Effect of nitrogen and energy supplementation on intake, digestibility and rumen fermentation efficiency in sheep fed poor quality *Eragrostis curvula* hay

By

Herman Mynhardt

Submitted in partial fulfilment of the requirements for the degree

PhD: Animal Science

In the Faculty of Natural & Agricultural Sciences

University of Pretoria

Pretoria

April, 15 2017

Outputs

Peer Reviewed Journals:

Mynhardt, H., Van Niekerk, W. A., Erasmus, L. J., Hassen, A. & Coertse, R. J., 2016. Substitution of rumen degradable nitrogen with urea in sheep fed low quality *Eragrostis curvula* hay. *Scientae Agricola 73, Volume 6: 498 – 504.*

International congresses:

Coxley, G., Van Niekerk, W.A., Hassen, A., Mynhardt, H. & Coertse, R. J., 2012. Substituting rumen degradable nitrogen portion of sunflower oilcake meal with urea on microbial protein supply, digestibility and intake of sheep fed on low quality forage. Conference: 8th International Symposium on the Nutrition of Herbivores (ISNH8), Aberystwyth, Wales.

Du Plessis, D., Van Niekerk, W.A., Hassen, A., Mynhardt, H. & Coertse, R. J., 2012. Supplementing different non-protein nitrogen to fermentable metabolisable energy ratios on microbial protein supply and digestibility to sheep fed poor quality forage. Conference: 8th International Symposium on the Nutrition of Herbivores (ISNH8), Aberystwyth, Wales.

Mentz, A., Hassan, A., Van Niekerk, W. A., Mynhardt, H. & Coertze, R. J., 2013. The effect of substituting urea for a commercial slow release urea as supplement to sheep fed a poor quality *Eragrostis curvula* hay. Conference: 4th International Symposium on Energy and Protein Metabolism and Nutrition, Sacramento, California, USA.

Mynhardt, H., Van Niekerk, W. A., Erasmus, L. J., Hassen, A & Coertze, R. J., 2015. Substitution of rumen degradable nitrogen with urea in sheep fed low quality *Eragrostis curvula* hay. Conference: 66th Annual Meeting of the European Federation of Animal Science, EAAP, Warsaw, Poland.

Mynhardt, H., Erasmus, L. J., Van Niekerk, W. A. & Coertze, R. J., 2017. ***Supplementation of different quantities of urea to wethers fed low-quality Eragrostis curvula hay.*** Conference: 68th Annual Meeting of the European Federation of Animal Science, EAAP, Tallin, Estonia.

Mynhardt, H., Van Niekerk, W. A., Erasmus, L. J. & Coertze, R. J., 2017. ***Synchronisation of energy and protein in wethers fed low-quality Eragrostis curvula hay.*** Conference: 68th Annual Meeting of the European Federation of Animal Science, EAAP, Tallin, Estonia.

Other publications:

Mynhardt, H., Van Niekerk, W. A. & Erasmus, L. J., 2016. The hassle with Highveld hay: supplementing low-quality sheep grazing grasses with urea and starch: Research and training. Red Meat / Rooivleis, Volume 7, Issue 6, Dec 2016, p. 80 – 83.

Declaration:

I, Herman Mynhardt, declare that the dissertation, which I hereby submit for the degree

PhD (Agric): Animal Science

At the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary intuition.

SIGNATURE: ……

DATE: …….

Acknowledgements

I wish to acknowledge the following persons whom had helped me and had an influence on me during my studies, trial work and writing.

My Promoter, Professor W. A. van Niekerk and co-promotor, Professor L. J. Erasmus. Without their knowledge and support, I would not have been able to finish this project.

Red Meat Producers Organization for the funding of the project.

Mr. R. J. Coertze for his assistance with the statistical analysis and with his general assistance.

Mrs. M. Smith for her statistical assistance especially on the meta-analysis.

Mss. Liandri Pretorius for the editing of this thesis.

Mrs. C. Swanepoel on the experimental farm for her assistance.

Caitlin A. Oosthuizen for her moral support and for all our discussions, you are a true friend.

Students and especially Georgina Croxford, D. du Plessis, L. Hansen, and A. Ments. I have learnt so much from you.

Nutrilab and everyone involved in the analyses of my samples.

Everyone who lived at the Experimental Farm, we have had such good times, and in particular the following people:

Casey Courtmann, Sune Myburg, Bonita Nortje

All my friends and family with whom this thesis would not have been possible.

I know how to live on nothing or with everything. I have learned the secret of living in every situation, whether it is with a full stomach or empty, with plenty or little. For I can do everything with the help of Christ who gives me the strength I need.

Philippians 4: 12 - 13

Contents

List of Tables

List of Figures

List of abbreviations

ADF	:	Acid detergent fibre
ADG	:	Average daily gain
ADL	:	Acid detergent lignin
ADP	:	Adenosine diphosphate
ADIN	:	Acid detergent nitrogen
AIC	:	Akaike information criteria
ANOVA	:	Analysis of variance
ATP	:	Adenosine triphosphate
BW	:	Body weight
C3	:	Temperate plants with three carbon photosynthetic pathway
C4	:	Tropical plants with four carbon photosynthetic pathway
C	:	Carbon
Ca	:	Calcium
CO_2	:	Carbon dioxide
CP	:	Crude protein
CVA	:	Canonical variate analysis
DIP	:	Digestible intake protein
DM	:	Dry matter
DMI	:	Dry matter intake
DMD	:	Dry matter digestibility
DOM	:	Digestible organic matter
DOMI	:	Digestible organic matter intake
ED	:	Effective degradability
EE	:	Ether extract
EMNS	:	Efficiency of microbial nitrogen synthesis
ENU	:	Efficiency of nitrogen use

ERDP	:	Effective rumen degradable protein
FME	:	Fermentable metabolisable energy
GI	:	Gastro intestinal tract
HE; LE; HN; LN	:	High energy; Low energy; High nitrogen; Low nitrogen
IVOMD	:	In vitro organic matter digestibility
L	:	Litre
LS; MS; HS	:	Low starch; Medium starch and High starch
LU; MU; HU; EHU	:	Low urea; Medium urea; High urea and Extra high urea
ME	:	Metabolisable energy
mg	:	Milligram
Mg	:	Magnesium
MJ	:	Mega Joule
mM	:	Millimolar
MNS	:	Microbial nitrogen synthesis
MNS:NI	:	Microbial nitrogen synthesised relative to available nitrogen intake
MP	:	Metabolisable protein
N	:	Nitrogen
NB	:	Nitrogen balance
NDF	:	Neutral detergent fibre
NDFI	:	Neutral detergent fibre intake
NDFD	:	Neutral detergent fibre digestibility
NFC	:	Non-fibrous carbohydrates
NI	:	Nitrogen intake
NPN	:	Non-protein nitrogen
O_2	:	Oxygen
OM	:	Organic matter
OMD	:	Organic matter digestibility
OMI	:	Organic matter intake

P	:	Phosphorus
PD	:	Purine derivatives
PEP	:	Phosphoenolpyruvate
QDP	:	Quickly degradable protein
RAN	:	Rumen ammonia nitrogen
RDN	:	Rumen degradable nitrogen
RDMI	:	Roughage dry matter intake
RDN	:	Rumen degradable nitrogen
RDP	:	Rumen degradable protein
Rubisco	:	Ribulose bisphosphate carboxylase oxygenase enzyme
RUBP	:	Ribulose bisphosphate
S	:	Sulphur
SDP	:	Slowly degradable protein
SEM	:	Standard error of mean
SFM	:	Sunflower meal
SI	:	Synchronisation index
TMR	:	Total mixed ration
UDP	:	Ruminally undegraded dietary crude protein
VFA	:	Volatile fatty acid

Summary

The overall aim of the study was to study the effects of starch and urea supplementation on roughage intake, digestibility, and microbial nitrogen synthesis (MNS) in sheep fed low-quality *Eragrostis curvula* hay (< 3% CP, 80% NDF; DM basis).

In Trial 1, urea partially substituted the rumen degradable nitrogen (RDN) fraction of sunflower meal (SFM). Neutral detergent fibre (NDF) digestibility was higher in the treatments where urea substituted 45% and 60% of the RDN fraction of SFM. Treatment did not affect roughage intake, rumen ammonia nitrogen (RAN), MNS or efficiency of MNS (EMNS), suggesting that urea could substitute up to 60% of the RDN supplemented by SFM in sheep fed low-quality *E. curvula* hay.

In Trial 2, the quantities of urea and starch supplemented to sheep differed. Urea supplementation did not affect roughage intake and digestibility, however, MNS: available Nitrogen intake (MNS:NI) improved from 2.21 to 0.88 as urea supplemented increased from 10.4 g urea/sheep/day to 32.4 g urea/sheep/day. Microbial N synthesis increased up to the highest level of starch supplemented (280 g/sheep/day). It was concluded that urea supplementation, as high as 26.4 g urea/sheep/day, coupled with starch supplementation, between 240 g and 280 g starch/sheep/day, could be supplemented to sheep (50 kg BW) consuming low-quality *E. curvula* hay.

In Trial 3, RDN and energy supplementation patterns differed in sheep fed low-quality *E. curvula* hay. Treatment did not affect roughage or N intake; however, roughage digestibility was higher in treatments where starch was supplemented, at least partly, during the morning (08h00) supplementation period. Urinary N excretion, MNS and EMNS were generally higher in the treatments where starch was supplemented twice daily. It was concluded that, while the most optimal rumen pH was achieved in the treatment where both urea and starch was supplemented twice daily, the supplementation frequency of starch was the more important parameter, compared to urea, stimulating roughage digestibility, MNS and EMNS in sheep fed low-quality *E. curvula* hay.

To conclude this research, a meta-analysis was conducted to study the importance of supplemental starch and/or urea on N efficiency in sheep fed low-quality *E. curvula* hay. Starch or urea supplementation did not affect roughage intake or digestibility. Starch supplementation affected MNS linearly while urea supplementation influenced RAN linearly and MNS:NI inversely, with MNS:NI decreasing as urea supplementation increased. A strong correlation was observed between starch: available CP and RAN, with RAN increasing exponentially as the ratio of starch to available CP decreased below 2:1. It was concluded that urea and starch supplementation, up to 0.5 g urea/kg BW and 2.2 g starch/kg BW respectively, were necessary to optimise N efficiency in sheep grazing low-quality *E. curvula* hay.

Problem Description

Diseases, parasites and nutritional constraints including seasonal droughts limit animal production in subtropical and tropical countries (Lamy *et al.*, 2012). These areas normally have a characteristic dry period during which the quantity and quality of the available roughage to the ruminant differs. In terms of quality, the quantity and extent of lignification increases during the dry period while the quantity of non-fibrous carbohydrates (NFC) and N compounds decreases (Meissner, 1997), resulting in a decreased neutral detergent fibre (NDF) degradability (Van Soest, 1994) of these roughages. Therefore, intake of digestible N and NFC by the host animal are significantly reduced (Leng, 1990).

Small stock during those dry periods might lose up to 25% of its summer body weight (BW) gain (Van Niekerk, 1996) and in severe cases, up to 30% of its total BW (Almeida *et al.*, 2006). Those weight losses, while having an economic consequence on its own, are also associated with an increased susceptibility for disease and parasitic infestations and decreased reproductive performances (Almeida *et al.*, 2007).

One such subtropical area is the high veldt of Southern Africa. Almeida *et al.* (2006) suggested that this area is one of the areas most affected by seasonal weight losses as it has a low and erratic rainfall with the distribution almost exclusively limited to the summer.

Supplementation is necessary to counteract the seasonal weight losses of ruminants grazing these tropical areas (Leng, 1990; Van Niekerk, 1996; Detmann *et al.*, 2009). However, current knowledge on supplementation strategies are mostly derived from studies conducted on temperate C3 grass species (Leng, 1990; NRC, 2007; Costa *et al.*, 2013) and

not on tropical C4 grasses. Due to differences between temperate C3 grasses and tropical C4 grasses (Bohnert *et al.*, 2011), supplementation recommendations from animal requirement tables derived from temperate grasses cannot be used on tropical C4 grasses. As such, Mullik (2007) in cattle observed that the efficiency of MNS of green cut pongola grass (C4 – tropical grass, *Digitaria eriantha*) was only 11.5 g MNS/kg digestible organic matter (MNS/kg DOM), which "was only 55% of the minimum value (20.8 g MCP/kg DOM) suggested for roughage based diets" based on the Australian feeding recommendations (SCA, 1990).

It is evident that not much information is available on firstly, the type and quantity of nutrients that are necessary to limit seasonal weight loss in ruminants consuming low-quality tropical grasses and secondly, the effects those nutrients might have on the productivity of these animals. The overall objective of this study is to investigate the effects of different nutrients and supplementation strategies on forage intake, digestibility, and microbial nitrogen synthesis (MNS) in sheep receiving poor quality *Eragrostis curvula hay* (tropical, C4 grass). In the literature review, the general differences between temperate C3 and tropical C4 grasses are discussed. The focus of this discussion then shifts to the nutritive values and differences between the two types of grasses for the ruminant. Thereafter, the review focuses on recent supplementation studies and publications in both cattle and sheep receiving low-quality roughages.

Following the literature review, a series of trials conducted in this study are discussed.

It is envisaged that the outcomes of this thesis will aid in a better understanding of the mechanisms involved when supplementing poor quality tropical roughage to sheep by enhancing the efficiency of utilisation of feed by the animal, thereby improving farmers' income and livelihood in Southern Africa.

Chapter 1

1.1 Introduction

Most of the developed countries are in the temperate climates. To be profitable, animal production in these countries needs to be maximised through high quality feed ingredients that are rarely deficient in nutrients (Leng, 1995). Thus, the main aim of research in these countries is more related to fine tuning nutrient balances arising from the relative high digestibility and absorbability of feed nutrients to maximise animal production (Leng, 1995). In contrast, most of the developing countries are in the tropical areas with the focus based more on survivability, both for the animal as well as the farmer. Animals in these countries generally need to consume locally available feed sources that are deficient in nutrients (Leng, 1995). Roughages from these areas generally are of a low-quality, with a CP analysis of roughly 6% or lower and high fibre levels and digestibility values lower than 50% (Leng, 1990). Therefore, the production levels of these animals are well below its genetic potential, even with the aid of supplementation (Leng, 1995).

Supplementation recommendations from current feeding evaluation systems are generally based on low-quality temperate grasses and not necessarily on tropical grasses (Costa *et al.*, 2013). In addition, most of the feeding evaluation systems do not describe the types of feed sources used in the developing countries as it is considered too low in nutritional value, such as straw, which is primarily used as bedding in temperate countries, whereas it frequently forms the basis of the diets in the tropical areas (Leng, 1995).

Leng (1995) suggested there is ample evidence that the production of ruminants, grazing low-quality tropical roughages, is low due to limiting or deficient nutrient profiles of the roughages, and not necessarily due to the low digestibility or degradability of the roughages *per se*. These deficiencies decrease the growth rates of the microbial population in the rumen, thereby decreasing fermentation and digestibility of the basal roughage. As such, the nutrients are used inefficiently by the ruminant, which increases the generated metabolic heat. The increase in metabolic heat might reduce the often already insufficient feed intake due to the general higher environmental temperatures and humidity associated with the tropical regions and the low nutritional value of the grasses (Leng, 1990). The authors further

suggested that by supplementing these deficient nutrients, a more efficient rumen environment could be created, resulting in an increased and more efficient usage of the available roughage by the ruminant.

It is therefore imperative that the correct nutrients and/or nutrient combinations need to be identified in supplementing ruminants consuming low-quality tropical roughages. Although some efforts have been made in identifying animal requirements under tropical conditions (Costa *et al.*, 2013), which had been incorporated into the more current feeding requirements (NRC, 2007), most of the feeds and feed principles are still based on temperate feeds and not tropical feeds (Costa *et al.*, 2013). Recent studies suggest that supplementation have different effects on the rumen milieu depending on the type of grass (low-quality tropical grasses or temperate grasses), even though the chemical analysis of the grasses might be similar (Bohnert *et al.*, 2011). In addition, it was established that different nutrients and/or nutrient levels used as supplements, could have different effects on roughage utilisation in ruminants consuming tropical grasses (Kanjanapruthipong and Leng, 1998; Detmann *et al.*, 2009) compared to temperate grasses (Bohnert *et al.*, 2011). Thus, supplementation strategies developed for ruminants consuming low-quality temperate grasses will differ to ruminants consuming low-quality tropical grasses (Bohnert *et al.*, 2011). These differences will be discussed in more detail in this review.

The classical aim of supplementation is to supply essential nutrients to rectify nutrient deficiencies in the rumen, thereby maximising or optimising rumen microbial growth and therefore roughage degradation and roughage intake (Leng, 1990; Leng, 1995). An additional aim of supplementation would be to balance the products of rumen degradation. Leng (1995) suggested that the protein to energy ratio of the "absorbable" nutrients are the major constraint limiting ruminant production in tropical areas and not necessary the low energy density or degradability of tropical roughages. The authors therefore recommended that nutrient deficiencies, in both the rumen as well as the post-ruminal "absorbable" nutrient profile, need to be improved through supplementation to reflect both the rumen microbes, as well as the ruminants' requirements, more closely. These objectives could be achieved by supplementation to optimise the rumen milieu for optimal MNS and secondly, through "bypass" protein sources to optimise the absorbable protein (metabolisable protein; MP) to energy ratio to the ruminant (Leng, 1995).

Traditionally, rumen efficiency in supplementation studies was indirectly measured by measuring the rate of roughage degradability of the basal roughage (Ørskov and McDonald, 1979) and/or roughage intake (Leng, 1990). The optimum level of supplementation was then determined as either the maximal rate of roughage degradability in the rumen and/or where roughage intake was maximised. It is of importance to note that the "original and more common" methods measuring rumen efficiency in ruminants consuming roughages (through roughage intake and/or degradability) does not necessarily indicate whether MNS in the rumen was maximised, but merely whether the conditions in the rumen were sufficient to maximise roughage intake and/or degradability. The development of a new, non-invasive method in calculating MNS (Chen and Gomes, 1992) through purine derivatives (PD) led to an increase in research papers (Gomes *et al.*, 1994; Kanjanapruthipong and Leng, 1998; Detmann *et al.*, 2009, 2014) where MNS had been calculated. Results from these studies suggest differences in rumen efficiencies between tropical C4 – and temperate C3 – grasses.

The question is now what the correct level of supplementation to the ruminant would be to optimise ruminant production in the tropical roughage fed ruminant. However, before that question is answered, it is important to look at the different types of nutrients that can be supplemented to the roughage fed ruminant that might improve animal production (Leng, 1995):

- Minerals and especially calcium (Ca). Some minerals might affect the rumen microbial activity in the ruminant grazing low-quality roughages. A Ca deficiency, for instance, might inhibit the Ca-dependantable cellulase activity of cellulolytic bacteria. Other important minerals include the macro-minerals, phosphorus, magnesium, sulphur, sodium and certain trace minerals. However, while one or more of these minerals might be deficient in roughages and needs to be supplied to optimise the rumen milieu, it is difficult to assess mineral deficiencies in the diet due to the following reasons:
    - The mineral status of many of the roughages in the tropical regions are not always known and needs to be measured in laboratories.
    - The effects of mineral supplementation are difficult to measure as it often occurs with other deficient nutrients (for example N).

  - Many of the supplements given to animals may contain, to various degrees, minerals (an example is molasses, which generally is high in various minerals including sulphur). The authors suggested the best option under these circumstances is a "shotgun" approach where all the minerals are supplied.

- Non-protein nitrogen (NPN) and sulphur (S) for microbial growth.

- "Bypass" protein to enhance the amino acid supply to the ruminant. In addition to the natural deficiency of N compounds (amino acids, peptides, NPN sources) associated with ruminants consuming low-quality tropical roughages, Leng (1995) noted that ruminants in the tropical areas might be more energy efficient than ruminants in the temperate areas. The higher energy efficiency is due the lesser quantity of energy needed by the ruminant to maintain its core temperature. Therefore, ruminants in the tropical environments need more RDN to satisfy their requirements than previously anticipated (Costa *et al.*, 2013). The newest NRC recommendations (NRC, 2007) considered these aspects where the protein requirements of sheep grazing in the tropical areas are adapted to take the higher energy efficiency into account (Costa *et al.*, 2013). Leng (1995) further noted that undegradable, digestible protein (UDP) supplementation has the potential to increase production (growth, milk production) and feed intake of ruminants consuming low-quality tropical roughages. However, the authors also commented that UDP improved production results to a higher degree in studies where the rumens of the animals were more efficient (due to NPN and mineral supplementation). As such, the maximum response of "bypass" protein supplementation to ruminants consuming low-quality tropical roughages is related to the digestibility characteristics of the basal feed, which is a function of the rumen efficiency. It is therefore important to satisfy the "rumen" protein requirements first, and then to optimise production in the ruminant by feeding UDP sources.

- Supplements that increase the overall digestible energy density of a low digestible basal feed. This includes roughages with higher digestibility values and/or starch/sugar products like molasses. High-energy supplements supply easy digestible energy for the rumen microbes to grow and to produce. This type of

supplement might improve total organic matter (OM) intake and digestibility and in some cases, even roughage intake and degradability (Henning *et al.*, 1980; Gomes *et al.*, 1994), leading to an improvement in the efficiency of the MNS to the lower gastro-intestinal (GI) tract (Leng, 1990; Poppi *et al.*, 1999).

In this thesis, the focus will be on meeting the maintenance requirements of the sheep and not on production *per se*. Therefore, although it is acknowledged that supplementation of the ruminant with "bypass" proteins might balance the digestible products arising from the rumen degradation of the basal roughage, thereby increasing the overall status of the animals, the emphasis in this thesis will be limited to supplementation, which might optimise the rumen milieu.

From the literature review, it is important to characterise the optimal rumen milieu in ruminants grazing low-quality tropical roughages and then to identify the effects of different nutrients used as supplements in achieving this optimal rumen milieu.

As stated earlier, an efficient rumen is a rumen where not only the rate of roughage intake and degradability is optimised (Leng, 1990), but also MNS. An increase in roughage intake, degradability and especially MNS might correct the nutrient imbalances often associated with the ruminant grazing poor quality tropical roughages (Leng, 1995). Rumen conditions for optimising these three aspects are not necessarily similar in ruminants grazing tropical roughages. The rumen conditions necessary for optimising these three aspects also differ between low-quality tropical grasses and low-quality temperate grasses (Bohnert *et al.*, 2011) due to anatomical differences between the two types of grasses (Bohnert *et al.*, 2011).

It is known that N is the most deficient nutrient in ruminants grazing tropical roughages (Kanjanapruthipong and Leng, 1998; Detmann *et al.*, 2009). Microbial protein potentially could supply most of the amino acids to sheep to meet its maintenance requirements (NRC, 2007). In addition, it could supply up to 72% of the protein reaching the small intestine (Beever and Siddons, 1992) with the amino acid profile of microbial protein resembling the animal's protein requirements (NRC, 2007). It is therefore more than likely that, by "optimising" the rumen environment through supplementation of rumen deficient nutrients, the maintenance requirements of the host ruminant could be met. However, recent studies suggested that nutrients other than N compounds could also be deficient in ruminants grazing low-quality tropical roughages (Gomes *et al.*, 1994; Leng, 1995). However, before these nutrients are discussed, it is important to look more closely at the characteristics and

nutritive value of low-quality roughages and especially at the differences between low-quality temperate and tropical grasses.

1.2 C3 and C4 Grass Species: Differences in Photosynthetic Pathways

The terms C3 and C4 in temperate and tropical grasses refer to the different photosynthetic pathways in which these plants produce sugars. In the temperate C3 plant, the first product of photosynthesis is a three-carbon molecule called phosphoglycerate while in the tropical C4 plant, the corresponding molecule is a four-carbon molecule called oxaloacetate (Ehleringer and Cerling, 2002).

In the C3 plant, carbon dioxide (CO_2) reaches the mesophyll cells where the chloroplast cells are located. This process happens through normal diffusion across the stomata, the intercellular air spaces, and the cell wall membranes. Thus, the partial pressures of CO_2 reaching the mesophyll cells where photosynthesis are taking place, are always lower than the ambient CO_2 pressure. In the mesophyll cells, ribulose bisphosphate (RuBP, a 5-carbon molecule) combines with the diffused CO_2 and water to form two molecules of phosphoglycerate (3C molecules). This reaction takes place under the influence of the ribulose bisphosphate carboxylase oxygenase (Rubisco) enzyme. Phosphoglycerate is then converted to starch via the Calvin cycle and stored in the plant (Ehleringer and Cerling, 2002).

The Rubisco enzyme can not only reduce RuBP through the carboxylation reaction, but it can also oxidise RuBP in the presence of oxygen (oxidation reaction). Rubisco has a very low affinity for atmospheric CO_2 levels. At low partial CO_2 concentrations, RuBP, under the influence of Rubisco, would bind to O_2 and be oxidised to form phosphoglycerate (3C molecule) and phosphoglycolate (2C molecule). This additional conversion reaction (from phosphoglycolate to phosphoglycerate), requires energy (ATP) which lowers the total efficiency of photosynthesis in the plant. In addition, CO_2 is released into the atmosphere (Ehleringer and Cerling, 2002).

Various factors affect the efficiency of photosynthesis and starch production in the temperate C3 plant. The first factor is the partial atmospheric CO_2 concentration. Under conditions of low CO_2 concentrations, the rate of photosynthesis in the C3 plants and grasses decreases due to the enzyme's (Rubisco) low affinity for CO_2 while the rate of

photorespiration increases as the oxidation reaction is favoured above the reduction reaction (Ehleringer and Cerling, 2002).

The second factor is light intensity or temperatures. During periods of high light intensity or temperatures, the rate of photosynthesis in C3 plants increases. Thus, CO_2 in the mesophyll cells is used at a faster rate and might drop in the mesophyll cells to levels where the affinity of the Rubisco enzyme for CO_2 might not be reached. Therefore, the rate of photosynthesis will start to decrease due to the enzyme's low affinity for CO_2. In addition, the rate of photorespiration will increase, thereby decreasing the overall efficiency of photosynthesis (Ehleringer and Cerling, 2002). As such, the efficiency of C3 plants in areas with a low ambient CO_2 concentration and/or a high light intensity or temperatures is severely inhibited and inefficient. Plants have adapted to those conditions by altering their photosynthetic processes and pathways. In tropical plants, the mesophyll cells surrounding the bundle sheath cells contain phosphoenolpyruvate (PEP) carboxylase with a higher affinity for CO_2 than Rubisco. This enzyme fixates CO_2 into oxaloacetate, a C4 acid. The oxaloacetate is transformed to malate which then diffuses to the bundle sheath cells, containing the chloroplast and Rubisco enzymes. Malate is then decarboxylate into CO_2 where photosynthesis occurs through the normal C3 pathway. Thus, CO_2 is delivered against a concentration gradient by PEP carboxylase from the mesophyll cells to the Rubisco enzyme. This increases the CO_2 concentration at the Rubisco enzyme complex several folds (Ehleringer and Cerling, 2002), increasing in the efficiency of photosynthesis as these plants can photosynthesise faster and more efficient than C3 grasses (less photorespiration) under higher temperatures and/or lower environment CO_2 concentrations (Ehleringer and Cerling, 2002).

It is evident that tropical grasses have a distinct advantage over temperate grasses in terms of efficiency of photosynthesis, especially in conditions where the ambient CO_2 concentration is relatively low and/or in areas with high temperatures and/or light intensities. However, these adaptations come with a cost. The additional metabolic pathways in the C4 system photosynthetic pathways, including the regenerating of PEP from pyruvate, need more energy to operate than the C3 photosynthetic pathways. Under ideal conditions, the C3 pathway requires 18 molecules of ATP for the synthesis of one molecule of glucose. The corresponding number for the C4 pathway is 30 molecules of ATP (Ehleringer and Cerling, 2002). The extra energy needs of the C4 photosynthetic pathways *versus* the inefficiency of the Rubisco enzyme of the C3 plants determine the cross-over temperature of C3 to C4

grasses. At the current mean world CO_2 pressure (35 Pa), the mean cross-over temperature between C3 grasses and C4 grasses is 22°C for the warmest month of the year, provided that there is sufficient precipitation (more than 25 mm) during the specific growth months (Collatz *et al.*, 1998). However, the optimum rate of photosynthesis for C4 plants, depending on the quantity of radiation and water availability, ranges between 35 to 40^{o}C (Collatz *et al.*, 1998). In contrast, the optimal temperature for photosynthesis in the C3 plants ranges between 10 to 25^{o}C (Black, 1971).

It is important to note that it is not temperature *per se* which determines the efficiency of the C4 photosynthetic pathway, but the level of radiation (which is independent of temperature). Photons within the chloroplast need to be energised to supply energy driving the photosynthetic processes. Tropical C4 plants will continue to increase CO_2 uptake as light intensity increases to nearly full sunlight (1.5 – 1.8 langleys, where langleys are units of solar radiation and full sunlight is approximately 1.5 to 1.8 langleys). In comparison, the photosynthetic pathways of C3 plants are saturated at 0.2 to 0.4 langleys. Thus, most of the C4 grasses are in the subtropical and tropical areas (below latitudes of 45°; Collatz *et al.*, 1998) where higher and more direct radiation is observed.

Due to the higher photosynthetic efficiency of tropical C4 grasses, these plants generally produce two- to threefold more DM than C3 grasses in relatively sunny, warm, dry climates (Black, 1971). However, as will be seen later in this review, this additional DM production generally is of a lower quality (in terms of herbivore nutrition) than temperate C3 grasses.

1.3 Anatomical Differences Between Temperate C3 Grasses and Tropical C4 Grasses and Its Effects on the Nutritive Value to Herbivores

Barbehenn *et al.* (2004) conducted a study investigating the chemical and anatomical differences between temperate C3 grasses (n = 7) and tropical C4 plants (n = 6) during normal and elevated CO_2 levels. The authors observed that CO_2 level did not affect the nutritional quality of the tropical C4 grasses to any significant degree. In contrast, an elevated CO_2 concentration decreased the nutritional quality of the C3 grasses. Despite the observed drop in nutritional quality of the C3 grass at the higher CO_2 levels, the nutritional quality of the temperate C3 grasses at all stages was better than that of the tropical C4 grasses. The

authors concluded that C4 grasses are nutritionally inferior to C3 grasses; and that C3 grasses will remain more nutritious than C4 grasses, despite environmental conditions (elevated CO_2 concentrations).

Barbehenn *et al.* (2004) and Ehleringer and Cerling (2002) observed that the spaces between the veins in the leaves of the C4 grasses were narrower compared to that of C3 grasses with the mean distance between veins in C4 grasses ranging between 25 to 70 micrometres. The corresponding numbers in C3 grasses were 75 to 130 micrometres (Collatz *et al.*, 1998). Wilson and Minson (1980) noted that the mean air spaces in the leaves of temperate grasses ranged between 10 – 35% of the leaf volume, while for tropical grasses, the corresponding number was 3 – 12%. The more "open spaced" configuration of temperate grasses allows rumen microbes a quicker access to larger surfaces areas compared to those of the tropical grasses, increasing the nutritive value of the C3 grass over the C4 grass (Bohnert *et al.*, 2011).

Berbehenn *et al.* (2004) further observed differences in the quantity and physical proportions of bundle sheath cells between the two types of grasses. Within leaves, each vein is composed of a vascular bundle surrounded by concentric layers of bundle sheath cells and mesophyll cells with four mesophyll cells between each bundle sheath cells in the C4 grasses. This configuration of cell types in the C4 plants is known as Krantz cells or Krantz cell morphology. In C3 grasses, the ratio is around 12 to 1 (Ehleringer and Cerling, 2002). The different ratios of mesophyll cells *versus* bundle sheath cells are due to the manner and location in which carbon fixation takes place between the two types of grasses, with carbon fixation occurring in the mesophyll cells in C3 grasses while it takes place in the bundle sheath cells of the C4 grasses (Bohnert *et al.*, 2011). Thus, the leaves of tropical grasses contain less of the more readily digestible mesophyll cells and more of the less digestible epidermis cells, vascular bundle cells and sclerenchyma cells compared to temperate grasses (Van Soest, 1994; Wilson and Minson, 1980).

Due to the more "open spaced" veins and lower ratios of mesophyll cells to bundle sheath cells, C3 grasses commonly contain higher levels of NFC, N, water, and lower levels of fibre compared to tropical grasses (Ehleringer and Cerling, 2002). Tropical C4 grasses also have lower levels of photosynthetic enzymes due to the C4 grasses' better photosynthetic efficiency (Berbehenn *et al.*, 2004) and greater efficiency in transporting CO_2 from the mesophyll cells to the bundle sheath cells (Ehleringer and Cerling, 2002). These N containing

compounds comprise a substantial portion of the total N fraction of the grasses, ranging between 20% and 40% of the total N found in the leaves of the grasses (Van Soest, 1994). As such, C4 grasses contain lower protein levels compared to C3 grasses. In addition, the location of the N containing compounds also differs between the two types of grasses. In the temperate grasses, the chlorophyll containing the Rubisco enzyme is in the mesophyll cells while it is in the bundle sheath cells in the tropical grasses. Thus, the N containing compounds are "more bound" by the less digestible bundle sheath cells in the tropical grass, which are more resistant to microbial adhesion and degradation (Van Soest, 1994).

In addition to the differences in ratios of cells between the two types of grasses, the bundle sheaths in the C4 grasses are thicker than in C3 grasses (Wilson and Minson, 1980; Berbehenn *et al.,* 2004), increasing its resistance to microbial degradation (Wilson and Minson, 1980). The leaves to stem ratio in temperate grasses is also more superior to that of the tropical grasses, especially during the dormant season (Wilson and Minson, 1980). As the rates of rumen degradation of leaves are more superior than that of stems (Ellis *et al.*, 1987), this factor, combined with the other factors as discussed earlier, generally explains the lower rates of roughage degradability of tropical grasses compared to temperate grasses, even at similar maturity stages (Bohnert *et al.*, 2011).

Under similar environmental conditions, tropical grasses produce more DM than temperate grasses due to a higher photosynthetic efficiency resulting in a more efficient N utilisation. Thus, the N concentration found in tropical grasses is generally lower than for the same maturity temperate grass. The lower N content, as will be discussed in more detail later in this review, might be a factor limiting DM intake of the roughage and subsequently animal production. An interesting comment from Wilson and Minson (1980) is that the application of N fertiliser in various studies did not always result in higher DM digestibility values in the tropical grasses. The main reason was that fertilisation frequently resulted in higher growth rates with a subsequent higher stem to leaf ratio. Stems in general are less digestible than leaves (Ellis *et al.*, 1987) as they contain more of the less digestible fibre fractions.

Bohnert *et al.* (2011) observed that the ratio of total carbohydrates (total non-fibrous carbohydrates plus fibre) relative to protein was higher in the leaves of the C4 grasses compared to the leaves of the C3 grasses. As discussed, this higher ratio is due to the more efficient N utilisation in the tropical C4 grass (Wilson and Minson, 1980). However, Barbehenn *et al.* (2004) observed significant differences in the differential carbohydrate

fractions between the two types of grasses. The authors observed that the hexose and fructan concentrations of the C3 grasses' leaves were higher compared to the leaves of the C4 grasses. Another significant observation was that the C3 plants contained on average 6% more water and 36% less fibre than the leaves of the C4 grasses. Poppi *et al.* (1999) concluded from a review that C4 grasses contain less water-soluble carbohydrates (WSC) or non-soluble carbohydrates (NSC) compared to C3 grasses. In addition, the leaves of the C4 plants were tougher (as measured through a spectrometer where the leaves were punched two mm holes) compared to the leaves of the C3 plants (Barbehenn *et al.,* 2004), probably due to the higher ratios of bundle sheath cells compared to mesophyll cells in tropical grasses as well as the relative differences in bundle sheath thicknesses (Ehleringer and Cerling, 2002).

Ehleringer and Cerling (2002) noted that plants with lower carbohydrate to N (C: N) ratios are generally more nutritious to vertebrate herbivores. The authors further noted that N is a limiting nutrient in grasses for herbivores when the ratio of C: N is higher than 7:1. In another study, Trevaskis *et al.* (2001) conducted a trial where planted fertilised kikuyu (C4 grass) were fed to sheep. The authors observed an increase in MNS when starch was supplemented in addition to the kikuyu and commented that a ratio of more than 2:1 (C: N) was necessary to optimise MNS. According to the authors, energy compounds at that stage might be the limiting nutrient inhibiting MNS and possibly animal production as the C4 grass was fertilised with N. As such, the optimal range of C: N of roughages consumed by ruminants vary between 2:1 and 7:1, with energy the deficient nutrient at the lower end spectra and N compounds at the higher end spectra.

In both C3 and C4 grasses, the ratio of stems to lea influences basal roughage intake and digestibility and therefore, the relative nutritive value of the roughage to the host animal (Van Soest, 1994; NRC, 2007). In general, the digestibility and nutritional value of leaves are better than stems due to the higher concentration of easier fermentable cell fractions and lesser concentration of the slowly and indigestible fibre fractions (Ellis *et al.,* 1987). During any maturity stage, the leaf to stem ratio of temperate grasses is superior to that of the tropical grasses, especially as the roughages mature during the dormant season (Wilson and Minson, 1980). The differences in relative proportion of stem: leaf between temperate and tropical grasses are due to the general higher growth rates of tropical grasses compared to temperate grasses, resulting in a higher stem elongation in tropical grasses (Wilson and Minson, 1980). The authors also noted that the stems of tropical grasses contain more vascular bundle cells (in relation to temperate grasses). In addition, the extent of lignification in the stems of the

tropical grasses was higher compared to the temperate grasses (Wilson and Minson, 1980). Lignin decreases the digestibility of roughages from 90% to 20% as its concentration increases from 5% to 15% in plant cells (NRC, 2007) as lignin the binds to cell wall polysaccharides, thereby restricting microbial access during degradation (NRC, 2007). Therefore, the digestibility of the tropical grasses decreases to a higher extent than that of the temperate grasses due these reasons and are less digestible than temperate grasses at all maturity stages (Wilson and Minson, 1980).

Bohnert *et al.* (2011) conducted a study in which the nutritive values of similar low-quality C3 (Kentucky blue grass, *Pao pratensis*) and C4 grasses (tallgrass Prairie) were compared in ruminants. The chemical analysis of both the temperate C3 grasses and tropical C4 grasses were similar (CP percentage and NDF percentage on DM basis were 6.3% and 66.4% *vs.* 5.7% and 69.8%, respectively, for the C3 and C4 grasses). The authors observed that, although the grasses were of similar quality, differences existed which affected the grasses' utilisation by the ruminant. One of the main differences was the non-fibre carbohydrate (NFC) fraction with the NFC of the C3 grasses significantly higher (14.1%) than that of the C4 grasses (8.8%). In addition, from *in sacco* degradation studies, the authors calculated that the rumen degradable nitrogen (RDN) fraction of the C3 grasses accounted for 84.7% of the total N found in the temperate grasses. The corresponding number in the tropical C4 grasses was only 66%. The higher percentage of RDN in the temperate grasses could be explained by the probable locations of the N compounds in the temperate *versus* the tropical grasses, as was discussed earlier. In addition, the general higher lignification of the stem fraction in the tropical plants probably reduced the availability of the N compounds in the stems of the tropical grasses compared to that of the temperate grasses.

It is evident that significant anatomical differences exist between tropical C4 grasses and temperate C3 grasses, which will have an influence on its respective nutritive values to the ruminant grazing these plants. In addition, these differences are magnified as the grasses mature, with the nutritional quality of the tropical grasses deteriorating faster than that of the temperate grasses.

Table 1.1 is a summary of the anatomical and chemical differences between mature, low-quality temperate C3 and tropical C4 grasses.

Table 1.1 Summary of the chemical and anatomical differences between low-quality temperate C3 and tropical C4 grasses

Parameter	***Temperate C3 plants***	***Tropical C4 plants***
Cell types	• More mesophyll cells and less bundle sheath cells, ratio of 12:1. • Less stem elongation, thereby leaf: stem ratio is always better.	• Decreased quantity of mesophyll cells, increased quantity of bundle sheath cells, (ratios of 4:1; Krantz cells configuration). Bundle sheath cells also are thicker. • More stem elongation, lignification of stems also to a higher degree.
Spatial configuration	More intra-cellular spaces, less compaction.	Less intra-cellular spaces, more cell compaction.
Nutrient distribution	Higher NFC content, more of the total N as chlorophyll in mesophyll cells, more sugars (sucrose, fructans).	Less NFC, N "bound" in bundle sheath cells, sometimes more starch in leaves, but always fewer sugars. However, starch is also bound in bundle sheath cells.
Fibre composition	Less lignification.	More lignification, cuticle, bundle sheath cells.

NFC, Non-fibrous carbohydrates; N, Nitrogen

1.4 Effects of the Anatomical Differences Between C3 and C4 Grasses on the Utilisation of Low-quality Roughages by Ruminants

Bohnert *et al.* (2011) conducted a study on steers and sheep consuming either a low-quality C3 grass (Kentucky blue grass, *Pao pratensis*) or low-quality C4 grass (tallgrass prairie). The quality and chemical analysis of both grasses were similar. The authors fed both grasses to the ruminants with or without protein supplementation, with protein being supplemented as soybean meal at 0.09% BW or 0.19% BW for steers and sheep, respectively. The authors observed differences in intake and digestibility values of the non-supplemented tropical C4 grasses *versus* the non-supplemented temperate C3 grasses in both the steers and sheep. The observed NDF intakes of the C4 grasses were 10.8 g/kg BW in the steers *versus* 15.6 g/kg BW for the C3 grasses. The corresponding values in the sheep were 17.8 g/kg BW and 20.0 g/kg BW, respectively. From *in sacco* studies, higher soluble a-fractions (soluble fraction disappearing instantaneously from the bags into the rumen) and rates of degradation (c-values) for both the DM and NDF fractions were observed in the C3 grasses compared to the C4 grasses. Thus, the effective degradability (ED) of the roughages was higher in the ruminants consuming the temperate grasses. The authors also observed that the total digestibility values (DM, OM and NDF) of the different non-supplemented grasses differed, with the observed digestibility of the C4 grasses at any stage between 4 – 8% lower than the corresponding digestibility values observed in the temperate grasses.

The authors argued that the differences were due to the arrangements and differential proportions of tissue cells found between the two different types of grasses, the different ratios of mesophyll cells and bundle sheath cells. In addition, the higher quantity of NFC (14.1% and 8.8% in the C3 and C4 grasses, respectively) as well as the easier "available" N compounds in C3 grasses might have contributed to the higher rates of observed degradability values in the temperate compared to the tropical grasses.

Ellis *et al.* (1987) discussed various factors influencing roughage intake by ruminants. One of the factors is the rate of particle size reduction of the plant material. Digesta particles need to be broken down to sizes less than one mm (critical size) to flow through the reticulo-rumen orifice in sheep (Ellis *et al.*, 1987). Large particles have high buoyancies due to entrapped gasses within the vascular tissues. Thus, these particles will "float" in the rumen, thereby forming a raft in the dorsal rumen, especially in particles of highly vacuolated tissues like stems (Ellis *et al.*, 1987). During fermentation and rumen mastication, entrapped gasses

are released and the specific gravity of the particles is increased, which will then sink to the ventral rumen.

Chewing and rumination are the most important actions in particle size reduction (Faverdin *et al.*, 1995). In addition, the authors suggested that roughage intake is related to 12 or 24-hour *in situ* degradability and/or the energy needed to grind the roughages. Any factor therefore that will increase the resistance in which plant material is broken down might have a negative effect on intake by the ruminant. This observation explains the high correlations commonly observed between NDF intake and DM intake in ruminants (Köster *et al.*, 1996) as the NDF fraction of a feed includes hemicellulose, cellulose and lignin, which increase the resistance of plant degradation (Van Soest, 1994). As discussed, the leaves of tropical grasses contain more vascular tissue cells and less mesophyll cells compared to the same low-quality temperate grasses. The rates of degradation of mesophyll cells are faster than that of bundle sheath cells (Barbehenn *et al.*, 2004). In addition, the intracellular spaces within the leaves of the temperate grasses are substantially more than in the tropical grasses. Bacterial invasion and adherences to the already more fermentable mesophyll cells therefore will be faster in temperate grasses compared to tropical grasses. These cells would reach the minimum critical size faster than the bundle sheath cells and therefore would have the opportunity to escape the rumen through the rumen reticulum orifice faster. Thus, the rumen retention time of mesophyll cells will be less than for bundle sheath cells. Feed intake *per se* would therefore be "stimulated" by the more fermentable fibres in the temperate grasses relative to the tropical grasses.

Another factor that could have influenced the observed intake differences between temperate and tropical roughages in the study of Bohnert *et al.* (2011) was the WSC content. Ciavarella *et al.* (2000) observed that sheep preferred grasses with a higher WSC content. As mentioned earlier, the WSC content of temperate grasses at all maturity stages generally is higher compared to tropical grasses (Bohnert *et al.*, 2011). Therefore, it is a possibility that roughage intake from ruminants consuming temperate grasses will be higher than the corresponding roughage intake from ruminants grazing tropical roughages due to this factor alone.

Bohnert *et al.* (2011) observed better roughage retention times and rumen dilution rates (about 40% better) in steers consuming non-supplemented C3 grasses compared to steers consuming non-supplemented C4 grasses. However, the authors did not observe any

differences in either the N balance (0.022 g/kg BW *vs*. 0.025 g/kg BW for C4 and C3 grasses, respectively) or RAN concentrations (0.64 mM RAN and 0.52 mM RAN, respectively). For both parameters, higher N and RAN recordings were expected in the ruminants consuming the low-quality temperate grasses compared to those consuming the tropical grasses due to the potential higher availability of the N compounds in the temperate grasses. The authors did not explain the observed similar N balance and RAN concentrations between ruminants receiving the two types of grasses. However, it is of interest to note that the N balance in both groups of ruminants were positive (although low). In the study, the maintenance requirements of sheep consuming low-quality roughages were not met (NRC, 2007) as the ruminants were not supplemented at that specific stage of the trial. This observation is in contrast with other studies where negative N balances were observed when non-supplemented low-quality roughages were fed to ruminants (Köster *et al.*, 1996; Detmann *et al.*, 2009). The observed positive N balances in the study of Bohnert *et al.* (2011) therefore might be suggestive that the availability of N compounds in both temperate as well as tropical grasses was probably sufficient to satisfy the needs of both sheep and cattle consuming these roughages. The observed RAN concentrations were within the general recommendations of Satter and Slyter (1974) of 5 – 20 mg/dL rumen fluid, suggesting that roughage intake and/or degradability probably was optimised (at 1 mM = 17 mg/dL rumen fluid, the respective RAN concentrations for the non-supplemented C4 and C3 grasses were 8.84 and 10.88 mg RAN/dL rumen fluid). However, results from more recent studies suggested that the observed RAN concentrations in the study of Bohnert *et al.* (2011) for ruminants consuming tropical grasses might not have been sufficient to maximise tropical roughage intake and/or MNS (Kanjanapruthipong and Leng, 1998; Detmann *et al.*, 2009). These aspects will be discussed in more depth and detail later in the literature review.

Microbial N synthesis and efficiency of MNS was not measured in the study of Bohnert *et al.* (2011). A direct comparison between ruminants consuming either tropical or temperate grasses was therefore not possible. However, Mullik (2007) conducted a study with cattle consuming tropical grasses and observed that MNS efficiency was 11.45 g MNS/kg DOMI. These values agree with values observed for 18.1 g MNS/kg DOMI for bermuda grass (*Cynodon dactylon*), 16 – 21 g MNS/kg DOMI for paspalum (*Paspalum plicatulum*) and kikuyu (*Pennisetum clandestinum*) and 9.6 – 16.0 g MNS/kg DOMI for Rhodes grass (*Chloris gayana*); all C4 grasses (SCA, 1990). The higher EMNS values in ruminants consuming temperate grasses are probably due to the anatomical differences between the two

types of grasses, resulting in more NFC and digestible N compounds in the temperate compared to the tropical grasses (Bohnert *et al.*, 2011). Dove and Milne (1994) also suggested that MNS efficiency is influenced by the WSC content of plants. In addition, SCA (1990) stated that plants with less than 90 g WSC/kg DM) have lower MNS efficiencies. In a review written by Poppi *et al.* (1999), the authors concluded that C4 grasses are generally deficient in WSC and NSC, and that MNS efficiency of tropical grasses is low and below what feeding tables would predict is theoretically possible per kilogram fermentable OM present in the plant.

It can be concluded that intake and digestibility (and degradability) values of the same (chemically) low-quality temperate and tropical grasses differ in ruminants. These differences are the result of the anatomical differences between the two types of grasses. In addition, the availability of these nutrients tends to be lower in tropical grasses compared to temperate grasses as a large quantity of potential nutrients are located within the bundle sheath cells of the tropical grasses. Therefore, low-quality C4 grasses generally provide a lower quantity of total digestible nutrients compared to low-quality C3 grasses, even at similar maturity levels. In addition, MNS and the efficiency of MNS to the ruminant consuming low-quality tropical roughages are below the levels observed in ruminants consuming low-quality temperate grasses (Mullik, 2007). Due to these differences (in nutrient content and availability), supplementation recommendations from current nutritional tables, which are primarily derived from low-quality temperate grasses, cannot be extrapolated to ruminants consuming low-quality tropical grasses (Leng, 1995; Mullik, 2007).

In the following section, the effects of supplementation of ruminants grazing or receiving tropical C4 grasses *versus* temperate C3 grasses are discussed.

1.5 Influence of Supplementation on Low-quality Tropical and Temperate Grasses: A Literature Overview

Bohnert *et al.* (2011) observed that N supplementation (soybean meal at 0.09% BW) to cattle increased the intake of tropical grass by 47%. In comparison, the corresponding increase for temperate grass was only 7%. As discussed, strong correlations exist between feed intake and NDF intake, with feed intake being maximised at a NDF intake of 1.25% BW in cattle (Köster *et al.*, 1996) and up to 1.7% BW in lambs (Bohnert *et al.*, 2002). Therefore,

it was expected that roughage intake by ruminants containing high concentrations of NDF would be lower than higher quality roughages containing less NDF. As such, an upper limit exists at which ruminants can ingest NDF, and that supplementation of deficient nutrients will only improve NDF intake if the upper limit has not been reached yet.

In general, roughage intake of non-supplemented C3 grasses is normally higher compared to tropical grasses (Bohnert *et al.*, 2002). Bohnert *et al.* (2002) observed an NDF intake of 13.0 g/kg BW in lambs consuming non-supplemented low-quality C3 grasses. With supplementation, NDF intake increased to 13.8 g/kg BW. In the study of Bohnert *et al.* (2011), NDF intake of tropical non-supplemented C4 grasses was 1.08% BW. In comparison, NDF intake was 1.56% BW for the non-supplemented temperate grass. These differences in intake between temperate and tropical grasses are due to anatomical differences between the two types of grasses, resulting in a higher bioavailability of nutrients as was discussed earlier. Thus, roughage intake in the study of Bohnert *et al.* (2011) was probably maximised, or near maximisation, in the steers consuming the low-quality temperate roughages. Therefore, the chances for improvement in roughage intake due to supplementation were much larger in the ruminants consuming tropical roughages compared to steers consuming temperate roughages.

In the same study, N supplementation increased digestibility by 21% for steers consuming low-quality C4 grasses, but only 9% for the steers consuming C3 grasses (Bohnert *et al.* 2011). In addition, N supplementation decreased rumen retention time by 46% and 10%, respectively, for the C4 and C3 grasses. In sheep, N supplementation did not improve intake; however, digestibility was improved by 18% and 7%, respectively, in the sheep consuming C4 and C3 grasses. Nitrogen supplementation increased RAN in the cattle receiving C3 and C4 grasses from almost the same base values by respectively 334% and 134%. The N balances in the sheep were markedly improved to the same extent. Supplementation did not improve the dilution rate or retention time in the steers consuming C3 grasses. However, both the parameters were improved (by 34% and 24%, respectively) in the steers consuming C4 grasses.

This study suggested that the effects of supplementation are different between the two types of grasses (at the same maturity level and quality). The anatomical differences between the two types of grasses could explain these differences, with the ratio of mesophyll cells to bundle sheath cells being an important parameter. In addition, the differences in nutrient content and availability between C3 and C4 grasses (the leaves of C3 grasses contain more

NFC and digestible N compared to the leaves of the C4 grasses) also might explain the relative low supplementation responses observed in the ruminants consuming low-quality C3 grasses compared to low-quality C4 grasses. A noticeable exception was the RAN concentration where N supplementation increased RAN (and N balance) significantly more in the steers receiving C3 grasses compared to the steers receiving C4 grasses. The higher degree of stimulation in the temperate grasses was probably the result of the initial higher quantity and bioavailability of N compounds found in the temperate C3 grasses, compared to the tropical C4 grasses. Supplementation of N compounds to the steers receiving the low-quality temperate C3 grasses therefore might have increased the pool of digestible N compounds to such levels as to exceed the ability of the bacteria to use the N compounds. In comparison, due to the lower quantity and bioavailability of the N compounds found in low-quality tropical roughages, the ability of the rumen bacteria to utilise the supplemental N compounds was probably not exceeded (or to a lesser extent compared to the steers receiving the temperate grass).

From the discussion, it is important to be cautious in interpreting data from ruminant supplementation studies if the type of roughage used in the study is not known or well described.

Table 1.2 gives a summary of the general differences between the "same" low-quality C3 and C4 grasses (<6% CP; >70% NDF) in terms of nutritive value and N supplementation.

Table 1.2 General effects of nitrogen supplementation on low-quality (0.8% N) temperate C3 grasses and low-quality tropical C4 grasses

Parameter and Response	***Reasons***
Forage intake The effects of N supplementation are generally higher in C4 grasses compared to C3 grasses.	1. Basal feed intake of C3 grasses is generally higher, compared to C4 grasses due to anatomical differences (more mesophyll cells, less bundle sheath cells, less lignification). There is thus more "room" to improve roughage intake in C4 grasses through supplementation. 2. C3 grasses contain more available nutrients (more NFC and more digestible N compounds). Nutrients supplied through supplementation in C3 grasses therefore would be used less

	efficiently as there are already more nutrients available from the plant. 3. Roughage intake depends on rate of degradability. Rates of degradability of non-supplemented C3 grasses generally are faster than C4 grasses. Therefore, N supplementation would have a lesser effect on roughage intake and effective degradability in ruminants consuming C3 grasses compared to those consuming C4 grasses.
Degradability/Digestibility Supplementation increased the rate of degradability and overall digestibility of tropical grasses more than in temperate grasses.	1. Basal rate of degradability of C3 grasses is higher due to the "easier" degradable cells (mesophyll *versus* bundle sheath cells) compared to C4 grasses. In addition, more open spaces are found in the leaves of the C3 grasses and less lignification in its stems compared to C4 grasses. As such, effective degradability in C3 grasses was generally higher compared to C4 grasses.
MNS and efficiency of MNS Supplementation generally increases total MNS for ruminants consuming low-quality C4 roughages. The effects on C3 grasses, however, are less than in C4 grasses.	1. Ruminants grazing non-supplemented C3 grasses generally have higher MNS values and produce MNS more efficiently than ruminants grazing non-supplemented C4 grasses. Reasons include the higher availability of N and carbohydrates located in the mesophyll and NFC fractions of temperate grasses. Therefore, the effects of supplementation might not be as apparent in C3 grasses as in C4 grasses.

NFC, Non-fibrous carbohydrates; N, Nitrogen

1.6 The Effects of Supplementation N Compounds in the Tropical Roughage Fed Ruminant

An efficient rumen is defined as a rumen in which roughage intake and/or degradability/digestibility is optimised. It could also be defined as a rumen where microbial growth is maximised per unit degradable organic matter intake (Leng, 1990; Chen and Gomes, 1992). A more efficient rumen in the tropical roughage fed ruminant therefore would not just cause an increase in the rate of degradability or feed intake, but also result in an improved ratio of metabolisable protein to energy (MP: E ratio) that are supplied to the lower GIT (Leng, 1990).

It is of interest to note that in most papers, only feed intake and/or roughage degradability (or more specifically, the rate of degradability) are considered as indices of rumen efficiency (Köster *et al.*, 1996, 1997). A possibility for the infrequent measurement or determination of MNS in supplementation studies was that it was argued by the researchers that it is not important to maximise MNS in ruminants consuming low-quality tropical roughages, but only to maximise forage intake and/or degradability. While this argument might be viable, it must be emphasised that the post ruminal MP: E ratio in ruminants consuming low-quality tropical roughages is considered one of the main reasons for the low animal production observed in tropical areas (Leng, 1995). It therefore follows that either UDP needs to be supplemented to optimise MP or alternatively, MNS needs to be optimised (Leng, 1995). The "production" of microbial N in the rumen is relative inexpensive compared to the supplementation of UDP as MNS could be maintained with NPN sources through RAN (Russell, 1984). Poppi *et al.* (1999) also suggested that increasing MNS is a more effective strategy to increase protein supply in the tropical forage fed ruminant.

Mullik (2007) and Poppi *et al.* (1999) commented that MNS efficiency differs between tropical and temperate grasses and that supplementation recommendations derived from current animal feed requirement tables cannot be used as a guide to optimise MNS production in the tropical roughage fed ruminant. In addition, more recent research indicates that higher RAN concentrations are necessary in ruminants consuming low-quality tropical roughages to maximise feed intake compared to ruminants consuming temperate roughages (Kanjanapruthipong and Leng, 1998; Detmann *et al.*, 2009).

The major deficient nutrient for ruminants grazing low-quality tropical roughages, especially during the dry dormant period is RDN compounds (Leng, 1990, 1995; Köster *et al.*, 1996; Detmann *et al.*, 2009; Lamy *et al.*, 2012; Poppi *et al.*, 1999). The N deficiency is not only due to the general lower N content of the tropical grasses (compared to temperate grasses), but also due to the lower availability of these compounds for microbial degradation in the rumen (Bohnert *et al.*, 2011). In addition, RDN is not only a limiting nutrient for rumen bacteria in ruminants consuming low-quality tropical roughages, but MP is also deficient to the host animal (Leng, 1995).

It is important to note that it is not only the quantity and/or frequency of RDN compound supplementation that need to be considered in the tropical roughage fed ruminant, but also the type of RDN compounds. Different supplementation studies in which different RDN compounds had been used, yielded different results and conclusions, resulting in many "rule of thumb" recommendations from researchers and advisors (Leng, 1995). Some of the results from the studies are discussed in the following section:

Köster *et al.* (1996) conducted a study with cattle consuming low-quality tropical prairie grass (tropical C4 grass). The animals were supplemented with supplements containing a combination of digestible intake protein (DIP, casein) and/or urea. Starch was added in incremental quantities as the level of urea was increased in the supplement to keep the supplements iso-nitrogenous and iso-energetic. Thus, the DIP of the treatment groups differed in the ratios of true protein (casein) to urea. The authors observed lower NDF digestibility values and digestible OM intake in the treatments where only urea was supplemented (100% urea treatment group). It was concluded that high levels of urea supplementation to the tropical roughage fed ruminant might have a negative effect on intake and total tract digestibility and that supplements need to contain some true protein in addition to urea to maximise roughage intake and/or digestibility. However, various authors (Mould *et al.*, 1983; Leng, 1990) suggested that starch might decrease the digestibility of poor quality roughages, possibly through a reduction in rumen pH (which favour amylolytic bacteria to the expense of cellulolytic bacteria; Mould *et al.*, 1983) or a carbohydrate effect which is independent of rumen pH (Mould and Ørskov, 1983). The increasing starch levels in the supplements containing the higher urea treatments in the study of Köster *et al.* (1996) therefore could have been responsible for the observed decreases in forage digestibility, a confounding effect that was also recognised by the authors.

In another study, Meissner *et al.* (1989) fed sheep either low or medium quality *E. curvula* hay. The chemical composition (*in vitro* organic matter digestibility, IVOMD and N concentration of the two qualities hays were 45% and 0.62% *versus* 52% and 1.21%, respectively. The authors observed that supplementation of casein (a true protein source) in addition to urea did not improve roughage intake or average daily gain (ADG) of the sheep consuming low-quality hay. However, roughage intake and ADG were improved when casein was supplemented in addition to urea to the sheep receiving the medium quality *E. curvula* hay. The authors commented that the observed response of casein was probably due to the higher concentration and availability of energy substrates within the medium quality hay compared to low-quality hay.

Griswold *et al.* (2003), using continuous cultures to study forage degradability and MNS, stressed the importance of having enough NPN sources (above true protein sources) in the rumen to maintain adequate ammonia levels to optimise MNS. The authors further stated that it is important to "first satisfy the RAN, then higher RDP levels could prove beneficial if there is maize in the diet". Various authors (Russell 1984, 1989; Leng 1990, 1995 and Kanjanapruthipong and Leng, 1998) stated that RAN is the preferred N source for fibrolytic bacteria growth in the rumen. In comparison, amylolytic bacteria need a higher supply of peptides and protein precursors, which are derived from true RDN sources (Russell, 1984).

From the discussion, rumen bacteria need peptide and preformed protein precursors in addition to RAN for growth and optimal efficiency. Literature studies, however, suggested that the requirements for peptides and protein precursors in the rumen could be met through microbial N turnover in the ruminant consuming low fermentable roughages (Kozloski *et al.*, 2007). In addition, true protein sources will only increase rumen efficiency with high fermentable diets, or where there is a source of easily fermentable compounds like starch or maize available to be fermented in the rumen (Griswold *et al.*, 2003).

As discussed, the leaves of tropical C4 grasses generally contain less NFC and RDN compounds than the leaves of low-quality C3 grasses (Bohnert *et al.*, 2011). In addition, these compounds are less available for rumen fermentation as most of these compounds are in the bundle sheath cells of the tropical grass compared to the mesophyll cells of the temperate grass. Due to these differences, low-quality C3 grasses (even at the same maturity stage and chemical analyses) have higher fermentation and degradation rates than low-quality tropical roughages (Bohnert *et al.*, 2011). Therefore, it could be speculated that ruminants consuming

low-quality C3 grasses must be supplemented with higher levels of true protein sources (and less NPN sources) to satisfy the rumen microbial needs compared to ruminants consuming low-quality C4 grasses. These differences could explain the different responses in supplementation studies where NPN sources were evaluated against true protein sources in ruminants receiving low-quality roughages.

The differences in potential protein utilisation between C3 and C4 grasses, again highlight the importance in distinguishing the roughages used in supplementation studies. However, in many of the supplementation studies, it is not mentioned whether the type of roughage used is either of temperate or tropical origin (C3 or C4). It is therefore not always possible to distinguish whether results observed could be applied to supplementation studies in ruminants consuming tropical roughages.

From a supplementation strategy viewpoint, it is more important to satisfy RAN concentration before the need for peptides and other protein precursors are met through true RDN sources in ruminants grazing low-quality tropical forages (Griswold *et al.,* 2003). Factors important in influencing the "breakpoint" at which true protein becomes more important *versus* NPN sources, are related to the quality of the tropical roughage with higher quality tropical roughages benefiting from supplementation with true protein sources (Meissner *et al.,* 1989) and even UDP sources (Poppi *et al.,* 1999). As such, the availability of additional starch products in a supplementation program necessitates the usage of true protein sources in the rumen (Russell, 1984; Köster *et al.*, 1996).

The importance of RDN supplementation is to increase the RAN concentration to levels that will optimise rumen bacterial growth and production which could result in an increase in roughage degradability, digestibility and therefore, possibly intake. In addition, RDN supplementation could also increase MNS to the lower GI tract, which will increase the post ruminal MP to energy ratio in these animals (Leng, 1995). It is therefore important to know or to define the optimal RAN concentrations necessary at which state roughage intake, degradability, MNS and/or efficiency of MNS is maximised in the tropical forage fed ruminant.

Satter and Slyter (1974), using a wide range of roughages, suggested that a RAN concentration ranging between 5 to 20 mg RAN/dL rumen fluid, optimises fibre degradability in the rumen. In addition, Erdman *et al.* (1986) suggested that RAN necessary for maximal feed degradability depends on the fermentability of the feed, with higher fermentable feeds

needing higher RAN concentrations to maximise feed degradability. Detmann *et al.* (2009), conducting a study with steers grazing low-quality tropical hay (3% CP, DM basis) observed a linear increase in the effective degradability (ED) of poor quality C4 hay as RAN was increased from 5 to 8 mg/dL rumen fluid. The authors also observed that roughage intake, MNS and ED were not optimised at the same RAN concentrations, with NDF intake and MNS being maximised at higher RAN concentrations (15 mg/dL rumen fluid). It is of interest to note that for both NDF intake and MNS, the relationships with RAN were not linear, with both parameters decreasing curvilinear as RAN increased above the optimal level of 15 mg/dL rumen fluid. In addition, RAN concentration within the rumens of cattle linearly increased from about 8 mg/dL to 10 mg/dL rumen fluid as the dietary CP concentration was increased from 50 g CP/kg DM to 110 – 120 g/kg DM. After this, RAN increased at a faster rate as the final CP concentration of the diet was increased. This observation could be indicative of energy that could have become deficient in the rumen at that specific stage, which would have limited MNS (Leng, 1990; Poppi *et al.*, 1999).

Incidentally, the optimal RAN concentration where ED of the roughage was maximised (8 mg RAN/dL rumen fluid) was achieved at a dietary CP concentration of 50 g CP/kg DM. This observation is very near to the recommendations set for cattle (NRC, 2000) and sheep (NRC, 2007) to meet maintenance requirements (6% CP DM basis). In contrast, NDFI and MNS were optimised at higher dietary CP concentrations (120 – 130 g CP/kg DM). It is of interest to note that the fibrous particle flow from the rumen peaked at similar RAN concentration (15 mg/dL), also corresponding to a dietary CP concentration ranging between 120 – 130 g CP kg/DM. These observations are not surprising, as a major factor determining feed intake is rate of fibre degradation and subsequently, the rate of particle outflow of the rumen (Ellis *et al.*, 1987). In addition, the quantity of MNS reaching the duodenum is a result of microbial production and degradation in the rumen (Firkins, 1996). Longer rumen retention times will result in more protein lyses within the rumen, thereby reducing the efficiency of MNS (Poppi *et al.*, 1999). Leng (1995) and Detmann *et al.* (2014) also suggested that the tropical forage fed ruminant needs to be supplemented with dietary CP to the level of 120 g CP/kg DM to optimise the use of available low-quality tropical roughage. It therefore seems that higher RAN concentrations (than required to maximise ED) and dietary CP concentrations are necessary to optimise low-quality tropical roughage intake and MNS in cattle.

An interesting conclusion made by Detmann *et al.* (2009) was that fibre degradation in tropical grasses could be considered a second order process where it is not only substrate characteristics that determine fibre degradation, but also enzymatic activity from ruminal microbes. As such, substrate characteristics determined ED at RAN concentrations above 8 mg/dL rumen fluid where RAN did not influence ED. At these RAN concentrations, enzymatic activity (quantity of rumen bacteria) probably was sufficient to maximise ED of the roughage. However, at RAN concentrations less than 8 mg/dL rumen fluid, there was a lack of enzymatic activity (not enough rumen bacteria) and ED was not maximised.

Kanjanapruthipong and Leng (1998) observed a similar type of observation in sheep (Merinos). The authors sprayed various levels of urea (5 to 20 g) on 750 g oaten chaff hay (0.8% N, C4 tropical hay) and fed it to sheep, weighing 42 – 48 kg. Using the data supplied in the paper, it could be calculated (with urea containing 46% N and a conversion factor of 6.25 from N to CP), that the quantity of CP supplied by the urea to the 750 g hay diet, ranged between 14.4 g CP (for the 5 g urea supplemented) up to 57.5 g CP (for the 20 g urea supplementation). Add together the hay's CP (0.8% N or 5% CP); the final quantity of CP fed to the animals ranged between 37.5 g CP/750 g hay (oaten hay, non-supplemented) to 95 g/750 g hay (750 g oaten hay, supplemented with 20 g urea). Therefore, the final calculated dietary CP percentages of the experimental diets ranged between 5% (non-supplemented hay) up to 12.7% (hay supplemented with 20 g urea/750 g hay). As such, the corresponding dietary CP values, where 5, 10 or 15 g of urea were supplemented to the experimental hay, were 6.9%, 8.8% and 10.8%, respectively.

The authors observed that between urea supplementation of 10 g/day and 15 g/day (from the above calculation at a CP percentage of 8.8% and 10.8%, respectively), RAN increased from 6.74 mg/dL rumen fluid to 16.78 mg/dL rumen fluid (from a base value of 0.38 mg/dL). The authors further observed that the *in sacco* degradability of the oaten chaff was maximised at a RAN concentration just less than 5 mg/dL, corresponding to the 5-g urea supplemented treatment or 6.9% CP. The *in sacco* degradability did not increase further at higher RAN concentrations, however, the purine derivatives (PD) excreted in the urine dipped initially as RAN increased and reached its lowest value at 3 mg RAN/dL rumen fluid. Thereafter, PD increased and remained constant between 10 – 17 mg RAN/dL rumen fluid. The PD increased for a second time as RAN reached 20 to 25 mg/dL rumen fluid. In addition, protozoal concentrations increased with increasing RAN concentration from a base value of 0.38 mg/dL rumen fluid up to 1.5 mg/dL rumen fluid where after it plateaued as RAN was

increased up to 16.8 mg/dL rumen fluid. However, protozoal numbers dropped again as RAN increased above 16.8 mg/dL rumen fluid.

Kanjanapruthipong and Leng (1998) concluded that rumen bacteria, and especially cellulolytic bacteria, could use RAN and the small quantities of amino acids derived from the incomplete protein metabolism by bacteria and protozoa, as well as from the lyses of microbes as N source. The authors also suggested that protozoa do not use urea as N source and require preformed amino acids to grow and to reproduce optimally. In addition, high RAN concentrations might be detrimental to protozoa. Rumen bacteria (and especially the cellulolytic bacteria) therefore might have an advantage over protozoa in competing for RAN at higher RAN concentrations, which might be beneficial to the roughage fed ruminant.

Based on these trials, it is evident that higher RAN concentrations were necessary to maximise roughage intake and MNS of low-quality tropical grasses (up to 15 mg RAN/dL rumen fluid) compared to maximising ED of tropical grasses (8 mg RAN/dL rumen fluid). The RAN concentrations necessary to maximise ED were obtained with a supplementation program supplying around 6% CP (in the form of RDN) to the host animal. This concentration agrees with the current NRC (2007) recommendations to meet maintenance requirements for ruminants consuming low-quality roughages. However, higher RAN and thus, dietary CP concentrations, up to 120 g CP/kg DM, were necessary to optimise roughage intake and MNS.

1.7 Fermentable Energy

Crude protein is the first limiting nutrient in ruminants grazing low-quality roughages. As such, protein supplements increase roughage intake and digestibility and improve performance of animals (Minson, 1990; Poppi and McLennan, 1995). However, while RDN supplementation stimulates low-quality roughage intake and/or digestibility, probably by increasing the RAN concentration to optimal levels (Detmann *et al.*, 2009), fermentable energy has the bigger influence on MNS in ruminants consuming low-quality roughages (Leng, 1990, 1995; Poppi *et al.*, 1999). In addition, N sources are utilised ineffectively in the rumen if appropriate energy sources are not available. Under such conditions, protein is deaminated and excreted in the urine in the form of urea (Caton and Dhuyvetter, 1997).

The concentrations of NSC and N compounds are less in low-quality tropical roughages compared to low-quality temperate roughages (Ehleringer *et al.*, 2002; Barbehenn *et al.*, 2004; Bohnert *et al.*, 2011). Poppi *et al.* (1999) furthermore concluded that C4 grasses have low NSC and WSC content, such that MNS efficiency is low and below what feeding tables would predict is theoretically possible to the level of fermentable OM present in the plant. The low NSC levels are due to the anatomical cell structures and conformation of the cells in tropical roughages as discussed. Coupled with the roughages' slow rate of rumen dry matter degradability (Bohnert *et al.*, 2011), the quantity and rate at which these energy sources could become available to the rumen microbes, is lower compared to temperate grasses. Therefore, supplementation of NSC to ruminants consuming tropical roughages is even more important to rectify fermentable energy deficiencies (Bohnert *et al.*, 2011), especially if ruminants are supplemented with easily available RDN sources (Leng, 1990; Poppi *et al.*, 1999). In addition, synchronisation of energy and RDN might be necessary to optimise MNS in the rumen (Sinclair *et al.*, 1993; Poppi and McLennan, 1995).

As discussed, supplementation of RDN sources frequently increases roughage intake and/or roughage digestibility in supplementation studies. However, responses varied in studies in which FME sources were supplemented to ruminants consuming low-quality roughages. Various authors (Hennesy *et al.*, 1983; DelCurto *et al.*, 1990; Matejovsky and Sanson, 1995) observed decreased roughage intakes and digestibility values when ruminants consuming low-quality roughages, were supplemented with NSC. In contrast, Henning *et al.* (1980) and Gomes *et al.* (1994) observed no, or even slight increases in roughage intake and digestibility values as NSC was supplemented to ruminants consuming low-quality tropical roughages. In addition, the level at which point supplemental NSC started to cause depressions in roughage intake and/or digestibility also varied between studies. Hennesy *et al.* (1983) for instance suggested that starch levels as low as 0.16% BW could depress roughage intake in cattle. Similarly, Matejovsky and Sanson (1995) observed adverse effects on roughage utilisation when maize was supplemented at levels higher than 0.25% BW (which is equivalent to a starch inclusion of 0.18% BW if it is assumed that maize contain 72% starch; Huntington, 1997). In contrast, Gomes *et al.* (1994) observed improved feed intakes and digestibility values in sheep receiving poor quality hay with starch concentrations up to 19% of the diet DM. Henning *et al.* (1980) also reported increased roughage intakes (maize straw) in sheep at low levels of maize supplementation (7.8% DM intake), with higher levels (more than 23% DM intake) reducing roughage intake.

It is evident that NSC supplementation could decrease roughage intake, especially at higher supplementation or dietary concentrations. However, it is also evident that roughage intake and/or digestibility values did increase under certain conditions when ruminants consuming low-quality tropical roughages, were supplemented with NSC. The degree and level at which point starch supplementation will inhibit roughage intake and/or digestibility is dependent on a few factors, including the quality of the diet. Henning *et al.* (1980) observed that reductions in hay intake were greater than reductions in straw intake in sheep fed either straw or hay and supplemented with increasing levels of maize. Other authors observed similar trends (Matejovsky and Sanson, 1995; Fieser and Vansant, 2004), suggesting that higher quality roughages could be substituted to a higher degree by easy FME sources than lower quality roughage.

Another factor influencing the effects of FME on low-quality roughage intake and/or digestibility, is the quantity of supplemental N sources. Caton and Dhuyvetter (1997) suggested that digestibility responsiveness to energy supplementation might be dependent on the protein concentration of the diet. Sanson *et al.* (1990) supplementing steers consuming low-quality meadow hay (tropical grass) with protein and various levels of maize, suggested that energy supplementation alone could worsen CP deficiencies. On the contrary, small quantities of carbohydrate supplementation might stimulate low-quality tropical roughage intake and even digestibility where CP is not limiting (Heldt *et al.*, 1999). The authors conducted two similar trials with cattle consuming low-quality tropical hay (72% NDF, 5.2% CP, DM basis). In both trials, different NFCs (glucose, fructose, starch, or sucrose at 0.3% BW) were supplemented to the steers. In Trial 1, CP supplementation was limited to 0.031% BW/day (as casein), which were insufficient to meet the requirements of the cattle while in Trial 2, CP supplementation at 0.122% BW was sufficient to meet the requirements of the cattle. In Trial 1, carbohydrate supplementation did not influence roughage intake; however, roughage digestibility was negatively affected due to the depletion of the RDN by the microbes by the readily available carbohydrates sources. According to the authors, this was the result of an insufficient RDN available for the microbes to ferment the more slowly fermentable fibre of the roughage. In Trial 2, carbohydrate supplementation did not affect roughage digestibility, however, roughage intake was stimulated relative to the control treatment.

In another study, Gomes *et al.* (1994) fed a low-quality barley straw diet containing urea and fishmeal (2% and 2.5%, respectively) to sheep. The N content of the experimental diet was 22 g N/kg DM and therefore not deficient. Starch was supplemented as maize and/or barley starch, varying from 0.2% (control group) to 9.4% and 18.6% (DM basis) for the experimental diets. The authors observed significant increases in DM intake (DMI; 910 g/day for the control diet to 1816 g DM/day for the experimental diet containing 18.6% starch) and organic matter digestibility (OMD, 50.0% to 61.2% for the control diet and diet containing 18.6% starch, respectively). The authors concluded that the increase in DMI and OMD observation could be explained by the high level of N compounds in the diet as the level of starch was increased in the diet.

As already discussed, the nutrient determining MNS the most is FME or NSC (Leng, 1990). In the study of Gomes *et al.* (1994), not only was DMI and OMD increased in the sheep where starch was supplemented, but MNS was increased from 5.7 to 18.2 g N/day for the control and experimental diet containing 18.6% starch, respectively. The efficiency of MNS was also improved from 12.8 g MNS/kg DOMI in the control, non-supplemented diet to 17.5 g MNS/kg DOMI in the diet containing 18.6% starch. The authors concluded that for a straw diet, starch supplementation up to 19% (of the diet DM) increased not only DMI but also the supply of microbial N to sheep, probably due to the larger quantity of OM available for microbial fermentation and growth. It must be noted though, that the RDN intake of that trial was 22 g N/kg DM (or 58 g N/kg DOMI) and as such, the experimental diets were not deficient in RDN.

Starch supplementation also has an influence on the rumen milieu. Mould and Ørskov (1983) and Mould *et al.* (1983) suggested that the activity of cellulolytic bacteria, and hence the digestibility of straw, would be reduced when rumen pH drops below 6.2. Declining ruminal pH associated with increasing dietary starch concentrations affects the ruminal bacteria population towards more amylolytic and lower cellulolytic populations, resulting in a reduced fibre digestion and roughage intake (Ellis *et al.*, 1987). However, Mould and Ørskov (1983) demonstrated that, by artificially raising the rumen pH of penned sheep fed high levels of concentrate with bicarbonate, *in sacco* roughage DM degradation failed to return to the controlled, non-supplemented treatments. The authors further observed that certain carbohydrates depressed roughage intake and digestibility to larger degrees than other carbohydrates. It was concluded that the reductions in roughage fibre digestibility and intake

in starch-supplemented ruminants were due to depressions in ruminal pH as discussed, as well as a not yet identifiable carbohydrate effect.

It is clear from the review that the effects of energy supplementation on tropical roughage fed ruminants depend on the quantity of RDN supplementation in the rumen. Under most situations, FME supplementation will decrease roughage intake and digestibility of low-quality roughages, especially at high NFC intakes and low supplemented RDN levels. However, when ruminants are adequately supplied with RDN, FME supplementation could stimulate low-quality forage intake and/or digestibility. Although lower roughage intakes are generally associated with increasing levels of FME sources, total OM intake is often improved in ruminants consuming low-quality roughages and supplemented with FME sources (Sanson *et al.*, 1990). In addition, the authors suggested that, as the level of concentrate increases in the diet, efficiency of energy use for both maintenance and gain, will increase due to an increase in the metabolisability of the diet, resulting in less energy wastage (McDonald *et al.*, 2011). Therefore, more energy is available for the rumen bacteria to produce microbial protein, resulting in increased concentrations of MNS and a better efficiency of MNS (Gomes *et al.*, 1994). It is also important to note that starch supplementation increased the rate of outflow (Chen *et al.*, 1992; Gomes *et al.*, 1994), which could further aid the efficiency of MNS.

1.8 Effects of Synchronisation of Nitrogen and Fermentable Energy in Ruminants Consuming Low-quality Roughages

Nutrient synchrony, per Hersom (2008), "would imply a parallel occurrence of nutrients for the ruminant animal to consume or be present in the diet and the rumen, so by supplying energy and nitrogen concurrently, an increase or optimisation of microbial efficiency would occur." Herrera-Saldana *et al.* (1990) suggested that protein and energy release must be complementary to elicit positive responses. The authors (Herrera-Saldana *et al.*, 1990) as well as Richardson *et al.* (2003) furthermore suggested that nutrient synchronisation often leads to an increase in rumen microbial efficiency. Leng (1990, 1995) suggested that, in the ruminant grazing low-quality tropical roughages, an increase in MNS efficiency theoretically should translate to an increase in animal production that would not have been observed if the provision of energy and protein had not been synchronised. From this description, the emphasis of nutrient synchronisation is on enhancing the efficiency of

MNS in the rumen of the roughage fed ruminant, cumulating to increased animal production in the tropical regions.

As discussed, the availability of nutrients differs between low-quality tropical and temperate grasses, even when the grasses are of similar chemical concentration (Bohnert *et al.*, 2011). It therefore follows that supplementation and the degree of nutrient synchronisation necessary to optimise animal production from ruminants consuming low-quality tropical roughages, might not be the same as in ruminants consuming low-quality temperate roughages. Care must therefore be taken in extrapolating results obtained from synchronisation studies conducted on ruminants consuming low-quality temperate roughages to ruminants consuming low-quality tropical roughages.

It must be noted that in most supplemental and nutrient synchronisation studies, the emphasis is frequently limited or based on energy and protein supplementation and/or synchronisation, as these two nutrients form the bulk of the diet and generally have the biggest effect on animal production (Hersom, 2008). However, other micronutrients, and especially minerals, also have some effects on microbial production and efficiency and ultimately animal production, and as such, need to be supplied for optimal animal production. Leng (1990) acknowledges this statement; however, the author suggested that it is difficult to study and to observe significant effects of these micro minerals in ruminants consuming low-quality roughages. Reasons include the variable roughage intake of the ruminant, various mineral interactions, and the general small influences that the mineral or micronutrients might have on various production parameters studied. A possible exception is sulphur (S), which has been proven to affect animal production, especially in ruminants grazing low-quality roughages (Weston *et al.*, 1988; Bal and Ozturk, 2006). Leng (1990) suggested a "shotgun" approach in which these micronutrients are supplied through a premix in adequate quantities to meet the animals' requirements.

There are three methods to achieve nutrient synchronisation in ruminants. The first method is to supply N or energetic compounds over various periods or time intervals (Huston *et al.*, 1999; Trevaskis *et al.*, 2001; Hersom, 2008). This is the most common method used to induce nutrient synchronisation in the roughage fed ruminant (Hersom, 2008). The basis of this method is to synchronise nutrients released from the roughage to the individual nutrients (energy and protein substrates) from the supplement.

The second method is to induce nutrient synchronisation through the type of supplements given. The basis of this type of synchronisation is that the rates of protein and/or energy release in the rumen from the different feedstuffs within the supplement vary within the rumen. For instance, results from *in sacco* trials undertaken by Sinclair *et al.* (1993) suggested that the rate of starch fermentation takes longer compared to sugar fermentation. The slow rate of starch fermentation might therefore explain the delay in drop of rumen pH (after five hours) generally observed in studies where roughage fed ruminants were supplemented with starch (Mould and Ørskov, 1983; Sinclair *et al.,* 1993). The rate of rumen degradability also varies for protein or NPN sources, with urea almost instantaneously dissolving in the rumen (Sinclair *et al.,* 1993). The choice of nutrients chosen as supplements in ruminants consuming low-quality roughages, is therefore based on the timing of the roughage intake as well as on the respective rates of rumen degradation or fermentation of both the roughage and supplement compounds within the rumen (Hersom, 2008).

A third method in which nutrient synchronisation could be achieved is to balance the release of energy and N compounds from various feed ingredients within the rumen. This method is used in animals (ruminants) receiving total mixed rations (TMR) and is strictly speaking, not applicable to the roughage fed ruminant supplemented with N and/or energy compounds.

In a study conducted by Sinclair *et al.* (1995) on sheep fed low-quality roughage, the authors formulated diets in which the hourly disappearance of N and OM in the rumen was either synchronous or asynchronous. In both diets, wheat and barley straw were used as energy sources, while urea was used as the N source in the asynchronous diet and rapeseed meal as the protein source in the synchronous diet. The total rumen degradability of the OM and carbohydrate fractions were similar between the two diets. However, the N:OM ratio ranged between 24.9 and 27.8 g N/kg OM in the synchronised diet compared to 12.8 g N/kg OM and 37.3 g N/kg OM in the asynchronous diet. Rumen ammonia N concentration in the sheep receiving both diets peaked one hour after feeding as expected. However, the peak RAN was 60% higher in the sheep receiving the asynchronous diet compared to those receiving the synchronous diet (16 mM and 10 mM for the sheep receiving the asynchronous and synchronous diets, respectively, 1 mM = 1.7 mg NH_3/dL rumen fluid). In both the dietary treatment groups, RAN dropped within three hours after feeding to 4 mM RAN. While rumen volatile fatty acid (VFA) concentrations did not differ between diets, both MNS and MNS efficiency, measured as MNS/kg OM fermented, was higher in the animals receiving the

synchronous diet compared to those receiving the asynchronous diet (27% and 13% higher for the MNS and MNS efficiency, respectively). It was also of interest to note that the authors observed a larger N recycling to the rumen of sheep fed the asynchronous diets. The authors concluded that synchronising the supply of N and energy substrates could improve microbial flow and the efficiency of MNS in the rumen.

Heldt *et al.* (1999) conducted two trials (within one study) using steers where different types of carbohydrates were used as supplements at different DIP levels. In both trials, steers were fed low-quality (CP 5.2%; NDF 72%) tallgrass prairie hay (C4 tropical grass). In Trial 1, steers were supplemented with urea to such levels that total DIP was 0.031% BW/day, which was insufficient to maximise low-quality tropical forage intake (Köster *et al.*, 1996). In Trial 2, urea was supplemented at levels high enough to induce total DIP intakes of 0.122% DIP BW/day, which was sufficient to maximise roughage intake. In both trials, one of four different carbohydrates sources (starch, glucose, sucrose, or fructose at 0.30% DM of BW) was supplemented together with the urea. The supplements were supplemented once daily directly into the rumen. In both trials, a negative control group, receiving no supplements, were used as reference. Supplementation, irrespective of the carbohydrate source, increased roughage OM intake in Trial 2 ($P = 0.05$) compared to the control diet. However, no differences were observed in Trial 1 between the treatment groups and the control group. A similar tendency was observed for digestibility where OM digestibility and NDF digestibility were higher in the supplemental treatment groups in Trial 2 ($P < 0.05$) compared to the control treatment. The authors concluded that carbohydrate sources did not affect roughage intake and digestibility in Trial 1 as DIP was deficient. In contrast, NDF and OM digestibility differed between the different carbohydrate treatments in Trial 2 with higher OMD and NDF digestibility values ($P < 0.05$) observed in the steers supplemented with sugars compared to those supplemented with starches. The steers supplemented with monosaccharides also resulted in a higher OM ($P = 0.02$) and NDF digestibility ($P = 0.03$) than those supplemented with sucrose in Trial 2. The authors concluded that, although carbohydrate sources did not affect roughage intake in Trial 2, glucose or fructose might have had a more positive effect on fibre digestion than starch or sucrose in the presence of sufficient DIP. The authors did not discuss possible reasons for the higher digestibility values observed in the steers supplemented with the sugars and monosaccharaides compared to the starches and disaccharide sugars. Hoover *et al.* (2006) conducted an *in vitro* study and observed that sugars (sucrose) fermented more rapidly

compared to starch. It was therefore a possibility that the release of energy compounds from the sugar based diets was more in synchronisation with the N release from urea in the rumen in the study of Heldt *et al.* (1999); hence, the higher digestibility values observed in the sugar and urea supplemented diets compared to the starch and urea supplemented diets. In addition, Mould and Ørskov (1983) observed in sheep that certain carbohydrate sources influenced feed intake and digestibility more than other carbohydrate sources. The authors concluded that a "carbohydrate" effect other than rumen pH might be responsible for the reductions of roughage intake and/or roughage degradation values through the supplementation of carbohydrates to ruminants consuming hay or barley. The authors did not discuss the nature of the possible carbohydrate "effects"; however, a possibility might be the degree of synchronisation between carbohydrate and DIP sources within the rumen.

Trevaskis *et al.* (2001) conducted a series of trials with cannulated sheep to test the hypothesis that synchronising carbohydrate and N availability in the rumen of sheep would increase MNS. The sheep were fed either planted ryegrass (*Lolium spp.*, C3 grass) or kikuyu (*Pennisetum clandestinum*, C4 grass) hay. The mean N concentration of the two roughages was 4.10% and 3.23% N respectively (25.6% and 20.2% CP). Rumen fluid was collected hourly and analysed for RAN. Allantoin concentrations in the urine were determined as an indicator of MNS (Chen and Gomes, 1992). Peak RAN concentrations were recorded at 66 mg/dL and 90 mg/dL, 3 – 4 hours and one hour after feeding respectively in the sheep fed ryegrass or kikuyu hay. In a follow-up study, sucrose was supplemented into the rumens of the sheep at the expected times of peak RAN concentration (4 hours post ingestion, synchronised treatment in the ryegrass fed sheep). In the asynchronous treatment, sucrose was supplemented at –1, +1 and +7 hours after feeding. The same procedure was applied to the sheep receiving the kikuyu hay, with sucrose infusions at +1 hour post ingestion (synchronous) and +7 hours post feeding (asynchronous). Synchronising sucrose infusion at the expected peak RAN concentration lowered peak RAN concentrations by between 20% and 27%. In addition, allantoin excretion in the urine increased from 6.6 mmol/sheep/day to 7.6 mmol/sheep/day in the synchronised kikuyu fed sheep. Sucrose infusion did not improve allantoin excretion at the asynchronous infusion periods. In another follow-up trial, fine rolled barley (*Hordeum vulgare*) was fed 1 – 2 hours before feeding kikuyu to sheep to synchronise the availability of the rumen fermentable carbohydrates in the grain with the N released from the hay and thus, the expected time of peak RAN concentration in the rumen. In addition, barley was fed 4 or 6 hours before feeding the kikuyu hay (asynchronous

treatments). The synchronised feeding of the fine rolled barley grain 2 hours before feeding kikuyu hay reduced peak RAN concentration in the rumen and increased the urinary allantoin excretion (from 10.1 to 11.8 mmol/sheep/day) in the urine. The authors concluded that synchronising of the available rumen fermentable carbohydrates with available N compounds in the rumen (and therefore with the RAN peak) stimulated a more efficient MNS usage by the microbial bacteria.

In contrast, Kolver *et al.* (1998) studied dairy cows fed harvested roughage and supplemented with a maize-based supplement. The supplement was given either at the same time the roughage was offered or 4 hours after the roughage was offered. No significant differences were observed in cow performances between the two treatments as BW change, milk yield or milk component yield did not differ. In addition, Henning *et al.* (1993) studied the effect of pulse dosing or gradual supply of protein and energy supplement combinations in sheep. While the authors observed some positive responses on microbial synthesis in sheep fed low-quality hay and synchronised in terms of energy and protein supply, feed intake, ruminal outflow, MNS and MNS efficiency were all improved to a higher extent by the gradual supply of energetic compounds compared to pulse dosing. In contrast, the supply pattern of protein did not affect any of the parameters. The authors concluded that it is more important to supply a constant supply of energy compounds to ruminants consuming low-quality roughages before synchronisation of energetic and protein nutrient supply should be considered.

Hersom (2008) in a review suggested that the timing of energy supply has a greater effect on nutrient synchronisation in the rumen compared to the timing of protein supply. This conclusion agrees with the general perception of synchronisation in the dairy cow (Hall and Huntington, 2008). Possible reasons include the inability of rumen bacteria to store energy or carbohydrate sources. Excess carbohydrates will lower the rumen pH, especially in N deficient diets, thereby favouring amylolytic bacteria at the expense of cellulolytic bacteria (Mould and Ørskov, 1983). In addition, energy (glucose) can be detrimental to rumen bacteria in other metabolic ways (Russell, 1989). In a catabolic reaction, ADP is converted to ATP using glucose as energy compound, which is then involved in anabolic reactions including bacterial growth and maintenance. In the absence of N (or any other compound limiting bacterial growth), and after the bacterial requirements for maintenance have been met, ATP concentrations will remain high in the cell while ADP will become limiting. With an excess of sugars, glucose carbon will be converted to methylglyoxal as the availability of

ADP that could be converted to ATP, becomes limited. This compound is highly toxic, causing potassium depletion as well as protein and DNA damage. Rumen bacteria have developed mechanisms to counteract this ATP – ADP imbalance by allowing protons to cross its membranes. As such, ATP is dissipated out of the cells allowing bacteria to "use" the excess energy substrates (Russell and Strobel, 1990, 2005).

The inability of rumen bacteria to store energetic compounds, coupled with the ruminants' ability to recycle urea N in N deficient diets, underlines the importance of energy supply to the rumen. It also explains the observations of Henning *et al.* (1993), suggesting that a gradual energy supply is more important than nutrient synchronisation *per se* in ruminants fed a low-quality roughage. In ruminants fed or grazing low-quality tropical roughages, the continuous supply of energy compounds might even be more critical as the roughages are generally lower in NFC than in the same quality temperate roughage (Bohnert *et al.*, 2011). In addition, these nutrients are generally less available compared to low-quality temperate grasses due to the location of the nutrients as well as the higher lignification generally found in low-quality tropical grasses.

In summary, it is evident that a constant supply of energy compounds to the roughage fed ruminant is essential in maximising MNS and MNS efficiency. However, FME and RDN need to be supplemented in conjunction with each other as energy supplementation alone or in excess to the available protein might worsen the N deficiency experienced by the tropical roughage fed ruminant. Moore *et al.* (1999) underline this statement, suggesting that N supplementation will only improve roughage intake when the total digestible nutrients (TDN) to CP ratio of the roughage is above seven, indicating a deficiency of N. In addition, Hoover *et al.* (2006) calculated that the ratio of NFC to DIP should be above two to optimise rumen microbial activity, as ratios less than 2:1 might be indicative of an energy deficiency in the rumen. These ratios however, were derived from ruminants consuming temperate roughages and as such, might not be applicable to the tropical roughage fed ruminant. However, Henning *et al.* (1993) as well as Trevaskis *et al.* (2001) observed improvements in MNS in sheep consuming tropical roughages and synchronised with protein and energy supplementation. Hersom (2008) summarised nutrient synchronisation in the roughage fed ruminant with the notation that synchronisation between energy and protein within the rumen needs to be continuous to elicit improved animal performances.

1.9 Additional Notes on Microbial Nitrogen Synthesis

Throughout the literature review, it is evident that MNS is an important parameter as it increases MP, which is frequently deficient in the tropical forage fed ruminant (Leng, 1995). Recent data suggested that MNS and MNS efficiency differ in ruminants consuming low-quality temperate and low-quality tropical roughages (Mullik, 2007), possibly due to nutrient availability differences between the two types of roughages (Bohnert *et al.*, 2011). The aim of this section is to highlight current knowledge on MNS and MNS efficiency in the tropical roughage fed ruminant and the influence of supplementation on MNS and MNS efficiency.

Microbial N can contribute up to two-thirds of the amino acid requirements of ruminants (Pathak, 2008) with an amino acid profile similar to the protein fractions in the main animal products (milk, meat) (NRC, 2007). As such, optimising of MNS to the lower GIT must be prioritised in the tropical roughage fed ruminant (Leng, 1995).

Microbial N synthesis in the general literature refers to the quantity of microbial protein as determined by PD in the urine (Chen and Gomes, 1992). Microbial N synthesis and microbial protein produced in the rumen are therefore two distinctive processes. Microbial N produced in the rumen could be twice or more times the actual MNS to the lower digestive tract, depending on diet, bacterial lyses, and rumen outflow of the rumen bacteria (Sniffen and Robinson, 1987). However, any factor influencing any of the parameters could potentially affects MNS and therefore the efficiency of MNS to the ruminant.

Efficiency of MNS is normally expressed as gram microbial protein/kg OM fermented in the rumen (Chen and Gomes, 1992; Hoover *et al.*, 2006). Care must be taken in comparing studies as microbial efficiency sometimes could be expressed in different units between scientific papers. One of the expressions or definitions frequently used is gram microbial protein/kg OM (*i.e.* including unfermentable OM) (Sniffen and Robinson, 1987). Nocek and Russell (1988) further suggested that it would be more appropriate to express MNS efficiency in terms of carbohydrates fermented within the rumen as fats and silage based feed constituents do not contribute to MNS. To draw comparisons between studies, it is important to take note of the expression of MNS efficiency within and between research papers. In this thesis, MNS efficiency is expressed as gram microbial N/kg DOMI.

Microbial protein is dependent on the supply of energy and RDN (Poppi *et al.*, 1999) with FME the nutrient determining MNS the most (Leng, 1990). The AFRC system (1993) encompassed this principle, where a fixed proportion of MNS is produced per unit FME at different dilution rates, providing the RDN requirements are met. In the AFRC (1993) system, MNS production is 9, 10 and 11 g microbial CP (MCP)/MJ FME, respectively (or 1.44, 1.60 and 1.76 g MNS/MJ FME; Poppi *et al.*, 1999) for maintenance, growth and lactating in ruminants at dilution rates of 0.02, 0.05 and 0.08/h. The mean efficiency of MNS varies between diets (SCA, 1990; Pathak, 2008). If it is assumed that ME averages 15.83 MJ ME/kg DOMI for forages (Poppi *et al.*, 1999) and using a constant conversion of 0.93 between ME and FME (forages), AFRC (1993) values equate to 21.1, 23.5 and 25.9 g MNS/kg DOMI at the three dilution rates (132, 147 and 162 g MCP/kg DOMI). It is of interest to note that typical MNS values vary between ruminants consuming tropical and temperate grasses with MNS efficiency generally lower (up to 55% the MNS efficiency) in ruminants consuming tropical roughages *versus* temperate roughages (Mullik, 2007; Bohnert *et al.*, 2011). In a review by Poppi *et al.* (1999), MNS efficiency of C4 grasses ranged between 60 – 100 g MCP / kg DOMI for Rhodes grass (*Choloris gayana*), 113 g MCP/kg DOMI (Bermuda grass, *Cynodon dactylon*) and 100 – 131 g MCP/kg DOMI (paspalum, *Paspalum plicatulum*). Data from SCA (1990) show that MNS efficiency range between 16 – 37.8 g MNS/kg DOMI with most forages ranging between 20.8 and 27.2 g MNS/kg DOMI. For roughage based diets, the mean MNS efficiency is 20.8 g MNS or 130 g microbial crude protein (MCP)/kg DOMI, 28.2 g MNS or 176 g MCP/kg DOMI for roughage and concentrate diets, and 21.1 g MNS or 132 g MCP/kg DOMI for concentrate diets.

The quantity and availability of easily fermentable carbohydrates fermented in the rumen is, from a dietary perspective, the main factor influencing MNS in the rumen and subsequently, MNS to the lower GI tract (Leng, 1990). In the tropical roughage fed ruminant, the total quantity and availability of NFC is lower compared to temperate grasses (Bohnert *et al.*, 2011), thereby reducing MNS (Dijkstra *et al.*, 1998; Leng, 1990) and MNS efficiency compared to the temperate roughage fed ruminant (Mullik, 2007). The Australian feeding system (SCA, 1990) suggested that grasses containing less than 90 g WSC/kg DM generally have lower MNS efficiencies compared to grasses containing more WSC. Poppi *et al.* (1999) in a review concluded that MNS efficiency from tropical C4 grasses is low due to WSC shortage and below feeding table suggestions possible for the level of fermentable OM present in the plant due to the lower NSC content.

Supplementation studies where NFCs were supplemented to ruminants fed low-quality roughages frequently led to an increase in MNS (Gomes *et al.*, 1994; Henning *et al.*, 1993). As such, Gomes *et al.* (1994) observed an increase in MNS from 5.67 g to 18.24 g N/day in sheep where the starch percentage in straw based diets was increased from 0.2% to 17.4% DM. Efficiency of MNS also increased from 12.8 g MNS/kg DOMI in sheep receiving the control diet (0.2% starch) to 17.8 g MNS/kg DOMI in sheep receiving the experimental diet containing 174 g starch/kg DM intake.

While FME supplementation can increase MNS and MNS efficiency in the roughage fed ruminant, MNS and MNS efficiency could be reduced at either extremity of FME supplementation. In the low-quality roughage fed ruminant, energy could become limited to the rumen microbial population due to the slow rate of carbohydrate fermentation (roughage degradation and fermentation), failing even to meet the maintenance requirements of the bacteria (Russell and Strobel, 1990, 2005). This could result in an impairment of bacterial growth, thereby reducing MNS (Pathak, 2008). Supplementation of FME frequently increased feed intake in ruminants receiving roughage-based diets (Gomes *et al.*, 1994; Heldt *et al.*, 1999), thereby increasing rumen particle and rumen fluid flow (Ellis *et al.*, 1987; Gomes *et al.*, 1994). Higher rumen flow could stimulate MNS and MNS efficiencies, as there is less time available for bacterial lyses in the rumen (Sniffen and Robinson, 1987). In addition, more of the available energy in the rumen is used for microbial growth, as less bacterial maintenance energy is needed. As such, Dijkstra *et al.* (1998) suggested that at a rumen outflow of 0.02/hour, which is typical of a low-quality roughage diet, only 45% of energy substrates within the rumen is used for bacterial growth. For higher producing animals with a rumen flow of 0.12/hour, the corresponding number is 85%. The rest of the available energy is used to maintain the bacterial population within the rumen (maintenance energy which, depending on the type of bacteria and the rumen environment, could range from 0.05 g to 0.15 g carbohydrate/g bacterial DM/hour) (Dijkstra *et al.*, 1998). However, energy is also used to “replace” microbial N due to bacterial lyses. In this regard, protozoal predation is one of the major contributors to bacterial lyses. By increasing rumen outflow, time spent within the rumen by the rumen bacteria decreases, leaving less time for bacterial predation. It is of interest to note that while MNS efficiency could be improved by defaunation ruminants due to less protozoal predation, OM degradability could be decreased by a lack of protozoal action, which could reduce roughage intake. As such, in studies conducted by Dijkstra *et al.*

(1998) defaunation did not always lead to an increase of total MNS due to a reduction in DOMI (Leng, 1990, 1995).

In contrast, an abundance of FME in the rumen could limit MNS or MNS efficiency. This reduction is due to a rumen pH decline as the rate of carbohydrate fermentation in the rumen increases (Mould *et al.*, 1983), favouring amylolytic bacteria above cellulolytic bacteria (Mould and Ørskov, 1983; Mould *et al.*, 1983). It is of interest to note that, although the effects of fermentable carbohydrates on rumen pH had been established as a factor limiting fibrolytic bacterial activity in the rumen (Mould and Ørskov, 1983; Mould *et al.*, 1983), other mechanisms exist whereby carbohydrates affect fibre intake and degradability and possibly MNS (Mould and Ørskov, 1983). While the authors did not speculate on the nature of "carbohydrate" effects, it is possible that it might be related to the rate and timing of nutrient release from the individual carbohydrates as discussed earlier. Lastly, energy abundance in the rumen might also decrease the efficiency of MNS due to an energy spilling effect (Russell, 1989; Russell and Strobel, 2005).

The question now could be asked at what stage the supplementation of FME becomes a burden to the production of MNS and not an asset to the low-quality tropical roughage fed ruminant. Literature review studies suggested that the optimal level of carbohydrate supplementation is related to the ruminant's RDN intake. Higher FME intake is possible where the RDN requirements of the ruminant are met without negatively affecting MNS and MNS efficiency (Gomes *et al.*, 1994).

As discussed, RDN is the first limiting nutrient in ruminants fed low-quality tropical roughages (Leng, 1990; Köster *et al.*, 1996; Kanjanapruthipong and Leng, 1998; Detmann *et al.*, 2009; Wickersham *et al.*, 2008, 2009) as RDN supplementation increased roughage intake and digestibility or rumen degradability in various studies (Köster *et al.*, 1996; Kanjanapruthipong and Leng, 1998; Detmann *et al.*, 2009). In contrast, energy supplementation alone or at DIP concentrations below certain critical concentrations, frequently decreased roughage intake and/or degradability (Heldt *et al.*, 1999). It is therefore important to meet the animals' requirements for this nutrient before energy supplementation should be considered. Results from supplementation studies in which N compounds had been supplemented to ruminants grazing low-quality tropical roughages, have been discussed earlier. Pathak (2008) summed up these results, suggesting that it is necessary to fully meet the needs of the rumen microbes for N compounds, either as degradable protein or by

metabolic N compounds. Due to the location and relative lower bioavailability of nutrients in tropical roughages compared to temperate roughages, the need for N supplementation in ruminants fed low-quality tropical roughages might be higher compared to ruminants fed low-quality temperate roughages (Bohnert *et al.*, 2011).

Another point necessary to highlight is the possible effects of nutrient synchronisation (energy and protein) on MNS. Non-synchronisation of RDN and FME in the rumen could result either in an abundance or deficiency of FME substrates relative to RDN during specific periods. An abundance of FME to RDN might be detrimental to the survivability of bacteria as rumen bacteria will spill energy (waste energy) to avoid toxic substances forming and destroying them (Russell and Strobel, 1990, 2005). In addition, an abundance of FME could reduce rumen pH, thereby reducing the survivability of fibrolytic bacteria (Mould and Ørskov, 1983; Mould *et al.*, 1983). On the contrary, periods of RDN abundances in the rumen relative to energy could result in decreasing FME availabilities for the rumen microbes to incorporate RAN into microbial protein. Hersom (2008) in a review article suggested that the rumen availability of these two nutrients need to be in constant synchronisation with each other to achieve maximal or optimal rumen fermentation and therefore MNS to the roughage fed ruminant.

It can be concluded that in the roughage fed ruminant, small changes in nutrient availability and the rumen milieu could result in large changes in MNS (Sniffen and Robinson, 1987; Chen and Gomes, 1992). Fermentable metabolisable energy is needed by rumen microbes to capture RAN to build microbial N and is a critical nutrient determining MNS and the efficiency of MNS (Leng, 1990, 1995). However, FME needs to be supplemented at a constant rate to the roughage fed ruminant, as FME cannot be stored in the rumen; nor recycled from the body to the rumen. In addition, N compounds are necessary to create sufficient RAN concentrations to be used as building blocks by rumen microbes to build microbial protein. This nutrient however, can be recycled to the rumen and as such, the constant supplementation of RDN is not as critical for microbial synthesis as fermentable carbohydrates. However, supplementation studies where the effects of N compound frequency were studied, suggested that MNS might be enhanced by more frequent supplementation strategies as roughage intake and/or digestibility is frequently increased. Increased MNS was also observed in supplementation studies where the release of energetic compounds was in synchronisation with the expected RAN in roughage fed sheep (Trevaskis *et al.*, 2001). Synchronisation therefore influences MNS in the roughage fed ruminant,

however, Hersom (2008) and Pathak (2008) suggested that synchronisation must be continuous to maximise MNS to the roughage fed ruminant.

1.10 Summary

The literature review highlights some of the most important aspects of supplementation as well as research needs in the supplementation of the tropical roughage fed ruminant.

1. Information on ruminants consuming low-quality tropical roughages is limited with only a few regions; Brasilia, Asia, Southern Africa, and Australia conducting research on these plant species. Most of the supplementation studies on ruminants consuming low-quality roughages were conducted on temperate species.

2. Nutrient composition and bioavailability differ between low-quality tropical grasses and low-quality temperate grasses, even though the chemical analyses might be similar. These differences arise from the relative location of these nutrients within each plant, which is a function of its photosynthetic pathway.

3. Current knowledge obtained from ruminants consuming low-quality tropical roughages suggests that N is the major deficient nutrient limiting animal production (Detmann *et al.*, 2009). However, the nutrient having the biggest effect on MNS in the tropical roughage fed ruminant, is energy (Leng, 1995).

4. Due to the different bioavailability values of nutrients between temperate and tropical grasses, supplementation responses differ between ruminants consuming low-quality temperate and low-quality tropical roughages. Thus, different supplementation programs are needed to fulfil the ruminant's requirements consuming these roughages. Recommendations derived from supplementation studies on ruminants consuming temperate roughages are not always applicable under tropical conditions (Mullik, 2007).

5. Higher quantities of RDN and FME are needed to be supplemented to the tropical roughage fed ruminant compared to the temperate roughage fed ruminant (Leng, 1990, 1995; Detmann *et al.*, 2009; Bohnert *et al.*, 2011).

6. Synchronisation of supplemental FME and RDN in the rumen of the roughage fed ruminant has the potential to increase MNS and MNS efficiency. The increased potential of MNS through FME and RDN supplementation could be significant in the tropical roughage fed ruminant, as the bioavailability of these FME and RDN sources from the tropical roughage might not be as high as in the temperate roughage. However, synchronisation studies on the tropical roughage fed ruminant are limited; and as such, information on this topic is limiting.

7. Despite the above knowledge, questions remain with a few being:

    a. Various authors (Kanjanapruthipong and Leng, 1998; Detmann *et al.*, 2009) observed strong correlations between RAN and various parameters (roughage intake, effective degradability, MNS and MNS efficiency) in the tropical roughage fed ruminant. In addition, the authors also observed a strong positive correlation between the supplementation of NPN sources and RAN. However, Köster *et al.* (1997) suggested that the supplementation of NPN and true protein is necessary to optimise intake and utilisation of tropical grasses in beef steers. Data regarding NPN and true protein supplementation on sheep fed low-quality tropical roughages, is limiting. As such, the question remains whether the supplementation of NPN alone or in combination with a true protein will have similar effects on RAN, roughage intake and/or degradability, MNS or MNS efficiency in the tropical forage fed sheep.

    b. Reliable recommendations on RDN requirements of sheep consuming low-quality tropical roughages is limiting, as is FME requirements and the ideal RDN to FME ratio of sheep consuming low-quality tropical roughages.

    c. Research suggested that RDN and FME synchronisation in the roughage fed ruminant could be beneficial (Hersom, 2008). However, data on the effects of RDN and FME synchronisation in the tropical forage fed ruminant, especially sheep, is limited. In addition, as nutrient bioavailability differs between tropical and temperate roughages (Bohnert *et al.*, 2011), the optimal degree of synchronisation of RDN and FME might also differ between tropical and temperate roughages in sheep.

Chapter 2 Substitution of rumen degradable nitrogen of sunflower meal with urea in supplements to sheep fed low-quality *Eragrostis curvula* hay

2.1 Abstract

The aim of the present study was to determine the effects of substituting the rumen degradable nitrogen (RDN) of sunflower meal (SFM) with urea, a non-protein nitrogen source on intake, rumen fermentation and microbial nitrogen synthesis (MNS) in sheep fed low-quality *Eragrostis curvula* hay. Five sheep were fed *ad lib*, low-quality *E. curvula* hay [2.7% crude protein (CP); 84.1% neutral detergent fibre (NDF), dry matter (DM) basis] and supplemented twice daily in equal proportions in the rumen, one of five iso-nitrogenous and iso-energetic supplements in a 5 x 5 Latin square format. The supplements differed in the ratios of RDN supplied by either SFM and/or urea, and were as follows (percentages indicate the level of RDN supplied by SFM and urea): T0 (100% SFM, 0% urea); T15 (85% SFM, 15% urea); T30 (70% SFM, 30% urea); T45 (55% SFM, 45% urea) and T60 (40% SFM, 60% urea). Roughage intake and total tract DM digestibility did not differ between treatments; however, roughage neutral detergent fibre (NDF) digestibility was higher in T45 and T60 compared to T15. Neither rumen pH nor total rumen VFA production differed between treatments. The mean rumen ammonia nitrogen (RAN) concentration of T60 was higher than T30 (9.35 mg RAN/dL rumen fluid *versus* 7.41 mg RAN/dL rumen fluid) respectively; however, no differences were observed in the MNS or MNS efficiency between treatments. Results suggest that up to 60% of the RDN supplied by SFM can be substituted with urea, without affecting intake, digestibility or MNS in sheep receiving low-quality tropical hay.

2.2 Introduction

Seasonal weight loss during the dry season limits ruminant production in the subtropics (Lamy *et al.,* 2012) with animals loosing up to 30% of their total BW (Almeida *et al.,* 2006). Typically, low-quality tropical roughages contain less than 60 g CP/kg DM (Detmann *et al.,* 2009; Leng, 1990), which is insufficient to meet the maintenance requirements of sheep (NRC, 2007). Nutritional deficiencies during these periods can be rectified by N and/or CP supplementation (Köster *et al.,* 1996; Ferrell *et al.,* 1999).

Urea is commonly used as a RDN supplement, as it is more cost effective than true protein per unit RDN. Russell (1984) suggested that cellulolytic bacteria only utilise RAN for growth while Arroquy *et al.* (2004) stated that rumen branched chain volatile fatty acids, derived from the deamination of amino acids, are essential to produce fibre-reducing bacteria. Supplementation studies on the use of NPN *versus* true protein sources for RDN in ruminants consuming tropical grasses (C4 grasses) are also contradictory and inconclusive. Kozloski *et al.* (2014) for instance observed no differences in low-quality grass hay (*Cynodon sp.* C4 grass) intake or fibre digestibility in sheep supplemented with either calcium caseinate (degradable true protein) *versus* NPN source. In contrast, Köster *et al.* (1997) observed an increase in OM and NDF digestibility in steers fed low-quality prairie grass (C4 grasses) supplemented with sodium caseinate compared to urea.

A possible explanation for the inconsistent results observed in supplementation studies could be differences in nutrient bioavailability between low-quality tropical and temperate roughages, resulting in differential intake and digestibility values between the grass species (Bohnert *et al.*, 2011). Low-quality temperate grasses contain higher levels of NFC and RDN sources compared to tropical grasses (Bohnert *et al.,* 2011), which might necessitate different supplementation requirements. Recent studies suggested that RAN concentration needed to optimise low-quality tropical roughage utilisation by ruminants differ to ruminants fed low-quality temperate grasses (Kanjanapruthipong and Leng, 1998; Detmann *et al.*, 2009). The aim of the present study was to investigate whether the form of RDN supplemented as a true protein or as a NPN source would affect RAN, roughage intake, digestibility and MNS in sheep receiving a low-quality tropical grass.

2.3 Hypothesis

It is hypothesised that substitution of SFM, a RDN source by urea, a NPN source, will affect roughage intake, ruminal degradability, ruminal VFA and RAN production as well as MNS and efficiency of MNS in sheep consuming low-quality *E. curvula* hay.

2.4 Materials and Methods

The Animal and Ethical Committee of the University of Pretoria, South Africa, approved the trial and protocol. Five rumen cannulated Merino sheep (58.1 ± 1.1 kg BW) were fed *ad lib* a low-quality [2.7% CP; 87.1% NDF; 93% OM, 49.6% acid detergent fibre (ADF), dry matter (DM)–basis] *Eragrostis curvula* hay. If it is assumed that the *E. curvula* hay contained 1.5% ether extract ((EE) NRC, 2007), the calculated non-fibre carbohydrate fraction of the *E. curvula* was 4.7% (calculated as DM – ash – EE- CP – NDF; Fox *et al.*, 2004, Table 2.1).

Table 2.1 Chemical composition of *Eragrostis curvula* hay fed to wethers and supplemented with urea and starch

Dry Matter (g/kg)	930
Ash (g/kg DM)	72
Nitrogen (g/kg DM)	4.2
Neutral detergent fibre (g/kg DM)	841
Acid detergent fibre (g/kg DM)	496
Acid detergent insoluble Nitrogen (% of CP)	45 %
***Non-Fibre carbohydrate (g/kg DM)**	47

*Non-Fibre carbohydrate was calculated as DM – ash – EE- CP – NDF (Fox *et al.*, 2004), assuming that low-quality hay contained 1.5% EE (NRC, 2007).

The sheep were assigned to five different supplemental treatments in a 5 x 5 Latin square design. As such, there were 25 experimental treatments with five periods per treatment.

The supplements, containing sunflower meal (SFM), urea, starch and a mineral supplement were supplemented in equal proportions, twice daily at 08h00 and 16h00, intra-ruminally via rumen cannulae. Due to the low N concentration of the hay, it was decided not to have a negative control treatment to reduce the possible risk of rumen stasis. Similarly, the decision not to replace all the RDN fraction of the SFM with urea (100% NPN treatment) was taken as the quantity of urea necessary to replace the total RDN fraction of the SFM would have been more than 16 g urea/sheep/day. This quantity would have been more than the general safety recommendations (14 g urea/sheep/day) for sheep weighing 50 kg. The supplements differed in the ratios of RDN supplied by SFM and urea and were assigned T0; T15; T30; T45 and T60, respectively, where T0, T15, T30, T45 and T60 indicated that 0%, 15%, 30%, 45% and 60% of the RDN fraction of SFM was substituted with urea, respectively.

The decision to include starch in the supplement was to reduce the chances of urea toxicity by providing urea alone with a slow fermenting, low-quality roughage (Köster *et al.*, 1997). Bohnert *et al.* (2011) showed that the quantity and availability of the NFC fraction of tropical grasses is significantly lower compared to temperate grasses, reducing the utilisation of RAN by the rumen microorganisms even to a higher extent in the tropical roughage fed ruminant (Leng, 1990). It was for this reason that it was decided to add supplemental starch to the supplements where urea substituted the RDN fraction of SFM. However, it could have been argued that the energy fraction of SFM differs to that of starch, containing fat, NDF and NFC (NRC, 2007). Therefore, while the ME values of the treatments were similar between treatments (Table 2.2), the possibility existed that the FME fractions were higher in the urea and starch supplements.

A mineral premix, locally manufactured by NuTec Pty (Ltd) (234 Royston Road, Willowton, Pietermaritzburg, RSA) and additional sulphur were added to each supplement to fulfil in the maintenance requirements of sheep (NRC, 2007). The ingredient composition of each supplement is given in Table 2.2.

Table 2.2 Composition of the five different supplementation treatments

	*Treatments				
	T0	***T15***	***T30***	***T45***	***T60***
Ingredients (g DM/day)					
Sunflower meal	130	111	91	72	53
Urea	0	2.4	4.8	7.2	9.5
Starch	0	12.6	25.1	37.7	50.3
****Mineral supplement**	24.2	24.2	24.2	24.2	24.2
Sulphur	1.8	1.8	1.8	1.8	1.8
Nutrient intake of supplements (as supplied)					
Metabolisable Energy (MJ ME/day)	1.29				
Nitrogen Intake (g N/day)	7.00				

*Treatments: T0 (100% SFM, 0% urea); T15 (85% SFM, 15% urea); T30 (70% SFM, 30% urea); T45 (55% SFM, 45% urea) and T60 (40% SFM, 60% urea).

**Mineral composition of the supplement: Macro minerals (g/kg DM): K (230); Ca (83); P (63); Mg (46); Na (29); Cl (26); Trace minerals (mg/kg DM): Co (4.58); Cu (166.53); I (33.31); Fe (333.06); Mn (726.48); Se (1.67) and Zn (1248.96).

Each experimental period consisted of 22 days, with a 10-day adaptation period where the sheep were adapted to the supplemented treatment, followed by a 5-day sampling period, 3-day *in sacco* collection period and a 4-day period in which two sets of rumen fluid samples were collected for RAN and VFA analyses. During the adaptation period, the sheep remained in an outside pen and were group fed *E. curvula* hay and supplemented with the trial supplement. On day 8 of the adaptation period, the sheep were allocated to individual metabolic crates where faecal bags were fitted.

Feed intake and faecal and urine output were recorded daily. Feed, orts and faeces were sampled and pooled over the five-day experimental period within each treatment to estimate total tract digestibility. Urine was collected from urine pans and transferred into urine bottles containing 5 mL sulphuric acid (H_2SO_4; 50% v: v) for preservation, and adjusted to a final pH below 3 with H_2SO_4, if required. Daily urine volumes were measured and diluted to 4000 mL. From this diluted volume, 50 mL sub samples were taken, pooled over the collection

period and frozen at –20°C for purine derivative (PD) analysis. Creatinine was determined from each urine sample to determine the corrected PD (Chen *et al.*, 1995), assuming that the daily excretion of creatinine as a proportion of muscle mass from the wethers was constant Broderick and Merchen, 1992; Chen *et al.*, 1995). The corrected PD was used in the estimation of MNS (Chen and Gomes, 1992).

Rumen fluid was collected at 12-hour intervals for four days from four predetermined locations within the rumen (top left and centre and bottom left and centre). After every day, there was a three-hour shift in sample collection time to obtain samples at 03h00, 06h00, 09h00, 12h00, 15h00, 18h00, 21h00 and 24h00. Rumen fluid pH was measured immediately after each collection period. The samples were pooled within the treatment and collection period. Five mL sulphuric acid (H_2SO_4; 10% v: v) and 4 mL phosphoric acid (H_2PO_4; 25% v: v) were added to 30 mL and 25 mL rumen samples, respectively, for RAN (Broderick and Kang, 1980) and VFA concentrations (Vanzant and Cochran, 1994). The samples were frozen at –20°C until analysis.

Feed and faecal samples were ground using a Wiley mill to pass a 1 mm screen. Hay, orts and faecal samples were dried for 24 hours at 105°C in a forced air oven to determine DM and then combusted for 8 hours at 450°C in a muffle furnace for OM determination (AOAC, 2000). The N content of hay, faeces and urine was determined by total combustion (Nitrogen Analyzer Model FP – 2000; Leco Corporation, St. Joseph, MI, USA). All hay, orts and faecal samples were analysed for NDF and ADF with the ANKOM Fibre Analyzer (ANKOM Technology, Fairport, NY, USA). *In vitro* organic matter digestibility (IVOMD) was determined using the Tilley and Terry method (Tilley and Terry, 1963) as modified by Engels and Van der Merwe (1967) for low-quality roughages under South African conditions. In this modification, N in the form of urea was added to each test tube (20 mg urea per test tube), simulating N recycling in ruminants consuming low-quality roughages.

In sacco rumen incubations were conducted to determine the roughage's effective degradability (ED) in the rumen, using ANKOM R510 bags (8 x 5 cm nylon bags, pore size 50 µm). Approximately 5 g hay (DM; ground through a 2 mm screen) was weighed into each bag. Duplicate bags were suspended in an opaque nylon stocking in the rumen (Cruywagen, 2006) and retrieved at two, four, eight, 12, 24, 48 and 72 hours. Following incubation, all bags as well as a 0-hour control bag, were washed under running tap water until water

obtained by gently squeezing the bags, was clear. The bags and contents were dried at 60^{o}C in a forced draught oven for 48 hours before NDF analyses were performed.

2.5 Calculations

Microbial N synthesis was calculated using corrected PD analysed from urine (Chen and Gomes, 1992; Chen *et al.*, 1995). Corrected PD was calculated as total daily PD (mmol/day) (Chen and Gomes, 1992) divided by the daily creatinine excretion corrected for metabolic weight. Chen *et al.* (1995) observed a relationship between PD: Creatinine (per kg $BW^{0.75}$) of 2.128 *PD, which was used to calculate the corrected PD. The model, $y = 0.84x + (0.15\ BW^{0.75}\ e^{-0.25x})$ was used to calculate daily microbial protein. In the model, y represented corrected PD found in daily urine, while x represented total microbial protein. The model corrected for the contribution of endogenous purine derivatives, which are represented by the component within the parentheses. The calculation of x from y based on the equation was made by means of Newton's iteration process (Chen and Gomes, 1992).

The non-linear model: $y = a + b\ (1 - e^{-ct})$ used for *in sacco* analyses was described by Ørskov and McDonald (1979). The disappearance values at different time intervals were used to calculate the degradation constants of NDF, where y = the disappearance of NDF at time t; a is the washing loss (rapidly soluble fraction); b the slowly degradable NDF fraction, and c the rate (/h) of degradation of fraction b. All fractions were expressed on g/kg DM basis. The degradation constants were used to estimate effective degradability (ED) of the hay following the model of Ørskov and McDonald (1979) where $ED = a + [bc / (k + c)]$. In this model, k is the passage rate from the rumen, assumed 0.02/h for ruminants consuming low-quality roughages.

2.6 Statistical Analysis

Data were subjected to an analysis of variance (ANOVA) using the Proc GLM procedure of SAS (Statistical Analysis System, 2015). The model fitted was:

$$yijk = \mu + rj + ck + ti + \varepsilon ijk,$$

where yijk is the response for the ijkth unit, μ is the overall mean, rj (j = 1...n) represents the row effects (number of periods), ck (k = 1...n) the column effects (number of

different animals), ti (i = 1...n) the main treatment effects, and εijk is the error variation for the ijkth unit.

Variables in the model were sheep, treatment and period. The repeated measures analysis of variance function with the GLM models were used for repeated measurements within each treatment and period. Significance was declared at $P < 0.05$ using Fischer's test (Samuels, 1989).

2.7 Results and Discussion

The high NDF and ADF concentrations of the *E. curvula* are indicative of the poor quality of the hay. In addition, the N concentration of the hay was well below the suggested level of 0.96% N necessary to meet maintenance requirements of the sheep (NRC, 2007), emphasising the necessity of N supplementation for ruminants consuming low-quality tropical roughages (Köster *et al.*, 1996, 1997; Kanjanapruthipong and Leng, 1998; Detmann *et al.*, 2009).

On a DM basis, the SFM contained 6.72% N, 38% NDF and 29% ADF. Based on the NRC (2007) tables, it was assumed that the RDN fraction of the SFM was 5.76% N (85% of N). As the different treatments were formulated to substitute the RDN fraction of SFM with urea, total N supplied to the sheep differed between the various supplemental treatments (8.67, 8.48, 8.40, 8.32 and 8.24 g N/day respectively for T0, T15, T30, T45 and T60). However, RDN supplied by the supplements was similar with mean intake of 7.50 g N/sheep/day.

Table 2.3 shows daily DM intake, diet digestibility and *in sacco* NDF degradability of *E. curvula* hay in sheep as affected by the substitution of sunflower meal with urea.

Table 2.3 Daily dry matter (DM) intake, diet digestibility and *in sacco* neutral detergent fibre degradability of *Eragrostis curvula* hay in sheep as affected by the substitution of the rumen degradable nitrogen fraction of sunflower meal with urea

	*Treatments					
***Parameters**	***T0***	***T15***	***T30***	***T45***	***T60***	****SEM***
Intake (g/day)						
Roughage DMI	1067	1008	1147	1066	1048	73.5
Roughage OMI	992	937	1067	991	974	62.5
Total DMI	1223	1260	1294	1209	1187	51.9
DOMI	687	698	718	703	667	58.9
NDF	841	789	885	858	838	55.9
NDF (g/kg BW)	14.6	13.8	15.2	14.6	14.3	0.90
Digestibility (%)						
DM	55	51	55	57	56	2.8
OM	59	55	58	62	61	2.6
NDF	60	57	59	65	63	2.2
Roughage NDF degradability						
a (%)	6.2	10.0	8.9	8.9	7.4	1.6
b (%)	76	57	58	58	70	8.4
c (%/hour)	2.0	2.0	1.5	1.8	1.7	0.04
ED (%)	35	33	31	33	33	0.7

Means within a row with similar alphabetically superscripts ($^{a, b, c}$) do not differ (P > 0.05).

*Treatments: T0 (100% SFM, 0% urea); T15 (85% SFM, 15% urea); T30 (70% SFM, 30% urea); T45 (55% SFM, 45% urea) and T60 (40% SFM, 60% urea).

**Parameters: DMI = Dry Matter Intake; OMI = Organic Matter Intake; DOMI = Digestible Organic Matter Intake; NDF = Neutral Detergent Fibre; BW = Body Weight; DMD = Dry Matter Digestibility; OMD = Organic Matter Digestibility; NDFD = Neutral Detergent Fibre Digestibility; a = soluble fraction; b = potential degradable fraction; c = rate of degradation; ED = Effective Degradability.

***SEM = Standard error of mean

Roughage intake and digestibility did not differ among treatments (P > 0.05; Table 2.3). This observation is in contrast with studies conducted by Köster *et al.* (1997), observing that incremental substitutions of RDN with NPN sources decreased feed intake and digestibility in cattle consuming low-quality prairie (tropical) hay. It is acknowledged that supplemental starch decreases roughage intake and digestibility of low-quality roughages (Caton and Dhuyvetter, 1997), probably through a reduction of rumen pH (Mould and Ørskov, 1983). In a study reported by Köster *et al.* (1997), incremental quantities of starch were added to the supplements as the levels of urea were increased to keep the supplements iso-energetic. As such, the decreased forage intakes and digestibility values observed by Köster *et al.* (1997) could have been due to the incremental starch supplementation as urea substituted true protein as RDN source. While starch was added in the present study in a similar manner compared to the study of Köster *et al.* (1997), the level of N intake in all treatments were at maintenance requirements of the sheep (11.52 g N/day; NRC 2007). None of the sheep during each experimental treatment therefore was protein deficient. Caton and Dhuyvetter (1997) in a review suggested that the negative effects of energy supplementation on roughage intake and/or digestibility is protein dependant. As such, roughage fed ruminants could "tolerate" higher levels of starch intake if low-quality roughages are supplemented with N supplements compared to non-supplemented treatments. In the present study, the highest daily starch supplementation (T60 treatment) was 50.3 g (DM basis; Table 2.2), representing less than 5% of the daily DMI (Table 2.3). This concentration is below the 20% level considered detrimental to roughage intake in ruminants fed roughages supplemented with protein to meet maintenance requirements (Gomes *et al.*, 1994; Henning *et al.*, 1980). In addition, starch was supplemented twice daily, together with urea, in equal proportions into the rumen. This would have decreased the potential negative effects of starch on the rumen milieu, which was then observed as the rumen pH (Table 2.5) among all periods and treatments was above the 6.2 level associated with optimal cellulolytic fermentation (Mould and Ørskov, 1983). Therefore, roughage intake and digestibility were similar among treatments (Table 2.3).

Köster *et al.* (1996) suggested that DMI is maximised when NDF intake is 12.5 g NDF/kg BW/day in cattle and that intake responses to N supplementation could only be expected if intake of low-quality roughages is below this value. Ferrell *et al.* (1999) and Bohnert *et al.* (2002) observed slightly higher corresponding values (13.0 and 14.8 g NDF/kg BW/day, respectively) in lambs consuming low-quality roughages and supplemented with

protein sources. In this study, NDF intake did not differ among treatments and ranged between 13.8 g NDF/kg BW/day in the T15 treatment to 15.2 g NDF/kg BW/day in the T30 treatment (Table 2.2). These NDF intakes are therefore suggestive that N supplementation was sufficient to maximise roughage intake among treatments and that additional N supplementation probably would not have resulted in an increased roughage intake.

The effective degradability (ED) of the hay did not differ ($P > 0.05$) among treatments (Table 2.3) and were similar to observations made by Flachowsky and Tiroke (1993) on Merino rams consuming wheat straw. The low ED of the hay in the present study (Table 2.3) could be attributed to the low rates of degradability (c value, ranging between 1.5% and 2.0% among treatments) and low rapid soluble fractions (a value, ranging between 6.2% and 10% among treatments) observed in the sheep.

Detmann *et al.* (2009) observed a linear increase in ED of low-quality prairie grass in cattle as RAN increased from 5 to 8 mg/dL rumen fluid where after it plateaued. The authors concluded that fibre degradation in tropical grasses could be considered a second order process in which it is not only substrate characteristics which determine fibre degradation, but also enzymatic activity from ruminal microorganisms. In the present study, the ED of the roughages did not differ among treatments (Table 2.3). The mean RAN concentration observed in the study was 8.27 mg/dL, with the only differences ($P < 0.05$) observed between T60 (9.35 mg/dL) and T30 (7.41 mg/dL; Table 2.5). Therefore, it is doubtful whether higher levels of N supplementation would have resulted in an increase in ED. Based on this observation, as well as the relative high NDF intake (expressed per kg BW) among treatments, it can be argued that ED of the hay in the present study was maximised or close to maximised ED.

Table 2.4 shows N intake and apparent N balance of the sheep as affected by the treatments.

Table 2.4 Nitrogen intake and balance of sheep consuming *Eragrostis curvula* hay as affected by the substitution of the rumen degradable nitrogen fraction of sunflower meal with urea

	*Treatments					
**Parameters	T0	T15	T30	T45	T60	***SEM
Intake (g N/day)						
Hay N intake	4.59	4.34	4.94	4.62	4.57	0.32
Sunflower meal N intake	8.69^{a}	7.39^{b}	6.08^{c}	4.78^{d}	3.47^{e}	–
Urea N intake	0^{e}	1.10^{d}	2.21^{c}	3.30^{b}	4.40^{a}	–
Total RDN intake	11.9	11.7	12.3	12.0	11.8	0.32
Total N intake	13.3	12.8	12.7	12.7	12.4	0.32
N excretion (g N/day)						
Faecal N	5.97	5.94	5.95	5.18	5.10	0.39
Urinary N	0.46	0.85	0.59	0.40	0.89	0.19
Apparent N balance (g/day)	6.87	6.06	6.14	7.10	6.42	0.39
Apparent N digestibility (%)	47^{b}	48^{b}	56^{a}	52^{ab}	51^{ab}	2.01

Means within a row with similar alphabetically superscripts ($^{a, b, c, d, e}$) do not differ significantly (P > 0.05).

*Treatments: T0 (100% SFM, 0% urea); T15 (85% SFM, 15% urea); T30 (70% SFM, 30% urea); T45 (55% SFM, 45% urea) and T60 (40% SFM, 60% urea).

**Parameters: Hay N intake = Hay N % * hay intake (g/day); Sunflower meal N intake = sunflower meal N % * Sunflower meal intake (g/day); Urea N intake = urea intake (g/day) x 46% (McDonald *et al.*, 2011); Total N intake = Hay N intake + Sunflower meal N intake + urea N intake. Total RDN intake = Total N intake, assuming that RDN fraction of the SFM was 85% of its total N (NRC, 2007); Apparent N balance = Total N intake – (faecal N + urinary N excretion); Apparent N digestibility (%) = apparent N balance/Total N intake

***SEM = Standard error of mean

The supplements were formulated to substitute the RDN fractions of the SFM with urea. Thus, RDN intake did not differ among treatments (P > 0.05; Table 2.4) as no

differences in roughage intake were observed among treatments (P > 0.05; Table 2.3). Chandrasekharaiah *et al.* (2012) fed finger millet straw (4.34% CP; 79.2 % NDF, DM basis) to sheep, suggested that 16 g supplemental RDN/kg DOMI might be sufficient for MNS and optimum digestibility in sheep fed straw diets. Supplemental RDN intake in the present study was 7.3 g RDN/sheep/day (Table 2.4, Total RDN intake – Hay N intake). Using the mean DOMI (0.677 kg) of sheep among treatments (Table 2.3, data not shown), RDN supplemented/kg DOMI equals 10.83 g RDN/kg DOMI, which was less than the recommendations suggested by Chandrasekharaiah *et al.* (2012). While the N balances of the sheep did not differ across treatments (P > 0.05), the mean total apparent N balance (6.51 g N/sheep/day) suggested that N intake of the sheep could have been insufficient to meet its maintenance requirements of 9.6 g N (NRC, 2007). As the sheep in this present study were supplemented according to the NRC (2007) recommendations, this observation highlights the potential difference in N supplementation requirements between ruminants fed low-quality temperate and tropical roughages.

Table 2.5 shows the rumen pH between treatments and times of sheep fed low-quality *E. curvula* hay.

Table 2.5 Rumen pH among treatments and time-periods of sheep fed *Eragrostis curvula* hay as affected by the substitution of the rumen degradable nitrogen fraction of sunflower meal by urea

	*Treatments						
Parameters	**T0**	**T15**	**T30**	**T45**	**T60**	**Mean**	****SEM**
Mean rumen pH	6.47	6.46	6.53	6.49	6.48	6.48	0.03
Rumen pH/ Periods							
00h00	6.34^{2}	6.34	6.46^{23}	6.40^{2}	6.48^{12}	6.40^{45}	0.04
03h00	6.38^{12}	6.34	6.44^{23}	6.56^{2}	6.32^{2}	6.37^{5}	0.05
06h00	6.52^{12}	6.44	6.54^{123}	6.64^{12}	6.40^{2}	6.48^{34}	0.03
09h00	6.52^{12}	6.56	6.62^{12}	6.52^{12}	6.52^{12}	6.56^{12}	0.04
12h00	6.58^{1}	6.56	6.74^{1}	6.64^{1}	6.64^{1}	6.63^{1}	0.03
15h00	6.46^{12}	6.46	6.36^{3}	6.52^{12}	6.50^{12}	6.44^{345}	0.04
18h00	6.56^{12}	6.46	6.54^{123}	6.44^{12}	6.48^{12}	6.48^{234}	0.04
21h00	6.34^{12}	6.48	6.52^{123}	6.52^{12}	6.46^{12}	6.51^{23}	0.04

Means within a column with similar numerical superscripts ($^{1, 2, 3, 4, 5}$) do not differ (P > 0.05).

*T0 (100% SFM, 0% urea); T15 (85% SFM, 15% urea); T30 (70% SFM, 30% urea); T45 (55% SFM, 45% urea) and T60 (40% SFM, 60% urea).

**SEM = Standard error of mean

Rumen pH did not differ (P > 0.05) among treatments, however, a time effect was observed with the rumen pH generally peaking between 09h00 and 12h00. In general, the lowest rumen pH was observed between midnight (00h00) and 03h00 in the morning. Results from *in sacco* degradation studies conducted by Sinclair *et al.* (1993) suggested that starch disappearance from ground maize in the rumen could take up to five hours to complete compared to the instantaneous disappearance of urea. While the rate of SFM degradation and pure starch was not determined in this study, the decrease in rumen pH during the night hours could be indicative of an increased starch fermentation during those hours. In addition, rumen fill was possibly at a low during those night hours, as most of the feeding would have taken place during the daytime, resulting in less chewing and saliva production that could have buffered rumen pH. It must be noted though that the observed differences in rumen pH among periods were biologically insignificant as the values, ranging between 6.32 and 6.74, were well within the range for optimal cellulolytic bacteria fermentation (Mould and Ørskov, 1983). Rumen conditions across all treatments therefore were optimal for fibre degradation (Mould and Ørskov, 1983) and probably were due to the supplementation pattern followed where supplementation occurred twice daily at set intervals (08h00 and 16h00).

Table 2.6 shows VFA production and profile, RAN, MNS and MNS efficiency of sheep as affected by the substitution of the RDN fraction of SFM by urea.

Table 2.6 Rumen fermentation parameters and microbial nitrogen supply of sheep fed *Eragrostis curvula* hay as affected by the substitution of the rumen degradable nitrogen fraction of sunflower meal by urea

	*Treatments					
**Parameters	T0	T15	T30	T45	T60	***SEM
Total volatile fatty acid (VFA) concentration (mmol/dL)	78.5	76.0	75.3	72.1	73.7	2.39
VFA percentages (% of total VFA)						
Acetate	79.4	77.9	79.7	79.0	79.8	0.68
Propionate	14.5^{ab}	15.3^{a}	14.2^{ab}	14.5^{ab}	14.0^{b}	0.41
Isobutyrate	0.63^{a}	0.62^{a}	0.53^{ab}	0.55^{ab}	0.46^{b}	0.04
Butyrate	4.71^{b}	5.73^{a}	5.15^{ab}	5.51^{ab}	5.36^{ab}	0.28
Valerate	0.57^{a}	0.51^{ab}	0.42^{ab}	0.47^{ab}	0.39^{b}	0.04
Acetate: Propionate (A: P)	5.47^{ab}	5.08^{b}	5.63^{ab}	5.46^{ab}	5.69^{a}	0.11
Mean rumen RAN (mg/dL)	7.84^{ab}	8.16^{ab}	7.41^{b}	8.60^{ab}	9.35^{a}	0.56
MNS (g N/day)	14.18	16.32	14.45	14.45	15.56	1.50
g MNS/kg DOMI	20.64	23.38	20.13	20.55	23.33	2.47

Means within a row with similar alphabetically superscripts ($^{a, b, c}$) do not differ (P > 0.05).

*T0 (100% SFM, 0% urea); T15 (85% SFM, 15% urea); T30 (70% SFM, 30% urea); T45 (55% SFM, 45% urea) and T60 (40% SFM, 60% urea).

Parameters: MNS = Microbial Nitrogen Synthesis ** g MNS/kg DOMI = g MNS per kg digestible organic matter intake.

***SEM = Standard error of mean

The VFA profile is typical of a high roughage diet with the acetate to propionate (A: P) ratios ranging between 5.08 and 5.69 (Table 2.6). Total VFA concentrations did not differ ($P > 0.05$) among treatments. The total VFA concentrations, varying between 72.1 and 78.5 mmol/dL between treatments were similar to concentrations observed by Fondevila *et al.* (1994) in sheep fed barley straw. While individual VFA composition differences were observed between treatments ($P < 0.05$; Table 2.6); those differences were inconsistent and biologically not significant. Thus, the observed differences ($P < 0.05$) in the ratio of acetate: propionate (A: P) among treatments were biologically not significant.

The NDF intake of the sheep among all treatments suggested that roughage intake probably was maximised in the present study (Bohnert *et al.,* 2002; Detmann *et al.,* 2009; Ferrell *et al.,* 1999). Results from various studies suggested that DM intake, MNS and ED in sheep (Kanjanapruthipong and Leng, 1998) and NDF intake, MNS and ED in cattle (Detmann *et al.,* 2009) consuming low-quality tropical grasses are affected by RAN with ED being maximised at 8 mg RAN/dL. However, higher RAN concentrations (15 to 20 mg RAN/dL) were necessary to maximise NDF intake in cattle (Detmann *et al.,* 2009) and DMI in sheep (Kanjanapruthipong and Leng, 1998) consuming low-quality tropical roughages. The RAN concentration observed in the present study ranged between 7.84 and 9.35 mg RAN/dL rumen fluid (Table 2.6). As such, it is doubtful whether higher RAN concentrations, obtained through higher N supplementation, would have resulted in higher roughage intake by the sheep in the present study.

Broderick and Merchen (1992) discussed various methods estimating MNS in the ruminant, including internal markers (diaminopimelic acid and individual purines), and external markers (^{15}N and ^{35}S). The authors stated that while MNS could be calculated from these markers if the digesta flow in the cannulated animals is known (or calculated using markers), these markers must account for bacterial as well as protozoal pools within both the fluid as well as the particular phase to be accurate. The authors also discussed calculation of MNS through PD as well as using the PD: creatinine ratio, assuming a constant excretion of creatinine in proportion of metabolic mass. The authors stated that no marker has proven completely satisfactory, and that yield estimates must be compared relatively *versus* absolute.

Microbial N synthesis in this study was calculated using the spot urine method as described by Chen *et al.* (1995), even though total daily urine excreted was collected. This method was preferred over the total collection method (Chen and Gomes, 1992) due to the

use of cannulated wethers in the trials (the chances of rumen fluid spilling into the urine pans during the opening and closing of the cannulas were high and would have distorted the PD and MNS results) as well as the dilution factor. In the total urine collection method, daily urine output was diluted to 4 000 mL with water. A 50-mL sample was then taken from the urine-water mix. Over 5 days, 250 mL of urine-water sample was collected from effectively 20 L of urine-water. During the analysis, a 10-mL subsample was taken from the 250-mL sample and analysed for PD. The chances were high that the 10-mL subsample was not uniformly mixed and that the proportions of urine and water in the subsample was not representative of the original daily samples. Chen *et al.* (1995) stated that within physiological boundaries (10 – 50 mmol PD: Creatinine/kg $BW^{0.75}$), the relationship between PD: Creatinine/kg $BW^{0.75}$ and corrected PD is 2.128* PD in sheep.

The MNS in the present study did not differ between treatments, ranging between 14.18 g MNS to 16.32 g MNS/sheep/day. Leng (1990) and Poppi *et al.* (1999) suggested that FME and non-structural carbohydrates (NSC) have a more pronounced effect on MNS compared to RDN. The NFC (non-fibre carbohydrates) fraction of the hay used in this study was 4.7%. The SCA (1990) suggested that the minimum NSC concentration value below which MNS efficiency reduced, is 90 g/kg DM. Even though the supplements were formulated to be iso-energetic (Table 2.3), the FME content of the treatments probably was different between treatments due to increasing quantities of starch supplemented in the supplements where urea replaced the RDN fraction of SFM. As such, it was expected that the additional supplementation of starch in the higher urea treatment treatments could have resulted in higher MNS values in those treatments (Gomes *et al.*, 1994). However, the highest starch intake (50.3 g starch DM/day) supplemented in the T60 treatment in this study corresponded to a daily starch intake just below 5% of total dietary intake (Table 2.2), which were significantly less than the starch levels supplied by Gomes *et al.* (1994) at 18.2 % DM. It can be concluded that FME did not differ to such an extent between treatments that it caused differences in MNS between treatments.

The efficiency of MNS in the present study, ranging between 20.13 g MNS/kg DOMI and 23.38 g MNS/kg DOMI between treatments, was higher compared to the MNS efficiencies (12.8 to 17.5 g MNS/kg DOMI) observed by Gomes *et al.* (1994). The MNS efficiency values observed in this study were well in the range (20.8 g MNS/kg DOMI) of MNS efficiencies for tropical grasses (SCA, 1990; AFRC, 1993; Detmann *et al.*, 2007) even though the quality of the hay was low. This observation is probably due to the

supplementation of the wethers as C4 grasses generally have low levels of MNS efficiency (SCA, 1990). While MNS values in the present study were lower than the maximal values observed by Gomes *et al.* (1994), the relative high MNS efficiency values calculated was probably due to the low OM digestibility of the diet, ranging between 55% and 62% (Table 2.3). The observation emphasises the general wide range (up to 4 times) of MNS efficiencies observed between studies (SCA, 1990; Chen and Gomes, 1992) as MNS efficiency is not only influenced by factors influencing MNS production *per se*, but also factors influencing OM intake and digestibility.

2.8 Conclusion

It is concluded that urea could substitute up to 60% of the RDN supplied by SFM without affecting intake, digestibility or MNS in sheep receiving low-quality *E. curvula* hay. While ED and roughage intake probably was maximised in this study, total N balance suggests that RDN supplementation probably was not optimal in this study.

Chapter 3 Supplementation of different levels of urea and starch to sheep fed low-quality *Eragrostis curvula* hay

3.1 Abstract

The primary objective of the study was to determine whether the level of supplemental urea would have any effect on roughage intake, roughage degradability and microbial nitrogen synthesis (MNS) in the tropical roughage fed ruminant. Four cannulated Döhne Merino sheep were fed low-quality *Eragrostis curvula* hay *ad libitum* and supplemented with different quantities of starch and urea. The sheep were supplemented twice daily with half of the total daily supplements supplemented during the morning (08h00) and afternoon (16h00) sessions. In total, three different starch and four different urea levels were used in three Latin square design trials. The levels of urea in the supplements used were as follows: 10.4 g urea/sheep/day (LU); 18.4 g urea/sheep/day (MU); 26.4 g urea/sheep/day (HU) and 32.4 g urea/sheep/day (EHU) where LU, MU, HU and EHU were used as acronyms for low, medium, high, and extra high urea respectively. The three starch levels used were as follows: LS (200 g starch/sheep/day), MS (240 g starch/sheep/day) and HS (280 g starch/sheep/day) where LS, MS and HS were used as acronyms for low, medium, and high starch respectively.

Urea supplementation did not affect roughage intake or NDF digestibility across all starch levels. However, nitrogen intake (NI) increased with increasing levels of urea as expected. The level of urea supplemented affected total N balance with the N balance of the sheep increasing from less than 2 g N/sheep/day in the LU treatment to over 11 g N/sheep/day in the EHU treatment across all starch levels. In addition, efficiency of N use, calculated as N retained relative to available N intake, increased from around 0.25 in the LU treatments to 0.60 in the HU treatments across all starch levels. Efficiency of N use did not differ between HU and EHU treatments across all starch levels. However, rumen ammonia N (RAN) differed with RAN increasing from around 7 mg RAN/dL rumen fluid to above 20 mg RAN/dL rumen fluid as urea supplementation was increased across all starch levels. Neither microbial N synthesis (MNS) nor efficiency of MNS differed between urea treatments across starch levels. The ratio of MNS: available N intake decreased from levels above two to less than one as the level of urea supplementation was increased from 10.4 g urea/sheep/day (LU treatment) to 32.4 g urea/sheep/day (EHU treatment). As ratios higher than one could be

indicative of a possible N deficiency in the rumen (Detmann *et al.*, 2014), the results suggested that sheep supplemented with 32.4 g urea/sheep/day (EHU treatment) and possibly the HU treatment (26.4 g urea/sheep/day) were the only urea treatments not deficient in N.

3.2 Introduction

Few supplemental studies exist where the effects of N and energy supplementation to sheep consuming low-quality tropical roughages were studied. Therefore, information on the ideal quantities of both N and energetic compounds necessary to optimise roughage intake, digestibility and MNS in the tropical roughage fed ruminant is limiting. It is well documented that RDN is the first and main nutrient deficient in the tropical roughage fed ruminant (Leng, 1990, 1995; Kanjanapruthipong and Leng, 1998; Detmann *et al.*, 2009). As such, N supplementation often increased roughage intake and digestibility in the tropical roughage fed ruminant (Leng, 1990, 1995; Detmann *et al.*, 2009) while energy supplementation generally has the opposite effect, reducing roughage intake and digestibility (Caton and Dhuyvetter, 1997). Reasons included the reduction of rumen pH which favours amylolytic bacteria (Ørskov *et al.*, 1983), a carbohydrate effect (Mould and Ørskov, 1983) and energy toxicity in the rumen (Russell, 1989). However, Heldt *et al.* (1999) observed that energy supplementation to the roughage fed ruminant would only reduce roughage intake and/or roughage digestibility when the experimental diets contained insufficient N.

Microbial N synthesis and MNS efficiency generally are increased as the level of RDN supplementation is increased (Detmann *et al.*, 2009; Kanjanapruthipong and Leng, 1998). However, in contrast to roughage intake and digestibility, FME supplementation generally increases MNS (Leng, 1990, 1995) and MNS efficiency (Gomes *et al.*, 1994).

Despite this knowledge, information quantifying the RDN and energy requirements for wethers fed low-quality tropical forages is lacking. The aim of this study is to study various levels of urea supplementation within fixed starch supplementations on roughage intake, digestibility and MNS efficiency.

3.3 Hypotheses

The aim of the study was to investigate the effects of supplemental N (urea) and fermentable energy (starch) supplementations in sheep in terms of roughage intake, digestibility, MNS and RAN. The following hypotheses were tested.

1. Non-protein N (urea) supplementation affects roughage intake, digestibility, RAN, MNS and MNS efficiency of sheep fed low-quality *E. curvula* hay and supplemented with urea and starch, within each starch level.

2. Non-protein N (urea) supplementation affects N balance of sheep fed low-quality *E. curvula* hay and supplemented with urea and starch, within each starch level.

3. Rumen ammonia N is correlated with roughage intake, digestibility, MNS and MNS efficiency of sheep fed low-quality *E. curvula* hay and supplemented with urea and starch.

3.4 Materials and Methods

Five rumen cannulated Merino sheep (58.1 ± 1.1 kg BW) were fed *ad lib* a low-quality [2.7% CP; 87.1% NDF; 93% OM, 49.6% acid detergent fibre (ADF), dry matter (DM) basis] *Eragrostis curvula* hay. If it is assumed that the *E. curvula* hay contained 1.5% ether extract (EE) (NRC, 2007), the calculated non-fibre carbohydrate fraction of the *E. curvula* was 4.7% (calculated as DM – ash – EE – CP – NDF; Fox *et al.*, 2004, Table 3.1).

Table 3.1 Chemical composition of *Eragrostis curvula* hay fed to wethers and supplemented with urea and starch

Dry Matter (g/kg)	930
Ash (g/kg DM)	72
Nitrogen (g/kg DM)	4.2
Neutral detergent fibre (g/kg DM)	841
Acid detergent fibre (g/kg DM)	496
Acid detergent insoluble Nitrogen (% of CP)	45 %
***Non-Fibre carbohydrate (g/kg DM)**	47

*Non-Fibre carbohydrate was calculated as DM – ash – EE – CP – NDF (Fox *et al.*, 2004), assuming that the hay contained 1.5% EE (NRC, 2007).

An earlier trial was conducted to determine the level and quantities of supplements to be given to the wethers. In that trial, forage intake of 50 kg sheep fed a similar quality *E. curvula* hay (3.0% CP) was 1100 g DM/day. Organic matter (OM) of the hay was 93% with

OM digestibility at 50%. Based on that dataset, digestible organic matter per kg DM (DOMD) was calculated using the following equation (AFRC, 1993):

Hay ME = 0.016 * DOMD **Eq. 3.1**

From this equation, hay ME was calculated to be 7.4 MJ ME/kg DM

[ME = 0.016*1000*0.93*0.50]

Fermentable energy (FME) intake was assumed to be 90% of hay ME intake (Robinson, personal communication).

Maintenance metabolisable protein (MP) requirements for 50 kg wethers is 62 g MP (NRC, 2007). Microbial protein can provide the maintenance MP requirements. However, microbial protein only contains 75% true protein (25% nucleic acids) and is 85% digestible (AFRC, 1993). Therefore, effective rumen degradable protein (ERDP) was calculated at 97 g RDP/day. At an expected forage intake of 1100 g/day, CP intake from the hay was 33 g/day, requiring another 64 g RDP/day or 10.2 g RDN/day to meet maintenance requirements.

Using the AFRC (1993) recommendations of 9 g RDP/MJ FME at maintenance, FME intake per day was calculated at 10.8 MJ FME/day. With an expected forage intake of 1100 g/day, FME intake of the hay equated to 7.4 MJ FME/day requiring another 3.4 MJ FME/day to meet maintenance requirements.

The calculated N supplementation necessary to meet the maintenance requirements of 50 kg sheep in the trial were met by the HU treatment (AFRC, 1993), based on the expected roughage intake (1100 g/day) of the sheep and chemical composition of the roughage. This calculation was conducted as follows:

Urea requirement (g/day) = RDN deficient (g/day) / (0.8 * 0.466) **Eq. 3.2**

where RDN = Rumen degradable N and the factors 0.8 and 0.466 representing the degradation of NPN in the rumen and the fraction of N in urea, respectively.

The levels of urea in the supplements were as follows: 10.4 g urea/sheep/day (LU); 18.4 g urea/sheep/day (MU); 26.4 g urea/sheep/day (HU) and 32.4 g urea/sheep/day (EHU) where the acronyms LU, MU, HU and EHU were used to describe low urea, medium urea, high urea, and extra high urea, respectively. The MU and EHU treatments deviated 20% from

the HU treatment with urea supplementation of the LU treatment at 10.4 g urea/sheep/day, 20% lower than the MU treatment.

The three starch levels used were as follows: LS treatment (200 g starch/sheep/day), MS (240 g starch/sheep/day) and HS (280 g starch/sheep/day) where LS, MS and HS were used as acronyms for low, medium, and high starch, respectively. The starch levels were chosen using the recommendations of the AFRC (1993) where the MS treatment (240 g starch/sheep/day) would have met the maintenance requirements of sheep weighing 50 kg as follows:

Starch requirement (g DM/day) = FME required * 1000/FME of starch Eq. 3.3

A mineral premix (24.0 g mineral mix/sheep/day) and additional sulphur (1.8 g S/sheep/day) was added to each supplement to fulfil the maintenance requirements of sheep weighing 50 kg (NRC, 2007). The mineral composition of the premix was as follows: Macro minerals (g/kg DM): K (230); Ca (83); P (63); Mg (46); Na (29); Cl (26); Trace minerals (mg/kg DM): Co (4.58); Cu (166.53); I (33.31); Fe (333.06); Mn (726.48); Se (1.67) and Zn (1248.96).

The supplements, containing urea, starch and a mineral supplement were supplemented in equal proportions, twice daily at 08h00 and 16h00, intra-ruminally, via rumen cannulae.

The sheep were adapted to the supplements for a period of 7 days, which was similar to adaptation periods used by Wickersham *et al.* (2008, 2009) for steers fed low-quality tropical prairie grass (tropical grass) and supplemented with various levels of N compounds. On the last day of the adaptation period, the sheep were transferred to metabolic cages where faecal harnesses were fitted. During the experimental period, feed intake and faecal and urine output were recorded daily. Feed, orts and faeces were sampled and pooled over the experimental period within each treatment to estimate total tract digestibility. Urine was collected from urine pans and transferred into urine bottles containing 5 mL sulphuric acid (H_2SO_4; 50% v:v) for preservation, and adjusted to a final pH below 3 with H_2SO_4, if required. Daily urine volumes were measured and diluted to 4000 mL. From this diluted volume, 50 mL sub-samples were taken, pooled over the collection period and frozen at –20^{o}C for purine derivative (PD) analysis. Creatinine was determined from each urine sample to determine the corrected PD (Chen *et al.*, 1995), assuming that the daily excretion of creatinine as a

proportion of muscle mass from the wethers was constant (Broderick and Merchen, 1992; Chen *et al.*, 1995). The corrected PD was used in the estimation of MNS (Chen and Gomes, 1992).

Rumen fluid was collected at 12-hour intervals for 4 days from four predetermined locations within the rumen (top left and centre and bottom left and centre). After every day, there was a 3-hour shift in sample collection time to obtain samples at 03h00, 06h00, 09h00, 12h00, 15h00, 18h00, 21h00 and 24h00. Rumen fluid pH was measured immediately after each collection period. Five mL sulphuric acid (H_2SO_4; 10% v: v) was added to 30 mL rumen samples for RAN (Broderick and Kang, 1980) concentrations. The samples were frozen at –20^{o}C until analysis commended.

Feed and faecal samples were ground using a Wiley mill to pass a 1 mm screen. Hay, orts and faecal samples were dried for 24 hours at 105^{o}C in a forced air oven to determine DM and then combusted for 8 hours at 450^{o}C in a muffle furnace for OM determination (AOAC, 2000). The N content of hay, faeces and urine was determined by the Kjehldahl method (AOAC, 2000). All hay, orts and faecal samples were analysed for NDF and ADF with the ANKOM-Fibre Analyzer (ANKOM Technology, Fairport, NY, USA). *In vitro* organic matter digestibility (IVOMD) was determined using the Tilley and Terry method (Tilley and Terry, 1963) as modified by Engels and Van der Merwe (1967) for low-quality roughages under South African conditions. In this modification, N in the form of urea was added to each test tube (20 mg urea per test tube), simulating N recycling in ruminants consuming low-quality roughages feed intake and faecal and urine outputs were recorded daily, sampled, and pooled over a 5-day digestibility period to estimate total tract digestibility.

The trial was conducted in three blocks with starch the differential blocks. Within each block, the sheep received four different urea treatments in a 4 x 4 Latin square (four animals, four treatments) format. As such, three Latin square trials were conducted in this experiment, with the level of starch supplemented to the sheep, differing between each trial. Statistical analysis was conducted using ANOVA from SAS (Statistical Analysis System, 2015). The model fitted per Latin square was:

$$yijk = \mu + rj + ck + ti + \varepsilon ijk$$

where yijk is the response for the ijkth unit, μ is the overall mean, rj (j = 1...n) represents the row effects (number of periods), ck (k = 1...n) the column effects (number of different animals), ti (i = 1...n) the main treatment effects (number of urea levels within each starch block), and εijk is the error variation for the ijkth unit.

Treatment differences were detected using the F test (Samuels, 1989) and was declared at $P < 0.05$. Comparisons were made between different urea treatments within each starch supplementation level.

3.5 Results and Discussion

Table 3.2 shows the mean roughage and N intakes and roughage and DM digestibility values of the four urea treatments within Starch 1 (starch supplemented at 200 g/wether/day or low starch; LS). Tables 3.3 and 3.4 show the same parameters as Table 3.2, but for Starch 2 (starch supplemented at 240 g/wether/day or medium starch; MS) and Starch 3 (starch supplemented at 280 g/wether/day or high starch; HS), respectively. In all three tables, total N intake (supplements included), N intake from the hay and available N is shown where available N was calculated as total N intake (hay and supplements) minus ADIN intake of the hay.

Table 3.2 Roughage, dry matter intake and nitrogen intake and feed digestibility of wethers fed low-quality *Eragrostis curvula* hay and supplemented with 200 g starch/day as affected by urea treatment

	*Treatment				
**Parameters	Urea 1	Urea 2	Urea 3	Urea 4	***SEM
RDMI	841	810	780	864	36
DMI	1035	1008	988	1078	38
DOMI	545^{b}	563^{ab}	567^{ab}	611^{a}	17
NDFI	612	589	574	630	37
NDFI BW	1.14	1.10	1.10	1.17	0.39
DOMI BW	1.02^{b}	1.05^{ab}	1.09^{a}	1.14^{a}	0.03
NI (Hay)	3.97	3.84	3.63	4.11	0.30
Total NI	8.75^{d}	12.30^{c}	15.78^{b}	19.02^{a}	0.30
Available N	6.77^{d}	10.38^{c}	13.97^{b}	16.96^{a}	0.30
NI (% DM)	0.85^{d}	1.23^{c}	1.63^{b}	1.78^{a}	0.05
DMD	52.0	56.2	58.7	58.2	2.41
NDFD	42.0	46.5	51.1	50.4	3.39
OMD	55.0	58.7	60.7	60.0	2.41

Means within a row with similar alphabetically superscripts ($^{a, b, c, d}$) do not differ (P > 0.05).

*Treatments: Urea 1 = 10.4 g urea/day; Urea 2 = 18.4 g urea/day; Urea 3 = 26.4 g urea/day; Urea 4 = 32.4 g urea/day

**Parameters: RDMI = Roughage dry matter intake (g/day); DMI = Dry matter intake (g/day); DOMI = Digestible organic matter intake (g/day); NDFI = Neutral detergent fibre intake (g/day); NDFI BW = NDFI expressed as percentage of BW; DOMI BW = DOMI expressed as percentage of BW; NI (Hay) = Nitrogen Intake = N % (Hay) * Hay intake (g/day); Total NI = Total N intake = NI (hay) + N intake from urea (g/day); NI (available) = N Intake – ADIN intake (Hay); NI (% of DMI) = Total NI (g) as percentage of DMI; DMD = Dry matter digestibility (%); NDFD = Neutral detergent fibre digestibility (%); OMD = Organic Matter digestibility (%)

***SEM = Standard error of mean

Table 3.3 Roughage, dry matter intake and nitrogen intake and feed digestibility of wethers fed low-quality *Eragrostis curvula* hay and supplemented with 240 g starch/day as affected by urea treatment

	*Treatment				
**Parameters	Urea 1	Urea 2	Urea 3	Urea 4	***SEM
RDMI	1082	1024	907	930	51
DMI	1347	1297	1198	1216	0.05
DOMI	781^{a}	719^{ab}	680^{ab}	665^{b}	30
NDFI	760^{a}	721^{a}	640^{b}	655^{ab}	35
NDFI BW	1.39	1.35	1.23	1.21	0.06
DOMI BW	1.43^{a}	1.35^{ab}	1.30^{ab}	1.23^{b}	0.56
NI (Hay)	5.02^{a}	4.86^{b}	4.22^{b}	4.64^{b}	0.31
Total NI	9.81^{d}	13.33^{c}	16.37^{b}	19.36^{a}	0.31
Available NI	7.30^{d}	10.90^{c}	14.26^{b}	17.14^{a}	0.31
NI (% DM)	0.74^{d}	1.04^{c}	1.38^{b}	1.60^{a}	0.05
DMD	56.8	55.1	57.7	54.9	2.27
NDFD	44.8	41.7	43.9	39.0	2.97
OMD	60.0	58.2	60.3	57.7	2.28

Means within a row with similar alphabetically superscripts ($^{a, b, c, d}$) do not differ (P > 0.05).

*Treatments: Urea 1 = 10.4 g urea/day; Urea 2 = 18.4 g urea/day; Urea 3 = 26.4 g urea/day; Urea 4 = 32.4 g urea/day

**Parameters: RDMI = Roughage dry matter intake (g/day); DMI = Dry matter intake (g/day); DOMI = Digestible organic matter intake (g/day); NDFI = Neutral detergent fibre intake (g/day); NDFI BW = NDFI expressed as percentage of BW; DOMI BW = DOMI expressed as percentage of BW; NI (Hay) = Nitrogen Intake = N % (Hay) * Hay intake (g/day); Total NI = Total N intake = NI (hay) + N intake from urea (g/day); NI (available) = N Intake – ADIN intake (Hay); NI (% of DMI) = Total NI (g) as percentage of DMI; DMD = Dry matter digestibility (%); NDFD = Neutral detergent fibre digestibility (%); OMD = Organic Matter digestibility (%)

***SEM = Standard error of mean

Table 3.4 Roughage, dry matter intake and nitrogen intake and feed digestibility of wethers fed low-quality *Eragrostis curvula* hay and supplemented with 280 g starch/day as affected by urea treatment

	*Treatment				
**Parameter	Urea 1	Urea 2	Urea 3	Urea 4	***SEM
RDMI	814	975	1004	908	68
DMI	1042	1212	1248	1159	72
DOMI	582	546	579	536	22
NDFI	574	688	709	638	37
NDFI BW	1.12	1.33	1.39	1.23	0.08
DOMI BW	1.19	1.28	1.41	1.19	0.08
NI (Hay)	3.82^{b}	4.69^{a}	4.72^{a}	4.37^{a}	0.31
Total NI	8.61^{d}	13.15^{c}	16.86^{b}	19.26^{a}	0.31
Available N	6.69^{d}	10.82^{c}	14.51^{c}	17.09^{a}	0.31
NI (% DM)	0.83^{d}	1.09^{c}	1.38^{b}	1.68^{a}	0.05
DMD	57.48	54.42	58.37	54.25	2.42
NDFD	45.27	44.32	47.39	42.28	3.62
OMD	60.2	57.2	60.9	56.7	2.32

Means within a row with similar alphabetically superscripts ($^{a, b, c, d}$) do not differ (P > 0.05).

*Treatments: Urea 1 = 10.4 g urea/day; Urea 2 = 18.4 g urea/day; Urea 3 = 26.4 g urea/day; Urea 4 = 32.4 g urea/day

**Parameters: RDMI = Roughage dry matter intake (g/day); DMI = Dry matter intake (g/day); DOMI = Digestible organic matter intake (g/day); NDFI = Neutral detergent fibre intake (g/day); NDFI BW = NDFI expressed as percentage of BW; DOMI BW = DOMI expressed as percentage of BW; NI (Hay) = Nitrogen Intake = N % (Hay) * Hay intake (g/day); Total NI = Total N intake = NI (hay) + N intake from urea (g/day); NI (available) = N Intake – ADIN intake (Hay); NI (% of DMI) = Total NI (g) as percentage of DMI; DMD = Dry matter digestibility (%); NDFD = Neutral detergent fibre digestibility (%); OMD = Organic Matter digestibility (%)

***SEM = Standard error of mean

Dry matter intake (DMI) and NDF intake (NDFI) did not differ ($P > 0.05$) between treatments within each starch treatment (Tables 3.2 – 3.4). In contrast, digestible organic matter intake (DOMI) differs ($P < 0.05$) between urea treatments in Starch 1 and 2 (Tables 3.2 and 3.3). In Starch 1, lower digestible organic matter intakes (DOMI) were observed in Urea 1 compared to Urea 4 (Table 3.2). In Starch 2, Urea 3 was lower ($P < 0.05$) compared to Urea 1 and 2 (Table 3.3). Digestible organic matter intake did not differ ($P > 0.05$) in Starch 3 (Table 3.3).

Nitrogen intake from the hay (g N/sheep/day), calculated as N concentration of the hay (% N) * roughage DM intake (g DM/sheep/day) did not differ ($P > 0.05$) between urea treatments within each starch period due to similar forage intakes (Tables 3.2 – 3.4). However, total NI and N, expressed as a percentage of DM, increased ($P < 0.05$) within each starch treatment as the quantity of urea supplemented was increased (Tables 3.2 – 3.4). These increases were expected as forage intake and NI from the hay were similar between urea treatments within each starch period as the quantity of urea supplemented was increased.

Dry matter digestibility (DMD), neutral detergent fibre digestibility (NDFD) and organic matter digestibility (OMD) did not differ ($P > 0.05$) among urea treatments within each starch period (Tables 3.2 – 3.4).

Various dietary factors affect roughage intake in ruminants consuming low-quality tropical roughages. In studies conducted by Detmann *et al.* (2009) on cattle and Kanjanapruthipong and Leng (1998) on sheep, RDN supplementation increased roughage intake in ruminants consuming low-quality tropical roughages. In the study of Detmann *et al.* (2009), roughage intake was maximised as RDN supplementation was increased up to 11% CP or 1.76% N of the final diet (DM). Similar results were obtained in the study of Kanjanapruthipong and Leng (1998) where roughage intake was maximised in sheep (45 kg) where urea was supplemented at 15 g urea/sheep/day, corresponding to 10.8% CP or 1.73% N (for a detailed discussion on the calculation, consult the literature review earlier in this thesis). In the present study, the least quantity of urea supplemented per sheep per day was 10.4 g for the LU treatment, increasing up to 32.4 g/day for the EHU treatment. Using the intake data of the sheep as basis (Tables 3.2 – 3.4), N intake (DM basis) in the present study as a percentage of total DM intake ranged between 0.84% N to 1.78% N in the low starch (LS) treatment (Starch 1), 0.73% N – 1.60% N in the medium starch (MS) treatment (Starch 2) and 0.845% N – 1.68% N in the high starch (HS) treatment (Starch 3; Tables 3.2 – 3.4).

Based on the studies of Kanjanapruthipong and Leng (1998) and Detmann *et al.* (2009), the possibility therefore existed that roughage intake might not have been maximised at the lower urea treatments within each starch period as the final dietary N concentrations were well below the suggested concentrations of 1.73% N maximising forage intake. However, roughage and NDF intake were not affected by urea supplementation in the present study (Tables 3.2 – 3.4; $P > 0.05$). In addition, NDF intake, expressed as a percentage of BW did not differ between urea treatments in any of the starch levels and ranged between 1.10% – 1.17% in Starch 1; 1.21% – 1.39% in Starch 2 and 1.12% – 1.39% in Starch 3 (Tables 3.2 – 3.4). These values suggest that NDF intake was maximised or near maximisation in the present study (Köster *et al.*, 1996; Ferrel *et al.*, 1999; Bohnert *et al.*, 2002). It is therefore doubtful whether higher urea supplementation treatments would have resulted in further increases in roughage intake under these experimental conditions.

Probable reasons for the relative high roughage DM intake observed in the present study at the relative low total dietary N concentrations compared to Detmann *et al.* (2009), might be related to the quality of the hay fed, combined with the quantity of urea supplemented to achieve the final dietary N concentrations. In the present study, the N concentration of the roughage (DM) was 0.4% N, with an ADIN fraction of 45%. Urea was supplemented at levels from 10.4 g urea/sheep/day (LU treatment) up to 32.4 g urea/sheep/day for the EHU treatment. In the study of Kanjanapruthipong and Leng (1998), roughage intake increased with urea supplementation levels up to 15 g/sheep/day. However, the hay used in both the studies of Detmann *et al.* (2009) and Kanjanapruthipong and Leng (1998) was of a higher quality (0.8% N) compared to the hay used in this study (0.4% N). It could be argued that the bioavailability and solubility of the N fraction of urea is substantially higher compared to the N fraction found in low-quality tropical roughages. As such, total diet N concentration needed to maximise roughage intake probably was lower in the present study compared to the studies of Detmann *et al.* (2009) and Kanjanapruthipong and Leng (1998), as more N was available from the urea. The presentation of the hay to the sheep could be a second possibility. The experimental hay was hammer milled through a 2.5 cm sieve, creating particles ranging from only a few millimetres long to about 5 cm long. While the milling action would not have changed the chemical composition of the hay nor the rate of degradability in the rumen (Ellis *et al.*, 1987), reducing the particle size through milling could have decreased the lag time during which rumen bacteria would have adhered to the particles (Ellis *et al.*, 1987). This could have caused an increase in roughage intake, as the particles

would have reached the critical size sooner at which point it could have passed through the rumen-reticulo-orifice to the lower GI tract (Ellis *et al.,* 1987), although rumen mat characteristics might limit roughage intake (Poppi *et al.,* 2001). As such, the potential increase in roughage DM intake associated with the milling action of the hay could have been independent to the type of supplementation given.

Another dietary factor that could have affected roughage intake in the trial is fermentable energy (FME) or non-fibrous carbohydrate (NFC) intake (Hennesy *et al.,* 1983; DelCurto *et al.,* 1990; Matejovsky and Sanson, 1995; Heldt *et al.,* 1999). The effect of energy supplementation on roughage intake in the ruminant is not consistent across studies with moderate starch supplementation inhibiting roughage intake in some studies (Hennesy *et al.,* 1983; Henning *et al.,* 1980), but stimulating it in other (Gomes *et al.,* 1994). In the present study, starch supplementation ranged from 200 g/sheep/day (LS treatment) to 280 g/sheep/day (HS treatment). This is equivalent to starch concentrations of 0.40% BW in the LS treatment, 0.48% BW for the MS treatment and 0.56% BW for the HS treatment. In terms of total feed intake, starch intake was similar at 19% DM in both the LS and MS treatments (200 and 240 g starch at 1027 and 1262 g DM intake, respectively, for the LS and MS treatments; Tables 3.2 and 3.3), increasing towards 24% in the HS treatment (280 g starch and 1165 g DM intake; Table 3.4). As such, starch supplementation in all treatments were at the upper limits at which point it could have had a negative effect on roughage intake and/or digestibility by either (i) a substitution effect (Caton and Dhuyvetter, 1997) and/or (ii) by a reduction of rumen pH (Mould and Ørskov, 1983; Mould *et al.,* 1983) and/or (iii) carbohydrate effect (Mould and Ørskov, 1983) and/or (iv) carbohydrate toxicity (Russell, 1984, 1989; Russel and Strobel, 1990, 2005). These aspects were explained in detail in the literature review.

Nitrogen intake from the hay (g N/sheep/day) ranged between 8.75 g N/wether/day – 19.02 g N wether/day between urea supplementation treatments in the LS period (Starch 1). Similar values were observed in Starch 2 (ranging between 9.81 g N/wether/day and 19.36 g N wether/day) and Starch 3 (8.61 g N wether/day – 19.27 g N wether/day; Tables 3.2 – 3.4). Interactions between urea treatments and NI were expected as predetermined daily quantities of urea were supplemented in the rumens of the sheep receiving each urea treatment. Nitrogen intake from hay alone (without supplements) ranged between 4 and 5 g N/wether/day across all urea and starch treatments (Tables 3.2 – 3.4), which was substantially lower than the maintenance requirements of sheep, weighing 50 kg, at 9.92 g N/ sheep/day

(NRC, 2007). Using 0.85 and 0.75 as benchmarks for the digestibility of microbial N and the ratio of microbial protein to true protein, respectively (McDonald *et al.*, 2011), the calculated microbial N intake needed to meet maintenance requirements of the sheep would have been 15.5 g N/sheep/day [9.92 / (0.75 * 0.85)]. Based on these values, only the wethers supplemented with 26.4 g urea/day (Urea 3 treatments) within each starch period received sufficient N to meet maintenance requirements.

It is of interest to note that if only available NI was considered for MNS, where NI is defined as total NI – ADIN (hay), only the maintenance N requirements of the sheep receiving the EHU treatment would have been met across all starch levels (Tables 3.5 – 3.7). This observation again highlights the deficiency of N in the basal diet as urea was supplemented at 32.4 g urea/sheep/day in the EHU treatment. This level of urea supplementation is more than twice the current safety recommendation of 14 g urea/day to sheep in the industry as a precaution to limit the occurrence of ammonia toxicity in sheep. However, the observation is in accordance with the recommendations of Preston and Leng (1987), suggesting that urea could be supplemented up to 3% of the dietary DM intake in cattle consuming low-quality tropical roughages.

Tables 3.5 – 3.7 shows the N excretion, N balance, efficiency of N usage (ENU), starch to available CP intake and average rumen ammonia N (RAN) over 24-hour time-period in the three starch levels as influenced by the different urea treatments within each starch period.

Table 3.5 Nitrogen excretion and balance and efficiency of N use of wethers fed low-quality *Eragrostis curvula* hay and supplemented with 200 g starch/day as affected by quantity of urea supplemented

	*Treatments				
**Parameters	Urea 1	Urea 2	Urea 3	Urea 4	***SEM
Faecal N excretion	5.70	5.36	5.04	5.07	0.28
Urinary N excretion	0.93^{c}	1.65^{b}	1.46^{b}	2.16^{a}	0.16
Total N excretion	6.62	7.01	6.50	7.23	0.35
N Balance	2.13^{d}	5.30^{c}	9.28^{b}	11.79^{a}	1.37
Hay N Balance	-2.66	-3.17	-2.86	-3.12	0.37
ENU	0.31^{c}	0.51^{b}	0.66^{a}	0.70^{a}	0.16

Means within a row with similar alphabetically superscripts ($^{a, b, c, d}$) do not differ (P > 0.05).

*Treatments: Urea 1 = 10.4 g urea/day; Urea 2 = 18.4 g urea/day; Urea 3 = 26.4 g urea/day; Urea 4 = 32.4 g urea/day

**Parameters: Total N excretion = Faecal N excretion + Urinary N excretion; N balance = Total N intake – Total N excretion (g/day); Hay N balance = Hay N intake – Total N excretion (g/day); ENU = Efficiency of N usage = Total N balance / Total N intake; MNS = Microbial N synthesis (g/day), EMNS = Efficiency of MNS (g MNS/kg DOMI); MNS: Available N = MNS / (Total N intake – ADIN intake).

**SEM = Standard error of mean

Table 3.6 Nitrogen excretion and balance and efficiency of N use of wethers fed low-quality *Eragrostis curvula* hay and supplemented with 240 g starch/day as affected by quantity of urea supplemented

	*Treatments				
**Parameters	Urea 1	Urea 2	Urea 3	Urea 4	***SEM
Faecal N excretion	6.94	7.36	6.26	6.45	0.28
Urinary N excretion	1.01^{b}	1.07^{b}	1.71^{a}	1.74^{a}	0.26
Total N excretion	7.95	8.43	7.97	8.19	0.35
N Balance	1.86^{d}	4.90^{c}	8.40^{b}	11.17^{a}	1.54
Hay N Balance	-2.93	-3.57	-3.74	-3.73	0.27
ENU	0.25^{c}	0.44^{b}	0.59^{a}	0.65^{a}	0.16

Means within a row with similar alphabetically superscripts ($^{a, b, c, d}$) do not differ (P > 0.05).

*Treatments: Urea 1 = 10.4 g urea/day; Urea 2 = 18.4 g urea/day; Urea 3 = 26.4 g urea/day; Urea 4 = 32.4 g urea/day

**Parameters: Total N excretion = Faecal N excretion + Urinary N excretion; N balance = Total N intake – Total N excretion (g/day); Hay N balance = Hay N intake – Total N excretion (g/day); ENU = Efficiency of N usage = Total N balance / Total N intake; MNS = Microbial N synthesis (g/day), EMNS = Efficiency of MNS (g MNS/kg DOMI); MNS: Available N = MNS / (Total N intake – ADIN intake).

**SEM = Standard error of mean

Table 3.7 Nitrogen excretion and balance and efficiency of N use of wethers fed low-quality *Eragrostis curvula* hay and supplemented with 280 g starch/day as affected by quantity of urea supplemented

	*Treatments				
**Parameters	Urea 1	Urea 2	Urea 3	Urea 4	***SEM
Faecal N excretion	5.52^{b}	6.99^{ab}	6.40^{ab}	7.28^{a}	0.48
Urinary N excretion	1.09^{b}	0.93^{b}	1.06^{b}	1.46^{a}	0.18
Total N excretion	6.64^{b}	7.92^{a}	7.42^{a}	8.64^{a}	0.35
N Balance	2.00^{d}	5.23^{c}	9.41^{b}	10.53^{a}	1.34
Hay N Balance	-2.78^{a}	-3.23^{a}	-2.74^{a}	-4.38^{b}	0.37
ENU	0.30^{c}	0.48^{b}	0.65^{a}	0.62^{a}	0.16

Means within a row with similar alphabetically superscripts ($^{a, b, c, d}$) do not differ (P > 0.05).

*Treatments: Urea 1 = 10.4 g urea/day; Urea 2 = 18.4 g urea/day; Urea 3 = 26.4 g urea/day; Urea 4 = 32.4 g urea/day

**Parameters: Total N excretion = Faecal N excretion + Urinary N excretion; N balance = Total N intake – Total N excretion (g/day); Hay N balance = Hay N intake – Total N excretion (g/day); ENU = Efficiency of N usage = Total N balance / Total N intake; MNS = Microbial N synthesis (g/day), EMNS = Efficiency of MNS (g MNS/kg DOMI); MNS: Available N = MNS / (Total N intake – ADIN intake).

**SEM = Standard error of mean

In general, faecal N excretion did not differ (P > 0.05) between urea treatments within each starch treatment (Tables 3.5 – 3.7) with the exception in Starch 3 where the faecal N excretion was higher (P < 0.05) in Urea 4 compared to Urea 1 (Table 3.7). In contrast, urinary N excretion was higher (P < 0.05) in the EHU treatments compared to the other urea treatments within each starch treatment (Tables 3.5 – 3.7). The exception was in Starch 2 (MS) where urinary N excretion of the EHU treatment was similar to that of the HU treatment which were both higher (P < 0.05) compared to the LU and MU treatments (Table 3.4). The higher urinary N excretion in the EHU treatment within each starch level could be indicative of a N abundance which was not used by the rumen microbes. Alternatively, it also could be argued that the quantity of FME supplemented in the high urea treatments was insufficient in relation to the level of urea supplemented, as microbial bacteria would not have been able to convert RAN into microbial N (Leng, 1990).

Despite the higher urinary N excretions observed at the EHU treatments within each starch treatment, total N excretion did not differ (P > 0.05) between urea treatments within each starch treatment (Tables 3.5 – 3.7). The exception was Urea 1 within Starch 3 where a lower (P < 0.05) total N excretion was observed compared to the other urea treatments (Table 3.7). Due to the similar total N excretions across all urea treatments, N balance differed between urea treatments (P < 0.05) within each starch period with N balance increasing as the level of urea supplementation was increased (Tables 3.5 – 3.7). In addition, efficiency of N use (ENU), calculated as apparent N retained (N intake – N excreted) divided by N intake, differed (P < 0.05) and increased as the level of urea supplemented across all starch levels was increased (Tables 3.5 – 3.7). The implication of these observations was that not only did the wethers supplemented with the higher urea treatments retain more N, but the ingested N was also retained more efficiently from about 30% in the LU treatments to above 60% in the EHU treatments.

Table 3.8 shows the rumen pH of wethers as affected by the different urea treatments within each starch period while Table 3.9 shows the effects of the different urea treatments within each starch period during different times.

Table 3.8 Rumen pH as affected by urea treatment in wethers fed low-quality *Eragrostis curvula* hay and supplemented with fixed quantities of starch

Treatment	Starch 1 (200 g/day)	Starch 2 (240 g/day)	Starch 3 (280 g/day)
Urea 1 (10.4 g/day)	6.13[b]	6.11[b]	6.36
Urea 2 (18.4 g/day)	6.28[a]	6.29[a]	6.25
Urea 3 (26.4 g/day)	6.26[a]	6.27[a]	6.33
Urea 4 (32.4 g/day)	6.25[a]	6.34[a]	6.28
Standard error of mean	0.04	0.05	0.05

Similar superscripts ([a, b]) within columns do not differ (P > 0.05).

Table 3.9 Rumen pH over time as affected by urea treatment in wethers fed low-quality *Eragrostis curvula* hay and supplemented with fixed quantities of starch

Treatment/Time	Starch 1 (200 g/day)	Starch 2 (240 g/day)	Starch 3 (280 g/day)
00h00	5.99^{de}	6.03^{cd}	6.00^{e}
03h00	5.96^{e}	6.01^{cd}	6.33^{bcd}
06h00	6.49^{ab}	6.49^{b}	6.29^{bcd}
09h00	6.58^{a}	6.70^{a}	6.72^{a}
12h00	6.40^{b}	6.66^{ab}	6.48^{b}
15h00	6.12^{cd}	6.09^{c}	6.39^{bc}
18h00	6.16^{c}	6.18^{c}	6.09^{e}
21h00	6.14^{cd}	5.87^{d}	6.16^{de}
Standard error of mean	0.06	0.07	0.07

Similar superscripts ($^{a, b, c, d, e}$) within columns do not differ (P > 0.05).

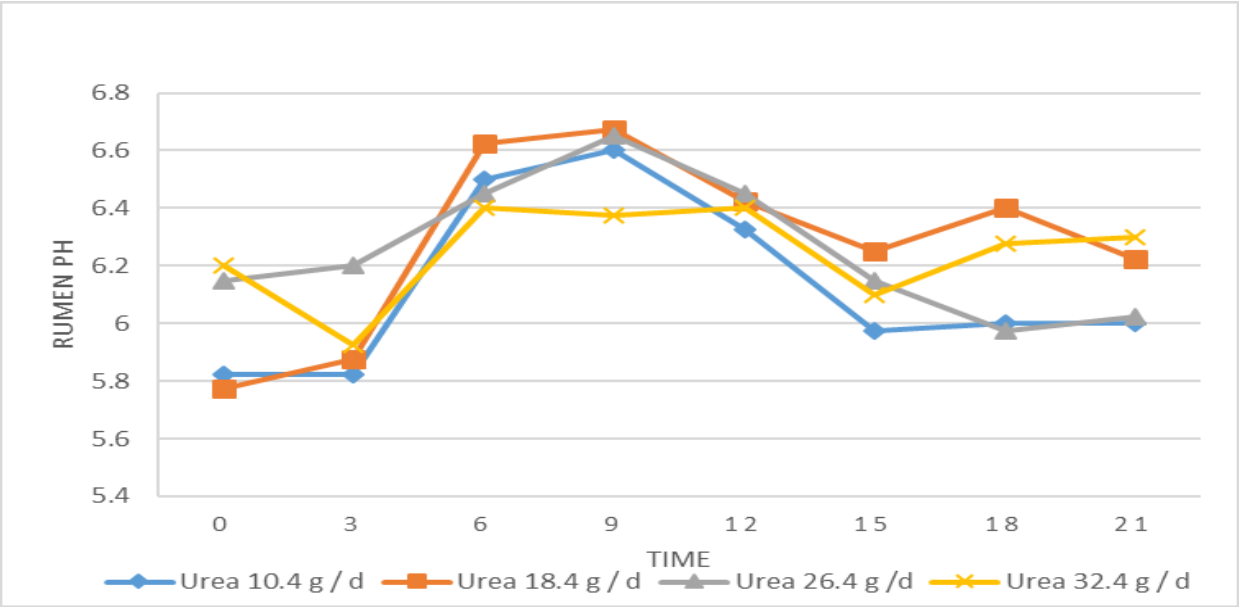

Figure 3.1 Rumen pH of wethers fed low-quality *Eragrostis curvula* hay and supplemented with 200 g starch per day per wether as influenced by urea supplementation over a 24-hour period

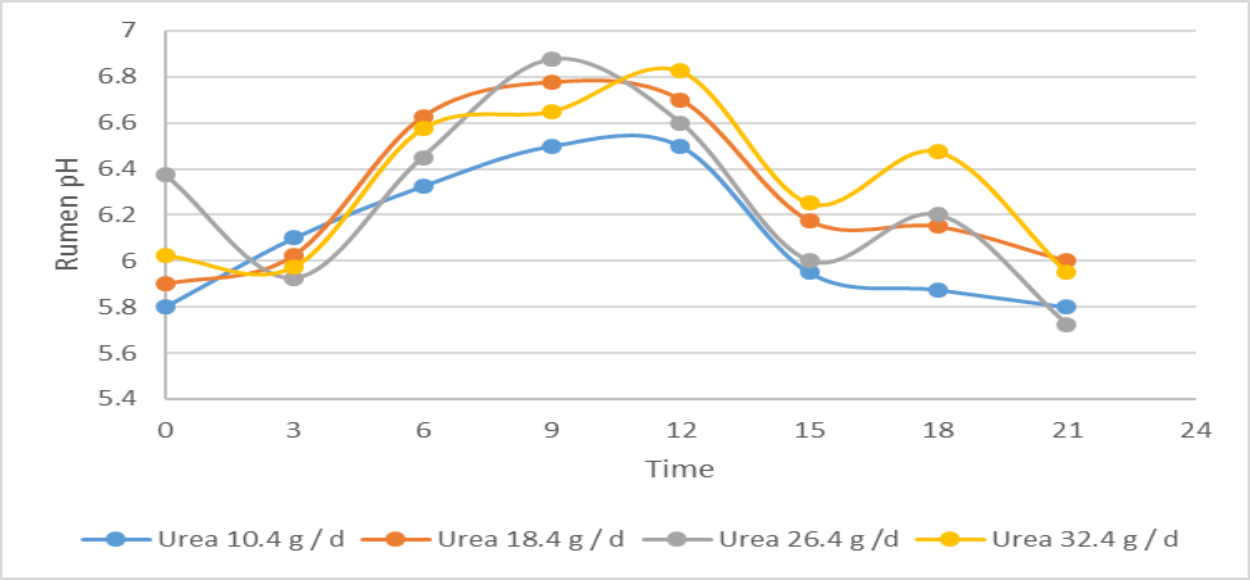

Figure 3.2 Rumen pH of wethers fed low-quality *Eragrostis curvula* hay and supplemented with 240 g starch per day per wether as influenced by urea supplementation over a 24-hour period

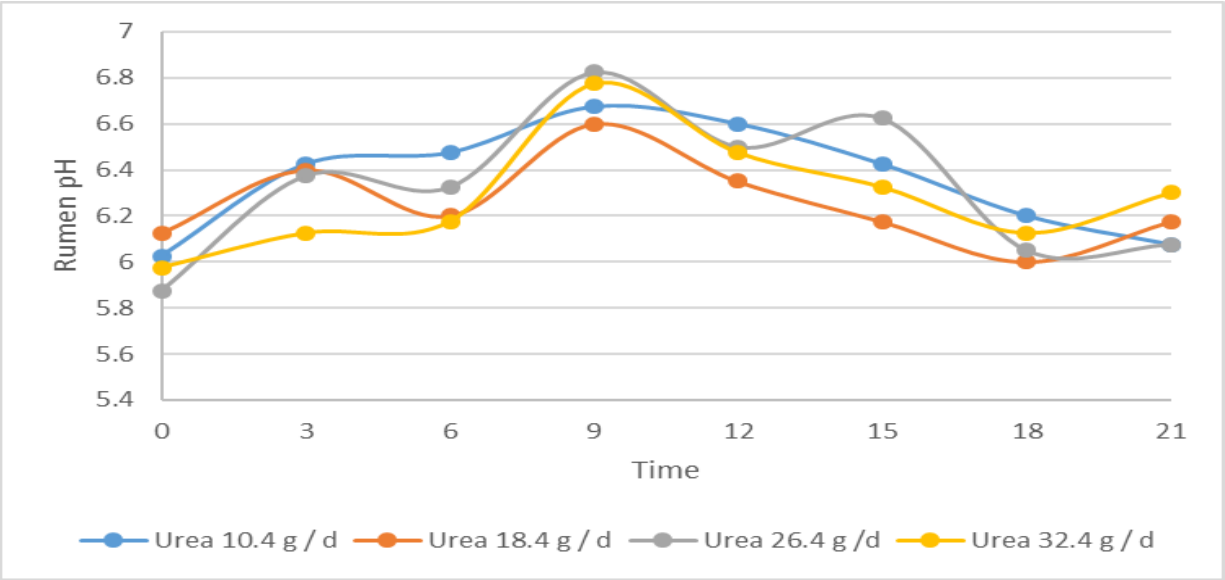

Figure 3.3 Rumen pH of wethers fed low-quality *Eragrostis curvula* hay and supplemented with 280 g starch per day per wether as influenced by urea supplementation over a 24-hour period

Rumen pH differed between urea treatments within Starch 1 and Starch 2 with the rumen pH of the wethers receiving Urea 1 being lower ($P < 0.05$) than those receiving the other urea treatments in both starch treatments (Table 3.8). No differences ($P > 0.05$) were observed between urea treatments in Starch 3 (Table 3.8). In both Starch 1 and Starch 2, the means of Urea 1 treatments were below the lower end range (pH 6.2) generally considered to be optimal for cellulolytic bacterial fermentation in the rumen (Mould and Ørskov, 1983; Mould *et al.*, 1983). However, roughage dry matter and NDFD did not differ between any urea treatment within each starch period (Tables 3.2 – 3.4). As such, the low rumen pH means observed in Urea 1 treatments within Starch 1 and 2 probably did not negatively affect roughage intake or digestibility. This was probably due to the diurnal rumen pH pattern of Urea 1 in both Starch 1 and 2 where the rumen pH was above 6.2 for at least 6 hours per day during the daytime during which time the wethers were fed (Figures 3.1 and 3.2).

During the day, rumen pH fluctuated across urea treatments within each starch treatment (Table 3.9; Figures 3.1 – 3.3). As such, during certain periods, the rumen pH of the sheep dropped below 6.2, which is the optimal rumen pH for cellulolytic bacterial fermentation (Mould and Ørskov, 1983; Mould *et al.*, 1983). As such, the lowest mean rumen pH across all starch levels was observed at midnight (00h00) while the highest mean rumen pH was generally observed at 09h00, 1 hour after the morning feeding and supplementation

(Table 3.8). The higher rumen pH observations at 09h00 could be explained by the supplementation and feeding pattern of the sheep at 08h00. Urea has a high solubility in the rumen while the rate of starch degradability is much slower and can take up to 5 hours to complete (Sinclair *et al.*, 1993). As both urea and starch were supplemented at 08h00, the higher solubility of urea probably would have resulted in an increase in RAN levels not coupled with starch availability yet, hence the increased rumen pH measured at 09h00. In addition, the highest RAN concentration also was measured at 09h00 (Table 3.10 – 3.12), corresponding to the higher rumen pH observations at those specific time-periods.

It is of interest to note that, except in Starch 3, the mean rumen pH at 18h00 was not different ($P > 0.05$) between 15h00 and 18h00 (Table 3.9), even though the sheep were supplemented at 16h00 with supplements containing urea and starch. There was a 2-hour delay during the afternoon supplementation and the measurement of the rumen pH at 18h00, compared to the 1-hour delay between the morning supplementation (08h00) and measurement period at 09h00 which could explain this observation. The lower rumen pH observed at 18h00 ($P < 0.05$) in Starch 3 (Table 3.9) compared to 15h00 not observed in Starch 1 and 2, could be explained by the higher starch supplementation of Starch 3 at 16h00 (280 g/day, therefore 140 g starch supplemented at 16h00) relative to the other starch treatments (200 g/day and 240 g/day, respectively, for Starch 1 and 2). However, statistical analyses across starch treatments were not performed in this trial as starch and period effect were correlated with each other. As such, differences between starch treatments cannot be commented on in this chapter, as it is unclear whether those differences were due to chance, period effect or treatment. However, a meta-analysis study was conducted in the last chapter (Chapter 5) of this thesis where the effects of starch and urea supplementation were studied.

Table 3.10 Rumen ammonia nitrogen, microbial nitrogen synthesis, efficiency of MNS and the ratio between MNS and available N as affected by urea treatment in wethers fed low-quality *Eragrostis curvula* hay and supplemented with 200 g starch/day

	*Treatments				
**Parameters	Urea 1	Urea 2	Urea 3	Urea 4	***SEM
RAN (average)	6.96^{c}	12.41^{bc}	18.35^{a}	16.87^{ab}	1.96
MNS	11.31^{b}	13.60^{a}	13.20^{a}	13.22^{a}	0.37
EMNS	13.03	15.15	15.04	13.50	0.79
MNS: Available N	1.68^{a}	1.31^{b}	0.95^{c}	0.78^{d}	0.03

Means within a row with similar alphabetically superscripts ($^{a, b, c}$) do not differ (P > 0.05).

*Treatments: Urea 1 = 10.4 g urea/day; Urea 2 = 18.4 g urea/day; Urea 3 = 26.4 g urea/day; Urea 4 = 32.4 g urea/day

**Parameters: RAN = Rumen ammonia N (mg NH_3-N / dL rumen fluid); MNS = Microbial N synthesis (g MNS/day), EMNS = Efficiency of MNS (g MNS/kg DOMI); MNS: Available N = MNS/ (Total N intake – ADIN intake).

**SEM = Standard error of mean

Table 3.11 Rumen ammonia nitrogen, microbial nitrogen synthesis, efficiency of MNS and the ratio between MNS and available N as affected by urea treatment in wethers fed low-quality *Eragrostis curvula* hay and supplemented with 240 g starch/day

	*Treatments				
**Parameters	Urea 1	Urea 2	Urea 3	Urea 4	***SEM
RAN (average)	5.64[b]	7.41[b]	18.30[a]	21.06[a]	0.88
MNS	15.71	15.85	14.45	14.88	0.55
EMNS	12.86	13.88	13.36	14.06	0.77
MNS: Available N	2.16[a]	1.46[b]	1.01[c]	0.87[d]	0.04

Means within a row with similar alphabetically superscripts ([a, b, c]) do not differ (P > 0.05).

*Treatments: Urea 1 = 10.4 g urea/day; Urea 2 = 18.4 g urea/day; Urea 3 = 26.4 g urea/day; Urea 4 = 32.4 g urea/day

**Parameters: RAN = Rumen ammonia N (mg NH_3-N / dL rumen fluid); MNS = Microbial N synthesis (g/day), EMNS = Efficiency of MNS (g MNS/kg DOMI); MNS: Available N = MNS/ (Total N intake – ADIN intake).

**SEM = Standard error of mean

Table 3.12 Rumen ammonia nitrogen, microbial nitrogen synthesis, efficiency of MNS and the ratio between MNS and available N as affected by urea treatment in wethers fed low-quality *Eragrostis curvula* hay and supplemented with 280 g starch/day

	*Treatments				
**Parameters	Urea 1	Urea 2	Urea 3	Urea 4	***SEM
RAN (average)	9.36^{c}	10.44^{c}	17.56^{b}	23.29^{a}	1.51
MNS	18.61^{ab}	18.75^{a}	16.97^{bc}	16.84^{c}	0.50
EMNS	19.90^{a}	17.79^{ab}	15.12^{b}	16.95^{ab}	0.98
MNS: Available N	2.79^{a}	1.74^{b}	1.17^{c}	0.98^{d}	0.04

Means within a row with similar alphabetically superscripts ($^{a, b, c}$) do not differ (P > 0.05).

*Treatments: Urea 1 = 10.4 g urea/day; Urea 2 = 18.4 g urea/day; Urea 3 = 26.4 g urea/day; Urea 4 = 32.4 g urea/day

**Parameters: RAN = Rumen ammonia N (mg NH_3-N / dL rumen fluid); MNS = Microbial N synthesis (g/day), EMNS = Efficiency of MNS (g MNS / kg DOMI); MNS: Available N = MNS / (Total N intake – ADIN intake).

**SEM = Standard error of mean

Rumen ammonia N of the wethers in the present study differed ($P < 0.05$) between the various urea treatments within each starch treatment (Tables 3.10 – 3.12), with RAN generally increasing as urea supplementation increased. As such, except for Urea 4 within Starch 1 which was similar ($P > 0.05$) to Urea 1 and Urea 2, RAN was higher in the wethers receiving Urea 3 (urea supplemented at 26.4 g/wether/day) and Urea 4 (urea supplemented at 32.4 g/wether/day) compared to Urea 1 (urea supplemented at 10.4 g/wether/day) and Urea 2 (urea supplemented at 18.4 g/wether/day) across all three starch treatments.

It is of interest to note that RDN supplementation in the studies of Detmann *et al.* (2009) and Kanjanapruthipong and Leng (1998) increased RAN as well as roughage intake, degradability and MNS in the tropical roughage fed ruminant. Detmann *et al.* (2009) commented that RAN is a prerequisite for rumen microbial efficiency as cellulolytic bacterial growth is dependent on RAN as a N source. As such, Detmann *et al.* (2009) suggested that a minimum RAN concentration is necessary to maintain microbial activity in the tropical forage fed ruminant to optimise rumen fermentation. However, the range of RAN in the literature apparently necessary to optimise the rumen of the roughage fed ruminant is widespread and not conclusive. Satter and Slyter (1994) for instance suggested that roughage intake and degradability in ruminants are maximised with RAN concentrations ranging between 5 – 20 mg RAN/dL rumen fluid. Detmann *et al.* (2009) using steers fed low-quality tropical roughages, observed that ED was maximised at RAN concentrations ranging between 5 and 8 mg RAN/dL rumen fluid. However, both Kanjanapruthipong and Leng (1998) in sheep and Detmann *et al.* (2009) in cattle fed low-quality tropical roughages, suggested that NDF intake as well as MNS are maximised at higher RAN concentrations ranging between 15 and 20 mg RAN/dL rumen fluid. A possible reason for the higher RAN concentrations needed to maximise roughage intake and MNS in tropical roughage fed ruminants is the lower nutrient availability of tropical roughages compared to temperate roughages (Bohnert *et al.*, 2011).

The stimulus of RAN by urea supplementation (Tables 3.10 – 3.12) in this study agrees with observations made by Kanjanapruthipong and Leng (1998) in sheep and Detmann *et al.* (2009) in cattle consuming low-quality tropical roughages. In the study of Detmann *et al.* (2009), RAN concentration remained constant, ranging between 8 and 10 mg RAN/dL rumen fluid as total dietary N was increased from 0.8% to 1.76% N (5 – 11% CP). However, RAN concentration almost doubled to 20 mg RAN/dL rumen fluid as total diet N percentage increased from 1.76% N to 2.24% N (11% CP to 14% CP). Kanjanapruthipong and Leng

(1998) observed similar results in sheep where RAN increased substantially from 6.7 to 16.8 mg RAN/dL rumen fluid as urea supplementation increased from 10 g/sheep/day to 15 g urea/sheep/day. In this study, the biggest increase in RAN concentration was observed between the MU treatment (18.4 g urea/wether/day) and HU treatment (26.4 g urea/wether/day) where RAN increased more than 50% in Starch 1 and Starch 3 and more than doubled in Starch 2 (Tables 3.10 – 3.12). These urea supplementations treatments corresponded with total dietary N percentages of 1.12% N (MU treatment) and 1.46% N (HU treatment), respectively (Tables 3.2 – 3.4), which were slightly lower than the dietary N percentages observed by Detmann *et al.* (2009) where RAN almost doubled. A possible explanation for the lower dietary N observed in the present study where RAN almost doubled, could be related to the higher degree of N supplementation in the present study. In both the studies of Detmann *et al.* (2009) and Kanjanapruthipong and Leng (1998), the quality of hay used was higher (0.80% N) compared to the hay used in the present study (0.43% N). Higher quantities of N supplementation were therefore necessary to obtain the same dietary N percentages in this study compared to the studies of Detmann *et al.* (2009) and Kanjanapruthipong and Leng (1998). It could be argued that the solubility and availability of N from urea is higher than the N compounds found within roughages. It therefore was a possibility that, due to the higher urea supplementation regime followed in this trial, the availability of the N compounds was higher than in the studies of both Kanjanapruthipong and Leng (1998) and Detmann *et al.* (2009), hence the lower total dietary N percentage in this study.

Urea supplementation affected MNS within Starch 1 and 3, but not in Starch 2 (Tables 3.10 – 3.12). In Starch 1, MNS was lower ($P < 0.05$) in Urea 1 compared to the other urea treatments (Table 3.10). In contrast, MNS of the wethers supplemented with 32.4 g urea/day (Urea 4) in Starch 3 was lower ($P < 0.05$) compared to the wethers receiving Urea 1 and Urea 2 (Table 3.12).

If it is assumed that the percentage of true protein in microbial protein is 75% with a digestibility of 85% (McDonald *et al.*, 2011) and that the maintenance requirements of 50 kg sheep for metabolic N is 9.9 g N/day (NRC, 2007), then MNS had to be 15.6 g N/day to fulfil the maintenance requirements of the wethers in this study [9.9 g N / (0.75*0.85)]. As such, even though two of the supplemental urea treatment groups (HU and EHU) were formulated to be sufficient in RDN to meet maintenance requirements of the sheep, none of the urea treatments in Starch 1 or 2 fulfilled the MNS requirements of the sheep (Tables 3.10 and

3.11). In contrast, at the highest starch supplementation treatment (280 g starch/wether/day; Starch 3), MNS was, based on the calculation, sufficient in all the urea treatments to fulfil maintenance requirements. Based on this observation, it therefore seems that the level of starch supplementation had a bigger effect on MNS compared to urea supplementation which agrees with Leng (1990) and Poppi *et al.* (1999), stating that FME had a more prominent effect on MNS compared to RDN in the tropical forage fed ruminant. As stated before though, statistical analyses were not conducted on the effects of starch supplementation on the various parameters as starch was confounded with periodic effect. However, the effects of starch, urea as well as starch*urea interactions were tested and studied in a meta-analysis described in Chapter 5.

Efficiency of MNS (EMNS) production did not differ ($P > 0.05$) between urea treatments in Starch 1 or Starch 2 (Tables 3.10 and 3.11). In Starch 3, wethers supplemented with 10.4 g urea/day (LU) had higher EMNS ($P < 0.05$) compared to wethers supplemented with 32.4 g urea/day (HU; Table 3.12). The higher observed EMS of the LU treatment within Starch 3 could be attributed to the numerical higher ($P > 0.05$) MNS observed in the wethers receiving the LU treatment compared to the HU treatment, combined with the numerical lower DMI ($P > 0.05$) of the LU treatment *versus* the HU treatment (Table 3.4). No differences ($P > 0.05$) were observed between LU and the other urea treatments or between HU and the other urea treatments within Starch 3. It is therefore unlikely that the observed EMNS difference between the LU and MU treatment within Starch 3 was the result of a treatment effect.

Pathak (2008) suggested that the average EMNS for roughage based diets is 20.8 g MNS/kg DOMI. These values agree with SCA (1990), citing values between 20.8 and 27.2 g MNS/kg DOMI for forages. However, Poppi *et al.* (1999) in a review concluded that warm season (C4) grasses have lower EMNS based on its digestible OM content compared to temperate C3 grasses. The authors stated further that the lower MNS efficiencies of C4 grasses compared to C3 grasses are due to lower NSC content (generally less than 90 DOM/kg DM) of tropical grasses which is considered as the minimum NSC necessary to optimise MNS efficiency. As such, EMSN values of 10 – 16 g MNS/kg DOMI for Rhodes grass (*Chloris gayana*) and 18.1 g MNS/kg DOMI for Bermuda grass (*Cynodon dactylon*) are quoted by the authors. In this study, NFC content of the *E. curvula* was calculated at 4.7%. While NFC theoretically is not equal to NSC (AOAC, 2000), it still could be argued that the NSC concentration of the *E. curvula* hay used in this trial was considerably less than the

minimum NSC (90 g/kg DOMI) required to optimise EMNS (SCA, 1990). Poppi *et al.* (1999) therefore suggested that energy supplementation could increase MNS supply and EMNS in the C4 grasses due to these deficiencies, including corn starch. In this trial, EMNS ranged between 13.03 g MNS/kg DOMI – 15.15 g MNS/kg DOMI in Starch 1, 12.86 – 14.06 g MNS/kg DOMI in Starch 2 and 15.12 – 19.90 g MNS/kg DOMI in Starch 3. As such, the mean EMNS quoted by SCA (1990) and Pathak (2008) for forages, was not reached across all starch levels in this trial. A possible reason includes the quality of *E. curvula* hay used in the trials. Poppi *et al.* (1999) stated that MNS and EMNS is not just limited by the NSC concentration in C4 grasses, but also the retention time of the grass in the rumen. Elliot *et al.* (1984) showed that sucrose supplementation to sheep fed low-quality C4 pongola grass (*Digitaria eriantha*) only improved roughage intake until a certain level (15 g NDF/kg BW). In addition, while MNS was increased ($P < 0.05$) by increasing levels of sucrose, EMNS did not differ ($P > 0.05$) between sucrose treatments. In addition, Poppi *et al.* (2001) stated that tropical forage intake is limited by the retention time of the small particles as well as the rumen mat structure where the particles are hindered to filter through. The lack of differences observed in EMNS can therefore be explained by the probable similar and low particle retention times between treatments (not measured) irrespective of starch content.

Despite the above argument, it is of interest to note that EMNS appears to have increased between Starch levels 1 and 2 and Starch level 3. This observation would agree with Leng (1990), Poppi *et al.* (1999) and SCA (1990), suggesting that NSC supplementation theoretically would increase EMNS in tropical C4 grasses due to their low concentration of NSC or WSC. This aspect is discussed in more detail in Chapter 5 where a meta-analysis study was conducted to investigate the effects of both starch and urea supplementation on MNS and EMNS.

A parameter not frequently used to describe MNS efficiency, is efficiency of feed N captured into MNS (MNS:NI; Detmann *et al.*, 2014). This ratio is used as an indirect indicator of the potential recycling of body N to the rumen as ratios above one (MNS is more than feed N intake) suggest that the ruminant used more N derived from body protein catabolism in addition to feed N for MNS (Detmann *et al.*, 2014). In this trial, available N instead of total N intake was used where available NI was calculated as total NI – ADIN intake as it was considered that the ADIN fraction of the hay would not have been available to the wethers.

The MNS: available NI differed ($P < 0.05$) across all urea treatments within each starch period (Tables 3.10 – 3.12) with the MNS: available N ratio decreasing as urea supplementation was increased. Theoretically, MNS:NI ratios less than one could be indicative that N intake was sufficient to maintain MNS and that less N was recycled to supply RDN to the rumen for MNS (Detmann *et al.*, 2014). It is of interest to note that the ratio drops below one at different urea supplementations within each starch period (Tables 3.10 – 3.12). As such, the MNS: available N ratio dropped below 1 at the HU treatment at the LS treatment, while at the MS and HS treatments, only the wethers supplemented with 32.4 g urea/day (EHU) had MNS: available N ratios below 1. Based on these observations, it seems that there could be an ideal ratio of starch to urea supplementation for wethers fed low-quality *E. curvula* hay under the experimental conditions. This hypothesis was tested in detail in the meta-analysis study described in Chapter 5 of this thesis.

3.6 Summary

The purpose of the trial was to study the effects of supplemental urea within different starch supplementations on roughage intake, digestibility, RAN and MNS in wethers fed low-quality tropical *E. curvula* hay. The levels of urea supplementation did not affect roughage intake and digestibility within each starch treatment. This was in contrast with observations made by Detmann *et al.* (2009) in cattle and Kanjanapruthipong and Leng (1998) in sheep, observing that RDN supplementation stimulated effective degradability and roughage intake up to certain levels.

In general, MNS was not influenced by urea supplementation. This observation contrasted with studies of Detmann *et al.* (2009) and Kanjanapruthipong and Leng (1998). A difference between this study and the studies of Detmann *et al.* (2009) and Kanjanapruthipong and Leng (1998) was that additional starch was supplemented to the wethers in the present study while no additional FME was supplemented in the studies of the mentioned authors. Both Leng (1990) and Poppi *et al.* (1999) suggested that the major nutrient stimulating MNS in the tropical forage fed ruminant is NSC or FME. Results from this study do suggest the abovementioned statements of Leng (1990) and Poppi *et al.* (1999). A similar observation was made for EMNS, suggesting that starch supplementation might be an important strategy to increase MNS and EMNS in wethers fed low-quality *E. curvula* hay. Urea supplementation improved the MNS: available NI ratio within each starch treatment,

with higher quantities of urea needed to be supplemented to keep the ratio below one as the level of starch supplementation was increased.

3.7 Conclusion

The ratio of MNS: available NI could be used as an indicator of N deficiency in the ruminant with ratios above one being indicative of a possible N deficiency. The results obtained in this study suggest that wethers fed low-quality *E. curvula* hay (0.4% N) and supplemented with starch needs to be supplemented with 26.4 g urea/day to have a favourable (less than one) MNS: available N ratio. These intakes also improved the N balance and the efficiency of N use. As the level of starch supplementation is increased, the quantity of urea to be supplemented should increase to keep the ratio below one. These results suggest that higher than recommended levels of N supplementation are necessary to optimise MNS in the tropical forage fed ruminant (NRC, 2007).

Chapter 4 Synchronisation of energy and protein supplementation in wethers fed low-quality *Eragrostis curvula* hay

4.1 Abstract

The primary objective of the study was to determine whether the pattern of starch and urea supplementation would impact on roughage intake, digestibility, and microbial nitrogen synthesis (MNS) in sheep fed low-quality [0.4% nitrogen (N), 83% neutral detergent fibre (NDF)] *Eragrostis curvula* hay. Seven, year-old rumen cannulated Döhne Merino sheep were assigned to a 7 x 7 Latin square design experiment. The sheep were fed low-quality *E. curvula* hay and supplemented twice daily at 08h00 and 16h00. The ingredients supplemented, except for treatment 7, were 120 g starch and 13.2 g urea. Treatment 7 contained 180 g molasses and 10.75 g urea. The distribution pattern of the starch and urea created the treatment differences. Starch and/or urea supplemented during either the morning or afternoon supplementation period, or divided into half (60 g for starch or 6.6 g for urea, respectively) and supplemented during both periods. In addition, in one treatment, both starch (120 g) and urea (13.2 g) were supplemented only every alternated day. Supplementation pattern did not affect roughage intake; however, roughage digestibility appeared to be higher in the treatments where starch was supplemented at least partly, during the morning supplementation periods. Neither N intake, nor faecal N excretion was affected by treatment. In contrast, urinary N excretion was higher in the treatments where starch was supplemented during both the morning and afternoon periods. Despite the apparently higher N excretion observed in those treatments, N balance did not follow a clear trend across treatments. The most consistent rumen ammonia N (RAN) concentration, with RAN concentrations appearing to be optimal during all times (ranging between 10 – 15 mg/dL rumen fluid), was achieved in the treatment where both starch and urea was supplemented during both the morning and afternoon periods. In general, MNS was higher in the treatments where starch was supplemented during both morning and afternoon supplementation periods. Efficiency of MNS (EMNS) followed the same trends as MNS across treatments, with the highest EMNS observed in the treatments where starch was supplemented more frequently (twice daily) compared to only once daily. It is concluded that, while the most consistent and apparent sufficient rumen RAN was observed in the treatment where both urea and starch was

supplemented twice daily, the supplementation frequency of starch tended to be the more important parameter compared to urea, stimulating forage digestibility, MNS and EMNS.

4.2 Introduction

Nutrient synchrony, per Hersom (2008), "would imply a parallel occurrence of nutrients for the ruminant animal to consume or be present in the diet and the rumen, so by supplying energy and nitrogen concurrently, an increase or optimisation of microbial efficiency would occur." Leng (1990, 1995) suggested that in ruminants grazing low-quality tropical roughages, an increase in microbial efficiency theoretically should translate to an increase in animal production that would not have been observed if the provision of energy and protein had not been synchronised. Despite the definition, synchronisation studies not always resulted in increased animal performances. Hall and Huntington (2008) commented in a review that, while nutrient synchronisation is sound in theory, it is elusive in practise. However, it must be noted that most nutrient synchronisation studies were conducted on high performance ruminants and dairy cows, where feed intake throughout the day is continuous where an asynchronous nutrient supply in one meal could be rectified by another meal within a short period (Hall and Huntington, 2008). In contrast, supplementation of ruminants consuming low-quality roughages is infrequent while roughage intake is continuous throughout the day. As such, Hersom (2008) noted that, to improve ruminant production in the roughage fed ruminant, it is important to achieve nutrient synchronisation throughout the day, and not just during certain periods.

Nutrient synchronisation studies conducted on sheep fed low-quality tropical roughages are limited. Leng (1995) in a review suggested that the general better responses observed in ruminants consuming low-quality tropical forages and supplemented with urea and molasses *versus* urea and maize (starches), could be due to a better synchronisation between energy release from sugars found in molasses and N release from urea *versus* energy release from starches and N release from urea.

The overall aim of the study was to evaluate the degree of RDN and FME synchronisation in sheep receiving low-quality *E. curvula* hay on daily RAN, roughage intake and digestibility, MNS and efficiency of N usage. Nutrient synchronisation of the supplements were obtained by altering the supplementation pattern of urea and/or starch between morning (08h00) and afternoon (16h00) supplementation if the "highest"

synchronisation would be obtained where starch and urea is supplemented at different time-periods (Sinclair *et al.*, 1993). In addition, the different supplementation patterns of urea and starch were compared with molasses and urea as supplement to test the hypothesis of Leng (1995).

4.3 Hypotheses

The following hypotheses were tested:

1. Starch and urea supplementation pattern will affect daily RAN concentration, with the highest RAN concentrations occurring in the non-synchronised treatments (where starch and urea is supplemented simultaneously).

2. Starch and urea supplementation pattern will affect daily rumen pH with the biggest rumen pH variations occurring in the non-synchronised treatments.

3. Starch and urea supplementation pattern will affect roughage intake and digestibility, MNS and efficiency of MNS.

4. Starch and urea supplementation pattern will affect N balance and N efficiency of the sheep with better N balance and N efficiency values observed in the synchronised treatments.

5. Microbial N synthesis, efficiency of MNS, roughage intake and/or roughage digestibility are higher in sheep supplemented once daily, with molasses and urea compared to sheep supplemented once daily, with starch and urea.

4.4 Materials and Methods

The Animal and Ethical Committee of the University of Pretoria, South Africa, approved the trial and protocol. The trial was conducted at the experimental farm of the University of Pretoria, South Africa, where seven rumen cannulated sheep (mean weight 40.5 kg) were used in a 7 x 7 Latin Square experimental design. The sheep received low-quality *E. curvula* hay *ad libitum* [2.7% CP; 84.1% NDF; 93% OM, 49.6% acid detergent fibre (ADF), dry matter (DM) basis] *Eragrostis curvula* hay. If it is assumed that the *E. curvula* hay contained 1.5% ether extract (EE) (NRC, 2007), the calculated non-fibre carbohydrate fraction of the *E. curvula* was 4.7% (Table 4.1). The sheep were supplemented intra-

ruminally with starch and urea or molasses and urea during either the morning (08h00) or afternoon (16h00) supplementation period, or both. Table 4.2 shows the composition of the supplements and the pattern of supplementation between the treatments, to sheep fed low-quality *E. curvula* hay.

The quantity of starch and urea needed to be supplemented to meet microbial requirements (AFRC, 1993) of the wethers fed the low-quality *E. curvula* hay (Table 4.1) had been calculated in Chapter 3. However, due to the high risk of urea toxicity in once-a-day supplementation regimes, it was decided to limit the urea to 13.2 g/day. At the expected forage intakes (1000 g/day), and using AFRC (1993) recommendations as discussed in Chapter 3 (9 g RDP/MJ FME), the quantity of starch needed to be supplemented with the urea was 120 g/day.

Table 4.1 Chemical composition of *Eragrostis curvula* hay fed to wethers and supplemented with urea and starch

Dry Matter (g/kg)	930
Ash (g/kg DM)	72
Nitrogen (g/kg DM)	4.2
Neutral detergent fibre (g/kg DM)	841
Acid detergent fibre (g/kg DM)	496
Acid detergent insoluble Nitrogen (% of CP)	45 %
*Non-Fibre carbohydrate (g/kg DM)	47

*Non-Fibre carbohydrate was calculated as DM – ash – EE – CP – NDF (Fox *et al.*, 2004), assuming that low-quality hay contained 1.5% EE (NRC, 2007).

Table 4.2 Composition and supplementation patterns of treatments in sheep fed low-quality *Eragrostis curvula* hay

Treatment/Time of supplementation	***Description***	***08h00***	***16h00***
SU^{2nd}	Starch and urea supplemented every second afternoon	None	13.2 g urea and 120 g starch supplemented every alternate day
Sm, Ua	Starch supplemented daily in morning, urea daily in afternoon	120 g starch	13.2 g urea
Sa, Um+a	Starch supplemented daily in afternoon, urea supplemented daily during both the morning and afternoon periods	6.6 g urea	6.6 g urea 120 g starch
Sm+a, Um+a	Both starch and urea were supplemented daily during the morning and afternoon supplementation periods	60 g starch 6.6 g urea	60 g starch 6.6 g urea
Sm+a, Ua	Starch supplemented daily during the morning and afternoon supplementation period, urea daily supplemented during the afternoon period	60 g starch	60 g starch 13.2 g urea
Sa, Ua	Starch and urea supplemented daily during the afternoon period	None	120 g starch 13.2 g urea
Molasses	Molasses and urea supplemented daily during the afternoon supplementation period	None	180 g molasses 10.75 g urea

In six of the seven treatments, the supplements contained (DM, daily basis) 120 g starch, 13.2 g urea, 24.02 g mineral mix and 1.8 g sulphur (S). Leng (1995) suggested that urea- and molasses-based supplements generally "outperform" urea and starch based supplements in tropical roughage fed ruminants as the fermentation of molasses, being a

sugar, might be more in synchronisation with urea compared to starch. It was therefore decided to have a "Molasses treatment", providing molasses and urea instead of starch and urea, to test this hypothesis against different urea and starch supplementation patterns.

Due to the lower metabolisable energy concentration of molasses compared to starch (10.5 MJ ME *versus* 14.9 MJ ME, respectively; NRC, 2007) and N concentration of the molasses (0.6% N or 4% CP), the quantities of molasses and urea supplemented was adjusted to 180 g molasses and 10.75 g urea per day per sheep, respectively. These quantities were adjusted to keep the daily molasses and urea supplement iso-nitrogenous (6.1% N) and iso-energetic (1.70 MJ ME) to the daily starch supplements. The chemical composition of the mineral mix is described in Chapters 2 and 3.

The supplementation pattern of the starch and urea was divided into morning (08h00) and afternoon (16h00) supplementation periods, where the quantities of urea and/or starch or molasses supplemented among treatments differed between time-periods. The treatments were as follows: SU2nd (120 g starch and 13.2 g urea supplemented every second afternoon at 16h00); Sm, Ua (120 g starch supplemented at 08h00, 13.2 g urea supplemented at 16h00. daily); Sa, Um+a (6.6 g urea supplemented at 08h00, 120 g starch and 6.6 g urea supplemented at 16h00, daily); Sm+a, Um+a (60 g starch and 6.6 g urea supplemented at 08h00, 60 g starch and 6.6 g urea supplemented at 16h00, daily); Sm+a Ua (60 g starch supplemented at 08h00, 60 g starch and 13.2 g urea supplemented at 16h00, daily); Sa Ua (120 g starch and 13.2 g urea supplemented at 16h00, daily); Molasses (180 g molasses and 10.75 g urea, supplemented at 16h00, daily).

The sheep were adapted to the supplements for a period of 7 days, which was similar to adaptation periods used by Wickersham *et al.* (2008, 2009) for steers fed low-quality tropical prairie grass (tropical grass) and supplemented with N compounds. On the last day of the adaptation period, the sheep were transferred to metabolic cages where faecal harnesses were fitted. During the experimental period, feed intake and faecal and urine output were recorded daily. Feed, orts and faeces were sampled and pooled over the experimental period within each treatment to estimate total tract digestibility. Urine was collected from urine pans and transferred into urine bottles containing 5 mL sulphuric acid (H_2SO_4; 50% v: v) for preservation, and adjusted to a final pH below 3 with H_2SO_4, if required. Daily urine volumes were measured and diluted to 4000 mL. From this diluted volume, 50 mL sub-samples were taken, pooled over the collection period and frozen at –20^{o}C for purine derivative (PD)

analysis. Creatinine was determined from each urine sample to determine the corrected PD (Chen *et al.*, 1995), assuming that the daily excretion of creatinine as a proportion of muscle mass from the wethers was constant Broderick and Merchen, 1992; Chen *et al.*, 1995). The corrected PD was used in the estimation of MNS (Chen and Gomes, 1992).

Rumen fluid was collected from four predetermined locations within the rumen (top left and centre and bottom left and centre) at 03h00, 09h00, 15h00 and 21h00. The collection procedure for the SU^{2nd} treatment was similar with the exception that each collection period occurred twice, on the supplemental and non-supplemental days. The rumen fluid samples of the SU^{2nd} treatment were pooled within time to obtain representative samples for supplemental and non-supplemental days. Five mL sulphuric acid (H_2SO_4; 10% v: v) was added to 30 mL rumen samples for RAN (Broderick and Kang, 1980) analyses. The samples were frozen at –20^{o}C until analyses commenced.

Feed and faecal samples were ground using a Wiley mill to pass a 1 mm screen. Hay, orts and faecal samples were dried for 24 hours at 105^{o}C in a forced air oven to determine DM and then combusted for 8 hours at 450^{o}C in a muffle furnace for OM determination (AOAC, 2000). The N content of hay, faeces and urine was determined by the Kjehdahl method (AOAC, 2000). All hay, orts and faecal samples were analysed for NDF and ADF with the ANKOM-Fibre Analyzer (ANKOM Technology, Fairport, NY, USA). *In vitro* organic matter digestibility (IVOMD) was determined using the Tilley and Terry method (Tilley and Terry, 1963) as modified by Engels and Van der Merwe (1967) for low-quality roughages under South African conditions. In this modification, N in the form of urea was added to each test tube (20 mg urea per test tube), simulating N recycling in ruminants consuming low-quality roughages. Feed intake and faecal and urine outputs were recorded daily, sampled and pooled over a 5-day digestibility period to estimate total tract digestibility.

Statistical analysis was performed using ANOVA from SAS (Statistical Analysis System, 2015) with a model suitable for a 7 x 7 Latin Square block design. The model fitted in this Latin Square layout was:

$$yijk = \mu + rj + ck + ti + \varepsilon ijk$$

where yijk is the response for the ijkth unit, μ is the overall mean, rj (j = 1...n) represents the row effects (number of periods), ck (k = 1...n) the column effects (number of different

animals), ti (i = 1...n) the main treatment effects (the individual treatments), and εijk is the error variation for the ijkth unit.

Treatment differences were detected using the F-test (Samuels, 1989) and significant differences were declared at $P < 0.05$.

4.5 Results and Discussion

An alternative way to study nutrient synchronisation in roughage fed ruminants is to change the ingredient composition of the supplements used as treatments (Hersom, 2008). A major disadvantage of this method is possible confounding effects between different ingredients. As such, Mould and Ørskov (1983) suggested that the negative effects of carbohydrate supplementation on roughage intake and digestibility are not only due to rumen pH effects caused by different carbohydrates, but also due to specific "carbohydrate" effects not yet identified. Due to possible confounding effects, the decision was made to use similar ingredients between treatments, with only the pattern of supplementation differing between treatments.

In contrast to the foretold discussion, an additional treatment, consisting of molasses and urea was created. Leng (1995) suggested that tropical roughage fed ruminants, receiving supplements containing molasses and urea, generally produced better than ruminants receiving supplements based on maize (starches) and urea. The authors commented that, while the better production responses observed could be attributed to a better mineral composition of the molasses compared to maize, better synchronisation between sugar and urea fermentation in molasses and urea *versus* starch and urea fermentation in starch and urea supplements, could also be an attributable factor to the better production responses.

In extensive sheep production in South Africa, supplementation normally occurs near watering holes during the late afternoon period. As such, it was decided to supplement accordingly with urea during the afternoon period (16h00). Fermentable energy in the form of starch or molasses was supplemented to improve the utilisation of the N fraction of urea (Köster *et al.*, 1996). The different treatments were developed through variation of the supplementation pattern of starch and some of the urea from the afternoon supplementation period (Table 4.2). A treatment that could have been included in the study, was the Sm Um treatment, where both starch and urea would have been supplemented during the morning supplementation period. It was decided not to include this treatment in the protocol as total

urea (13.2 g urea) supplementation during the morning before feeding, could have increased the risk of rumen ammonia toxicity as rumen content would have been at a minimum and secondly, it was assumed that the Sa Ua treatment, where both urea and starch were supplemented during the afternoon period, would give similar results compared to the Sm Um treatments in terms of synchronisation.

In an earlier trial (Chapter 3), the supplements were divided in half and supplemented twice-daily intra-ruminally (at 08h00 and 16h00). The reason for the twice-daily supplementation was the possibility of urea toxicity if the supplemented urea quantities (varying between 10.4 and 32.4 g urea/day/sheep) had been supplemented once daily. In this trial, the quantities of urea and starch supplemented per day, per sheep, was similar among treatments. To prevent or limit potential urea toxicity in the sheep, the quantity of urea supplemented was limited to 13.2 g/sheep/day as some treatments only had one urea supplementation period.

To calculate the starch necessary to "optimise" the sheep's roughage diet, roughage intake was estimated at 700 g DM/day/sheep (at 1.5% BW) based on intake data from an earlier trial (Table 3.4, Chapter 3). With urea supplementation at 13.2 g/day, rumen available N intake was estimated at 7.7 g N/day (N intake from hay and supplements – ADIN intake; Chapter 3). Using AFRC (1993) recommendations as discussed in Chapter 3, starch supplementation was calculated at 120 g starch/day/sheep (DM basis).

Table 4.3 shows intake, excretion, and digestibility data of sheep fed low-quality *E. curvula* hay and supplemented with urea and starch or molasses during the morning and/or afternoon supplementation period.

Table 4.3 Total and roughage intake of sheep fed low-quality *Eragrostis curvula* hay as affected by the supplementation pattern of urea and starch or molasses

	*Treatments							
**Parameters	SU^{2nd}	Sm, Ua	Sa, Um+a	Sm+a, Um+a	Sm+a, Ua	Sa, Ua	Molasses	***SEM
Roughage Intake								
(g DM/day)	727.1	699.8	664.6	762.3	679.8	702.7	731.1	40.53
DMI (g/day)	787.7^{b}	808.8^{b}	773.6^{b}	871.2^{ab}	788.8^{b}	811.7^{b}	911.1^{a}	43.53
OMI (g/day)	699.1^{c}	738.8^{abc}	708.1^{bc}	783.5^{ab}	723.1^{bc}	741.4^{abc}	796.0^{a}	35.99
DOMI (g/day)	395.8^{c}	443.8^{ab}	368.2^{c}	468.1^{a}	405.0^{bc}	407.8^{bc}	419.0^{b}	19.84
NDFI (g/day)	486.1	479.6	448.9	509.2	461.1	481.2	524.3	28.94
NDFI (% BW)	1.21	1.15	1.10	1.25	1.16	1.19	1.27	0.08
DOMI (% BW)	0.98^{b}	1.06^{b}	0.90^{c}	1.15^{a}	1.00^{b}	1.00^{b}	1.00^{b}	0.03
DMD (%)	53.3^{abc}	56.6^{a}	49.2^{c}	56.2^{ab}	52.9^{abc}	51.2^{bc}	49.7^{c}	1.87
NDFD (%)	43.3^{ab}	50.3^{a}	37.3^{b}	49.3^{a}	42.1^{b}	41.7^{b}	41.4^{b}	2.47
OMD (%)	56.6^{ab}	60.1^{a}	52.0^{b}	59.8^{a}	56.0^{ab}	54.2^{b}	52.6^{b}	1.88

Data within a row with similar alphabetical superscripts ($^{a; b; c}$) do not differ (P > 0.05).

*Treatments: $SU2^{nd}$ (120 g starch and 13.2 urea supplemented every 2^{nd} day at 16h00); Sm Ua (120 g starch supplemented daily at 08h00, 13.2 g urea daily at 16h00); Sa Um+a (6.6 g urea supplemented daily at 08h00, 120 g starch and 6.6 g urea supplemented daily at 16h00); Sm+a Um+a (60 g starch and 6.6 g urea supplemented daily at 08h00 and at 16h00); Sa Ua (120 g starch supplemented daily at 08h00, 13.2 g urea supplemented daily at 16h00); Molasses (180 g molasses, 10.75 g urea supplemented daily at 16h00).

**Parameters: DMI = Dry matter intake; OMI = Organic Matter intake; DOMI = Digestible organic matter intake; NDF = Neutral detergent fibre; NDFI = Neutral detergent fibre intake; DMD = Dry matter digestibility; NDFD = Neutral detergent fibre digestibility; OMD = Organic matter digestibility.

***SEM, Standard error of mean

Roughage and NDF intake was not affected by treatment (Table 4.3; $P > 0.05$). The mean roughage intake across treatments was 709.6 g DM/sheep/day, constituting a NDF intake (NDFI) of 1.23% BW. Köster *et al.* (1996) in a study conducted on steers fed low-quality tropical grass and supplemented with RDN, observed that RDN supplementation would only increase NDF intakes up to 1.25% BW and that RDN supplementation would not stimulate NDF intake above this level. Bohnert *et al.* (2002) observed similar, but higher observations in lambs fed low-quality forages, where NDF intakes were maximised at 1.7% BW, irrespective of supplementation, however, those observations were made in cool season (temperate) grasses.

Using the observations of Köster *et al.* (1996) as basis, roughage intake was near to maximal in the sheep in this study. It must be noted though that molasses meal *per se* contains up to 20% NDF (NRC, 2007) while pure starch contains no NDF. This could explain the numerical higher NDF intake observed in the sheep receiving the Molasses treatment; however, these differences were not significant ($P > 0.05$).

Dry matter intake (DMI) of the sheep in the Molasses treatment (911.1 g/sheep/day) was higher than the DMI of the sheep receiving the starch and urea treatments (ranging between 773.6 g/sheep/day and 811.7 g/sheep/day, Table 4.3). The exception was the sheep supplemented daily during the morning and afternoon sessions with both urea and starch (Sm+a Um+a treatment, $P > 0.05$). These observations were expected as starch was supplemented at 120 g/day while molasses was supplemented at 180 g/day to keep the supplements iso-energetic. The organic matter intake (OMI) of the SU^{2nd} treatment (supplementation only every alternate afternoon) was lower compared to the Molasses and Sm+a, Um+a treatments ($P < 0.05$) as starch and urea was only supplemented every alternate day in the SU^{2nd} treatment. However, no differences were observed ($P < 0.05$) in OMI between this treatment (SU^{2nd}) and the other treatments due to similar roughage intakes between treatments (Table 4.3).

Dry matter digestibility (DMD) differed among treatments ($P < 0.05$; Table 4.3) with the highest DMD values generally observed in the treatments where some, or all starch were supplemented during the morning supplementation period at 08h00 (Sm Ua; Sm+a Ua and Sm+a Um+a; treatment groups, Table 4.3, $P < 0.05$). The DMD of these treatments were 56.6%, 52.9% and 56.2%, respectively. The exception was the SU^{2nd} treatment (53.3%) where starch and urea were supplemented every alternate afternoon, which did not differ ($P >$

0.05) to any of the treatments (Table 4.3). The DMD of the Molasses treatment (49.7%) and the treatment groups where starch was supplemented only during the afternoon supplementation period (Sa Um+a and Sa Ua treatments at 49.15% and 51.2%, respectively) did not differ (P > 0.05, Table 4.3). The NDF digestibility (NDFD) of the sheep receiving starch during the morning supplementation periods (Sm Ua; Sm+a Um+a) were higher (P < 0.05) compared to the sheep where starch was supplemented only during the afternoon supplementation periods (Sa Um+a, Sa Ua) (Table 4.3). An exception was the Sm+a Ua treatment where starch was supplemented during both the morning and afternoon supplementation period. The NDFD of this treatment at 42.1% was lower (P < 0.05) than the other morning starch supplemental treatments (Sm Ua and Sm+a Um+a at 50.3% and 49.3%, respectively). As was observed with the DMD of the SU^{2nd} treatment, the NDFD of this treatment did not differ (P > 0.05) among treatments (Table 4.3).

Sinclair *et al.* (1993) conducting synchronisation studies in sheep, observed that *in sacco* disappearance of maize in the rumen of sheep could take up to 5 – 6 hours to complete, whereas it is almost instantaneously for urea. It could be argued that the rate of rumen fermentation of maize differs to that of starch and therefore cannot be used as an indicator of the rate of starch fermentation in the rumen. However, supplementing all, or a fraction of the total starch in the morning in the present study with urea in the afternoon session generally resulted in higher NDFD values compared to starch and urea supplementation only during the afternoon session (Table 4.3). This observation could suggest that synchronisation of urea and starch to sheep consuming low-quality *E. curvula* hay, by supplementing starch at 08h00 and urea at 16h00, might be beneficial in optimising roughage digestibility. It is of interest to note that, while NDF intake of the sheep receiving the Molasses treatment did not differ (P > 0.05) to other treatments (Table 4.3), the NDFD of the Molasses treatment was lower (P < 0.05) at 41.4% compared to the morning starch treatment groups (Sm Ua; Sm+a Um+a; 50.3% and 49.3%, respectively). However, it was similar to the treatments where starch was supplemented only during the afternoon supplementation period (Sa Um+a, Sa Ua; 37.3% and 41.7%, respectively). The nutrient content of molasses is different to starch, containing N, NDF and sugars (NRF, 2007), which could have affected the fermentation and rate of fermentation in the rumen. This aspect will be discussed in more detail later in the chapter.

An interesting point to consider is the potential NDFD of the hay as obtained from the sheep receiving the Molasses treatment. As discussed, molasses *per se* contains 20% NDF (NRC, 2007). At a daily supplementation rate of 180 g molasses, NDF supplied by the

molasses would have been 36 g/sheep/day, constituting to about 7% of the total NDF intake observed in the Molasses treatment. Assuming that the entire NDF fraction of the molasses meal was digestible, NDF digestibility from the hay alone would have been 36.9%. The possibility therefore existed that the NDFD of the hay fraction in the sheep receiving the molasses based supplement could have been significantly different from the NDFD data presented in the table. However, it is doubtful whether this "correction" would have resulted in a different interpretation as the recalculated NDFD is only slightly lower than the NDFD observed in the sheep receiving the Sa Um+a treatment (37.3%, data not shown), which did not differ ($P > 0.05$) with the NDFD of the Molasses treatment.

The OM digestibility (OMD) of the morning starch supplemented treatments (Sm Ua, Sm+a Um+a) generally were higher ($P < 0.05$) than the afternoon starch and Molasses supplemented treatments (Sa Ua, Sa Um+a, Molasses; Table 4.3). No differences ($P > 0.05$) were observed between the Molasses treatment and the afternoon starch supplemented sheep (Sa Ua, Sa Um+a). The OMD of the SU^{2nd} treatment where urea and starch was supplemented only alternate day did not differ ($P > 0.05$) to any treatment.

The results of the study suggest that the pattern of nutrient supplementation did not affect roughage intake in sheep fed low-quality *E. curvula* hay. Dry matter and OM intake differences between treatments were due to the quantities of supplements given to the sheep. However, supplementation pattern influenced DMD, NDFD and OMD with digestibility values generally higher in the sheep receiving starch during the morning supplementation period (or part thereof), coupled with urea (or part thereof), during the afternoon (16h00) session. As such, the digestible OM intake (DOMI) of the Sm+a Um+a treatment (468.1 g DOMI/sheep/day) was, apart from the Sm Ua treatment (443 g DOMI/sheep/day) higher ($P < 0.05$) compared to the other treatments (ranging between 368.2 and 419.0 g DOMI/sheep/day, Table 4.3). The DOMI of the Molasses and Sa Ua treatment was similar (407 and 419 g DOMI/sheep/day, respectively) while the DOMI of the SU^{2nd} and Sa Um+a treatments were lower at 395.8 and 368.2 g DOMI/sheep/day, respectively, compared to the other treatments. Based on the weight of the sheep, the highest ($P < 0.05$) DOMI was observed in the Sm+a Um+a treatment (1.15%) where both starch and urea was supplemented during the morning and afternoon supplementation periods.

Table 4.4 Nitrogen intake and excretion and nitrogen balance of sheep fed low-quality *Eragrostis curvula* hay as affected by the supplementation pattern of urea and starch or molasses

	*Treatments							
**Parameter	SU[2nd]	Sm, Ua	Sa, Um+a	Sm+a, Um+a	Sm+a, Ua	Sa, Ua	Molasses	***SEM
N intake (g/day)								
NI (Hay)	5.2	5.1	4.9	5.2	5.0	4.9	4.9	0.2
NI (Total)	9.2[b]	11.2[a]	11.0[a]	11.3[a]	11.1[a]	11.0[a]	11.0[a]	0.3
N excretion (g/day)								
Faeces	5.2	4.6	5.2	5.1	5.0	5.2	5.6	0.4
Urine	1.5[bc]	1.5[bc]	1.3[c]	2.1[ab]	2.3[a]	2.1[ab]	1.6[bc]	0.2
Total	6.7[b]	6.1[b]	6.5[b]	7.2[ab]	7.3[a]	7.4[a]	7.3[a]	0.5
N Balance (g/day)								
N Balance (Hay)	–1.4[ab]	–1.0[a]	–1.6[ab]	–1.9[ab]	–2.3[ab]	–2.4[b]	–2.4[b]	0.5
N Balance (Supplements)	1.6[c]	5.1[a]	4.5[ab]	4.2[ab]	3.7[b]	3.6[b]	3.7[b]	0.6
Efficiency of N Balance (ENU)	0.18[c]	0.46[a]	0.41[a]	0.37[b]	0.34[b]	0.33[b]	0.34[b]	0.05

Similar alphabetical superscripts ([a, b, c]) within a row do not differ (P > 0.05).

*Treatments: SU2[nd] (120 g starch and 13.2 urea supplemented every 2[nd] day at 16h00); Sm Ua (120 g starch supplemented daily at 08h00, 13.2 g urea daily at 16h00); Sa Um+a (6.6 g urea supplemented daily at 08h00, 120 g starch and 6.6 g urea supplemented daily at 16h00); Sm+a Um+a (60 g starch and 6.6 g urea supplemented daily at 08h00 and at 16h00); Sa Ua (120 g starch supplemented daily at 08h00, 13.2 g urea supplemented daily at 16h00); Molasses (180 g molasses, 10.75 g urea supplemented daily at 16h00).

**Parameter: NI (Hay) = Nitrogen Intake from hay = Hay intake (g DM/day/sheep * Hay N %); NI (Total) = NI (Hay) + NI (supplements); N Balance (Hay) = NI (Hay) (g/day) – Total N excretion (g/day); N Balance (Supplements) = NI (Total) (g/day) – Total N excretion (g/day). Apparent efficiency of N Balance (ENU) = N Balance (Suppl. Incl.) / Total N Intake. **SEM, Standard error of mean

Nitrogen intake from the hay alone did not differ ($P > 0.05$) between treatments (Table 4.4) due to similar hay intakes (Table 4.3). Total N intake (NI) was more than 10% lower ($P < 0.01$) in the SU^{2nd} treatment (9.2 g N/sheep/day) compared to the other treatments (ranging between 11.0 – 11.3 g N/sheep/day) (Table 4.4) and was due to the supplementation frequency where urea and starch was supplemented every alternate day (*versus* a daily supplementation in the other treatments). The similar N intake between the daily supplemented treatments was due to a similar roughage intake among treatments (Table 4.3), as the quantity of supplements did not vary among treatments.

Faecal N excretion did not differ ($P > 0.05$) between treatments, even though total NI differed between the SU^{2nd} and other treatments (Table 4.4). The similar faecal N excretion among treatments was due to similar roughage intake observed between treatments (Table 4.3). In contrast to faecal N excretion, urinary N excretion differed ($P < 0.05$) between treatments (Table 4.4). Higher urinary N excretion ($P < 0.05$) were observed in the sheep receiving the Sm+a Ua treatment (2.3 g N/day) compared to the sheep receiving the Molasses, SU^{2nd}, Sm Ua or Sa Um+a treatments (ranging between 1.3 and 1.6 g N/day). Urinary N excretion of the Sa Um+a treatment (1.3 g N/day) differed ($P < 0.05$) to the urinary N excretion of the sheep receiving the Sm+a Um+a or Sa Ua treatments (2.1 g N/sheep/day for both treatments). While no clear trend was observed between treatments, in general the urinary N excretions was higher in treatments where starch was supplemented during both the morning (08h00) and afternoon (16h00) supplementation periods (Sm+a Ua; Sm+a Um+a; Table 4.4), possibly indicating that more ingested N was excreted in the urine as starch supplementation frequency increased. Potthast *et al.* (1977) feeding N free diets to sheep, observed that N recycling towards the rumen increased as sucrose supplementation was increased. It was a therefore a possibility that N recycling to the rumens of sheep supplemented twice daily with starch was higher compared to the sheep supplemented only once daily with starch, resulting in the higher urinary N excretion as observed. However, N recycling was not measured in the present study, and as such, it is not clear whether the higher urine N excretion from the more frequent starch supplemented treatments were due to higher N recycling towards the rumen.

The N balance across treatments suggested that the sheep would have been in a negative N balance if only hay was to be fed, ranging between a minimum of –2.4 g N/sheep/day (Sa Ua) to a maximum of –1.0 g N/sheep/day (Sm Ua) (Table 4.4). The theoretically negative N balance from the hay alone emphasises the poor quality of the hay

and the general need for N supplementation to ruminants consuming low-quality tropical roughages. Due to differences in NI and excretion among treatments, N balance (Supplements), calculated as total N intake (supplements included) – N excretion (from both urine and faeces), differed ($P < 0.05$) between treatments (Table 4.4). The N balance of the sheep supplemented every second day (SU^{2nd} treatment) was, as expected, more than 50% lower at 1.6 g N/sheep/day compared to the sheep receiving supplements daily (ranging between 3.6 to 5.1 g N/day). The N balance (Treatments) of the Sm Ua (5.1 g N/day) was higher ($P < 0.05$) compared to the Sm+a Ua (3.7 g N/day), Sa Ua (3.6 g N/day) or Molasses (3.7 g N/day) treatments (Table 4.4). Apparent ENU ranged between 0.18 for the SU^{2nd} treatment to 0.46 for the Sa Um+a treatment (Table 4.4) and generally were collated with N Balance of the sheep, supplements included. The apparent ENU of the Sm Ua and Sa Um+a treatments were higher ($P < 0.05$) at 0.46 and 0.41 respectively while the apparent ENU of the SU^{2nd} at 0.18 was lower ($P < 0.05$) compared to the other treatments.

In the present study, total NI of the sheep in the SU^{2nd} treatment was 22.8% lower compared to the other treatments (9.2 g N/sheep/day *versus* 11.0 to 11.3 g N/sheep/day for the rest of the treatments; Table 4.4). However, the apparent ENU of the SU^{2nd} treatment was more than 50% lower ($P < 0.05$) at 0.18 compared to the rest of the treatments, ranging between 0.33 and 0.46 (Table 4.4). In an earlier study conducted on sheep fed low-quality *E. curvula* hay (Chapter 3), the level of urea supplementation had a marked effect on apparent ENU. Higher apparent ENU values (increasing from 0.22 to 0.58) were recorded as the level of urea was increased from 10.4 g urea/sheep/day to 32.4 g urea/sheep/day (Table 3.7). As in the present study, the higher apparent values were the result of similar faecal N excretion among treatments (Table 3.7). Based on the results of these two studies, it can be concluded that supplementation of urea not only increases the "pool" of N compounds to the ruminant, but also the fraction or percentage of N to be retained in the body in sheep receiving low-quality *E. curvula* hay.

The apparent ENU values of the Sm Ua and Sa Um+a treatments were higher ($P > 0.05$) at 0.46 and 0.41, respectively, compared to the other treatments (ranging between 0.33 and 0.37 where the supplements were supplemented daily; Table 4.4). As NI and faecal N excretion among treatments were similar, the higher apparent ENU values observed for these treatments were due to lower urinary N excretions in these treatments (Table 4.4). Sinclair *et al.* (1993) suggested that synchronisation of nutrients within the rumen could improve nutrient utilisation in the ruminant. The authors further observed that the rate of fermentation

between maize and urea differ (Sinclair *et al.*, 1993). While the rate of fermentation of maize cannot be used as an indicator of the rate of starch fermentation, it could be argued that, due to the high solubility of urea, a time difference had existed between the release of fermentation products from starch and urea in the rumen when supplemented simultaneously. As such, it was a possibility that the higher apparent ENU of the Sm Ua treatment was the result of starch supplementation during the morning period (08h00) while urea was supplemented at 16h00, resulting in a better utilisation of the individual nutrients within the daily supplement. The same argument could be given for the Sa Um+a treatment where starch supplementation in the afternoon was followed by urea supplementation the following morning. It is of interest to note that the apparent ENU of the Sm+a Um+a and Sm+a Ua treatments, where starch supplementation was divided, were lower ($P < 0.05$) at 0.37 and 0.34 compared to the apparent ENU of the Sm Ua and Sa Um+a treatments. As such, a more frequent (from once daily to twice daily) supplementation of starch did not increase the apparent ENU in sheep fed low-quality *E. curvula* hay and more research is necessary.

Table 4.5 shows the RAN concentration (mg RAN/dL rumen fluid) of sheep fed low-quality *E. curvula* hay as affected by the supplementation pattern of urea and starch or molasses.

Table 4.5 Rumen ammonia nitrogen concentration of sheep fed low-quality *Eragrostis curvula* hay as affected by the supplementation pattern of urea and starch or molasses (mg NH_3-N/dL rumen fluid)

	*Treatments								
Time	***SU^{2nd}***	***Sm, Ua***	***Sa, Um+a***	***Sm+a, Um+a***	***Sm+a, Ua***	***Sa, Ua***	***Molasses***	**Mean**	****SEM**
03h00	6.4^{d}	$11.1^{bc}{}_{2}$	$7.1^{cd}{}_{3}$	11.0^{bc}	$18.7^{a}{}_{1}$	$14.8^{ab}{}_{2}$	7.8^{cd}	11.0_{1}	1.7
09h00	6.7^{c}	$6.7^{c}{}_{3}$	$25.5^{a}{}_{1}$	13.6^{b}	$11.9^{b}{}_{2}$	$10.6^{bc}{}_{3}$	9.4^{b}	12.5_{1}	2.4
15h00	4.1^{c}	$3.9^{c}{}_{4}$	$12.0^{a}{}_{2}$	11.8^{a}	$6.8^{b}{}_{3}$	$6.6^{b}{}_{3}$	5.6^{b}	7.3_{2}	1.3
21h00	5.6^{d}	$14.2^{c}{}_{1}$	$13.2^{c}{}_{2}$	12.8^{c}	$20.5^{a}{}_{1}$	$18.6^{b}{}_{1}$	7.2^{d}	11.6_{1}	2.1
Mean	5.8^{d}	8.9^{c}	14.5^{a}	12.3^{b}	14.5^{a}	12.7^{b}	7.5^{cd}	10.6	0.5
*****SEM***	0.6	2.3	3.9	0.6	3.2	2.6	0.8		

Similar alphabetical superscripts ($^{a, b, c}$) within a row do not differ ($P > 0.05$). Similar numerical subscripts ($_{1, 2, 3}$) within a column do not differ ($P > 0.05$). Treatment and treatment*time interactions differed ($P < 0.05$), time differed ($P < 0.05$).

*Treatments: SU2nd (120 g starch and 13.2 urea supplemented every 2nd day at 16h00); Sm Ua (120 g starch supplemented daily at 08h00, 13.2 g urea daily at 16h00); Sa Um+a (6.6 g urea supplemented daily at 08h00, 120 g starch and 6.6 g urea supplemented daily at 16h00); Sm+a Um+a (60 g starch and 6.6 g urea supplemented daily at 08h00 and at 16h00); Sa Ua (120 g starch supplemented daily at 08h00, 13.2 g urea supplemented daily at 16h00); Molasses (180 g molasses, 10.75 g urea supplemented daily at 16h00).

**SEM, Standard error of mean

The lowest RAN concentration was observed in the SU^{2nd} treatment. This was expected as starch and urea was supplemented every second day, therefore, the wethers receiving the SU^{2nd} supplements only were effectively supplemented half of the daily starch and urea. In addition, RAN observation in the SU^{2nd} treatment was the result of pooled rumen fluid measurements on the supplementation and non-supplementation days. It was therefore a possibility that, on the supplementation days, RAN could have been comparable with the other treatments (and even lower on the non-supplemental days).

In contrast to the SU^{2nd} treatment, the highest mean RAN concentrations were observed in the Sa Um+a and Sm+a Ua treatments (14.5 mg RAN/dL rumen fluid for both treatments; Table 4.5). This observation was expected in the Sa Um+a treatment as urea was

supplemented twice daily during the morning (08h00) and afternoon (16h00) supplementation periods, especially at 09h00 where 6.6 g urea was supplemented at 08h00 without starch in the Sa Um+a treatment. In contrast to the Sa Um+a treatment, starch was supplemented twice daily in the Sm+a Ua treatment, and not urea. It is therefore less obvious why the mean RAN concentration of this treatment was so high compared to the other treatments. In a study conducted by Potthast *et al.* (1977) on sheep receiving N free diets, the authors observed that up to 9.5 g N (59.4 g CP) recirculated to the rumen when sucrose was supplemented intra-ruminally. It is a possibility that, due to the more frequent starch supplementation pattern in the Sm+a Ua treatment, more N was recycled towards the rumen compared to the other treatments, resulting in the higher mean RAN concentration.

The RAN concentration of the sheep receiving the Sa Ua treatment was, compared to the sheep receiving the Molasses treatment, consistently higher ($P > 0.05$) across all periods. Therefore, mean RAN concentrations differed ($P < 0.05$) between the two treatments with the Molasses treatment being 40% lower than the Sa Ua treatment (7.5 and 12.7 mg RAN/dL rumen fluid, respectively; Table 4.5). In both these two treatments, supplementation of both urea and the energy happened during the afternoon supplementation period (16h00). Timing of supplementation therefore cannot explain differences observed between the two treatments. The quantity of urea supplemented between the two treatments, however, differed, with the Sa Ua treatment being supplemented having 23% more urea than the Molasses treatment (13.20 g and 10.75 g urea, respectively). The higher quantity of urea supplementation at 16h00 could explain the RAN differences between the two treatments, especially as RAN at 21h00 of the Sa Ua treatment was almost three times higher ($P < 0.05$) compared to the Molasses treatment (20.5 and 7.2 mg RAN/dL rumen fluid, respectively; Table 4.5). However, RAN of the Sa Um+a and Sm+a Um+a treatments, where only 6.6 g urea was supplemented at 16h00, was 45% higher at 21h00 (13.2 and 12.8 mg RAN/dL, respectively) compared to Molasses treatment (7.5 mg RAN/dL rumen fluid; Table 4.5). It is therefore doubtful whether quantity of urea supplementation alone could explain the differences in RAN observed between the Molasses and Sa Ua treatment, especially at 21h00. Other factors that also could have contributed to the lower RAN concentrations observed in the Molasses treatment compared to the Sa Ua treatment, include the nutrient and chemical composition of molasses compared to starch. Molasses contain 0.6% N and 20% NDF (NRC, 2007). It could be argued that the availability and rate of N degradation of molasses is lower compared to the N fraction of urea, which could have contributed to a more even distribution

of RAN throughout the day in the Molasses treatment. As such, the rumen microbes could use N more efficiently for MNS. Another factor that could explain the observed RAN differences between the Molasses treatment and the Sa Ua treatment is the type of carbohydrates found in molasses *versus* starch. Molasses contain more sugars with a higher rate of fermentation compared to starch (Leng, 1995). As such, it could have been more in synchronisation with N degradation from urea (Leng, 1995) resulting in the lower and more consistent RAN concentrations observed in the Molasses *versus* the Sa Ua treatment.

The mean RAN concentrations of the sheep across periods were relatively constant, with only the mean RAN at 15h00 being lower ($P < 0.05$) than the mean RAN at the other measured periods (7.3 mg RAN/dL rumen fluid at 15h00 *versus* 11.0 – 12.5 mg RAN/dL rumen fluid for the other periods; Table 4.5). The low RAN concentrations observed at 15h00 could be explained by the relative time interval between morning supplementation at 08h00 and the measured time at 15h00. However, the relative high RAN concentration measured across treatments at 03h00 (Table 4.5) is less clear. It is a possibility, as discussed in the previous trial (Chapter 3), that rumen fill probably was at a minimum at 03h00 as the sheep would not have eaten to the same level during the night hours, compared to the day hours. This could have resulted in less rumen fluid (relative to during the day period), resulting in elevated RAN concentrations. In addition, urea was supplemented during the afternoon supplementation period in all the treatments, while during the morning supplementation period; urea was supplemented in only two of the treatments (Sa Um+a and Sm+a Um+a). Rumen ammonia N of those two treatments at 15h00 (12.0 and 11.8 mg RAN/dL rumen fluid, respectively) was almost double ($P < 0.05$) the RAN of the other treatments at 15h00, ranging between 3.9 and 6.8 mg RAN/dL rumen fluid; Table 4.5). These observations suggest that, even though the solubility of urea is almost instantaneous and degraded within an hour (Sinclair *et al.*, 1993), the effects of urea supplementation on RAN were still observed 7 hours after supplementation in this trial (between 08h00 and 15h00).

Within treatments and time, the highest RAN concentration at 09h00 was observed in the Sa Um+a treatment (25.5 mg RAN/dL rumen fluid; Table 4.5) where, during the morning supplementation period, only urea was supplemented (starch and urea was supplemented during the afternoon supplementation period). This was to be expected, as the disappearance of urea in the rumen is almost instantaneous, with most of the urea disappearing within an hour (Sinclair *et al.*, 1993, 1995). In contrast, at 21h00, the highest RAN concentration (20.5 mg RAN/dL rumen fluid) was observed in the Sm+a Ua treatment (Table 4.5) where 13.2 g

urea and 60 g starch was supplemented at 16h00. It could be argued that the effects of urea supplementation at 16h00 on RAN would have been diminished by 21h00 as the solubility of urea is almost instantaneous. However, as discussed earlier, urea supplementation stimulated RAN for long periods, up to 7 hours and as such, elevated RAN concentrations observed at 21h00 could have been the result of urea supplementation at 16h00. However, if RAN were only determined by timing of urea supplementation, the highest RAN at 21h00 would have been observed from the Sm Ua treatment where all the starch was supplemented at 08h00 and all the urea (13.2 g urea) at 16h00. However, RAN of this treatment at 21h00 was 30% lower at 14.2 mg RAN/dL rumen fluid compared to the Sm+a Ua treatment, suggesting that the pattern of starch supplementation influenced RAN as well.

The lowest RAN generally were observed at 15h00, with RAN of the SU^{2nd} and Sm Ua treatments at 4.1 and 3.9 mg RAN/dL rumen fluid, respectively, the lowest at that hour (Table 4.5). This was to be expected, as urea was not supplemented during the morning supplementation periods in both the Sm Ua and SU^{2nd} treatments. In addition, supplementation (of both urea and starch) only occurred every second day during the afternoon supplementation period (16h00) in the SU^{2nd} treatment. It is of interest to note that RAN of both the Molasses treatment as well as the Sa Ua treatment was similar, but higher ($P < 0.05$) than the SU^{2nd} and Sm Ua treatments, at 5.6 and 6.8 mg RAN/dL rumen fluid, respectively, at that hour. In both treatments, as with the SU^{2nd} and Sa Ua treatments, urea was supplemented only during the afternoon supplementation period. As such, it was expected that RAN in both these treatments would have been like that of the sheep receiving the Sm Ua treatment. Lastly, it is noticeable that the most constant RAN concentration across all periods were observed in the Sm+a Um+a treatment where the variation in RAN concentration between the highest RAN measured (13.6 mg RAN/dL rumen fluid at 09h00) and the lowest RAN measured (11.0 mg RAN/dL rumen fluid) was 19%. In addition, the observed RAN concentrations in this treatment is within the recommendations suggested by Detmann *et al.* (2009), optimising roughage intake, degradability and MNS in tropical roughage fed ruminants. Based on these RAN observations, in can be concluded that the most optimal supplementation pattern in sheep receiving low-quality *E. curvula* hay is a supplementation pattern in which there is a constant supply of both urea and starch (Sm+a Um+a).

Table 4.6 Microbial nitrogen synthesis (MNS): available nitrogen incorporated into microbial nitrogen (MNS:NI) and efficiency of microbial nitrogen synthesis (EMNS) of sheep fed low-quality *Eragrostis curvula* hay as affected by the supplementation pattern of urea and starch or molasses

[1] *Treatments*	*SU[2nd]*	*Sm, Ua*	*Sa, Um+a*	*Sm+a, Um+a*	*Sm+a, Ua*	*Sa, Ua*	*Molasses*	[3]*SEM*
[2]**MNS (g/day)**	7.08^{c}	8.28^{bc}	6.39^{c}	10.83^{a}	9.75^{ab}	7.69^{bc}	10.17^{ab}	0.87
[2]**MNS:NI (available)**	1.09	0.96	0.75	1.25	1.14	0.91	1.17	0.07
[2]**EMNS (g MNS/kg DOMI)**	10.99^{c}	11.60^{bc}	11.35^{c}	15.16^{abc}	15.86^{ab}	13.82^{abc}	17.16^{a}	1.56

Similar alphabetical superscripts (a, b, c) within a row do not differ significantly (P > 0.05).

[1]Treatments: SU2[nd] (120 g starch and 13.2 urea supplemented every 2[nd] day at 16h00); Sm Ua (120 g starch supplemented daily at 08h00, 13.2 g urea daily at 16h00); Sa Um+a (6.6 g urea supplemented daily at 08h00, 120 g starch and 6.6 g urea supplemented daily at 16h00); Sm+a Um+a (60 g starch and 6.6 g urea supplemented daily at 08h00 and at 16h00); Sa Ua (120 g starch supplemented daily at 08h00, 13.2 g urea supplemented daily at 16h00); Molasses (180 g molasses, 10.75 g urea supplemented daily at 16h00).

[2]MNS = Microbial nitrogen synthesis (g/day); EMNS = Efficiency of MNS (g MNS/kg DOMI where DOMI = Digestible organic matter intake; MNS:NI (available) is the ratio of MNS: available N intake, where available N intake = N intake from hay + urea N intake – ADIN intake.

[3]SEM = Standard error of mean

The mean MNS across treatments was 8.60 g N/day/sheep which is almost 50% lower than the values recorded in sheep fed low-quality *E. curvula* hay in an earlier study (15.3 g N/day/sheep; Chapter 3, Table 3.12). However, the sheep used in the present study were smaller (mean 40.5 kg) compared to the sheep used in the earlier study (mean 50 kg). In addition, starch supplemented in this trial was 120 g starch/sheep/day (3 g starch/kg BW), while it ranged between 240 g to 280 g starch/sheep/day in the earlier study (4.8 to 5.6 g starch/kg BW). As starch or FME supplementation stimulates MNS in the roughage fed ruminant (Leng, 1990, 1995), a statement also confirmed by the results obtained from the earlier study (Table 3.13), the lower quantity of FME or DOMI in this trial compared to the earlier trial could have been responsible for the lower MNS observed in this trial compared to the earlier trial, even though the wethers were lighter (20%).

Microbial N synthesis differ ($P < 0.05$) among treatments with MNS of sheep receiving Molasses (10.17 g N/day/sheep), Sm+a Um+a (10.83 g N/day/sheep) and Sm+a Ua (9.75 g N/day/sheep) treatments higher than the sheep receiving SU^{2nd} (7.08 g N/day) or Sa Um+a (6.39 g N/day) treatments. While the results from the Molasses treatment is an exception, results do suggest that a more constant supply of starch (FME) might result in higher MNS outputs in ruminants consuming low-quality *E. curvula* hay. This observation agrees with Leng (1990), stating that MNS is stimulated by energy supplementation in the roughage fed ruminant. Russell (1989) also suggested that ruminants do not have the capacity to store excess energy in the rumen as FME forms toxic compounds (methylglyoxal) in the rumen when not used. In addition, energy cannot be recirculated to the rumen (Russell and Strobel, 2005). Fermentable energy therefore needs to be supplemented constantly to maximise MNS in the roughage fed ruminant, which agrees with results obtained by Henning *et al.* (1993) in sheep fed maize straw.

The lowest MNS were observed in the SU^{2nd} and Sa Um+a treatments (7.08 and 6.39 g MNS/sheep/day, respectively). The MNS of these treatments were lower than the MNS of the Molasses treatment and the treatments where starch was supplemented twice daily (Table 4.6). This observation again highlights the importance of a more frequent supplementation of starch compared to urea to maximise MNS production in the topical roughage fed ruminant.

The MNS of the Molasses treatment (10.17 g MNS/sheep/day) and Sa Ua treatment (7.69 g MNS/sheep/day) did not differ ($P > 0.05$). Leng (1995) suggested that tropical forage fed ruminants “perform” better when supplemented with urea and molasses *versus* urea and

maize (starch) due to a better synchronisation between sugars found in molasses and urea compared to starches and urea. In terms of MNS production, results obtained from the present study do not confirm this hypothesis, even though a tendency was observed in favour of the Molasses treatment.

The ratio of MNS:NI (available) in this study was used as an indicator of the relative incorporation of available N taken by the sheep (measured as total N intake – ADIN intake) into MNS. Due to inefficiencies in the rumen, where microbial bacteria incorporate ammonia into microbial N, the MNS:NI ratio should be a below one, but preferably as low as 0.69 (Detmann *et al.*, 2014). Ratios higher than one are indicative that body N was used to produce MNS (Detmann *et al.*, 2014). The mean MNS:NI (available) across treatments was 1.03 and did not differ ($P > 0.05$) between treatments (Table 4.6), indicating that the wethers were not protein deficient (Detmann *et al.*, 2014). The observed MNS:NI (available) ratio was the result of the experimental supplements formulation as stated by the AFRC (1993) recommendations for wethers fed low-quality *E. curvula* hay. The Molasses treatment was formulated to be iso-energetic to the rest of the starch treatments, explaining the similar MNS:NI (available) ratio observed compared to the other starch treatments (Table 4.6).

The mean efficiency of MNS (EMNS), measured as MNS per unit digestible OM intake (DOMI), was 13.71 g MNS/kg DOMI. This value is well below the average EMNS (20.8 g MNS/kg DOMI) for tropical forages (SCA, 1990). The low EMNS is probably related to the quality of the hay used, even though starch or molasses and urea was supplemented. The rate of NDF degradation of a similar *E. curvula* hay was 2%/h in an earlier trial conducted on wethers (Table 2.3), which could have limited the EMNS (Poppi *et al.*, 1999). In addition, in the formulation of the supplements, the MP requirements of the wethers were not met (AFRC, 1993) as the quantity of urea to be supplemented had to be limited to reduce the possible occurrence of ammonia toxicity. As such, the quantity of starch supplemented was reduced as well based on the AFRC (1993) ratio of 9 g RDP/MJ FME. The lower intakes of both urea and starch in terms of requirements could have depressed EMNS (as well as MNS) as FME (Leng, 1990) and especially NSC or WSC is a prerequisite of MNS (Poppi *et al.*, 1999).

Efficiency of MNS was higher ($P < 0.05$) in sheep receiving the Molasses treatment compared to the sheep receiving the Sm, SU^{2nd} and Sa Um+a treatments (Table 4.6). The

EMNS of the sheep receiving supplements only every second day (SU^{2nd}) and the Sa Um+a treatment was lower ($P < 0.05$) than the sheep receiving the Sm+a Ua treatment.

The relative lower daily energy supplementation of this treatment compared to the other treatments could explain the lower EMNS values observed in the SU^{2nd} treatment. Energy drives MNS in the tropical forage fed ruminant (Leng, 1990). As energy supplementation only occurred every second afternoon in the SU^{2nd} treatment (*versus* daily supplementation in the other treatments), MNS production was not optimal (Table 4.6), thereby reducing EMNS (Table 4.6). The lower ($P < 0.05$) EMNS observed in the Sa Um+a compared to the Sm+a Ua treatment suggested that a higher frequency of energy (starch) *versus* N (urea) supplementation might stimulate EMNS more in sheep fed low-quality *E. curvula* hay. No differences ($P > 0.05$) were observed between the sheep receiving the Molasses treatment, Sm+a Um+a; Sm+a Ua or Sa Ua treatments (Table 4.6). The similarity in EMNS between the sheep receiving the Molasses and Sa Ua treatments suggests no benefit in supplementing ruminants with molasses and urea compared to starch and urea.

4.6 Summary and Conclusion

The purpose of this trial was to study the effect of nutrient synchronisation through supplementation in sheep receiving low-quality *E. curvula* hay. While roughage intake was similar among treatments, roughage digestibility appeared to be higher in the treatments where starch was supplemented at least partly, during the morning supplementation periods. Treatment did not influence nitrogen intake; neither was N excretion through faeces. In contrast, urinary N excretion was higher in the treatments where starch was supplemented during both the morning and afternoon supplementation period compared to the other treatments. Despite the higher N excretion observed in those treatments, N balance did not follow a clear trend across treatments except for the SU^{2nd} treatment, which was lower due to lower levels of daily N supplementation. However, despite the lower N balance observed in this treatment, only 18% of the total N intake was retained. In contrast, the percentage N retained was considerably higher in the rest of the treatments, ranging between 33% and 46%. In addition, more N was retained in the body in the treatments where starch was only supplemented once daily with urea 12 hours later (Sm Ua and Sa Um+a treatments). The most consistent RAN concentration, apparently sufficient to create the optimal rumen milieu in the tropical roughage fed ruminant (Detmann *et al.*, 2009), was achieved in the Sm+a Um+a treatment where both starch and urea was supplemented during both the morning and

afternoon supplementation sessions. Microbial N synthesis in general was higher in the Molasses treatment and treatments where starch was supplemented during both morning and afternoon supplementation periods. Efficiency of MNS generally followed the same trend as MNS across treatments, with the highest EMNS generally observed in the Molasses and treatments where starch was supplemented more frequently (twice daily) compared to only once daily. It is concluded that the most optimal rumen milieu was achieved in the treatment where both urea and starch was supplemented twice daily. However, the supplementation frequency of starch was the more important parameter compared to urea, as supplementation of starch during both the morning and afternoon supplementation period tended to stimulate MNS and EMNS more compared to urea supplementation frequency. In addition, there is merit in the statement that sheep receiving low-quality tropical roughages and supplemented, once daily with molasses and urea compared to starch, might perform better due to a higher synchronisation between sugars and urea compared to starch and urea. However, differences were not always consistent, and more research is necessary to test these hypotheses.

Chapter 5 Review of supplementation studies conducted with sheep fed low-quality *Eragrostis curvula* hay at the University of Pretoria during 2007 – 2013 using meta-analytical techniques

5.1 Introduction

In an earlier study conducted (Chapter 3), it was established that wethers "require" more RDN than suggested in the feeding tables (NRC, 2007) to fulfil maintenance requirements. As such, supplementation intakes of urea up to 26.4 g urea/sheep/day for 50 kg wethers were needed to optimise roughage intake, digestibility and MNS in sheep grazing low-quality (0.4% N, 80% NDF) *E. curvula* hay (tropical hay). However, while it seemed that energy (starch) supplementation had a positive effect on MNS and EMNS, which is in accordance with suggestions by Leng (1990) and Poppi *et al.* (1999), the effects of starch supplementation on these parameters could not be tested as starch was correlated with period effect.

Results from a single experiment cannot be used as basis for prediction purposes because the conditions under which observations were made are narrow and specific to that study. In a series of studies conducted during 2007 – 2013 at the University of Pretoria's experimental farm, the quantities of urea and/or starch supplemented to sheep receiving poor quality *E. curvula* hay, differed. As such, experiments were repeated to verify the generality and repeatability of the observations that were made, as well as to challenge the range of applicability of the observed results and conclusions. In this context, there was a need to summarise the findings across all the studies, using a meta-analytical approach.

The objective of this review was to combine the results of those studies to evaluate relative influences of supplemental starch and urea on the efficiency of N utilisation in sheep fed low-quality *E. curvula* hay, using meta-analytical methods.

5.2 Hypotheses

The following hypotheses were tested:

1. Starch supplementation affected roughage intake and digestibility, MNS and EMNS and N balance of sheep fed low-quality *E. curvula* hay and supplemented with urea and starch.

2. Urea supplementation affected roughage intake and digestibility, MNS and EMNS and N balance of sheep fed low-quality *E. curvula* hay and supplemented with urea and starch.

3. In addition to starch supplementation, urea affected roughage intake and digestibility, MNS and EMNS and N balance of sheep fed low-quality *E. curvula* hay and supplemented with urea and starch.

4. In addition to urea supplementation, starch affected roughage intake and digestibility, MNS and EMNS and N balance of sheep fed low-quality *E. curvula* hay and supplemented with urea and starch.

5. Starch*urea interactions affected roughage intake and digestibility, MNS and EMNS and N balance of sheep fed low-quality *E. curvula* hay and supplemented with urea and starch.

5.3 Materials and Methods

The dataset used to evaluate the nutritional characteristics and the efficiency of N utilisation, was compiled from four studies and six trials (Chapters 2 and 3; Du Plessis, 2011; Mentz *et al.*, 2013) totalling 123 data points, using linear mixed model meta-analysis, also known as REML analysis (Payne, 2012). Meta-analytical principles, *versus* ANOVA, were used to analyse the data to minimise biases between datasets (Mrs M. Smith, personal communication, marie.smith@stats4science.com).

Inclusion criteria for datasets include species sheep weighing between 37 kg BW and ranging between 37 kg and 60 kg BW) and basal feeding. Among all trials, sheep were fed low-quality tropical hay (ranging between 0.4% N and 0.7% N, > 65% NDF). In addition, all sheep were supplemented with urea and starch. The trials were all conducted at the same

location (experimental farm of the University of Pretoria, Hatfield, South Africa). The following variables were considered: digestible organic matter (DOM) and N concentrations (N%) of the diets, mean daily RAN, roughage dry matter intake (RDMI), neutral detergent fibre intake (NDFI), neutral detergent fibre digestibility (NDFD), starch intake, urea intake, DOM intake (DOMI), N intake (NI) and MNS. Apparent nitrogen balance (NB) was calculated as NB = NI – (faecal N + urinary N).

The mean daily RAN concentrations were calculated from pooled rumen fluid samples collected over a 24-hour period. In trials where RAN were observed over time-periods, daily RAN concentration was calculated as the mean of these observations. The production of MNS was estimated by urinary excretion of purine derivatives as described by Chen and Gomes (1992). To compare individual animals within and across treatments, intakes (NI, urea, and starch, RDMI, DOMI and NDFI) were expressed as a percentage of the bodyweight.

To minimise interference and bias between trials due to differences in intake and size of the sheep used between trials, the variables associated to the efficiency of N utilisation were expressed as ratios as described by the following equations:

MNS:NI

NB = NI – N apparently excreted (faecal + urinary)

ENU = NB:NI

EMNS = MNS: DOMI

where MNS:NI is the relative production of MNS to NI. Apparent NB was calculated as NI – N excreted in faeces and urine. The apparent efficiency of N utilisation (ENU) is the apparent N balance (NB) of the sheep (g/d) to NI (g N retained / g N intake) while efficiency of microbial N synthesis (EMNS) is MNS production relative to DOMI (g MNS/kg DOMI).

5.4 Statistical Analysis

Linear mixed model meta-analysis, also known as REML analysis (Payne, 2012), was applied to MNS, NDFI, EMS and NDFD data from four different trials. Prior to meta-analysis, the discriminating variables, NI, starch intake, RAN, NDFD and MNS (the rest were noise variables), were evaluated using canonical variate analysis (CVA), also known as linear discriminant analysis (GenStat® CVA procedure, 2012), to verify if there were differences between the four trials, or if some trials should be merged (Detmann *et al.*, 2014).

The CVA plot indicated that the four trials were different at the 5% level, as the 95% confidence regions around the canonical variate mean scores did not overlap. Therefore, in the meta-analysis, different error models were specified for each trial. The fixed effects were specified as starch, urea and starch by urea interaction, and the random effect was specified as trial. Akaike's information criterion (AIC, Akaike, 1973), as well as the R-square of the adjusted models, calculated as the square of the correlation between the predicted and observed values (Detmann *et al.*, 2014).

The statistical program GenStat® (Payne, 2012) was used for the analysis of data.

5.5 Results and Discussion

The overall purpose of the meta-analysis was to study the relative effects of starch and/or urea used as supplements, on roughage intake and digestibility, MNS and N efficiency in sheep fed low-quality *E. curvula* hay.

Meta-analytical studies are normally conducted on means from trials with different statistical designs and number of observations, resulting in different standard errors. Data therefore needs to be “weighed” using various criteria, including standard error of the mean, size of the trials or quality or precision of the individual trials (Sauvant *et al.*, 2008). However, individual raw data from the different trials could also be used in meta-analysis. Sauvant *et al.* (2008) suggested that such meta-analysis, conducted from primary raw data, is preferred to a meta-analysis study conducted on summary statistics. Data from such studies also need to be weighed (Sauvant *et al.*, 2008) which was performed in this meta-analysis using CVA analysis and different error models for each trial. In addition, individual intakes

were expressed as percentages of individual body weights across trials while N efficiency was expressed relative to intake (Detmann *et al.*, 2014)

Table 5.1 gives an overall description of the dataset derived from supplementation trials conducted on sheep fed low-quality *E. curvula* hay and supplemented with N and fermentable metabolisable energy at the experimental farm of the University of Pretoria during 2007 – 2013.

Table 5.1 Description of the dataset derived from supplementation trials conducted on sheep fed low-quality *Eragrostis curvula* hay and supplemented with nitrogen and fermentable metabolisable energy at the experimental farm of the University of Pretoria during 2007 – 2013

Identifier	*Minimum*	*Mean*	*Maximum*	*% Difference*	*Number*
NDFI (g/kg BW)	8.1	13.3	20.9	61.0	123
RDMI (g/kg BW)	10.4	17.3	26.3	60.3	123
NI (g/kg BW)	1.4	2.4	4.3	67.1	123
DOMI (g/kg BW)	7.1	11.5	18.2	60.8	123
N: DOM	12.61	23.09	38.74	67.4	123
N (% DM)	0.70	1.19	2.00	64.8	123
Starch (g/kg BW)	1.407	3.052	5.210	73.0	123
Urea (g/kg BW)	0.186	0.353	0.701	73.5	123
RAN (mg RAN/dL rumen fluid)	2.60	10.00	28.26	90.8	123
Starch: available CP (g/g)	1.46	2.61	5.79	74.7	123
NB (g N/day)	–12.48	17.94	37.50	132.1	123
NDFD (%)	31.26	52.77	75.45	58.6	123
MNS (g/kg BW)	0.129	0.235	0.416	69.0	123
MNS:NI (g/g)	0.653	1.263	2.990	78.2	123
EMNS (g MNS/kg DOMI)	7.136	16.40	35.33	79.8	123
ENU (g/g)	–0.3208	0.4352	0.6912	100.5	123

NDFI = neutral detergent fibre intake; RDMI = roughage dry matter intake; NI = Nitrogen intake; DOMI = digestible organic matter intake; N:DOM = N % relative to the digestible organic matter of the total diet (% N/kg DOM), NI (% DM) = Nitrogen % relative to the total diet DM intake; Starch = starch intake; Urea = urea intake; RAN = mean rumen ammonia N concentration measured over 24 hours (mg RAN/dL rumen fluid); Starch: available CP = starch supplemented to available CP where available CP = (Hay N intake + Urea N intake – ADIN intake) * 6.25 (g starch / g available CP); NB = Apparent N balance (N intake – N excreted faeces and urine) (g N/day) (McDonald *et al.*, 2011); NDFD = neutral detergent fibre digestibility (%); MNS = microbial N supply (g/day) expressed as g/kg BW; MNS:NI = efficiency of MNS over N intake (g MNS/g NI/day); EMNS = efficiency of MNS per kg digestible organic matter intake (g MNS/kg DOMI); ENU = efficiency of N use, calculated as NB / NI (g N retained / g NI).

Detmann *et al.* (2014) conducted a similar meta-analysis on cattle fed low-quality tropical roughages in Brazil. The values of the parameters observed in this meta-analysis generally agree with the values observed by Detmann *et al.* (2014). However, in the study conducted by Detmann *et al.* (2014), the cattle were only supplemented with N compounds, while both urea and starch were supplemented to sheep in this meta-analysis.

The differences between the lowest (minimum) and highest (maximum) values (expressed as a percentage of the maximum values) within each parameter (Table 5.1), highlight the differences between trials. The similarities between the trials included the type of animals (sheep), basal feed (low-quality *E. curvula* hay), supplemental regimes (urea and starch) and location (experimental farm of the University of Pretoria, Hatfield, South Africa). Despite these similarities, differences between and within the different trials resulted in differences observed within the parameters. These included the age of the sheep (one to five years), weight (ranging between 37 kg and 60 kg), the differences in nutrient quality of the hay offered between trials (ranging between 0.4% N and 0.7% N) and the relative quantities of supplements supplemented into the rumen. As such, starch supplementation ranged between 1.4 g starch/kg BW/day to 5.2 g starch/kg BW/day while urea supplementation ranged between 0.19 g urea/kg BW/day to 0.70 g urea/kg BW/day between treatments (Table 5.1).

Table 5.2 summarises the effects of starch and urea supplementation on roughage intake and roughage digestibility in sheep fed low-quality *E. curvula* hay and supplemented with urea and starch.

Table 5.2 Linear mixed meta-analysis describing roughage intake and neutral detergent fibre digestibility in sheep fed low-quality *Eragrostis curvula* hay and supplemented with urea and starch

**Parameter*	*Nutrient*	*P-value*	*Nutrient*	*P-value*	*Nutrient*	*P-value*
NDFI (g/kg BW)	Starch	0.081	Urea	0.901	Starch*Urea	0.676
RDMI (g/kg BW)	Starch	0.075	Urea	0.884	Starch*Urea	0.708
NDFD (%)	Starch	0.258	Urea	0.898	Starch*Urea	0.725

*Parameter: NDFI, Neutral detergent fibre intake; RDMI, Roughage dry matter intake, NDFD, neutral detergent fibre digestibility

The mean NDF intake (NDFI) across treatments was 1.33% BW, ranging between 0.81% BW and 2.09% BW (Table 5.1). Köster *et al.* (1996) suggested that NDFI of low-quality roughages in ruminants generally is maximised at 1.25% BW and up to 1.7% BW in lambs (Bohnert *et al.*, 2002). Köster *et al.* (1996) further suggested that N supplementation generally would not stimulate NDF intake when the upper limit NDF intakes were already reached in ruminants. As such, the NDF observations in this meta-analysis (Table 5.1) indicate that, while roughage intake in general had been maximised in sheep, NDF intake at the lower end spectra were well below the optimal levels, and that NDF intake (and roughage intake) probably was not maximised in all treatments across the trials.

Neither starch nor urea affected NDFI or RDMI. In addition, neither NDFI, nor RDMI were affected by the urea*starch interactions (Table 5.2).

These observations are in contrast with results obtained from various research studies (Detmann *et al.*, 2009, 2014; Kanjanapruthipong and Leng 1998; Wickersham *et al.*, 2008, 2009) where supplementation of N compounds stimulated RDMI in ruminants consuming low-quality tropical roughages. In a meta-analysis conducted by Detmann *et al.* (2014) on steers fed low-quality tropical roughages, roughage intake was maximised at CP: digestible organic matter (DOM) ratio of 288 g CP/kg DOM or 145 g CP/kg DM. In this meta-analysis, N intake was well below the values observed by Detmann *et al.* (2014), ranging between 78.8 g and 242.1 g CP/kg DOM (Table 5.1). As NDF intake was not maximised in all treatments in this meta-analysis and based on the observations of Detmann *et al.* (2014), it was expected that urea supplementation could have stimulated NDFI and RDMI in this meta-analysis. A

possible explanation for urea not influencing forage intake is that urea was supplemented to all treatments in this meta-analysis, while in the studies of Detmann *et al.* (2014), control treatments existed where N was not supplemented to the steers. The decision to supplement all the sheep during all trials, and not to have a control, non-supplemented treatment, was made to reduce the risk of possible rumen stasis in the non-supplemented groups as the quality of the roughages was poor (0.4% N – 0.7% N, > 65% NDF). Thus, all sheep were supplemented with at least 10.4 g urea/sheep/day, ranging between 0.186% BW to 0.701% BW (Table 5.1). Urea is a highly absorbable and available compound, with more than 90% disappearing in the rumen within an hour (Sinclair *et al.*, 1993). In contrast, the disappearance rate of the N fraction of tropical roughages is not as rapid compared to urea, as most of the N is located within the bundle sheath cells of tropical grasses (Bohnert *et al.*, 2011). The lack of RDMI response relative to the level of urea supplemented therefore might be due to the relative high level of urea supplemented, at even the lowest N diets, coupled with its relative high availability to the rumen bacteria. The high availability of N compounds from urea (relative to plant N) could have affected microbial bacteria population, stimulating roughage intake sufficient in this meta-analysis.

Energy supplementation to ruminants consuming low-quality roughages often decreased RDMI (Henning *et al.*, 1980; DelCurto *et al.*, 1990; Matejovsky and Sanson, 1995), through a reduction in rumen pH (Mould and Ørskov, 1983) and/or through a yet non-identifiable carbohydrate effect (Mould *et al.*, 1983). However, energy supplementation also stimulated low-quality roughage intake in ruminants under certain conditions (Gomes *et al.*, 1994; Elliot *et al.*, 1984). Heldt *et al.* (1999) suggested that energy supplementation to the roughage fed ruminant would only reduce RDMI if the total roughage diet was deficient in N. In the study of Gomes *et al.* (1994), N was supplemented in the form of fishmeal (2%) and urea (2.5% inclusion, DM basis) for a dietary concentration of 22 g N/kg DM. Nitrogen intake therefore was not deficient as is often experienced in ruminants fed low-quality tropical roughage diets. In contrast, Henning *et al.* (1980) did not supplement additional RDN sources to sheep receiving low-quality tropical roughages and supplemented with maize. The higher starch levels included in the present study and the study of Gomes *et al.* (1994) without negatively impacting on roughage intake and/or digestibility, could therefore be explained by the higher N intake in both those studies. It also agrees with studies conducted by Elliot *et al.* (1984) observing that NDF intake of sheep fed low-quality (1.39% N, 75.3% NDF) pongola hay (*Digitaria eriantha*) and sucrose (up to 60% DM) and urea (up to 2.5%

BW) was not affected by treatment. The authors concluded that the passage rate of small particles through the rumen might not be the main limitation on retention time as thought previously, but the rumen mat structure, where the movement of the particles through the rumen raft is limited. In addition, the resistance of the rumen raft to collapse under digestion might also limit forage intake (Poppi *et al.*, 2001). Elliot *et al.* (1984) further suggested that NDF intake of tropical grasses would be less compared to temperate grasses even at similar NDF concentrations and that the addition of sucrose cannot be manipulated the intake of tropical grasses without manipulating the underlying characteristics of the NDF fraction.

Roughage digestibility, measured as NDFD, was not affected by either of the supplements (starch or urea) or by the urea*starch interaction (Table 5.2). Detmann *et al.* (2009) and Kanjanapruthipong and Leng (1998) suggested that roughage degradability is maximised at lower dietary N concentrations (around 1.58% N) compared to maximising RDMI (2.30% N) in ruminants fed low-quality tropical roughages. In the present study, dietary N concentrations ranged between 0.70% N and 2.00% N (DM basis, Table 5.2). Based on these concentrations, the possibility therefore existed that, at the lower spectra of dietary N percentages, roughage digestibility would not have been maximised and that there could have been a correlation between the level of urea intake and NDF digestibility. As discussed earlier, there was not a control treatment in any of the trials used in the meta-analysis where RDN was not supplemented to sheep to minimise the risk of rumen stasis due to the poor quality of the hay. In the studies of Detmann *et al.* (2009), roughage degradability increased from the control, non-supplemented treatments until it plateaued at 6% dietary CP. In this meta-analysis, it was therefore a possibility that, roughage degradability was already maximised even at the treatment with the lowest quantity of urea supplementation, explaining the similar roughage digestibility across treatments. In addition, RDMI, requiring a higher level of urea supplementation to be maximised compared to NDFD (Detmann *et al.*, 2009), was not affected by the level of urea supplementation to sheep in the present study. As such, the lack of urea supplementation influencing NDFD in this study was not surprising.

Table 5.3 summarises the effects of starch and urea supplementation on MNS, efficiency of MNS (EMNS), MNS production relative to the total N intake (MNS:NI) and RAN in sheep fed low-quality tropical roughages and supplemented with urea and starch. Table 5.4 shows the results of regression analyses conducted on MNS, MNS:NI and RAN as affected by starch and urea supplementation to sheep fed low-quality *E. curvula* hay.

Table 5.3 Linear mixed meta-analysis describing microbial nitrogen synthesis (MNS), efficiency of MNS (EMNS) production, MNS production relative to nitrogen (N) intake (MNS:NI) and rumen ammonia nitrogen (RAN) in sheep fed low-quality *Eragrostis curvula* hay and supplemented with urea and starch

Parameter	*Nutrient*	*P value*	*Nutrient*	*P-value*	*Nutrient*	*P-value*
MNS	Starch	< 0.001	Urea	< 0.001	Starch*Urea	< 0.001
EMNS	Starch	0.685	Urea	0.758	Starch*Urea	0.547
MNS:NI	Urea	< 0.001	Starch	0.944	Starch*Urea	< 0.001
RAN	Urea	< 0.001	Starch	0.011	Starch*Urea	< 0.001

*MNS = Microbial nitrogen synthesised expressed as % BW; EMNS = Efficiency of MNS expressed as MNS divided by kg digestible organic matter intake; MNS:NI = efficiency of N usage for MNS (g MNS/g NI); RAN, rumen ammonia nitrogen; Starch and Urea expressed g/kg BW.

Table 5.4 Regression analyses of factors best describing microbial nitrogen synthesis (MNS), MNS production relative to nitrogen (N) intake (MNS:NI) and rumen ammonia nitrogen (RAN) in sheep fed low-quality *Eragrostis curvula* hay and supplemented with urea and starch

**Parameter (y)*	*Nutrient (x)*	*Equation*	*P-value*	***S. E.*	*R-square*
MNS (g/kg BW)	Starch (g/kg BW)	$y = 0.0453 (\pm 0.0825) x + 0.096 (\pm 0.082)$	$P < 0.05$	3.95	0.602
MNS:NI	Urea (g/kg BW)	$y = -0.796 (\pm 0.475) \ln x + 0.394 (\pm 0.182)$	$P < 0.05$	3.90	0.402
RAN	Urea (g/kg BW)	$y = 22.8 (\pm 4.776) x + 3.38 (\pm 1.34)$	$P < 0.05$	3.67	0.585
RAN	Starch: available CP	$y = 52.9 (\pm 1.776) / x^{2.22(\pm 2.89)} + 0.834 (\pm 0.470)$	$P < 0.05$	3.90	0.761

*Parameter: MNS = Microbial nitrogen synthesised (g/kg BW); MNS:NI = efficiency of N usage for MNS (g MNS/g NI); RAN = rumen ammonia nitrogen.

**S. E. Standard error

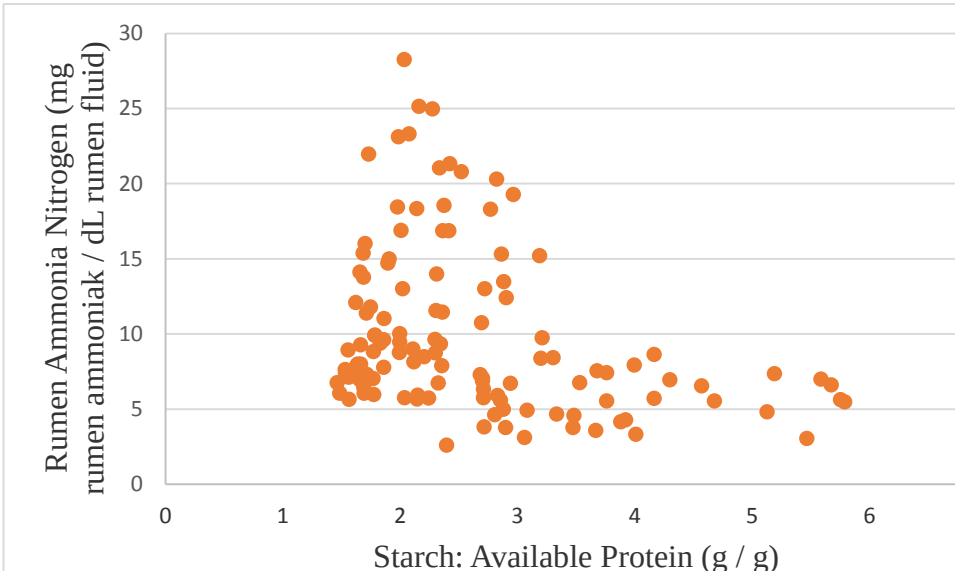

Figure 5.1 Mean rumen ammonia nitrogen concentration in sheep consuming low-quality *Eragrostis curvula* hay as affected by starch:available protein ratio (see Table 5.4 for graph information)

Starch supplementation affected MNS of sheep fed low-quality tropical hay and supplemented with urea and starch ($P < 0.001$; Table 5.3). The addition of urea as well as starch*urea to starch also affected MNS ($P < 0.001$), however, an AIC analysis suggested that the addition of urea and/or urea*starch interaction to starch did not affect MNS sufficiently to merit their inclusion in the model describing MNS to sheep fed low-quality *E. curvula* hay. This observation agrees with Kanjanapruthipong and Leng (1998), showing strong positive correlations between dietary RDN (Detmann *et al.*, 2009) or urea (Kanjanapruthipong and Leng, 1998) supplementation and MNS in ruminants grazing low-quality roughages. Leng (1990), Poppi *et al.* (1999) and SCA (1990) suggested that the nutrient having the most dominant effect on MNS in the tropical roughage fed ruminant, is FME.

A linear relationship was observed between MNS (g MNS/kg BW) and starch supplementation (g starch supplemented/kg BW; Table 5.4), where MNS increased linearly as starch supplementation was increased.

The NRC (2007) maintenance N requirements for sheep (40 kg BW) is 7.7 g N/day, for sheep weighing 50 kg it is 9.9 g N/day and for 60 kg sheep, it is 11.2 g N/day. Based on the regression equation obtained between MNS (g MNS/kg BW) and starch supplementation (g starch supplemented/kg BW) in this meta-analysis (Table 5.4), sheep (50 kg BW) fed low-quality *E. curvula* hay (< 0.7% N) needed to be supplemented with 2.2 g starch/kg BW/day

(110 g starch/day) to produce 9.8 g MNS/day. Similarly, 2.2 g/kg BW starch concentrations yielded 7.8 g MNS/day for sheep weighing 40 kg and 11.7 g MNS/day for sheep weighing 60 kg. Based on these calculations, sheep fed low-quality *E. curvula* hay (< 0.7% N) and supplemented with urea and starch needed to be supplemented with at least 2.2 g starch/kg BW/day to fulfil maintenance N requirements through MNS.

Poppi *et al.* (1999) in a review concluded that, while theoretically there seems to be differences in the utilisation of FME from different energy sources (sugars *versus* starch) for MNS, in practise no differences in MS and EMNS were observed between FME sources for MNS and EMNS in tropical forage fed ruminants. Therefore, while starch supplementation influenced MNS, it could be argued that it was due to the increased FME that was being supplemented by the starch which stimulated MNS. This aspect is discussed in Chapter 6 where the models derived from this thesis are discussed in terms of the current feeding standards.

An important parameter is efficiency of MNS (EMNS), expressed as MNS/kg DOMI where DOMI is digestible organic matter intake as it influences the post-rumen MP to energy ratio. This ratio is important in ruminants grazing low-quality tropical forages as low ratios frequently limit production (Leng, 1990). In this meta-analysis, neither starch, nor urea explained EMNS (Table 5.4). This observation contrasts with observations made by Gomes *et al.* (1994) observing that EMNS increased as the level of starch supplemented was increased in sheep fed low-quality straw.

In this meta-analysis, EMNS ranged between 7.136 and 35.33 g MNS/kg DOMI with an average of 16.40 g MNS/kg DOMI (Table 5.4). The mean value agrees with mean values quoted for tropical grasses of 18 g MNS/kg DOMI (bermuda grass; *Cynodon dactylon*), 16 – 21 g MNS/kg DOMI for paspalum (*Paspalum plicatulum*) and kikuyu (*Pennisetum clandestinum*) and 9.6 – 16.0 g MNS/kg DOMI for Rhodes grass (*Chloris gayana*) (SCA, 1990). Temperate grasses have higher NFC and digestible N concentrations compared to tropical grasses (Bohnert *et al.*, 2011). Poppi *et al.* (1999) in a review concluded that MNS efficiency of tropical grasses is low and below what feeding tables would predict is theoretical possible per unit fermentable OM present in the plant. In addition, SCA (1990) stated that WSC limits EMNS and that at least 90 g WSC/kg DM is required to optimise MNS and EMNS. This statement is supported by Mullik (2008), commenting that MNS efficiency of C4 grasses was almost 50% lower in a study conducted by the author than the

suggested Australian feeding recommendations (SCA, 1990) for temperate C3 grasses (20.8 g MNS per/kg DOMI).

As such, it was expected that starch supplementation would have increased EMNS in this study. However, no correlation was observed between any of the supplements and EMNS. This could be the result of the low-quality of the *E. curvula* hay used, constituting 4.7% NFC and 0.4% N. In addition, no control treatments (non-supplemental treatment) were used in the studies used in this meta-analysis due to the low-quality of the hay. Therefore, starch supplementation was relatively high, ranging between 1.407 – 5.210 g/kg BW. Similarly, urea supplementation ranged between 0.186 – 0.701 g/kg BW. It was therefore possible that the effects of starch and/or urea supplementation on EMNS was masked by the starch and/or urea supplementation at the lower levels, resulting in the lack of correlation observed between the supplements and EMNS.

The ratio of MNS to N intake (MNS:NI) is an important parameter as it is an indicator of the efficiency of N usage in the ruminant (Detmann *et al.*, 2014). Ratios of MNS:NI higher than one could be indicative of a dietary N deficiency as more microbial N is synthesised and delivered to the lower GI tract compared to N intake (Detmann *et al.*, 2014). As such, MNS:NI ratios above one are indicative of a much higher dependency on recycled N to sustain microbial growth in the rumen (Detmann *et al.*, 2014). Results from this meta-analysis suggested that the sheep were severely N deficient as the MNS:NI ratios ranged between 0.653 and 2.99, with a mean of 1.26 across treatments (Table 5.1). Interestingly, the MNS:NI ratios in this meta-analysis were almost twice as high compared to the MNS:NI ratios observed by Detmann *et al.* (2014), ranging between 0.31 and 1.63 with a mean of 0.67. The high MNS:NI ratios observed in this meta-analysis probably was due to the relative high quantities of starch supplemented in the present study (ranging between 1.407 and 5.210 g starch/kg BW; Table 5.1), stimulating MNS as was discussed.

Supplementation of urea impacted MNS:NI ($P < 0.001$; Table 5.3, Figure 5.1). Starch supplementation, as well as the starch*urea interaction, in addition to urea, did not have an additional effect on MNS:NI ($P > 0.05$). An inverse relationship was then observed between urea supplementation (per kg BW) and MNS:NI (Table 5.4) where MNS:NI decreased as urea intake increased.

In a meta-analysis conducted by Detmann *et al.* (2014) on steers fed low-quality tropical roughages, the authors observed a correlation between MNS:NI and urea

supplementation, which agrees with observations in this meta-analysis. Using the recommendations of Detmann *et al.* (2014), suggesting that MNS:NI ratios above one could be indicative of ruminants being N deficient, the minimum quantity of urea needed to be supplemented to the sheep in this meta-analysis was 0.5 g urea/kg BW, corresponding to 25 g urea/day for 50 kg sheep (Table 5.4). Assuming a roughage intake of 1 kg/day for sheep weighing 50 kg (NRC, 2007), the urea concentration of the final feed needed to be a minimum of 2.5% (DM basis). This observation agrees with suggestions of Leng (1995) from a review that urea intakes as high as 3% DM could be necessary to meet maintenance requirements of ruminants grazing low-quality tropical forages.

Rumen ammonia nitrogen (RAN) is often used as an indicator of rumen efficiency. Satter and Slyter (1974) suggested that the optimal RAN concentration necessary to maximise intake and digestibility in the forage fed ruminant ranges between 5 and 20 mg/dL rumen fluid. In studies conducted by Detmann *et al.* (2009) on cattle and Kanjanapruthipong and Leng (1998) on sheep receiving low-quality tropical forages, effective degradability of tropical roughages was maximised at 8 mg RAN/dL rumen fluid. However, Detmann *et al.* (2014) suggested that the optimal RAN concentration to maximise tropical forage intake is higher at 13 mg/dL rumen fluid while for MNS, it is 20 mg/dL rumen fluid.

Urea and starch supplementation per kg BW as well as the urea*starch interaction affected RAN (Table 5.3). Based on the regression analysis conducted between RAN and urea supplementation per kg BW, RAN increased linearly as urea supplementation increased (Table 5.4). Detmann *et al.* (2014) observed an exponential relationship between RAN and urea supplementation in a meta-analysis conducted on steers fed low-quality tropical forages. The authors however, commented that a threshold normally existed where the accumulation of ammonia becomes more intense as microbial N uptake becomes saturated. According to the authors, the threshold point normally (in steers) ranges between 100 – 140 g CP/kg DM (16 – 22.4 g N/kg DM). Below this threshold, the relationship between RAN and dietary CP concentration becomes linear. In this study, N percentage of the final diets after supplementation ranged between 0.70 – 2.00% N (7.0 – 20 g N/kg DM). This concentration is below the threshold value observed by Detmann *et al.* (2014), which could explain the linear relationship observed between RAN and urea supplemented/kg BW.

Both starch supplementation, as well as the starch*urea interaction influenced the RAN model. Results suggested a relationship between RAN and the starch supplemented to

available CP, where available CP is calculated as total CP intake excluding the ADIN intake*6.25 (Figure 5.1). In this meta-analysis, the relationship was tested and a regression equation was developed (Table 5.4) describing the relationship between RAN and the ratio of starch supplemented and available CP. From this regression equation, RAN was inversely related to the starch: available CP ratio, with RAN decreasing from concentrations as high as 25 mg RAN/dL rumen fluid to concentrations between 5 and 10 mg RAN/dL rumen fluid as starch: available CP increased from 1.5 to 6. In addition, a threshold was observed where RAN increased exponentially as the starch: available CP ratio dropped below 2:1, possibly indicating that microbial ammonia utilisation was exceeded and that FME was deficient. This observation agrees with observations made by Hoover et al. (2006) that the NFC: DIP ratio should be above two to optimise rumen microbial activity, as ratios less than 2:1 might be indicative of an energy deficiency in the rumen.

Summary and Conclusion

The aim of this meta-analysis was to determine whether roughage intake, apparent digestibility and MNS is affected by starch and/or urea in sheep fed low-quality tropical roughages. Datasets from six trials in four supplemental studies, totalling 123 experimental units were used in constructing this meta-analysis. Neither starch nor urea supplementation influenced roughage intake and digestibility, probably as urea was supplemented in all treatments to minimise the possible risk of rumen stasis across treatments. However, starch supplementation stimulated MNS, with MNS per kg BW increasing linearly as starch supplementation per kg of BW increased. In contrast, urea supplementation affected the MNS:NI ratio, with MNS:NI inversely related to urea supplementation per kg BW. Urea supplementation was also correlated with RAN, with RAN increasing linearly as urea supplementation per kg BW increasing. In addition, RAN was also inversely related to the starch: available CP ratio, with a threshold observed at starch: available CP ratio of 2:1. At ratios less than this threshold ratio, RAN increased exponentially, possibly indicating that FME intake was deficient relative to available CP. It is concluded from this study that for sheep fed low-quality *E. curvula* hay (< 0.7% N, NDF > 65% NDF), urea and starch supplementation should be a minimum of 0.5 g/kg BW (corresponding to 1.45 g CP/kg BW or 72 g CP/50 kg) and 2.2 g/kg BW (corresponding to 0.03 MJ FME/kg BW or 1.35 MJ FME/kg BW). The relationship between RAN and starch: available CP ratio suggested that the optimal ratio of starch supplemented and available CP is 2:1, indicating that sheep fed low-quality *E. curvula* hay must be supplemented with both RDN (urea) and FME (starch) sources to optimise ruminal fermentation.

Chapter 6 Practical aspects on rumen fermentation

Various models exist where nutrients and nutrient interactions are described and discussed in mathematical modelling. These models are used to predict animal responses under various conditions using different feeds. The NRC (2007), Cornell Net Carbohydrate and Protein System (CNCPS) and the SCA (1990) are such systems or models. The emphasis of this study was ultimately related to rumen fermentation, and the effects of supplementation of sheep on microbial N synthesis and efficiency. These effects were tested against the NRC (1996), SCA (1990) and AFRC (1993) models.

In this thesis, the wethers were fed low-quality *E. curvula* hay with an ED ranging 30 – 35% with the rate of degradability at 2% (Table 2.2). In addition, the NFC concentration of the hay was calculated at 4.7% while the N and ADIN fractions were 0.4% and 45%, respectively. Intake of the hay was in the range of 1000 g/wether/day (DM). Urea supplementation ranged between 9.4 and 32.4 g urea/day while starch supplementation ranged between 70 to 280 g/day starch based on 50 kg wethers (Table 5.1).

Urea supplementation did not affect MNS, however, starch supplementation influenced MNS as follows:

y = 0.0453 (± 0.0825) x + 0.096 (± 0.082) (Table 5.4) **Eq. 6.1**

where y = MNS (g MNS/kg BW) and x = starch supplemented (g starch/kg BW).

Using mean roughage intake as basis (17.3 g/kg BW; Table 5.1), roughage intake of 50 kg wethers was 865 g/day DM, ranging between 515 g/day DM (10.4 g/kg BW) and 315 g/day DM (26.3 g/kg BW). Digestible organic matter intake ranged between 355 g/day (7.1 g/kg BW) and 910 g/day (18.2 g/kg BW) with a mean of 575 g/day (11.5 g/kg BW) for 50 kg wethers (Table 5.2).

Metabolisable energy in forages was calculated from the digestible organic matter per kg DM (DOMD, McDonald *et al.*, 2011) as follows:

ME = 0.016 DOMD **Eq. 6.2**

In the series of trials described in this thesis, organic matter digestibility (OMD) ranged between 55% – 62% (Tables 2.3 and 3.3). However, the calculations used include starch which was supplemented in all treatments, and is therefore theoretically not the

DOMD of the hay. To calculate DOMD of the hay, starch intake was subtracted from DOMI, if starch digestibility was complete. By default, the remaining DOMI originated from the hay. Using these assumptions as basis as well as the minimum, maximum and mean values in Table 5.1, hay DOMI and hay digestible organic matter/kg DM (DOMD) was calculated as follows:

Table 6.1 Calculation of digestible organic matter intake in 50 kg wethers fed low-quality *Eragrostis curvula* hay and supplemented with urea and starch *(*data from Table 5.1)

*Parameters	Minimum	Mean	Maximum
RDMI (g/kg BW)	10.4	17.3	26.3
DOMI (g/kg BW)	7.1	11.5	18.2
Starch intake (g/kg BW)	1.407	3.052	5.210
Urea Intake (g/kg BW)	0.186	0.353	0.701
DOMI (Hay) (g/kg BW)	5.693	8.448	12.990
DOMD (Hay)	547	488	494
(g digestible OM/kg roughage DM)			
MNS (g/kg BW)	0.129	0.235	0.416

*RDMI = Roughage dry matter intake; DOMI = digestible organic matter intake; DOMI (hay) = DOMI – starch intake; DOMD = Digestible organic matter per kg roughage DM calculated as DOMI (Hay)/RDMI; MNS = Microbial N synthesis per day.

Using 14.99 MJ ME/kg DM for starch (NRC, 2007) and FME = 0.90 * ME for both starch and roughage (Robinson, personal communication), mean FME intake from both the hay and starch for 50 kg wethers used in the trials was calculated as set out in Table 6.2. In addition, urea intake, N intake from urea and MNS (g/day) was calculated for 50 kg wethers, using data from Table 5.1 as reference.

Table 6.2 Calculation of fermentable metabolisable energy of 50 kg wethers fed low-quality *Eragrostis curvula* hay and supplemented with urea and starch (data from Table 5.1)

*Parameters	Minimum	Mean	Maximum
RDMI (g/day)	520	865	1315
Starch intake (g/day)	70	153	261
DOMD (Hay) (g digestible OM/kg roughage DM)	547	488	494
Hay ME (MJ/kg DM)	8.75	7.81	7.90
Hay ME intake (MJ ME/day)	4.55	6.76	10.39
FME intake (Hay) (MJ FME/day)	4.10	6.08	9.35
ME (Starch) (MJ ME/day)	1.05	2.29	3.90
FME intake (Starch) (MJ FME/day)	0.95	2.06	3.51
Urea intake (g/day)	9.3	17.7	35.0
N from urea (g/day)	4.3	8.1	16.1
MNS (g/day)	6.45	11.75	20.8

*Parameters: All parameters are calculated for 50 kg wethers, using Table 6.1 as basis, RDMI = Roughage dry matter intake; DOMD = Digestible organic matter per kg roughage DM; ME (Hay) = Metabolisable energy from hay, calculated as DOMD (hay) * 0.016 (McDonald *et al.*, 2011); ME intake (Hay) = Metabolisable energy intake from hay, calculated as ME (Hay) * RDMI; FME (Hay) = ME intake (Hay) * 0.90 (Robinson, personal communication); ME (Starch) = Starch intake (Table 6.1) * 14.99 MJ/kg DM (Robinson, personal communication); FME intake (Starch) = ME intake (Starch) * 0.90 (Robinson, personal communication); N from urea = Urea intake * 46% (McDonald *et al.*, 2011); MNS = Microbial N synthesis.

For the purposes of this chapter, the mean values were used to compare with the feeding standards discussed below.

6.1 Agricultural and Food Research Council (AFRC, 1993)

The development of the AFRC (1993) system is based on Agricultural Research Council (ARC, 1980). In this system FME is defined and calculated as follows:

$$\textbf{FME = Metabolisable Energy (ME) – ME}_{\textbf{Fat}} \textbf{ – ME}_{\textbf{Fermentation}} \qquad \textbf{Eq. 6.1.1}$$

where FME is fermentable metabolisable energy (MJ/kg DM), ME is metabolisable energy (MJ/kg DM), ME_{Fat} is ME from fat, and $ME_{Fermentation}$ is ME from fermentation acids.

Level of feeding above maintenance influences MCP per MJ FME from the following equation:

$$y = 7 + 6 * (1- e^{-0.35L}) \qquad \textbf{Eq. 6.1.2}$$

where L is the level of intake.

In the AFRC system, MNS is assumed to be limited by the supply of effective ruminally degraded protein (ERDP), which is the sum of 80% of quickly and slowly degraded protein (QDP and SDP, respectively). The QDP is defined as the cold water extracted N while the SDP is computed from an equation based on water-soluble N content, potentially degradable N, degradation rate, and rumen outflow rate. The outflow rate of the diet is a function of level of feeding. Recycled N is not included, and is assumed to compensate for losses of degraded protein above the QDP adjustment.

In this study, urea was supplemented to the wethers fed low-quality *E. curvula* hay (0.4% N, 45% ADIN) with a mean intake of 17.7 g urea/day calculated for 50 kg wethers (Table 6.2). Urea contains 46% N (McDonald *et al.*, 2011) and this value could be used as the QDP value in the AFRC (1993) system (N*6.25). In this series of trials, QDP in the 50 kg wethers is calculated as follows:

$$\textbf{QDP = 17.7 g urea * 6.25 = 110.6 g/day} \qquad \textbf{Eq. 6.1.3}$$

The hay contained 0.4% N of which 45% was in the ADIN form. At the mean roughage intake for 50 kg wethers (Table 6.2), N intake from the hay was 3.5 g N/day. With ADIN at 45%, it could be argued that the N intake from the hay was negligible relative to the urea N intake. Therefore, for this chapter, only N intake from urea was considered in the models. Effective rumen degradable protein (ERDP) is therefore calculated as 80% of QDP.

ERDP = 80% * 110.6 g QDP/day = 88.5 g/day **Eq. 6.1.4**

If it is assumed that MCP is 85% digestible and contained 75% protein, then microbial N synthesis is calculated as follows:

MNS = MCP (rumen)*0.75*0.85 / 6.25 **Eq. 6.1.5**

The FME intake from the hay for the 50 kg wethers was calculated at 6.76 MJ FME/day (Table 6.2). Using Eq. 6.1.2 with a feed intake of maintenance, MCP computed to 8.8 g MCP/MJ FME (or 1.40 g MNS/MJ FME). As such, mean FME intake from the hay alone equates to 58.7 g MCP synthesized in the rumen and MNS from hay alone is calculated at 6 g MNS/day using Eq. 6.1.5.

Starch supplementation ranged between 1.407 g/kg BW to 5.210 g/kg BW with a mean of 3.052 g/kg BW (Table 6.1). For 50 kg wethers, these intakes equate to 70, 153 and 261 g starch/day, respectively (Table 6.2). Using Eq. 6.1 derived from this thesis, total MNS is calculated as follows (Table 6.3):

Table 6.3 Comparison of microbial nitrogen synthesis prediction in 50 kg wethers fed low-quality *Eragrostis curvula* hay *versus* AFRC (1993) prediction

*Parameters			**MNS prediction		***AFRC (1993) prediction
Starch intake (g/kg BW)	**Starch intake (g for 50 kg wether)**	**FME intake (starch)**	**MNS (g/kg BW)**	**MNS (50 kg wether)**	**MNS (50 kg wether)**
0	0	0	0.10	4.80	6.02
1	50	0.67	0.14	7.07	9.78
2	100	1.35	0.19	9.33	13.57
3	150	2.02	0.23	11.60	17.36
4	200	2.70	0.28	13.86	21.14
5	250	3.37	0.32	16.13	24.92

*Parameters: FME intake (starch) = Fermentable Metabolisable Energy from starch intake (50 kg wether) where FME (starch) = starch intake (g) / 1000 * 14.99 MJ ME/kg DM * 90% (Robinson, personal communication); MNS = Microbial nitrogen synthesis; AFRC = Agricultural and Food Research Council.

**Thesis prediction: MNS is predicted using Eq. 6.1.

$$y = 0.0453 (\pm 0.0825)\ x + 0.096 (\pm 0.082)$$

where y = MNS (g MNS/kg BW) and x = starch supplemented (g starch/kg BW)

***AFRC (1993) prediction of MNS = [Mean FME (hay) intake for 50 kg wether (Table 6.2) + FME intake (starch, Table 6.3)] * MCP (rumen) per MJ FME at maintenance (Eq. 6.1.2) * 0.85*0.75/6.25 accounting for MCP rumen digestibility and true protein and conversion to N.

The AFRC (1993) model overpredicts MNS compared to the MNS model obtained in this thesis (Eq. 6.1). Possible reasons include:

1. The MNS model obtained in this thesis predicts MNS from the *E. curvula* hay for 50 kg wethers at 4.8 g MNS/day while AFRC (1993) predicts MNS 25% higher at 6.0 g MNS/day. This observation agrees with Mullik (2007) stating that current feed tables cannot be used to predict MNS production from tropical grasses as it is derived mainly from temperate grasses. In addition, these observations confirm statements from Poppi *et al.* (1999) and SCA (1990) that MNS production in ruminants fed tropical grasses are generally lower per kg digestible organic matter intake compared to temperate grasses as WSC is generally lower in tropical grasses *versus* temperate grasses.

2. The difference between successive 50 g starch intakes in Table 6.3 is 2.27 g MNS and 3.78 g MNS, respectively, for the model derived from the thesis and the AFRC (1993) model. The calculated NFC content of the *E. curvula* hay fed to the sheep in this trial was 4.7%. It was therefore expected that starch supplementation would have increased MNS in the wethers. However, the observed MNS increase was almost 40% lower than the AFRC (1993) prediction per unit FME.

6.2 Feeding standards of Australian livestock (SCA, 1990)

In the SCA (1990) system, microbial crude protein yield is 170 g kg/DOM for the first growth of temperate legumes and grasses, 130 g/kg DOM for all other fresh and dried forages and mixed diets, and 95 g/kg DOM for silages. Microbial protein adjusted for 20% nucleic acids is assumed to have a 70% intestinal digestibility, giving a 56% MP value to microbial CP. Inefficient capture of N from ruminally degraded protein is assumed to be compensated for by recycled N. SCA (1990) acknowledges that although recycling N can offset intermittent inadequacies of RDP, it will not sustain the animal through a chronic inadequacy of N, which is consistent with Van Soest (1994) and NRC (2000).

Table 6.4 is a calculation and comparison between MNS as predicted in Table 5.4 and SCA (1990).

Table 6.4 Comparison of microbial nitrogen synthesis prediction of 50 kg wethers fed low-quality *Eragrostis curvula* hay from this thesis to SCA (1990)

*Parameters					
Starch intake (g/kg BW)	**Starch intake (g for 50 kg wether)**	**DOMI (hay + starch, g/day)**	**MCP (SCA)**	**MNS (SCA)**	**MNS (model prediction)**
0	0	422	54.9	8.79	4.80
1	50	467	60.8	9.72	7.07
2	100	512	66.6	10.66	9.33
3	150	557	72.5	11.59	11.60
4	200	602	78.3	12.03	13.86
5	250	647	84.2	13.47	16.13

*Parameters: DOMI = Digestible organic matter intake, MCP = Microbial crude protein (g/day); MNS = Microbial nitrogen synthesis (g/day); SCA = Feeding standards of Australian livestock.

**Thesis prediction: MNS is predicted using Eq. 6.1.

The SCA (1990) model overpredicts MNS where starch was not supplemented to the wethers. However, a few aspects are important to note:

1. There was not a control treatment where starch was not supplemented in this series of trials. Therefore, the MNS predicted by Eq. 6.1 (derived from this study) falls outside the data used to compute the model.

2. The *E. curvula* hay used in this study was poor and probably would not have been used as an animal feed under most situations. Poppi *et al.* (1999) and SCA (1990) stated that MNS in the tropical grasses is less per kg DOMI compared to temperate grasses due to a deficiency of WSC or NSC. According to the SCA (1990), MNS in tropical grasses would be impaired if the NSC is less than 90 g/kg DM. In this study, the calculated NFC of the *E. curvula* hay was 17 g/kg DM. It is therefore not surprising that the MNS model obtained from this study predicted such a low MNS compared to SCA (1990) at the "control, non-supplemented" treatment. The differences between the predicted SCA (1990) model and the MNS model obtained from this study decreases as the level of starch supplemented was increased until a starch supplementation of 150 g starch for 50 kg wethers (3 g/kg BW; Table 6.4). The

mean roughage DM intake for 50 kg wethers was 865 g/day (Table 6.2). At 150 g starch supplementation, the starch percentage as a total of DM intake was 14.7%. Add together the calculated NFC of the hay (4.7% DM), and total NFC is calculated at 16.4% which is 70% above the minimum NSC level required to optimise MNS (Poppi *et al.*, 1999). It was therefore expected that MNS would have been increased to a higher level as the level of starch supplementation was increased as FME intake has the biggest influence on MNS (Leng, 1990; Poppi *et al.*, 1999). However, the effective degradability (ED) of the *E. curvula* hay fed to the wethers was below 35% while the rate of degradation was 2% (Table 2.2). Poppi *et al.* (1999) suggested that not only is NSC or WSC limiting MNS in ruminants consuming low-quality tropical forages, but also of rumen retention time.

3. At starch supplementation levels above 150 g/day, SCA (1990) under predicts MNS in this study compared to the regression obtained in this study. While starch supplementation did not affect RDMI or NDF digestibility in this study (Table 5.4), bacterial growth rates are faster for non-fibre bacteria compared to fibre bacteria. The higher MNS observed in this thesis compared to SCA (1990) predictions could be explained by faster bacterial growth rate of non-fibre bacteria *versus* fibre bacteria (Fox *et al.*, 2004) and possible higher dilution rate of the non-fibre bacteria as starch supplementation was increased above 150 g/day. It must be remembered that SCA (1990) predicts MNS from tropical forages without starch supplementation with only the NFC available from the forage itself. Therefore, at the higher DOMD values, the typical forage being described by SCA (1990) contains more fibre in the organic matter fraction *versus* low-quality *E. curvula* hay used in this study which was supplemented with starch.

6.3 National Research Council (1996)

The NRC (1996) fermentation model is a refinement of the model described by Russel *et al.* (1992) which included the limitations of the model. This model assumes that bacterial growth yields are a constant function of total digestible nutrient intake. As such, NRC (2000) used a static efficiency of 130 g MCP/kg TDN to determine microbial yield for forages. However, TDN includes digestible protein, fats, and lipids, as an energy source, although most microbial bacteria can only use carbohydrates as energy source for growth (Nocek and Russell, 1988).

The fat content of the *E. curvula* hay used in the trials was low at 1.5%. Therefore, it could be argued that the TDN would be equal to the digestible organic matter per kg DM (DOMD). Using this as basis, a similar equation as in the calculation of MNS through SCA (1990) is being obtained as described in Table 6.4.

It is of interest to note that, in all three models, EMNS is assumed to be constant at maintenance intakes. AFRC (1993) however, suggested that EMNS increases as the level of feed intake relative to maintenance increases (Eq. 6.1.2) while SCA (1990) has separate efficiencies for temperate (160 g MCP/kg DOMD) and tropical grasses (130 g MCP/kg DOMD).

In this study, EMNS was not influenced by either starch or urea supplementation Table 5.3). This observation agrees with Bowen *et al.* (2016) observing that only high quantities of RDP (270 g RDP/kg DOMI), in the form of true protein (casein), increased EMNS in steers fed low quality tropical forages. Poppi *et al.* (1999) stated that MNS is not only limited by NSC content of tropical forages (Leng, 1990; SCA, 1990) but also the retention time of the particles. In a study conducted by Elliot *et al.* (1984), sucrose was supplemented (up to 60% DM) and urea (up to 2.5% BW) to sheep fed low-quality (1.39% N, 75.3% NDF) pongola hay (*Digitaria erianthra*). The authors observed that the potential of improving roughage intake and digestibility was limited and concluded intake of tropical grasses cannot be manipulated with sucrose without manipulation of the NDF structure. Poppi *et al.* (2001) further stated that passage of small particles through the rumen may not be the main limitation of retention time in the tropical forage fed ruminant, but also the rumen mat or raft structure, hindering the particle movement and its resistance to collapse under digestion. It is therefore possible that, despite the supplementation of both urea and starch in this study to the wethers consuming the *E. curvula* hay, the rate of particle passage through the rumen was still the main factor limiting EMNS as the passage rate of the microbes would have been limited, explaining the lack of influence of both starch and urea on EMNS.

Minimum starch supplementation needed to meet MNS maintenance requirements was calculated at 2.2 g/kg BW, or 110 g per 50 kg wether/day (Table 5.4). The calculated seemingly optimal starch: available CP ratio obtained in this study for wethers fed the low-quality *E. curvula* hay and supplemented with starch and protein is 2:1. Below this ratio, RAN increased exponentially, possibly indicating that microbial ammonia utilisation was

exceeded and that FME was deficient (Figure 5.1, Table 5.4). Available protein for the purposes of this study was calculated as total N intake (hay + urea) – ADIN intake, where the ADIN fraction was assumed not to be available for fermentation in the rumen, multiplied by 6.25 to convert from N to CP. Available CP and starch intake was expressed on gram to gram DM basis. Based on these data, urea needed to be supplemented at 25 g/day for 50 kg wethers, equating to 85 g available CP [urea intake (g/day) * 0.466 *6.25 to covert to N and CP respectively + hay CP intake, calculated as hay intake in g/day *2.7% CP * 55% as 45% of the N fraction was in the ADIN form]. As such, starch needed to be supplemented at a minimum of 170 g starch per day to have an optimal starch: available protein intake ratio in this study, which is higher than the minimum starch required to meet MP maintenance requirements through MNS.

Lastly, applying the MNS:NI equations derived in Table 5.4 to 50 kg wethers, the total CP intake required is calculated at 97 g/day (available CP intake as calculated above + unavailable fraction). With the mean forage intake at 17 g/kg BW (Table 5.1) and a starch intake at 170 g/day needed to balance the CP intake, total intake equates to roughly 1000 g/day. Therefore, the CP% of the diet is calculated at 9.7%, which was slightly lower than the recommendations stated by Detmann *et al.* (2009), observing that forage intake and MNS was maximised in steers fed low-quality tropical forages at levels higher than 12% CP (DM). However, the aim of this study was to meet maintenance requirements and not necessarily to maximise forage intake or MNS. In addition, no additional starch or energy was supplemented in the study of Detmann *et al.* (2009) while the quality of the hay fed was also superior to the hay fed in this study.

Summary and Conclusion

Supplementation studies conducted on ruminants grazing low-quality tropical roughages are limited (Costa *et al.*, 2013; Mullik, 2007). Bohnert *et al.* (2011) conducting studies on ruminants consuming tropical and temperate roughages, observed lower production in ruminants consuming tropical roughages compared to ruminants consuming temperate roughages. These lower productions were, according to the authors, due to anatomical differences existing between temperate and tropical roughages, resulting in lower nutrient content and availability of tropical roughages compared to temperate roughages (Bohnert *et al.*, 2011). It was furthermore observed that supplementation responses differ between ruminants fed low-quality tropical and low-quality temperate grasses, even at similar chemical composition grasses (Bohnert *et al.*, 2011).

Compiled in this thesis, a series of trials were conducted to study the effects of various levels and timing of starch and urea supplementation on tropical roughage intake and digestibility, RAN, MNS and EMNS in sheep. In addition, the effects of these nutrients on MNS:NI, where MNS:NI was defined as the ratio of g MNS per g NI, were also studied.

The purpose of Trial 1 was to investigate whether substitution of the RDN fraction of SFM, with urea, a NPN source, would have an impact on roughage intake, digestibility, MNS and MNS efficiency. Rumen degradable N was supplied at 46.9 g N/sheep/day to meet maintenance requirements of the sheep (NRC, 2007). The source of RDN (urea or SFM) had no impact on roughage intake, apparent DM digestibility, rumen pH, RAN or total rumen volatile fatty acid (VFA) production. It was concluded that urea could substitute up to 60% of the RDN supplied by SFM without affecting intake, digestibility or MNS in sheep receiving low-quality *E. curvula* hay. However, total N balance among all treatments suggested that the overall RDN supplementation was probably not optimal, suggesting that higher RDN supplementations than the current NRC (2007) recommendations had to be supplemented to sheep fed low-quality *E. curvula* hay.

The purpose of Trial 2 was to investigate the effects of various levels of urea within starch supplementation for sheep, weighing 50 kg, consuming low-quality *E. curvula* hay. Starch was supplemented at 200 g, 240 g, or 280 g starch per sheep per day while urea was supplemented at 10.4, 18.4, 26.4 or 32.4 g urea per day. The supplementation level of urea stimulated MNS within each starch period; however, it seemed that starch was more

influential than urea, stimulating MNS. As such, MNS increased from 12.8 g MNS to 17.8 g MNS as starch supplementation increased from 200 g starch/sheep/day to 280 g starch/sheep/day. Urea supplementation had no effect on roughage intake or roughage digestibility in Trial 2, possibly, as there were no control treatments where urea was not supplemented. As such, it was a possibility that urea supplementation, even at the lowest level of supplementation (10.4 g urea/wether/day) already stimulated and optimised rumen fermentation sufficiently to maximised roughage intake and digestibility. However, MNS: available NI ratio improved in all three starch levels (from above two to less than one as the level of urea supplemented to the sheep was increased from 10.4 g urea/sheep/day to 32.4 g urea/sheep/day. While MNS in this study was maximised at urea levels as low as 10.4 g/day, the ratio between MNS and available NI implied that higher urea levels, up to 26.4 and even 32.4 g/sheep/day, were necessary to meet the total N requirements of the sheep. Based on these observations, it was concluded that urea as high as 32.4 g urea/sheep/day coupled with starch, as high as 240 g starch/sheep/day, could be supplemented to sheep, weighing 50 kg, consuming low-quality tropical hay.

The emphasis of Trial 3 was on supplemental RDN and starch synchronisation in sheep fed low *E. curvula* hay. This was achieved by keeping the nutrient composition of the supplements similar, but to vary the supplementation patterns of the urea and starch between the morning (08h00) and afternoon (16h00) supplementation periods. Leng (1995) suggested that the production of ruminants grazing tropical forages and supplemented with molasses and urea is frequently better than ruminants grazing tropical forages and supplemented with starch and urea supplements. The authors further stated that one of the possible reasons could be due to a better synchronisation between the release of energy found in molasses (sugar) and N from urea compared to energy release from starch and N release from urea. As such, a "Molasses treatment" was created, providing molasses and urea as supplement to be compared with the starch-urea treatments. Synchronisation did not affect roughage intake, N intake or faecal N excretion. In contrast, urinary N excretion was higher in the treatments where starch was supplemented during both the morning and afternoon supplementation periods. More N was retained in the body in the treatments where starch was only supplemented once daily with urea 12 hours later. The most consistent RAN concentration, apparently sufficient to maximise tropical forage intake and MNS (Detmann *et al.*, 2009) was obtained in the treatment where both starch and urea was supplemented during both the morning and afternoon supplementation period. Microbial N synthesis and EMNS in general

were higher in the treatments where starch was supplemented during both the morning and afternoon supplementation periods, as was MNS of the sheep receiving the Molasses treatment. Interestingly, the EMNS of the Molasses treatment was comparable to the EMNS of the treatments where starch was supplemented twice daily.

It was concluded that, while the most consistent RAN was achieved in the treatments where both urea and starch were supplemented twice daily, the supplementation pattern of starch was the more important parameter compared to urea, as the supplementation of starch during both the morning and afternoon supplementation period stimulated roughage digestibility, MNS and EMNS the most. In addition, there is merit in the statement that sheep receiving low-quality tropical roughages and supplemented, once daily with molasses and urea *versus* starch, might perform better due to a more even synchronisation between sugars and urea compared to starch and urea. However, differences were not always consistent, and more research is necessary to test this hypothesis.

To conclude the series of experiments in this dissertation, a meta-analysis was conducted to study the importance of starch and/or urea on the various production parameters (roughage intake, digestibility, EMNS, MNS and MNS:NI) using data from supplementation studies conducted over a ten-year period at the experimental farm of the University of Pretoria. Neither starch nor urea supplementation affected roughage intake and digestibility; neither were any starch*urea interactions observed for this parameter. Urea was supplemented to all sheep to reduce the risk of rumen stasis, which could have influenced the intake and digestibility results. In contrast to roughage intake and digestibility, MNS was affected by starch and urea as well as by the starch*urea interactions. In developing a model predicting MNS, starch was the more important nutrient compared to urea in this meta-analysis, which agrees with the statements of Leng (1990). As such, MNS (expressed as MNS/kg BW) increased linearly as starch supplemented/kg BW increased. While MNS:NI was affected by both urea and the urea*starch interaction, only urea influenced the MNS:NI model, with MNS:NI inversely related to urea supplemented/kg BW. Detmann *et al.* (2014) suggested that MNS:NI ratio should be below one as ratios above one could be indicative of a N deficiency. As such, the regression equation developed for MNS:NI as influenced by urea intake, suggested that sheep needed to be supplemented with at least 0.5 g urea/kg BW not to be N deficient. A strong correlation was observed between the ratio of starch supplemented and available CP and RAN, with RAN increasing exponentially from levels as low as 5 mg

RAN/dL rumen fluid to 30 mg RAN/dL rumen fluid as the ratio of starch supplemented to available CP dropped below 2:1.

It was concluded from this study that both energy (starch) and N (urea) compounds were necessary to optimise tropical roughage utilisation and ruminant production under tropical conditions with the optimal ratio of starch supplemented to available CP ranging between 2:1 and 3:1.

Lastly, the recommendations and regression equations derived for MNS in this study were compared to the AFRC (1993), SCA (1990) and NRC (1996) models. AFRC (1993) overpredicted MNS under these experimental conditions while SCA (1990) and NRC (1996) overpredicted MNS at starch supplementation levels less than 150 g/day for 50 kg wethers (3 g starch/kg BW/day). At levels above 150 g starch/day (3 g/kg BW), SCA (1990) and NRC (2000) however, underpredicted MNS.

This thesis can briefly be summarised by the following statements:

1. The availability and bioavailability of the nutrients from low-quality tropical grasses are less compared to low-quality temperate grasses. This lower bioavailability is not only limited to the N fraction of the roughages *per se*, but also the energetic compounds, with low-quality tropical grasses containing less NFC than low-quality temperate grasses (Bohnert *et al.*, 2011).

2. Production responses of the tropical roughage fed ruminant is lower compared to the temperate roughage fed ruminant (Leng, 1990). Leng (1995) furthermore suggested that the lower production responses of the tropical roughage fed ruminant were due to nutrient imbalances and not necessarily due to the lower digestibility of tropical roughage compared to temperate roughages.

3. Supplementation responses with RDN observed from various studies conducted on ruminants consuming low-quality tropical roughages were different compared to studies conducted on ruminants consuming low-quality temperate roughages (Bohnert *et al.*, 2011). As such, higher RDN supplementations were necessary to optimise roughage intake and MNS in the tropical roughage fed ruminant compared to the temperate roughage fed ruminant (Detmann *et al.*, 2009; Bohnert *et al.*, 2011).

4. Current knowledge on the supplementation of the tropical roughage fed ruminant is mainly limited to RDN supplementation. Therefore, the series of studies conducted as part of this thesis is to our knowledge, one of the only studies where supplementation of various quantities of starch and urea (RDN) were studied in the tropical roughage fed ruminant.

5. The results obtained from the first study suggested that up to 60% of the RDN fraction of a true protein source could be substituted with urea without affecting roughage intake, digestibility or MNS. However, results from the latter studies suggested that even higher levels of NPN (urea) supplementation (up to 32.4 g urea/sheep weighing 50 kg/day) were necessary to optimise MNS, EMNS and MNS:NI. These observations agree with observations made by other researchers (Kanjanapruthipong and Leng, 1998; Detmann *et al.*, 2009, 2014) conducting trials on tropical roughage fed ruminants. These results are also in agreement with suggestions made by Leng (1995) that up to 3% urea could be included to low-quality tropical roughages.

6. The ratio of starch supplementation to available CP is a reliable indicator of starch needed to optimise MNS:NI and RAN in the tropical roughage fed ruminant. As supplemental starch and RDN intake (urea) influenced the MNS:NI ratio and RAN, with RAN decreasing and flattening out as the ratio increased. Therefore, even with high urea intakes above NRC (2007) recommendations, low RAN might be indicative of a better efficiency in the rumen.

7. Data obtained from the synchronisation study suggest that a more constant supply of both urea and starch generally result in a more even RAN throughout the day sufficient to optimise tropical roughage degradability, tropical roughage intake and MNS. However, the major improvements in MNS were obtained by a more frequent (twice daily) supplementation of starch to the tropical roughage fed ruminant. This observation agrees with suggestions made by Henning *et al.* (1993) that a more even supply of FME to the rumen could stimulate MNS more than synchronisation *per se*.

8. Data obtained from the meta-analysis suggested MNS is stimulated predominantly by starch supplementation in sheep fed low-quality *E. curvula* hay. However, RDN supplementation (urea) affected MNS:NI and RAN, with increasing levels of urea supplementation reducing the MNS:NI ratio while stimulating RAN. In addition, the

ratio of starch supplementation to available CP was also correlated with RAN and is an important factor in formulating supplementation programs for the tropical roughage fed ruminant. It is recommended that sheep fed low-quality *E. curvula* hay (< 0.70% N, > 65% NDF) needed to be supplemented with at least 0.5 g urea/kg BW to maintain a MNS:NI below one, which is the critical ratio suggested by Detmann *et al.* (2014) indicating potential N deficiencies. In addition, to produce sufficient MNS to meet maintenance N requirements of the sheep, starch supplementation needed to be 2.2 g starch/kg BW. In addition, a starch: available CP ratio ranging between 2:1 and 3:1 appears necessary to optimise RAN in the tropical roughage fed ruminant.

9. Despite the above recommendations, pure maize starch was predominantly used in the studies, which is a purified source from which almost the entire protein matrix was removed. As such, the rate and extent of fermentation of this source will differ from that of intact maize grain (Huntington, 1997). In addition, starch and urea were supplemented twice daily directly into the rumen of sheep. As such, care must be taken when extrapolating the recommendations derived from this series of trials to practical farm feed formulation and supplementation.

10. Due to the lower nutrient content and availability of tropical roughages compared to temperate roughages, the RDN and FME requirements of ruminants consuming low-quality tropical roughages differ to that of ruminants fed low-quality temperate forages. As such, higher quantities of both FME (starch) and RDN are needed to meet the maintenance requirements of tropical roughage fed ruminants compared to temperate roughage fed ruminants. In addition, in evaluating ruminant production under tropical conditions, it is important to not only study roughage intake and digestibility, but also to incorporate and monitor MNS and MNS:NI in such studies. While the objective of supplementation would be to maximise MNS in the tropical roughage fed ruminant (Leng, 1995), care should be taken in maximising MNS at the "expense of the ruminant" through body protein catabolism. As such, the MNS:NI ratio is an important parameter to distinguish between N from catabolism and dietary N intake and to optimise the efficiency of dietary NI for MNS.

11. Microbial N synthesis comparisons with existing feeding tables show that the MNS prediction equations derived from this thesis were comparable to the prediction models of the SCA (1990) for tropical forages.

References

AFRC, 1993. Energy and protein requirements of ruminants: an advisory manual. Technical Committee on Responses to Nutrients. *By Alderman, G. & Cottrill, B. R., Wallingford, Oxon, UK, CAB International.*

ANKOM^{RF} Gas Production System Operator's manual. Rev E 3/14/11. www.amkom.com

Akaike, H., 1973, Information theory and an extension of the maximum likelihood principle, *2nd International Symposium on Information Theory, Tsahkadsor, Armenia, USSR, September 2 – 8, 1971 (editors - B.N. Petrov, F. Csáki) 267-281 (Budapest: Akadémiai Kiadó).*

Almeida, A. M., Schwalbach, L. M. J, de Waal, H. O., Greyling, J. P. & Cardoso, L. A., 2006. The effect of supplementation on productive performance of Boer goat bucks fed winter veld hay. *Trop. Anim. Health Prod.* 38, 443 – 449.

Almeida, A. M., Schwalbach, L. M. J., Cardoso, L. A. & Greyling, J. P. C., 2007. Scrotal, testicular and semen characteristics of young Boer bucks fed winter veldt hay: The effect of nutritional supplementation. *Small Rum. Res. 7, 216 – 220.*

Arroquy, J. I., Cochran R. C., Wickersham T. A., Llewellyn D. A., Titgemeyer E. C., Nagaraja T. G. & Johnson, D. E., 2004. Effects of type of supplemental carbohydrate and source of supplemental rumen degradable protein on low-quality forage utilisation by beef steers. *Anim. Feed Sci. & Technol. 115, 247 – 263.*

Association of Official Analytical Chemistry - AOAC (1990). Official methods of analysis, 15th ed. AOAC International, Arlington.

Bal, M. A. & Ozturk, D., 2006. Effects of sulphur containing supplements on ruminal fermentation and microbial protein synthesis. *Res. Vet. Sci. 1, 33 – 36.*

Barbehenn, R. V., Chen, Z., Karowe, D. N. & Spickard, A., 2004. C3 grasses have higher nutritional quality than C4 grasses under ambient and elevated atmospheric CO_2. *Global Change Biol. 10, 1565 – 1575.*

Beever, D. E. & Siddons, R. C. 1986. Digestion and metabolism in the grazing ruminant. Pages 479 – 497 in L. P. Milligan, W. C. Grovum and A. Dobson, eds. Control of digestion and metabolism. Pentice-Hall, Englewood Cliffs, New Jersey.

BioAssay Systems. QuantiChrom™ Creatinine Assay Kit (DICT-500). www.bioassaysys.com

Black, C. C., 1971. Ecological implications of dividing plants into groups with distinct photosynthetic production capacities. *p. 87 – 114. In: J.B. Cragg, (Ed.) Advances in Ecological Research. Academic Press, New York, N.Y.*

Bohnert, D. W., Schauer, C. S., Bauer, M. L. & DelCurto, T., 2002. Influence of rumen protein degradability and supplementation frequency on performance and nitrogen use in ruminants consuming low-quality forage, I. Site of digestion and microbial efficiency. *J. Anim. Sci. 80, 2967 – 2977.*

Bohnert, D. W., DelCurto, T., Clark, A. A., Merril, M. L., Falck, S. J. & Harmon, D. L., 2011. Protein supplementation of ruminants consuming low-quality cool-or warm-season forage: Differences in intake and digestibility. *J. Anim. Sci. 89, 3707 – 3717.*

Bowen, M. K., Poppi, D. P. & McLennan, S. R., 2016. Effect of quantity and source of rumen nitrogen on the efficiency of microbial protein synthesis in steers consuming tropical forage. *Animal Production Science - https://doi.org/10.1071/AN15739 Submitted: 21 October 2015. Accepted: 20 October 2015 Published online: 2 December 2016*

Bowes, G. & Ogren, W. L., 1972. Oxygen inhibition and other properties of soybean ribulose 1, 5-diphosphate carboxylase. *J. Biol. Chem. 247, 2171 – 2176.*

Broderick, G. A. & Kang, J. A., 1980. Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and in vitro media. J. *Dairy Sci. 63, 64 – 75.*

Broderick, G. A. & Merchen, N. R., 1992. Markers for quantifying microbial protein synthesis in the rumen. *J. Dairy Sci. 75, 2618 – 2632.*

Cannas, A., Tedeschi, L. O., Fox, D. G., Pell, A. N. & Van Soest, P. J., 2004. A mechanistic model for predicting the nutrient requirements and feed biological values for sheep. *J. Anim. Sci. 82, 149 – 169.*

Chen, X. B. & Gomes, M. J., 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives. An overview of technical details. *Technical report, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, UK.*

Chen, X. B., Mejia, A. T., Kyle, D. J. & Ørskov, E. R., 1995. Evaluation of the use of the purine derivative: creatinine ratio in spot urine and plasma samples as an index of microbial protein supply in ruminants: studies in sheep. *J. Agric. Sci. Cambr. 125, 137 – 143.*

Caton, J. S. & Dhuyvetter, D. V., 1997. Influence of energy supplementation on grazing ruminants: requirements and responses. *J. Anim. Sci. 75, 533 – 542.*

Chandrasekharaiah, M., Thulasi, A. & Sampath, K. T., 2012. Effect of different rumen degradable nitrogen levels on microbial protein synthesis and digestibility in sheep fed on finger millet straw (*Eleucine coracana*) based diet. *Small Rum. Res. 102, 151 – 156.*

Ciavarella, T. A., Dove, H., Leury, B. J. & Simpson, R. J., 2000. Diet selection by sheep grazing *Phalaris aquatica L.* pastures of differing water-soluble carbohydrate content. *Austr. J. Agric. Res. 51 (6), 757 – 764.*

Collatz, G. J., Berry, J. A. & Clark, J. S., 1998. Effects of climate and atmospheric CO_2 partial pressure on the global distribution of C4 grasses: present, past, and future. *Oecologia 114,441 – 454.*

Costa, H. H. A., Rogério, M. C. P., Muir, J. P., Alves, A. A., Galvani, D. B., Pompeu, R. C. F. F., Landim, A. V., Carneiro, M. S. & Campos, W. E., 2013. Nutritional evaluation of lamb diets in a tropical setting formulated according to NRC (1985) and NRC (2007) specifications. *Small Rum. Res. 113, 20 – 29.*

Cruywagen, C. W., 2006. Technical note: A method to facilitate retrieval of polyester bags used in *in sacco* trials in ruminants. *J. Dairy Sci. 88, 1028 – 1030.*

Currier, T. A., Bohnert, D. W., Falck, S. J. & Bartle, S. J., 2004. Daily and alternate day supplementation of urea and biuret to ruminants consuming low-quality forage: I. Effects of cow performance and nutrient use in wethers. *J. Anim. Sci. 82, 1528 – 1535.*

DelCurto, T., Cochran, R. C., Haerman, D. L., Beoharka, A. A., Jacques, K. A., Towne, G. & Vanzant. E. S., 1990. Supplementation of dormant tallgrass-prairie forage: I. Influence of

varying supplemental protein and (or) energy levels on forage utilization characteristics of beef steers in confinement. *J. Anim. Sci. 68, 515 – 531.*

Detmann, E., Valente, E. E. L., Batista, E. D. & Huhtanen, P., 2014. An evaluation of the performance and efficiency of nitrogen utilization in cattle fed tropical grass pastures with supplementation. *Livest. Sci. 162, 141 – 153.*

Detmann, E., Paulino, M. F., Mantovani, H. C., Filho, S., Sampaio, C. B., De Souza, M. A., Lazzarini, I. & Detmann, K. S. C., 2009. Parameterization of ruminal fibre degradation in low-quality tropical forage using *Michaelis-Menten* kinetics. *Livest. Sci. 126, 136 – 146.*

Dijkstra, J., France, J. & Davies, D. R., 1998. Different mathematical approaches to estimating microbial protein supply in ruminants. *J. Dairy Sci. 81: 3370 – 3384.*

Dove, H. & Milne, J. A. 1994. Digesta flow and rumen microbial protein production in ewes grazing perennial ryegrass. *Aust. J. Anim. Sci. 20, 130 – 135.*

Du Plessis, D., 2011. The effect of supplementing different ratios of non-protein nitrogen to fermentable metabolisable energy on digestibility, intake and microbial protein yield in sheep. *M. Sc. Dissertation, University of Pretoria.*

Ehleringer, J. R. & Cerling, T. E., 2002. C3 and C4 Photosynthesis. The Earth system: Biological and ecological dimensions of global environmental change. *In: Harold A. Mooney and Dr Josep G. Canadell (eds), Ted Munn John Wiley & Sons, Ltd (Editor-in-Chief). Encyclopaedia of Global Environmental Change (ISBN 0-471-97796-9), Volume 2, 186 – 190.*

Ellis, W. C., Matis, J. H., Lascano, C., Mahloogi, M. & Pond, K., 1987. Size reduction, fermentation and passage of forage particles and forage intake by cattle. *In: Symposium Proceedings: Feed intake by beef cattle. November, 20 – 22, 1986. Animal Science Department, Agricultural Experiment Station, Division of Agriculture, Oklahoma State University, 81 – 95.*

Elliot, R., McMeniman, N. P., Norton, B. W., & Calderon Cortes, F.J. 1984. The food intake response of sheep fed five roughage sources supplemented with formaldehyde treated casein with and without urea. *Anim. Prod. Austr. 15, 337 – 340.*

Engels, E. A. N. & Van Der Merwe, F. J., 1967. Application of an *in vitro* technique to South African forages with special reference to affect certain factors on the results. *S. A. J. Agric. Sci. 10, 983 – 995.*

Erdman, R. A., Proctor, G. H. & Vandersal, J. H., 1986. Effect of rumen ammonia concentration on *in situ* rate and extent of digestion of feedstuffs. *J. Dairy Sci. 69, 2312 – 2320.*

Farmer, C. G. R., Cochran, R. C., Simms, D. D., Klevasahl, E. A., Wickersham, T. A. & Johnson, D. E., 2001. The effects of several supplementation frequencies on forage use and the performance of beef cattle consuming dormant tallgrass prairie forage. *J. Anim. Sci. 79, 2276 – 2285.*

Faverdin, P., Baumont, R. & Ingvartsen, K. L., 1995. Control and prediction of feed intake in ruminants. *In: M. Journet, E. Grenet, M – H. Farce, M. Thériez, C. Dermarquilly (eds). Recent developments in the Nutrition of Herbivores. Proceedings of the IVth International Symposium on the Nutrition of Herbivores, 95 – 120. INRA Edition, Paris.*

Ferrell, C. L., Kreikemeier, K. K. & Freetly, H. C., 1999. The effect of supplemental energy, nitrogen, and protein on feed intake, digestibility, and nitrogen flux across the gut and liver in sheep fed low-quality forage. *J. Anim. Sci. 77, 3353 – 3364.*

Fieser, B. G. & Vanzant, E. S., 2004. Interactions between supplement energy source and tall fescue hay maturity on forage utilization by beef steers. *J. Anim. Sci. 82, 307 – 318.*

Firkins, J. L., 1996. Maximizing microbial protein synthesis in the rumen. *J. Nutr. 126, S1347 – S1354.*

Flachowsky, G. & Tiroke, K., 1993. Influence of type of feeding and rumen incubation time on *in sacco* dry matter degradability of ryegrass, straw and concentrate in sheep and goats. *Small Rum. Res. 9, 321 – 330.*

Fox, D. G., Tedeschi, L. O., Tylutki, T. P., Russel, J. B., Van Amburgh, M. E., Chase, L. E., Pell, A. N. & Overton, T. R. 2004. The Cornell net carbohydrate and protein system model for evaluating herd nutrition and nutrient excretion. *Anim. Feed Sci. & Technol. 112, 29 – 78.*

Griswold, K. E., Apgar, G. A., Bouton, J. & Firkins, J. L., 2003. Effects of urea infusion and ruminal degradable protein concentration on microbial growth, digestibility, and fermentation in continuous culture. *J. Anim. Sci. 81, 329 – 336.*

Goering, H. K. & Soest, P. J., 1988. Forage Fiber Analyses (Apparatus, reagents, procedures and some applications). *In: Agriculture Handbook No. 379. USDA-A.R.S., U.S. Dept. of Agric. Washington, DC, USA.*

Gomes, M. J., Hovell, F. D. DeB., Chen, X. B., Nengomasha, E. M. & Fikremariam, D., 1994. The effect of starch supplementation of straw on microbial protein supply in sheep. *Anim. Feed Sci. & Technol. 49, 277 – 286.*

Hall, M. B. & Huntington, G. B., 2008. Nutrient synchrony: Sound in theory, elusive in practice. *J. Anim. Sci. 86, E287 – E292.*

Heldt, J. S., Cochran, R. C., Stokka, G. L., Farmer, C. G., Mathis, C. P., Titgemeyer, E. C., & Nagaraja, T.G., 1999. Effects of different supplemental sugars and starch fed in combination with degradable intake protein on low-quality forage use by beef steers. *J. Anim. Sci. 77, 2793 – 2802.*

Hennessy, D. W., Williamson, P. J., Nolan, J. V., Kempton, T. J. & Leng, R. A., 1983. The roles of energy- or protein-rich supplements in the subtropics for young cattle consuming basal diets that are low in digestible energy or protein. *J. Agric. Sci. 100, 657 – 666.*

Henning, P. H., Van der Londen, Y., Matthorpe, M. C., Nauhaus, W. K. & Swarts, H. M., 1980. Factors affecting the intake and digestibility of roughage by sheep fed maize straw supplemented with maize grain. *Proc. Wes. Sect. Am. Soc. Anim. Sci. 46, 514 – 525.*

Henning, P. H., Steyn, D. G. & Meissner, H. H., 1993. Effect of synchronization of energy and nitrogen supply on ruminal characteristics and microbial growth. *J. Anim. Sci. 71, 2516 – 2528.*

Herrera-Saldana, R., Gomez-Alarcon, R., Torabi, M. & Huber, J. T., 1990. Influence of synchronizing protein and starch degradation in the rumen on nutrient utilization and microbial protein synthesis. *J. Dairy Sci. 73, 142 – 148.*

Hersom, M. J., 2008. Opportunities to enhance performance and efficiency through nutrient synchrony in forage fed ruminants. *J. Anim. Sci. 86, E306 – E317.*

Hoover, W. H., Tucker, C., Harris, J., Sniffen, C. J. & De Ondarza, M.B., 2006. Effects of non-structural carbohydrate level and starch: sugar ratio on microbial metabolism in continuous culture of rumen contents. *Anim. Feed Sci. & Technol. 128, 307 – 319.*

Hume, I. D., 1970a. The response of microbial protein in the rumen. II. A response to higher volatile fatty acids. *Austr. J. Agricult. Sci. 21, 297 – 304.*

Hume, I. D., 1970b. Synthesis of microbial protein in the rumen. III. The effect of dietary protein. *Austr. J. Agricult. Sci. 21, 305 – 314.*

Huntington, G. B., 1997. Starch utilization by ruminants: From basics to the bunk. *J. Anim. Sci. 75, 852 – 867.*

Huston, J. E., Lippke, H., Forbes, T. D. A., Holloway, J. W. & Machen, R. V., 1999. Effects of feeding interval on adult cows in western Texas. *J. Anim. Sci. 67, 1360 – 1366.*

Jung, H. G., Mertens, D. R. & Payne, A. J., 1997. Correlation of acid detergent lignin and lignin with digestibility of forage dry matter and neutral detergent fiber. *J. Dairy Sci. 80, 1622 – 1628.*

Kanjanapruthipong, J. & Leng, R. A., 1998. The effects of dietary urea on microbial populations in the rumen of sheep. Amer. *J. Anim. Sci. 11 (6), 661 – 672.*

Kolver, E., Muller, L. D., Varga, G. A. & Cassidy, T. J., 1998. Synchronisation of ruminal degradation of supplemental carbohydrate with pasture nitrogen in lactating dairy cows. *J. Dairy Sci. 81, 2017 – 2028.*

Köster, H. H., Cochran, R. C., Titgemeyer, E. C., Vanzant, E. S., Abdelgadir, I. & St-Jean, G., 1996. Effect of increasing degradable intake protein on intake and digestion of low-quality, tallgrass-prairie forage by beef cows. *J. Anim. Sci. 74, 2473 – 2481.*

Köster, H. H., Cochran, R. C., Titgemeyer, E. C., Vanzant, E. S., Nagaraja, T. G., Kreikemeier, K. K. & St Jean, G., 1997. Effect of increasing proportion of supplemental nitrogen from urea on intake and utilization of low-quality, tallgrass-prairie forage by beef steers. *J. Anim. Sci. 75, 1393 – 1399.*

Kozloski, G. V., Reffatti, M. V., Sanchez, B., Lima, L. D., Cadorin Jr, C. J. & Fiorentini, G., 2007. Intake and digestion by lambs fed a low-quality grass hay supplemented or not with urea, casein or cassava meal. *Anim. Feed Sci. & Technol. 136, 191 – 202.*

Lamy, E., Van Harten, S., Sales-Baptista, E., Guerra, M. M. & Almeida, A. M., 2012. Factors influencing livestock productivity. *In S. Veerasamy Sejian, S.M.K. Naqvi, T. Ezeji, J. Lakritz & R Lal, eds. Environmental Stress and Amelioration in Livestock Production, pp. 19 – 51. Berlin, Germany. Springer-Verlag. ISBN: 978-3-642-29204-0*

Leng, R. A., 1990. Factors affecting the utilization of 'poor-quality' forages by ruminants particularly under tropical conditions. *Nutr. Res. Rev. 3, 277 – 303.*

Leng, R. A., 1995. Evaluation of tropical feed resources for ruminant livestock. *In: Tropical feeds and feeding system. First FAO electronic conference on tropical feeds and feeding systems: www/fao.org/ag/AGA/agap/FRG/ECONF95? contents.htm pp. 23 – 53.*

Leury, B. J., Siever-Kelly, C., Gatford, K. L., Simpson, R. J. & Dove, H., 1999. Spray-topping annual grass pasture with glyphosphate to delay loss of feeding value during summer. IV. Diet composition, herbage intake, and performance in grazing sheep. *Aust. J Agric. Res. 50, 487 – 495.*

Matejovsky, K. M. & Sanson. D.W., 1995. Intake and digestion of low-, medium-, and high-quality grass hays by lambs receiving increasing levels of corn supplementation. *J. Anim. Sci. 73, 2156 – 2167.*

McDonald, P., Edwards, R. A., Greenhalgh, J. P. D., Morgan, C. A., Sinclair, L. A. & Wilkinson, R. G., 2011. Animal Nutrition. 7th Ed. Longman. NY.

Meissner, H. H., Ponelat, J. & Spreeth, E. B., 1989. Effect of casein supplementation and hay quality on non-ammonia N flow to the abomasum in sheep. *S.-Afr. Tydskr. Veek. 19 (1), 52 – 54.*

Meissner, H. H., 1997. Recent research on forage utilization by ruminant livestock in South Africa. *Anim. Feed Sci. & Technol. 69,103 – 119.*

Mentz, A. M., Hassen, A., Van Niekerk, W. A., Mynhardt, H. & Coertze, R., 2013. The effect of substituting urea for a commercial slow release urea as supplement to sheep fed a poor quality *Eragrostis curvula* hay. *In:* Energy and protein metabolism and nutrition in sustainable animal production 134, 99 – 100. *4th International Symposium on Energy and Protein Metabolism and Nutrition Sacramento, California, USA 9-12 September 2013. Ed*: *Oltjen, J. W., Kebreab, E. & Lapierre, H.*

Mertens, D. R. & Loften, J. R., 1980. The effect of starch on forage fiber digestion kinetics *in vitro*. *J. Dairy Sci. 63, 1437 – 1446.*

Minson, D. J., 1990. Forage in Ruminant Nutrition. Academic *Press. San Diego, C. A.*

Moore, J. A., Brant, M. H., Kunkle, W. E. & Hopkins, D. I., 1999. Effects of supplementation on voluntary forage intake, diet digestibility, and animal performance. *J. Anim. Sci. 77, (2), 122 – 135.*

Mould, F. & Ørskov, E. R., 1983. Manipulation of rumen fluid pH and its influence on cellulolysis *in sacco*, dry matter degradation and the rumen microflora of sheep offered either hay or concentrate. *Anim. Feed Sci. & Technol. 10, 1 – 14.*

Mould, F., Ørskov, E. R. & Man, S. O., 1983. Associative effects of mixed feeds. 2. The effect of dietary additions of bicarbonate salts on the voluntary intake and digestibility of diets containing various proportions of hay and barley. *Anim. Feed Sci. & Technol. 10, 15 – 29.*

Mullik, M. M., 2007. Efficiency of microbial protein synthesis in steers fed freshly harvested tropical grass. *Tropentag, University of Kassel-Witzenhausen and University of Göttingen. Conference on International Agricultural Research for Development.*

Nocek, J. E. & Russell, J. B., 1988. *In situ* and other methods to estimate ruminal protein and energy digestibility: a review. *J. Dairy Sci. 71, 2051 – 2069.*

NRC, 2000. Nutrient Requirements of Beef Cattle. 7th updated ed. *The National Academy Press, Washington, DC.*

NRC, 2007. Nutrient Requirements of small ruminants, sheep, goats, cervids, and new world camelids. 2007. *The National Academies Press, Washington, DC.*

Ørskov, E. R. & McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements of feed weighted according to rate passage. *J. Agric. Sci. 92, 499 – 503.*

Payne, R. W. (Ed.) Introduction to GenStat® *for Windows*™ (15th Edition), © 2012 VSN International, Hemel Hempstead, Hertfordshire, UK. Website: http://www.genstat.co.uk/

Poppi, D. P. & McLennan, S. R., 1995. Protein and energy utilization by ruminants at pasture. *J. Anim. Sci. 73, 278 – 290.*

Poppi, D. P., McLennan, S. R., Bediye, S., De Vega, A. & Zorrilla-Rios, J. 1999. Forage quality: Strategies for increasing nutritive values of forages. In: Proceedings of the XVIII International Grassland Congress. *XVIII International Grassland Congress,* Winnepeg and Saskatoon, Canada, (302 – 322). June 8 – 17, 1997.

Poppi, D. P., Ellis, W. C., Matis, J, H. & Lascano, C. E. 2001. Marker concentration patterns of labelled leaf and stem particles in the rumen of cattle grazing Bermuda grass (*Cynodon dactylon*) analysed by reference to a raft model. *Br. J. Nutr. 85, 553 – 563.*

Potthast, V., Price, H. & Pfeffer, E., 1977 (Abstract). Untersuchungen zur dynamik der N-unsetzungen beim Schaf. *Z. Tierphysiol. Tierernaehr Futtermittelkd. 38, 338 – 339.*

Preston, T. R. & Leng, R. A., 1987. Matching ruminant production systems with available resources in the tropics and sub-tropics. *Armidale, Australia: Penambul Books.*

Richardson, J. M., Wilkinson, R. G. &. Sinclair, L. A., 2003. Synchrony of nutrient supply to the rumen and dietary energy source and their effects on the growth and metabolism of lambs. *J. Anim. Sci. 81, 1332 – 1347.*

Russell, J. B., 1984. Factors influencing completion and composition of the rumen microbial flora. *In: Gilchrist, F.M.C., Mackie. R.I. (Eds.), Herbivore Nutrition. The Science Press, Craighall, South Africa, pp. 3351 – 3561.*

Russell, J. B., 1989. Strategies that ruminal bacteria use to handle excess carbohydrate. *J. Anim. Sci. 76, 1955 – 1963.*

Russell, J. B. & Strobel, H. J., 1990. ATPase-dependent energy spilling by the ruminal bacterium, *Streptococcus bovis. Archives Microbiol. 153, 378 – 383.*

Russell, J. B., O'Connor, J. D., Fox, D. G., Van Soest P. J. & Sniffen C. J., 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *J. Anim. Sci. 70, 3551 – 3561.*

Russell, J. B. & Strobel, H. J., 2005. Microbial Energetics. Chapter 8, 229 – 262. *In: Quantitative aspects of ruminant digestion and metabolism. Edited by J. Dijkstra, Forbes, J. M. & France, J. 2nd ed. CABI – Publishing: ISBN 0 85199 8143.*

Samuels, M. L., 1989. Statistics for the life sciences. Collier MacMillan Publishers, London.

Sanson, D. W., Clanton, C. & Rush, I. G., 1990. Intake and digestion of low-quality meadow hay by steers and performance of cows on native range when fed protein supplements containing various levels of corn. *J. Anim. Sci. 68, 595 – 603.*

Satter, L. D. & Slyter, L. L., 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. *British J. Nutr. 32, 194 – 208.*

Sauvant, D., Schmidely, P., Daudin, J. J. & St-Pierre, N. R., 2008. Meta-analyses of experimental data in animal nutrition. *Animal (2008), 1203 – 1214.*

SCA, 1990. Feeding Standards for Australian Livestock. *Standing Committee on Agriculture, CISRO, Publications. Melbourne, Australia.*

Sinclair, L. A., Garnsworthy, P. C, Newbold, J. R. & Buttery, P. J., 1993. Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermentation and microbial protein synthesis in sheep. *J. Agric. Sci. 120, 251 – 263.*

Sinclair, L. A., Garnsworthy, P. C., Newbold, J. R. & Buttery, P. J., 1995. Effects of synchronizing the rate of dietary energy and nitrogen release in diets with a similar carbohydrate composition on rumen fermentation and microbial protein synthesis in sheep. *J. Agric. Sci. 124, 463 – 472.*

Sniffen, C. J. & Robinson, P. H., 1987. Microbial growth and flow as influenced by dietary manipulations. In: Symposium: Protein and fiber digestion, passage, and utilization in lactating cows. *J. Dairy Sci. 70, 425 – 441.*

SAS, 2015. Statistical Analysis Systems Institute user's guide (Version 9.3). SAS Institute. Inc., Raleigh, North Carolina, USA.

Tilley, J. M. & Terry, R. A., 1963. A two stage technique for the *in vitro* digestion of forage crops. *J. Brit. Grassl. Soc., 18, 104 – 111.*

Trevaskis, L. M., Fulkerson, W. J. & Gooden. J. M., 2001. Provision of certain carbohydrate-based supplements to pasture-fed sheep, as well as time of harvesting of the pasture, influences pH, ammonia concentration and microbial protein synthesis in the rumen. *Aust. J. Exp. Agric. 4, 21 – 27.*

Van Niekerk, B. D. H., 1996. Limiting nutrients in supplementary feeding. Bull. *Grassland. Soc. Sth. Afr. 7 (1), 51 – 56.*

Van Soest, P. J., 1994. Nutritional ecology of the ruminant, *2nd ed. Cornell. University Press, Ithaca.*

Vanzant, E. S. & Cochran, R. C., 1994. Performance and forage utilization by beef cattle receiving increasing quantities of alfalfa hay as a supplement to low-quality, tallgrass-prairie forage. *J. Anim. Sci. 72, 1059 – 1067.*

Webster, A. J. F., 1989. Bioenergetics, bioengineering and growth. *Anim. Prod. 48, 249 – 269.*

Weston, R. H., Lindsay, J. R., Purser, D. B., Gordon, G. L. R. & Davis, P., 1988. Feed intake and digestion responses in sheep to the addition of inorganic sulphur to a herbage diet of low sulphur content. *Aust. J. Agric. Res. 39, 1107 – 1119.*

Witt, M. W., Sinclair, L. A., Wilkinson, R. G. & Buttery. P. J., 1999. The effects of synchronizing the rate of dietary energy and nitrogen supply to the rumen on the metabolism and growth of ram lambs given food at a restricted level. *Anim. Sci. 69,627 – 636.*

Wickersham, T. A., Titgemeyer, E. C., Cochran, R. C., Wickersham E. E. & Moore E. S., 2008. Effect of frequency and quantity of rumen-degradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. *J. Anim. Sci. 86, 3089 – 3099.*

Wickersham, T. A., Titgemeyer, E. C., Cochran, R. C. & Wickersham, E. E., 2009. Effect of undegradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. *British J. Nutr. 101, 225 – 232.*

Wilson, J. R. & Minson, D. J., 1980. Prospects for improving the digestibility and intake of tropical grasses. *Trop. Grasslands 14 (3), 253 – 259.*