157 g/kg; NSC 477 g/kg), CF2 (CP 179 g/kg; NSC 508 g/kg) and CFC (CP 139 g/kg; NSC 455g/kg); a negative control (CON) treatment was included to represent lambs with no creep feed access but *ad libitum* access to suckling with dams on a kikuyu pasture.

The growth trial and accompanied carcass composition results accounted for those differences found in literature between lambs reared on pasture and those reared on concentrates (creep feed). Differences between the creep feed treatments were however harder to find. Lambs on CF1 performed similar to lambs on CFC with virtually no significant differences between these treatments. Lambs on CF2 seemed to perform the lowest, in all parameters (except for dressing percentage), of the three creep feed treatments.

The similarities between CF1 and CFC were attributed to the fact that initial limiting EAA requirement was underestimated for lambs and that the calculation of EAA requirement should have included MCP amino acid contributions. Lambs compensated for the lower amount of EAA by consuming more creep feed than predicted. As MCP is deficient in certain amino acids it was anticipated that certain limiting amino acids will still be in deficient supply. These amino acids were calculated to be His, Thr, and Met.

The growth results indicated that the FCR of lambs on CF1 was less efficient than lambs on CFC. In Chapter 5 the ME values depicted that lambs utilized CF1 more efficiently than CFC. The effect of energy on improved protein utilization was further clearly evident from the much higher nitrogen balance of the CF2 treatment.

Although the current study did investigate what the probable milk consumption is for the nursing lambs it did not investigate milk consumption as pertinently as feed intake. It is therefore the author's opinion and suggestion that future research on amino acid requirements for nursing lambs or calves should entail the full quantification of milk consumption. Milk not only contributes substantial amounts of amino acids, but it also has a considerable impact on the growth performance of lambs. Although the experimental design of this study limited the milk consumption of the creep feed treatment lambs, it could still be argued that certain treatment lambs consumed more milk during nursing or reunion phases. Such lambs typically waited for nursing to meet their "hunger" needs instead of consuming creep feed; other lambs consumed more creep feed and less milk. This "behaviour" can be quantified when milk consumption is determined. It is believed that this "behaviour" affected the FCR results as lambs consuming more milk and less feed showed to have more efficient FCR's than other lambs consuming more feed. In the current study FCR calculations did not take into account the amount of milk consumed to gain a unit of body weight, therefore the illusion that FCR was better for the creep feed treatment where feed consumption was lowest.

If this future research employs a similar experimental design as the current study the differences in milk consumption between creep feed treatments may show small differences and even no significant differences. Nevertheless, this data could account for the similar differences found for growth performance between creep feed treatments. Quantifying milk consumption for each lamb is however very time consuming and requires more workers as the *Lamb suckling weight differential technique* would be the simplest method to employ in a similar experimental design as the current study.

It is also from this point where the author wishes to suggest that the lambs from the commercial treatment showed some compensatory growth. The efficient FCR and similar growth performance of CFC compared to CF1 and CF2 could be attributed to this compensatory growth. This speculation is based on the observation that lambs from CFC had the lowest creep feed consumption at four weeks of age but the highest creep feed consumption at nine weeks of age. As the ewes started to wean the lambs near the end of the trial the lambs were forced to consume creep feed rather than milk. The increase in feed consumption beyond the consumption of CF1 and CF2 in turn resulted in compensatory growth and lead to the further observation that lambs from the creep feed treatments performed similar to lambs on the commercial treatment.

From blood urea nitrogen results it was concluded that the CP level of CF2 was set to high as BUN levels were much higher than the other treatments and the levels recommended by literature. Although the ME and nitrogen balance values favour CF2, growth and carcass results further agree that this CP level of CF2 was too high. Excess amino acids are deaminated and its carbon skeletons used for gluconeogenesis while the nitrogen containing molecules are excreted as urea via the urine. It could be surmised that the poorer growth rate of lambs in CF2 is due to energy wastage in deaminating the amino acids while the carbon skeleton availability resulted in higher fat deposition, hence the higher dressing percentage. Based upon the better ME and nitrogen balance values it is suggested that the energy to protein ratio factor plays an important role here.

Apart from these results, continued research is still required to delve into what the correct required ratio of protein to energy is for nursing lambs. An experimental design is suggested where creep feeds are formulated to be isocaloric with incremental CP levels accompanied by a second study where the creep feeds are isonitrogenous with incremental NSC levels. The former study may simultaneously endeavour to test for the effect of protein quality within a certain CP level (where protein quality of course is determined by balancing for limiting amino acids). Results from above mentioned study will provide useful data to include or expand nutrient prediction models such as the Cornell net carbohydrate and protein system (CNCPS) model for sheep and the Ruminant nutrition system (RNS).

From the rumen development results it was concluded that the feeding method employed in this study not only circumvented parakeratosis but also allowed the simultaneous development of papillae and the rumen wall. With regards to papillae length it was indicated that papillae length increases and width decreases as the NSC level increases. Papillae therefore becomes thinner the longer it becomes, hence an increased absorption surface area. This increase in absorption surface is thought to increase the apparent digestibility of the creep feeds as more nutrients disappear from the GIT. Digestibility followed the same pattern as papillae length, where digestibility increased as the NSC level increased.

A similar study for future research in rumen development should venture to determine the proportions of volatile fatty acids (VFA) accompanying the trend of rumen development due to dietary NSC levels, especially for butyrate proportions. The VFA proportion can be determined from rumen inoculum sampled from the rumen whilst taking the rumen wall sample after slaughter. It is further suggested that such a study should also investigate NSC level *per se* on rumen development by way of designing creep feed diets with distinct incremental levels of NSC. In addition, further value could be added by microbial population counts as well a study that pertinently links absorption surface area with rumen digestion.

Meat quality characteristics indicated that creep feeds did not have any effect on the quality of meat. The muscle amino acid content was unaffected by the diets with different amino acid compositions. This further emphasises that the amino acid composition of the WEB is the best starting point to determine the amino acid requirement of lambs. Lamb producers may further benefit economically from feeding optimised creep feed mixtures without any deterioration in carcass and meat quality characteristics

In conclusion, EAA supplementation of creep feeds at certain NSC levels is believed to elicit greater production responses with accompanied better feed efficiency over commercial available creep feeds. The commercial creep feed was, however, very well formulated and proved to be equally advantageous. Intensive sheep production systems will benefit from feeding optimised creep feeds such as CF1 because lambs will perform better while providing lean carcasses. Feed is also utilised more efficient thereby lowering feed wastage and overall feed costs. This optimised diet will also reduce the amount of nitrogen excreted into the environment thereby lowering the negative impact of

grain fed ruminants on the environment - something which has become a worldwide global concern. The producer could also market his/her lambs as “environmental-friendly grain fed lambs” similar to the Certified Natural Lamb marketing initiative.