Effect of diets differing in rumen soluble nitrogen on poor quality roughage utilization by sheep

by
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Contents

Declaration

I, Anna Margarietha Jooste declare that this dissertation, which I hereby submit for the degree MSc(Agric) Animal Nutrition 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 institution.

Signature:.....
July 2012

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Summary

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The aim of this study was to determine whether a rapid release N source can be substituted with a slow release N source without having any negative effects on intake, digestibility, rumen fermentation and microbial protein synthesis, if sheep are fed a poor quality roughage. Five rumen cannulated wethers were used in the trial in a 5x5 latin square design. Cannulated wethers were assigned to different treatments after each experimental period. The treatments studied had different proportions of urea to Optigen®II, with the same inclusion level of starch and a mineral premix between treatments. The five different treatments were: 100% urea; 75% urea:25% Optigen®II; 50% urea:50% Optigen®II; 25% urea:75% Optigen®II and 100% Optigen®II. Significant differences ($P<0.05$) between the 25% urea:75% Optigen®II and the other treatments in terms of intake suggested that a combination of urea and Optigen®II might be the preferred supplementation due to a significantly higher dry matter intake (DMI), organic matter intake (OMI), neutral detergent fibre intake (NDF intake) and digestible organic matter intake (DOMI). The intake variables of 100% urea and 100% Optigen®II did not differ ($p>0.05$). No differences ($P>0.05$) were recorded for dry matter digestibility (DMD), organic matter digestibility (OMD) and neutral detergent fibre (NDF) digestibility between treatments. However, the 100% Optigen®II treatment had a significant ($P<0.05$) lower apparent nitrogen digestibility, which might be the result of a slower rumen NH_3-N release and higher nitrogen excretion than the other treatments. No differences were observed for pH and VFA between different treatments. The rumen NH_3-N concentration of the 100% Optigen®II treatment was significantly ($P<0.05$) lower than the 100% urea treatment at 2 and 4 hours after infusion. The effective degradability of both DM and NDF did not differ ($P>0.05$) between treatments. Neither were there differences between treatments for total microbial crude nitrogen (MCN) production. Based on biological evaluation, it could be suggested that urea might be substituted with Optigen®II in supplements. From an economical point of view, urea might still be the preferred NPN source, as urea is cheaper than Optigen®II in terms of R/kg nitrogen.

List of abbreviations

ADF	**A**cid **D**etergent **F**ibre
ADIN	**A**cid **D**etergent **I**nsoluble **F**ibre
ADL	**A**cid **D**etergent **L**ignin
a	**S**oluble fraction
BCVFA	**B**ranched **C**hain **F**atty **A**cids
BW	**B**ody **W**eight
b	**I**nsoluble potential degradable fraction
CNCPS	**C**ornell **N**et **C**arbohydrate and **P**rotein **S**ystem
CNS	**C**entral **N**ervous **S**ystem
CP	**C**rude **P**rotein
Cr	**C**reatinine
c	**D**egradation rate
DM	**D**ry **M**atter
DMD	**D**ry **M**atter **D**igestibility
DMI	**D**ry **M**atter **I**ntake
DOMI	**D**igestible **O**rganic **M**atter **I**ntake
ED	**E**ffective **D**egradability
IVOMD	*In Vitro* **O**rganic **M**atter **D**igestibility
MCN	**M**icrobial **N**itrogen
MCP	**M**icrobial **P**rotein
NDF	**N**eutral **D**etergent **F**ibre
NH_3-N	**A**mmonia- **N**itrogen
N	**N**itrogen
NSC	**N**on **S**tructural **C**arbohydrates
NPN	**N**on **P**rotein **N**itrogen
NRC	**N**ational **R**esearch **C**ouncil

OM	**O**rganic **M**atter
OMD	**O**rganic **M**atter **D**igestibility
OMI	**O**rganic **M**atter **I**ntake
PD	**P**urine **D**erivatives
RDP	**R**umen **D**egradable **P**rotein
RUP	**R**umen **U**ndegradable **P**rotein
SC	**S**tructural **C**arbohydrate
SE	**S**tandard **E**rror
SRU	**S**low **R**elease **U**rea
VFA	**V**olatile **F**atty **A**cids

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Chapter 1

General introduction

Animal production in South Africa is mostly based on extensive grazing systems. Grazing throughout the country differs in terms of quality and quantity. Seasonal changes in rainfall is the largest contributing factor to differences in quality and quantity of veld. The nutritional value of sweet veld is the least affected by seasonal change. However, sour veld's nutritional value can change drastically with seasonal change. Sour veld usually occurs in the higher rainfall areas, whereas sweet veld is most common in low rainfall areas.

Nutrient requirements of grazing animals are often not met with the low and variable nutritional qualities of veld (especially in the sour veld regions of South Africa). Therefore, additional nutrients should be supplemented to animals to maintain and/or improve production.

Nitrogen and/or protein supplementation is mostly practiced on extensive grazing systems during the winter months when protein levels of veld are low and unavailable for use by animals. By providing supplemental nitrogen and/or protein during these difficult times to ruminants, intake and digestibility of the veld may improve. This in turn, will minimize production losses (Köster *et al.*, 1996; Ferrell *et al.*, 1999; Bohnert *et al.*, 2002).

A variety of protein sources can be used as the main source of protein in a supplemental lick. Plant proteins and animal proteins are some of the best protein sources to be used, but are very expensive and not always economically justified. Non protein nitrogen (NPN) sources are most commonly used in protein supplements due to the ability of ruminants to utilize the nitrogen, its high nitrogen density and low cost per unit nitrogen.

Often, improving roughage intake and digestibility from a NPN supplement, is a challenge due to unsynchronization of available energy from roughage and supplemental non protein nitrogen. This is because urea is hydrolysed at a fast rate in the rumen, therefore the availability of ammonia to rumen bacteria might be limited by energy sources for the efficient fermentation of the roughage (Galo *et al.*, 2003). When urea enters the rumen, it is broken down to ammonia. This ammonia is utilized by the rumen bacteria for the synthesis of microbial protein. But due to the rapid release of ammonia from urea, the most of the ruminal ammonia is absorbed through the rumen wall into the blood stream. The ammonia is then carried to the liver where it is converted into urea. Most of the urea is excreted in the urine and some is recycled to the rumen via saliva or through the rumen wall (Huntington *et al.*, 2006). To overcome this obstacle various other non protein nitrogen products have been developed with the characteristics of nitrogen (N) being slowly released in the rumen (Harrison & Karnezos, 2005).

Optigen®II is an example of a slow release non protein nitrogen product. It is a blended, controlled urea source, coated in polyester polyurethane, which allows the diffusion of urea through micro-pores, that slows down the rate of nitrogen release in the rumen (ICF Consulting, 2004).

The objectives of this study was to determine whether urea can be substituted by Optigen®II or if a combination between the two NPN sources would result in maximum intake, rumen fermentation, neutral detergent fibre (NDF) degradability and rumen microbial protein synthesis.

Chapter 2

Literature review

2.1 Natural veld

Extensive grazing on natural veld is mainly utilized for sheep production around the world, as well as in South Africa. The utilization of natural veld is often a low-input and low-cost management system for wool- and mutton production. Natural veld in South Africa is extremely diverse due to biophysical constraints, such as soil conditions and rainfall (O'Reagain & McMeniman, 2002). South Africa is a semi-arid country that has an erratic and seasonal rainfall, with prolonged droughts not being uncommon (Van Niekerk, 1996). Spatial and temporal variability occur within natural veld regarding forage quality and quantity. Variabilities in quality and quantity are caused by season, erratic rainfall and specie diversity (O'Reagain & McMeniman, 2002). Due to the variabilities in quality and quantity of natural veld, supplementation of protein, energy and some minerals is often required to optimize animal production.

2.1.1 Factors affecting the nutritive value of natural veld

2.1.1.1 Specie diversity

A great variety of grass species are present in a grass veld region. These grass species vary widely in their nutritive value and structural characteristics between and within each other (O'Reagain & McMeniman, 2002). Specie diversity is mainly associated to the photosynthetic pathways. The two common photosynthetic pathways that occur within a plant are the C3 and C4 pathways. Temperate grasses are known as C3 grasses and tropical grasses as C4 grasses

(Coleman & Henry, 2002). Tropical grasses (C4) have a higher tensile strength than temperate grasses (C3) due to a higher lignin concentration, densely packed mesophyll cells (McDonald *et al.*, 2002) and a low concentration of non-structural carbohydrates (NSC) (Poppi *et al.*, 1999) when mature. These characteristics of tropical grasses lead to decreased mechanical degradation and microbial protein production in the ruminant (McDonald *et al.*, 2002). Tropical grasses (C4) also produce more dry matter (DM) with a lower nutritive value than temperate grasses (C3) when mature (Minson, 1990).

Legumes and grasses also differ due to structural differences. Legumes are generally higher in protein, lower in cell wall content and have more localized and core lignin than grass species. The localization of the lignin in legumes enables the rumen microbes to degrade carbohydrates more than in grass species (Minson, 1990; Coleman & Henry, 2002). Legumes generally also have a higher voluntary intake than grass species at the same digestibility (Coleman & Henry, 2002). This is due to the quicker rumen degradation of legumes, because of the localized lignin, that result in a shorter retention time in the rumen than in grass species (Coleman & Henry, 2002).

2.1.1.2 Season and rainfall

The grass veld regions of South Africa are situated in the summer rainfall area with most of the rain falling during spring and summer. The growing season of grass starts in spring, with most of the growth occurring during summer and late summer. During winter, growth is limited due to low temperatures and low rainfall (Tainton, 1999).

After the first rains there is a rapid increase in the nutritive value of grass. As soon as the first frost appears, the nutritive value drops rapidly. A variable and prolonged dry and cold season coincides with the winter period, which might lead to a shortage of DM-grazing during late winter and early spring. Variation in the nutritive value, from a high nutritive value in summer to a low nutritive value after the first frost, is more pronounced in the sour grass veld than in the sweet grass veld. The sour grass veld regions are at a higher altitude and receives a higher rainfall than the sweet grass veld regions (Van Niekerk, 1975).

Leaching of minerals in the high rainfall areas, also has an impact on the nutritive value of

grass. In the higher rainfall areas leaching of minerals is more pronounced than in the low rainfall areas, therefore decreasing the mineral content of grass growing in the higher rainfall areas (sour grass veld regions).

2.1.1.3 Temperature

Temperature as well as light also influences the nutritive value of grass (Nelson & Moser, 1994). Lignification of cell walls is less pronounced at low temperatures and more pronounced at high temperatures. Consequently, grasses grown at high temperatures will have a decreased digestibility due to the accumulation of cell wall materials (Coleman & Henry, 2002). During low temperatures cell content deposition is increased in leaf fractions. The deposition of cell content in the leaf is more pronounced in temperate grasses than in tropical grasses during low temperatures (Nelson & Moser, 1994).

2.1.1.4 Growth stage

Cell wall, cell content and the growth stage (Nelson & Moser, 1994) influences the quality of the plant. Immature grass is lush, highly digestible and has a high moisture, low dry matter (DM), high protein and low fibre concentration. As soon as grass matures, DM and fibre concentrations increase and protein concentration decreases in grass (McDonald *et al.*, 2002). Cherney *et al.* (1993) and Arthington & Brown (2005) both found that neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin increases with maturation. Lignin is the major factor affecting the digestibility of forages. A negative correlation is found between lignin content and fibre digestibility (Cherney *et al.*, 1993). The higher the lignin content and the less localized it is in the cell wall, the lower the fibre digestibility. Lignin increases with maturity and fibre digestibility decreases with maturity (Cherney *et al.*, 1993). The cell wall consists out of cellulose and hemicellulose (fibre content) with reinforced lignin. The reinforced lignin decreases the digestibility of cellulose and hemicellulose as grass matures. During maturation the reinforced cell walls increase and the cell content, consisting out of protein and water soluble carbohydrates, decreases (McDonald *et al.*, 2002) and therefore decreasing the digestibility and degradability of the grass (Coleman & Henry, 2002). In the study done by Arthington & Brown (2005), the authors found that the CP concentration of tropical grasses decreased over a 10 week regrowth period. In contrast with tropical grasses examined by Arthington & Brown (2005), Chaves *et al.* (2006) found that with a temperate grass specie (rye grass), the CP degradation rate was unaffected by

maturation, but fibre degradation rate was slower. Fonnesbeck *et al.* (1981) concluded that the most important factor influencing the nutritive value of grass is the chemical composition.

Changes in the plant's leaf:stem ratio and cell wall content during maturation has an effect on the digestibility and dry matter intake (DMI) of grass (Van Soest, 1994). A decline in the nutritive value as grass matures, is also explained by the decrease in the leaf:stem ratio (McDonald *et al.*, 2002). Early growth is characterized by rapid leaf production, which is highly nutritive. Leaves and stems of immature grass are equally digestible and are digested to the same extent. When grasses mature the stem's digestibility decreases at a faster rate than the leaf fraction (Nelson & Moser, 1994). This is related to the higher lignin content in the stems (Coleman & Henry, 2002) and the high crude protein (CP) content in the leaves' mesophyll cells, which do not form highly lignified cell walls (Nelson & Moser, 1994).

2.1.1.5 Fertilization

Fertilization and the type of soil influences the mineral content of plants and thus the nutritive value of the plant. Mineral deficiencies in plants are dependant on the type of soil. The acidity of soils has a great influence on the absorption and availability of minerals to the plant. By applying fertilizers, the deficiencies can be overcome and therefore improve the availability and absorption of minerals. Nitrogenous fertilizers encourage the growth of grasses but also depresses the cell content of temperate grasses (McDonald *et al.*, 2002).

2.1.1.6 Grazing management

Grazing systems might have a large influence on the nutritive value of pastures. Animals, especially sheep, are selective grazers. At low stocking rates the animals are more selective. This allows that the rate of growth of certain grass species exceed the rate of harvesting. The non-selected grass accumulates and matures, thus decreasing their nutritive value (McDonald *et al.*, 2002). With high stocking rates the animals are forced to be less selective. This results in an uniformly grazed pasture, with regrowth that is high in nutritive value (Coleman & Henry, 2002).

2.2 Intake and digestibility

Voluntary intake is the most important factor determining performance. Intake of low quality forage by ruminants is usually not enough to meet the nutrient requirements of the animals for optimum production. Voluntary intake is a complex phenomenon and is determined by various physical and physiological factors, that is controlled by the hypothalamus in the brain (Weston, 2002). Voluntary intake is regulated by the energy needs and the physical constraints of a ruminant (Forbes, 2003). It is assumed that animals eat to meet their energy requirements (Meissner & Paulsmeier, 1995; Decruyenaere *et al.*, 2009). As presented in Fig. 2.1, Forbes (2003) confirmed this assumption by concluding that intake is controlled by energy and bulk sensing mechanisms. When a ruminant is offered a diet with increasing energy concentration or digestibility, feed intake will increase until the ruminant's energy requirements are met, thereafter intake will decrease to maintain the energy requirements of the ruminant. Forbes (2003) confirmed this assumption by concluding that intake is controlled by energy and bulk sensing mechanisms. In Fig 2.1 it can be seen that when a diet of low nutritive value and low digestibility is consumed, rumen distension will limit intake. As soon as the ruminant is offered a diet with increasing energy concentration or digestibility, physiological control mechanisms will limit feed intake to maintain energy requirements of the ruminant (Montgomery & Baumgardt, 1965).

There are a few factors that influence the ability of the ruminant to consume enough low quality forage to meet its nutrient requirements. These factors include: i) physical capacity (rumen fill), ii) bulkiness of the forage, iii) digestion rate, iv) palatability and v) physiological mechanisms that are dependant on the environmental factors, N-status, production status and the energy demand of the animal (Grovum, 1984; Ferrell *et al.*, 1999; Weston, 2002). The physical mechanisms are short term intake regulators and the physiological mechanisms are usually long-term intake regulators (Grovum, 1984). As illustrated in Fig 2.2, all these factors may influence voluntary intake via the hypothalamus and central nervous system. An energy deficit usually initiates intake, a hunger signal is sent to the central nervous system which in turn stimulates forage intake. A ruminant will then eat to meet its energy requirements (it is explained in the preceding paragraph), but natural veld usually fails to meet the energy requirements of a ruminant, therefore intake is inhibited by ruminal constraints that send a satiety signal to the central nervous system and hypothalamus. Digesta load are affected by the digestibility, the passage rate and the size of the particles. As soon as some of the digesta load is cleared from the rumen, intake will be initiated again due to the energy deficit (Weston, 2002).

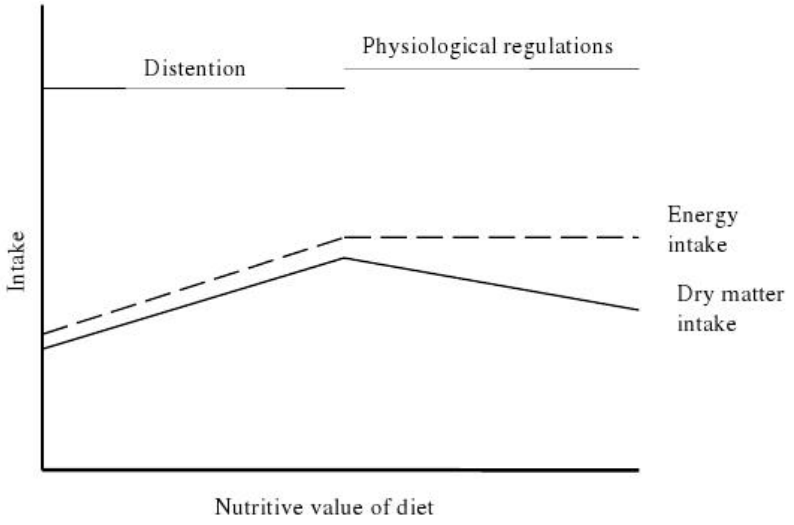

FIGURE 2.1 The effect of dietary energy concentration on the regulation of voluntary intake in ruminants (Montgomery & Baumgardt, 1965)

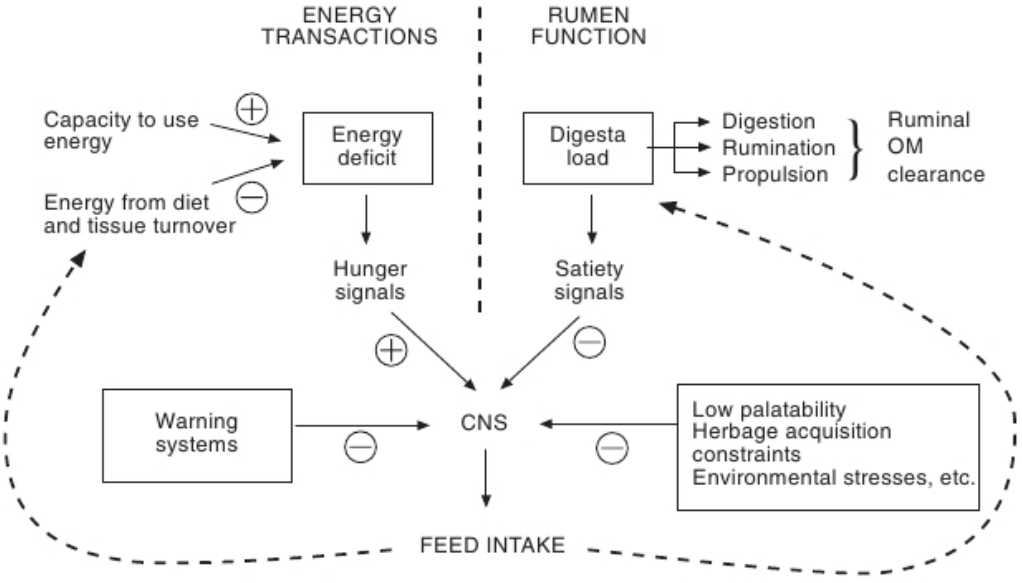

FIGURE 2.2 An illustration of how pasture intake is regulated in the ruminant (Weston, 2002)

Rumen fill is one of the main factors responsible for regulating forage intake and is dependant on the forage bulkiness, digestion rate and the passage rate of the forage consumed. Grounded ingredients, such as concentrates, are less bulky than forages, therefore more concentrate can be consumed than forage before rumen fill will decrease intake. The intake of diets containing concentrates usually increases with decreased energy content. In this case where concentrates are included in the diet and enough energy can be consumed by the ruminant, physiological mechanisms will limit intake before rumen fill (Weston, 2002). Forage, especially grass species, are digested slower than legumes or concentrates, due to its buoyancy, low specific gravity and

rigid cell walls. Legumes are more buoyant, have a higher specific gravity and have more localized lignin than grass species which makes the digestion and passage rate of the legume particles quicker (Romney & Gill, 2000). Due to the slower rate of digestion of grass, less grass is required to stimulate a satiety signal, due to rumen fill, than legumes. Voluntary intake between grass species also differ. The intake of temperate forages are higher compared to tropical forages at the same maturity. This is due to the higher digestibility and nutritive value of temperate forage at a certain age of maturity. Voluntary intake of tropical and temperature forages at the same digestibility (same protein levels) are similar (Decruyenaere *et al.*, 2009). Compensation for the low nutritive value and digestibility of tropical forages occur by the reduction of forage particle size through prolonged chewing and rumination (Assoumaya *et al*., 2007 as cited by Decruyenaere *et al*., 2009). A high moisture content of more than 80% might also inhibit intake due to rumen fill. Immature forages or regrowth usually have a high moisture content. When ruminants consume immature forages or regrowth, the rumen is filled with a large amount of water which initiates a decreased intake due to physical discomfort (Meissner & Paulsmeier, 1995). On the other hand, mature forages, high in fibre, such as grass hay, result in a decreased dry matter intake (DMI) due to its bulkiness, slow digestion and buoyancy that takes up a lot of space in the rumen. Mature forages have a low protein, high cell wall and lignin content and therefore, increasing the retention time and decreasing forage intake in the rumen, due to a reduced forage digestibility (Arthington & Brown, 2005). Intake of forages can be increased by changing the physical form of the forage, by grounding or chopping or by supplementation. Grounding and chopping will improve the passage rate, therefore making the forage less bulky, but it also resulted in a decrease in digestibility (Romney & Gill, 2000). Digestibility is negatively affected due to the fact that the forage is only available for a short period of time to microbial degradation in the rumen.

To illustrate the effect of bulkiness on DMI, Schettini *et al.* (1999) placed different amounts and weights of tennis balls into the rumen of steers fed a low quality forage. These authors found that increasing the number of tennis balls (volume and mass), DMI decreased. Therefore indicating that both mass and volume of rumen contents are correlated to DMI. In another study, Gregorini *et al.* (2007) found that rumen fill affected short-term forage intake and changed the way forage was consumed. These authors reported a decrease in bite size, bite rate and time spent grazing. On the other hand, an increase in rumen fill and bite depth were recorded by the authors. Distribution and availability of forage on extensive grazing are some of the environmental factors that have a great influence on forage intake (Garcia *et al.*, 2003). Due to erratic

rainfall, forages are often scattered and sparse over the veld and only small quantities of forage are sometimes available to the ruminant in a certain area. The animal has to walk vast distances to fulfil their requirements by increasing intake. In these circumstances the animals reduce biting frequency and intake rate, but compensate for it by increasing time spent grazing (Roguet *et al*., 1998 as cited by Decruyenaere *et al*,. 2009). Bite size, bite rate and grazing time are important factors influencing forage intake on grazing. Forage must be evenly distributed and not far apart to maximise bite rate. Dense, short forage allows the animal to maximise bite size and bite rate. With tall, sparse forage, bite size is limited (McDonald *et al.*, 2002).

Other environmental factors affecting voluntary intake are temperature, day length and humidity. Forage intake decreases when temperatures are above the thermoneutral zone and increases when temperatures are below the thermoneutral zone of the animal (McDonald *et al.*,2002; Weston, 2002). The reduction and increase in forage intake depending on environmental temperature, is due to the fact that all mammals need to maintain their body temperature to be able to survive. Various factors influence heat input and heat dissipation, that are necessary to maintain body temperatures by ruminants. Metabolic heat production and solar radiation generates heat (Weston, 2002). Heat generation of ruminants grazing on pasture is generally higher than of those fed indoors. This is due to the fact that ruminants on pasture generate more locomotion heat and graze for longer times to meet their nutrient requirements (Weston, 2002). The ability of the ruminant to dissipate the heat that is generated, is dependant on temperature and humidity (Weston, 2002). At high temperatures and humidities ruminants are less able to dissipate heat. In these circumstances voluntary intake will decrease until more favourable conditions occur. Forage digestion also generates more heat than concentrates or total mixed rations, due to its physical structure that makes it difficult to digest. The length of the day influences intake by the time which is available to spend grazing. The longer the day length the more time can be spent grazing, therefore intake should be more than on shorter days (McDonald *et al.*, 2002).

Forages containing low levels of protein also depresses intake. Rumen bacteria requires ammonia nitrogen for the degradation of low quality forage. By supplementing a non-protein nitrogen source, intake can be increased (McDonald *et al.*, 2002). Forage DMI increases with increasing rate of digestion, as well as increased passage rate. The high NDF content in low quality forage, is the principal nutrient responsible for a slow rate of digestion and therefore a depressed intake (Forbes, 2003).

2.3 Rumen fermentation

Feed entering the rumen undergoes fermentation by rumen bacteria before it is digested in the small intestine. The rumen bacteria require energy and nitrogen for the digestion of feed and microbial protein synthesis, therefore it is necessary to provide the correct nutrients for rumen bacteria as well as for digestion in the small intestine to ensure optimum production (Russell *et al.*, 1992). By providing the nutrients in the correct ratio according to the rumen bacteria's requirements, volatile fatty acid (VFA) production and microbial protein synthesis will be maximized, due to an increased growth rate of rumen bacteria. The rumen bacteria present during fermentation determines the quality and quantity of the production of VFA and microbial protein (Citron *et al*., 1987 as cited by Russell *et al*., 1992). The VFA are the primary source of energy to ruminants. Feed type and quality have an influence on the type of rumen bacteria present during fermentation (Russell *et al.*, 1992).

According to Russell *et al.* (1992) the Cornell Net Carbohydrate and Protein System (CNCPS) divides the rumen bacteria population into two groups regarding fermentation: i) those that ferment non-structural carbohydrates (NSC) and ii) those that ferment structural carbohydrates (SC). Rumen bacteria fermenting NSC is known as amylolytic bacteria which ferment starch, pectin and sugars. Amylolytic bacteria require ammonia, amino acids and peptides as a nitrogen source for the production of propionate and ammonia. Amylolytic bacteria has a faster growth rate than cellulolytic bacteria, that enable the amylolytic bacteria to utilize the available nitrogen before cellulolytic bacteria, therefore, resulting in reduced availability of nitrogen for NDF degradation (Heldt *et al.*, 1999). Cellulolytic bacteria (fibrolytic bacteria) are known to ferment cell walls (SC) such as cellulose and hemicellulose. These rumen bacteria require ammonia for microbial protein synthesis, fermentation and the production of acetate (Russell *et al.*, 1992).

Rumen bacteria utilize ammonia for fermentation and for the synthesis of microbial protein. In most cases, ammonia production exceeds the ability of rumen bacteria to utilize the ammonia. This excess ammonia results in an increased nitrogen excretion and energy loss due to urea synthesis, it may even cause urea toxicity (Russell *et al.*, 1992). The synthesis of VFA and microbial protein is influenced by the rate of degradation of feed consumed by the ruminant. When the rate of protein degradation exceeds carbohydrate fermentation, nitrogen excretion increases. However, when the rate of protein degradation is exceeded by carbohydrate fermentation, microbial protein synthesis will be inhibited. If the degradation rate of feed (especially forages) is

slow, intake will decrease due to rumen fill and some of the feed particles may enter the small intestine without fermentation (Nocek & Russell, 1988).

2.4 Supplementation

The nutritive value of grass deteriorates as soon as the vegetative growth stops (O'Reagain & McMeniman, 2002). In the summer rainfall areas, vegetative growth stops during late summer, therefore any unconsumed grass that is left for winter grazing is of low nutritive value (O'Reagain & McMeniman, 2002). Grazing during winter is of low quality and contains less than 6% CP (Currier *et al.*, 2004a). To prevent a 25-30% loss in body weight of sheep grazing winter veld in the sour grass veld regions, supplementation of the limiting nutrients must be given. Thus, the basic objectives of supplementation can be summarized as follows: i) to identify and supplement the nutrient that limits production (Van Niekerk, 1996), ii) and to supply the nutrients required by rumen bacteria for the degradation of the available forage. Low-quality forage can only be utilized by the ruminant if the cellulolytic bacteria are active (Bohnert *et al.*, 2002). By providing the limiting nutrient to an animal in a lick form, it is possible to improve digestion and intake of forage. However, you have to consider the season, quality and quantity of the grass and the productive state of the animal when formulating a lick (Van Niekerk, 1996). In South Africa, early winter licks are usually high in protein, due to the low protein availability of winter veld. An energy lick is often given to ruminants during late winter, when a shortage of poor quality pasture is common. During spring an energy lick is still appropriate due to the lush green pastures, which is high in soluble proteins, low in dry matter and energy. During summer most farmers supply only a phosphate lick that is high in minerals such as phosphorous and calcium (Van Niekerk, 1996). The productive state of the animal is very important when formulating a supplement. For example, a lactating animal may require some rumen undegradable protein to improve milk production, therefore, requiring a production lick containing some natural protein and energy to meet her requirements (Noftsger & St-Pierre, 2003). To supplement, one must make sure it is nutritionally and cost effective so that it is economically sound. There are a few factors that needs to be taken into account when formulating a supplement for ruminants on pasture, which will be discussed in the following section.

2.4.1 Reasons for providing a supplement to ruminants

As outlined in Fig. 2.3, supplements are usually offered to sheep for the following reasons: i) to negate/neutralize the negative effects of anti-nutritional factors, ii) to overcome a deficiency of a limiting nutrient, iii) to improve the efficiency of utilization and the nutrient supply (Dove, 2002)

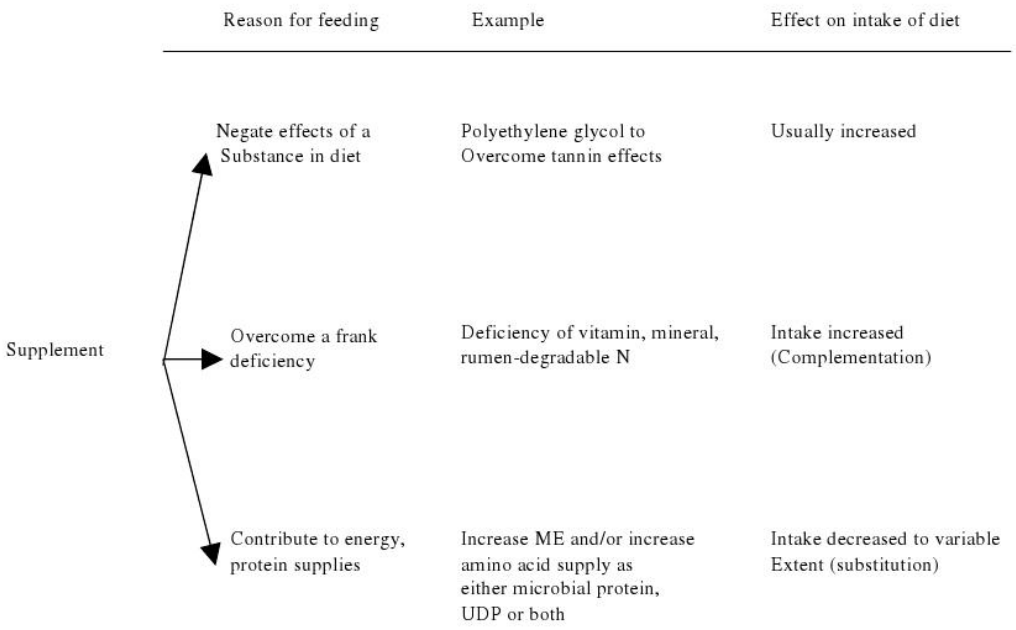

FIGURE 2.3 Three reasons for supplementary feeding on pastures (Dove, 2002)

The first circumstance where a supplement may be required, as shown in Fig 2.3, is where supplements are used to negate or neutralize the effects of anti-nutritional factors. An example where this might arise is in the bush veld areas, where browse species are common. Browse species often contain tannins, that might limit the intake and digestibility of the forage. To overcome the negative effects of tannins, polyethylene glycol can be used. Polyethylene glycol forms a complex with the tannins, that improves digestibility and protein availability to the ruminant and therefore, improving intake (Dove, 2002). The second circumstance is where a supplement is given to overcome a specific deficiency (Dove, 2002). Deficiencies that might occur from veld grass vary with season, rainfall and type of soil (Van Niekerk, 1996). The most common deficiencies that might occur are those of rumen degradable protein, phosphate, various other minerals, trace minerals and vitamins. During winter the sour veld regions in South Africa are deprived of protein, energy and phosphorous. Protein is the primary limiting nutrient, followed by energy. To improve production in circumstances like these, the primary and

secondary limiting nutrients must first be alleviated before phosphorous will have an influence on production (Van Niekerk, 1996). Therefore, it is important to identify the primary limiting nutrient before formulating a supplement. The third circumstance is where nutrients can be supplied to improve production of ruminants on veld. These type of supplements usually include nutrients such as energy and/or rumen undegradable protein. The supplementation of energy is often used during breeding times for optimum reproductive performance. Rumen undegradable protein are often used to improve milk production and growth (Dove, 2002). Each of these circumstances usually has a specific outcome on veld intake, which will be discussed under the following heading.

2.4.2 The outcomes of supplementation

When supplementation is given to grazing ruminants, it might have three different outcomes depending on the type of supplementation given. The three outcomes include: i) supplementation, ii) complementation and iii) substitution (Dove, 2002). Supplementation is when a supplemental feed is given to ruminants, but the intake of the supplement does not reduce pasture intake (Dove, 2002). Complementation usually occurs where low quality forages are supplemented with the deficient nutrients in the forage. In this case, pasture intake usually increases (Dove, 2002). An example of complementation is mostly seen when supplements are provided to ruminants on winter veld, which is generally of low quality and quantity. Winter veld often has a deficit of N. By providing a protein lick to the ruminants on winter veld the deficiency can be overcome (Van Niekerk, 1996). The nitrogen available from the lick allows the rumen bacteria to function optimally by improving the fermentation and digestion of the forage, therefore increasing the passage rate and intake. Complementation is the result of i) an increased energy intake, ii) the available fermentable energy, ammonia-nitrogen and amino acids for the production of microbial protein (MCP) by the rumen bacteria and iii) the provision of additional amino acids by RUP to the small intestine (Ferrell *et al.*, 1999). The third outcome is substitution and is the most unwanted outcome when a supplement is provided. Substitution is where the animals rather consume the supplement given and DMI from grazing declines (Dove, 2002). High energy, low CP supplements usually result in reduced forage intake (Ferrell *et al.*, 1999). Substitution usually occurs when licks are rich in energy and protein. The ruminants will rather meet their energy requirements by consuming more of the lick than to go out and graze (Bohnert *et al.*, 2002; Dove, 2002).

2.5 Protein supplementation

Energy and protein are usually the first limiting nutrients for ruminants (Blackwood & Duddy, 2009), but protein (RDP) is considered the first limiting nutrient for ruminants grazing pasture of a low quality (Köster *et al.*, 1996; Currier *et al.*, 2004a). During the maturation process of veld, the fibre and lignin content of the veld increases and the CP content decreases. This results in a decreased forage intake and energy intake due to slower rates of digestion and passage rate (Romney & Gill, 2000). To improve the digestion and passage rate of grass, the rumen bacteria requires ammonia for effective digestion of the grass (Köster *et al.*, 1996). Therefore, an energy shortage might occur due to a nitrogen shortage by the rumen bacteria. Many animals are not able to meet their nutrient requirements for maintenance as well as production on poor quality roughage, due to the low CP content and unavailability of energy from the roughage. To minimise production losses during times when roughage are of low quality and quantity, RDP supplements must be given to grazing animals (Köster *et al.*, 1996; Wahrmund *et al.*, 2007). Many advantages can be achieved when protein is supplemented to ruminants on low quality forage. Increased forage intake, digestibility (Köster *et al.*, 1996; Ferrell *et al.*, 1999) and reduced body weight loss or even an increase in body weight (Ferrell *et al.*, 1999; Bohnert *et al.*, 2002) are some of the advantages that can be achieved. Supplements can be adjusted according to the animals' physiological state (lactating, dry or growing). Lactating and growing ruminants usually require both non-protein nitrogen (NPN) and natural protein. Natural protein provides amino acids to the small intestine which might increase milk production and therefore growth of lambs and calves. Non-protein nitrogen (NPN) is sufficient for animals on maintenance and is usually a cheaper source of nitrogen than natural protein sources such as soy-bean oil cake meal. Feed costs can be reduced if natural protein can be substituted completely or partially by a NPN source (Chizzotti *et al.*, 2008).

2.6 Urea

Feed grade urea is most commonly used in supplements as a NPN source for ruminants, and is cheaper than true protein per unit nitrogen. Feed grade urea is manufactured as a spherical white solid consisting out of amides containing about 46% nitrogen. Urea was accidentally discovered in the 1800's by Friederich Wohler when he attempted to synthesize ammonium cyanate with silver cyanate (Saltzman, 1999). Urea is highly soluble and upon entering the rumen is rapidly

hydrolysed by rumen bacteria to rumen ammonia. The rapid rate of urea hydrolysis may exceed the ability of the rumen bacteria to use the rumen ammonia, resulting in a loss of nitrogen for the utilisation of microbial protein synthesis and rumen fermentation (Löest *et al.*, 2001; Galo *et al.*, 2003; Golombeski *et al.*, 2006). Most of the rumen ammonia is rapidly absorbed through the rumen wall into the blood stream, converted to urea by the liver and excreted as urinary nitrogen. The danger with overfeeding urea might result in high concentrations of ammonia in the blood that may cause reduced feed intake and performance, ammonia toxicity and death (Huntington *et al.*, 2006).

2.6.1 Effects of urea supplementation on intake and digestibility

According to the study of Currier *et al.* (2004a), no increase in forage intake was observed for the steers when a low quality forage (4%CP) was supplemented with urea or biuret. The authors explained the lack of response in forage intake when urea or biuret was given as a NPN source, to the NDF intake (13.2g/kg BW) of the hay used in the study, which was higher than the suggested NDF intake (12.5g/kg BW) to maximize DM intake (according to Currier *et al.*, 2004 as cited by Mertens *et al.*, 1985,1994). Although Currier *et al.* (2004a) didn't observe an increase in forage intake with urea or biuret, the authors found an increase in OM intake, N intake and total tract digestibility when urea was used as a NPN source. Köster *et al.* (1997) found that the substitution of rumen degradable true protein with urea does not have a negative impact on the forage intake of beef steers. Forage intake was not significantly affected by the substitution of casein with 0, 25, 50, 75 or 100% urea as the RDP source. However, urea levels above 75% (99g DM/day) depressed NDF and OM digestibility and therefore the DOMI. Apparent CP digestion increased with increasing levels of urea and the total tract CP digestion did not differ between treatments according to Köster *et al.* (1997).

2.6.2 Effect of urea supplementation on ruminal fermentation

2.6.2.1 Rumen NH_3-N concentration

Low levels of rumen ammonia nitrogen (NH_3-N) are often observed when low quality forage is consumed by ruminants. When low quality forages are being consumed by ruminants, the rumen NH_3-N concentrations might decrease below 2mg/dL and therefore impaired microbial protein synthesis and rumen fermentation (Satter & Slyter, 1974). To overcome this low rumen

NH_3-N concentration, urea, which is an excellent source for the provision of rumen NH_3-N to rumen bacteria, could be included into a supplement. According to Currier *et al.* (2004b) rumen NH_3-N increased when low quality forage (4% CP) was supplemented with urea or biuret. A disadvantage of using urea in a supplement, is the rapid hydrolysation of urea to rumen NH_3-N. Rumen NH_3-N production from urea usually exceeds the ability of the rumen bacteria to utilize the available nitrogen (N) for microbial protein synthesis and other functions of rumen fermentation. The excess rumen NH_3-N are then excreted through the urine or recycled to the rumen via saliva or blood (Russell *et al.*, 1992), therefore some of the N might be lost for microbial protein synthesis and rumen fermentation. Köster *et al.* (1997) reported similar results to Currier *et al.* (2004b), in relation to the effect of increasing levels of urea on poor quality roughage as the RDP source. Both the authors found an increase in rumen NH_3-N concentrations with increasing levels of urea as supplement. In a study done by Rihani *et al.* (1993) where the authors studied the influence of the level and method of urea supplementation on the digestion of high fibre diets by sheep, it was found that rumen NH_3-N increased with higher levels of urea intake when rumen NH_3-N concentration was above 5.0mg/dL. A higher level of rumen NH_3-N concentration was observed during this study at feeding and infusion than at 6 hours after feeding and infusion. This suggests that a N influx into the rumen existed when rumen NH_3-N levels started to decrease in the rumen. Ruminants are able to recycle N to the rumen via saliva or the blood stream (Huntington *et al.*, 2006). According to Vercoe (1969) (as cited by Rihani *et al.*, 1993) a net N influx occurred when rumen NH_3-N concentrations were below $<$5.5mg/dL. The N influx and rumen NH_3-N absorption were dependent on rumen pH (Bloomfield *et al.*, 1963 as cited by Rihani *et al.*, 1993). With the intake of urea as supplement, the rumen pH increases due to the rapid hydrolysis of urea to rumen NH_3-N. At a high pH, rumen NH_3-N is present in the rumen in its unionised form (NH_3) and is able to be absorbed through the rumen wall into the bloodstream (Bloomfield *et al.*, 1963 as cited by Rihani *et al.*, 1993).

2.6.2.2 Volatile fatty acid concentration

Köster *et al.* (1997) found that total VFA production was not affected by increasing levels of urea, with the constant rumen pH levels confirming this observation. Earlier Rihani *et al.* (1993) found similar results than Köster *et al.* (1997) in relation to VFA concentration with the supplementation of different levels of urea. Rihani *et al.* (1993) also found that the acetate:propionate

ratio was relatively high, but concluded that the low intake level of the study might have been the reason for this observation.

2.7 Synchronisation of dietary energy and nitrogen

Rumen degradable protein sources provide rumen NH_3-N, peptides and amino acids to rumen bacteria for growth and microbial protein synthesis. Most of the amino acids (70-100%) supplied to the small intestine of a ruminant is synthesized by rumen bacteria. Microbial protein synthesis and microbial growth are influenced by factors such as pH, passage rate, digestibility and intake. For the optimization of microbial protein synthesis both nitrogen (rumen NH_3-N) and carbohydrates are required by the rumen bacteria. By synchronising the available nitrogen and energy for rumen bacteria, efficiency of microbial protein synthesis may be improved (Harrison & Karnezos, 2005). An improvement in the efficiency of NPN incorporation into microbial nitrogen and the use of nitrogen for microbial growth may occur when dietary energy and nitrogen are synchronized. Microbial growth and degradation in the rumen is a slow process, therefore the rapid increase in rumen NH_3-N after urea intake, may cause a loss in nitrogen for microbial growth and microbial protein synthesis (Taylor-Edwards *et al.*, 2009b).

Due to the rapid hydrolysis of urea to rumen NH_3-N, asynchrony may occur between nitrogen and energy available from forages to rumen bacteria for growth and microbial protein synthesis. Fermentable energy from forages are released slowly during degradation by rumen bacteria (Löest *et al.*, 2001). Therefore, the possibility of asynchrony between available rumen NH_3-N from urea and energy from the forage, exists. It might be possible to improve intake, digestion and rumen fermentation of low quality forages by providing a slow release nitrogen source, such as RDP, in a supplement. By providing a slow release NPN source, synchronisation may be achieved between the available nitrogen from the NPN source and the slow release of fermentable energy from forages (Löest *et al.*, 2001, Huntington *et al.*, 2006).

Various studies have been done on the effects of synchronisation of different nitrogen sources and energy in the rumen, but the results that have been obtained are inconsistent (Henning *et al.*, 1993). In a study done by Rihani *et al.* (1993) the authors concluded that it is impractical to supplement ruminants with a slow release nitrogen source. These authors reported that neither microbial growth nor microbial protein synthesis and microbial efficiency, were improved by

slow releasing urea (through continuous infusion). In a study conducted by Herrera-Saldana *et al.* (1990), microbial growth and microbial protein production were increased with the synchronization of dietary energy and nitrogen. Instead, according to Henning *et al.* (1993), synchrony of energy and nitrogen did not have an effect on microbial growth, and suggested that the responses of microbial efficiency may be due to an improvement in the energy and nitrogen balance in the rumen. Henning *et al.* (1993) also suggested that N recycling in the ruminant makes it possible for the ruminant to synchronise N and slow release energy in the rumen without the necessity of synchronising dietary nitrogen and energy. These authors concluded that microbial yield is not increased with the synchronisation of dietary nitrogen and energy but, suggested that nitrogen should be provided in adequate quantities, after an even level of ruminal energy supply is achieved.

2.8 Slow release NPN source

Various technological methods have been conducted over the years to improve synchrony between available energy and nitrogen in the rumen to maximise microbial growth and microbial protein synthesis. Most of the technologies were developed by controlling the NH_3-N release from urea to be closely correlated to carbohydrate degradation. Problems usually encountered with slow release NPN sources, are a too slow release, too quick release or hardly any release of NH_3-N into the rumen (Harrison & Karnezos, 2005, Taylor-Edwards *et al.*, 2009b). Therefore, a slow release NPN source might even decrease the ability of rumen bacteria to incorporate rumen NH_3-N into microbial protein (Galo *et al.*, 2003). Slow release NPN sources might be used without the detrimental effects caused by urea such as NH_3-N toxicity and decreased feed intake (due to poor palatability). One of the advantages suggested by Golombeski *et al.* (2006) of a slow release NPN source, is the time it provides for more effective utilization of rumen NH_3-N by rumen bacteria. The main objective of a slow release NPN source is to maintain and to provide a sustained NH_3-N concentration in the rumen and reduce absorption of NH_3-N into the blood stream. Efficient utilization of rumen NH_3-N as a result of decreased rumen NH_3-N absorption will decrease the cost of energy necessary for urea synthesis. Therefore, a slow release NPN source might have the ability to improve the energy balance in ruminants (Highstreet *et al.*, 2010).

2.8.1 Effect of a slow release NPN source on intake and digestibility

According to Golombeski *et al.* (2006) a decrease in DMI was observed when urea or a slow release urea source was offered to dairy cows, but milk production was not affected, therefore indicating that feed efficiency was improved. Löest *et al.* (2001) found no difference in forage and total tract digestibilities of DM, OM and NDF between urea versus urea/biuret. Neither did the authors find any difference between DMI, OMI and NDF intake of forage when urea/biuret supplements were provided. The CP digestibility was also lower for urea/biuret supplements, than for urea alone. This might indicate that some of the supplement containing biuret passed through to the small intestine without being hydrolysed in the rumen. Therefore, less nitrogen was available for microbial growth and microbial protein synthesis.

2.8.2 Effect of a slow release NPN source on rumen fermentation

2.8.2.1 Rumen NH_3-N concentration

Polymer-coated urea sources are the most common slow release NPN source used these days in animal feed. With slow release NPN sources, a sustained rumen NH_3-N concentration is obtained due to the slower rate of NH_3-N release when entering the rumen (Taylor-Edwards *et al.*, 2009b) and thus, providing a sustained level of N, to improve microbial growth and microbial protein synthesis. The only downside expected with slow release NPN sources, is that NH_3-N might be released too slow for effective microbial growth and microbial protein synthesis. This disadvantage of a slow release NPN source may be more pronounced in ruminants with production demands (Taylor-Edwards *et al.*, 2009b). Golombeski *et al.* (2006) did not find a difference in rumen NH_3-N concentrations when cows were supplemented with a rapid release urea source or with a slow release urea (SRU) source.

2.8.2.2 Volatile fatty acid concentration

According to the studies of both Garrett *et al.* (2005) and Taylor-Edwards *et al.* (2009b), the urea source (feed grade urea or slow releasing urea) did not affect VFA production and composition. In the study of Löest *et al.* (2001), urea/biuret had a lower total VFA concentration than urea. These authors suggested that this may be due to the lower availability of nitrogen for microbial

growth from the supplement containing biuret, consequently limiting ruminal fermentation and VFA production.

2.9 Optigen® II

Optigen ®II (Alltech Inc.) is a blended, controlled release urea source. Urea is coated in a polyester polyurethane coating which allows the diffusion of the urea through micro-pores, that slows down the rate of nitrogen release in the rumen (ICF Consulting, 2004). The idea of Optigen® II is to give a slow even release of nitrogen over 24 hours, to meet the rumen bacteria requirements when rumen NH_3-N levels are low. To provide a sustained level of N, N efficiency and microbial protein production would increase (Harrison & Karnezos, 2005).

TABLE 2.1 Nutritive value of Optigen®II (g/100g)

	DM	CP	N	Lipids	CF	Ash
DM basis	100	258.59	41.41	12.12	0	0
ˋˋAs is ´´	99	256	41	12	0	0

The expected uniqueness of Optigen® II as illustrated in Fig.2.4, lies in its ability to have a similar nitrogen disappearance as Optigen® 1200 (274% CP on DM) relative to soybean oil cake meal. According to the illustration in Fig.2.4, urea has a rapid linear increase in N disappearance, suggesting that urea has a rapid N release rate, where Optigen® 1200 has a slower N release rate similar to that of soybean oil cake meal (Akay *et al.*, 2004). In comparison with soybean oil cake meal (53% CP on DM basis), 1g of Optigen® 1200 will provide the same amount of nitrogen from RDP than 6g soy-bean oil cake meal (Harrison et al., 2005). According to Tikofsky & Harrison (2006), 1g of Optigen®II (256% CP on DM basis) will give a similar amount of N as that of 7.1g of soybean oil cake meal (53% CP on DM basis) on a RDP basis.

The value of a slow release urea product lies in its nitrogen density (Harrison & Karnezos, 2005, Tikofsky & Harrison, 2006). In Table 2.1, it is demonstrated that Optigen®II is a N dense product (41.41g N/100g) and contains an energy value in the form of lipids (12.12g/100g). The space created with a high nitrogen dense diet in the rumen may be used to counteract or improve other nutritional shortcomings. The following objectives may be achieved by using the created rumen space: i) increasing the efficiency of microbial protein synthesis, ii) increasing the diet's nutrient density, iii) improving rumen health and iv) reducing ration cost (Harrison & Karnezos,

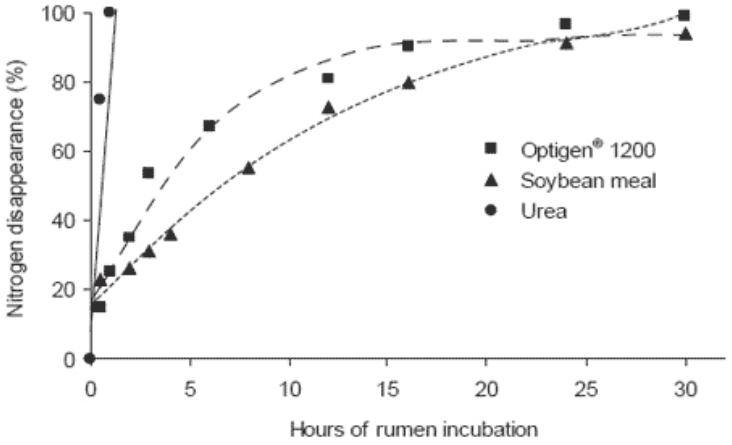

FIGURE 2.4 *In situ* nitrogen disappearance of Optigen 1200, soybean oil cake meal and feed grade urea (Akay *et al.*, 2004)

2005; Tikofsky & Harrison, 2006). Rumen fermentation and microbial protein synthesis may be optimized, by providing a RDP source,. Rumen microbes require rumen NH_3-N for growth and incorporation into microbial protein. Tikofsky & Harrison (2006) concluded that Optigen® II (nitrogen dense and slow release NPN source) improved the efficiency of microbial protein synthesis.

Nitrogen density is a characteristic that is seen as useful when ruminants are under stress. Ruminants under stress may not consume enough feed to meet their nutrient requirements due to reduced intake. By using a nitrogen dense source, the space created can be used to improve the diet's nutrient density by adding energy and RUP. Therefore, enabling the animals to have a lower DMI, but still meeting their nutrient requirements (Tikofsky & Harrison, 2006). A slow release NPN and/or high nitrogen dense source, provides space that can be filled with a cheaper high quality forage. Costs of the ration can be reduced by still maintaining the production level. Plant protein sources such as soy-bean oil cake meal is very expensive. This also provides the opportunity to include more effective fibre to maintain a healthy ruminal environment (Harrison & Karnezos, 2005).

The objectives of this study were to determine whether urea can be substituted with Optigen®II and if synchrony between different proportions of Optigen®II, urea and fermentable energy were achieved to obtain maximum intake, rumen fermentation, NDF degradability and microbial protein synthesis.

2.10 Hypothesis

H_0: That different proportions of a slow and rapid release NPN source will affect DM intake, roughage digestibility, rumen fermentation and microbial protein synthesis.
H_1: That different proportions of a slow and rapid release NPN source will not affect DM intake, roughage digestibility, rumen fermentation and microbial protein synthesis.

H_0: Urea can be substituted with Optigen®II without influencing DM intake, roughage digestibility, rumen fermentation and microbial protein synthesis.
H_1: Urea can not be substituted with Optigen®II without influencing DM intake, roughage digestibility, rumen fermentation and microbial protein synthesis.

Chapter 3

Materials and Methods

3.1 Introduction

A 5x5 Latin Square design was used in the experiment. Five Merino rumen cannulated wethers were randomly assigned to five different treatments during the experiment. The trial consisted out of 90 days. The trial was run in five experimental periods. Each experimental period lasted for 18 days. The first 10 days were used for adaptation and the last 8 days for collection of samples. After each experimental period the wethers were allocated to a different treatment.

The experiment was conducted on the Hatfield Experimental Farm of the University of Pretoria. The experiment was approved by the ethics committee, nr. EC004-10.

3.2 Animals

Five rumen cannulated wethers, with an average body weight of 59kg (SE ±0.43) were used in the experiment. The wethers were treated for internal parasites and hooves were clipped before the onset of the 90 day experimental period. Treatment for internal parasites was also done during the experimental period, according to the FAMACHA method. Animals were treated immediately if any symptoms of disease or discomfort were shown.

During the 10 day adaptation period, the animals were kept in a pen for 8 days. *Eragrostis curvula* hay (CP 4.44%, NDF 85.0%, ADF 51.0%, ADL 8.99% on a DM basis) was used as the

basal feed for the experiment. The *Eragrostis curvula* hay and water were available at all times. Supplements were infused into the rumen via the rumen cannulae at 08:00 and 16:00 throughout the adaptation and collection periods. The wethers were placed individually into metabolic cages in a metabolic house on day 8. Each metabolic cage was equipped with its own water and feed troughs. Animals were fed every morning and afternoon to ensure that they had *ad lib* access to the *Eragrostis curvula* hay and water. Urine pans were fitted under each metabolic cage for urine collection during the collection period. Faecal bags were fitted to each animal on the day they were placed into the metabolic crates. Cannulaes were cleaned and disinfected before and after each collection period. Wool around the cannulae was also clipped regularly. Animals were weighed before and after each experimental period. After each experimental period, the wethers were left to walk freely around and graze natural pastures for a day without any supplementation given. Adaptation for the next supplement treatment started the following day in a pen where the wethers were able to move freely.

3.3 Experimental treatments

3.3.1 Preliminary intake trial

A preliminary intake trial was conducted on the poor quality roughage, to calculate the voluntary intake level of the roughage, so that the amount of supplements (Urea, Optigen®II and starch) that is required to meet the requirements of a 60kg wether could be determined. Six wethers in metabolic cages were fed individually. 1500g of roughage and 400g of Voermol Premix 450 lick (see composition in Table 3.2) per day was fed, and water was available at all times. Voermol Premix 450 is a typical commercial protein concentrate used to supplement sheep grazing poor quality winter pastures in South Africa. Orts were weighed back the following morning to determine intake. Intake of the last five days was used to determine the average intake of the six wethers. The average intake of roughage and lick was 1118g/day and 274.73g/day, respectively.

The roughage was analysed to determine its quality (See Table 3.1). Dry matter (DM), nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), acid detergent insoluble nitrogen (ADIN), *in vitro* organic matter digestibility (IVOMD) and ash concentrations were determined according to the A.O.A.C. (2000).

TABLE 3.1 Roughage analysis (g/100g DM)

DM	Ash	N	CP	NDF	ADF	ADL	ADIN	IVOMD
100	3.83	0.71	4.44	85.0	51.0	8.99	37.22g/100g CP	35.8

TABLE 3.2 Nutrient composition of Voermol - Premix 450 (g/kg DM)

Nutrient	Inclusion
Crude protein	450
% Protein excluding NPN	94
Urea	131.2
Calcium	12
Phosphorus	21

3.3.2 Experimental diets

The experimental design consisted of a 5x5 cross-over design, consisting of five replicates (wethers) and five treatments (diets). The diets presented in Table 3.3 were randomly assigned to the animals and was not allocated in a fixed sequence. The wethers were fed *Eragrostis curvula* hay and a daily supplement. Treatments consisted of different ratios of urea and/or Optigen® II, and each treatment received additional fermentable metabolizable energy (starch) and a mineral premix. The mineral premix (See Table 3.4) was manufactured by Nutec (234 Royston Road, Willowton, Pietermaritzburg, RSA). The mineral premix of Nutec, had sulphur levels below what is been recommended by the NRC (2001) for wethers at maintenance level of feeding. An additional 1.8g sulphur/day was thus added to each treatment. Wethers had access to water and *Eragrostis curvula* hay (See Table 3.1) at all times. The *Eragrostis curvula* hay was milled by a hammer mill with a 3cm sieve. Treatments were infused ruminally at 08:00 and 16:00 every day, throughout the adaptation and sampling periods. These times were chosen because grazing sheep are usually at the water points at these times, and that is where and when they will consume the supplemental feed. The supplemental treatments' nutrient composition is presented in Table 3.5.

The following calculations were done to determine the supplement composition for each treatment used in the experiment:

Eragrostis curvula intake:	1118g/d/sheep (as is basis) DM basis = 1118*92.20% = 1030.80g/d
Eragrostis curvula CP:	4.44g CP in 100g hay (DM basis) 45.77 g CP in 1030.80g hay But ADIN is 37.22%,thus 37.22% of 45.77 g CP is not available Thus, 45.77*37.22% = 17.036g CP is not available Available CP from roughage = 45.77-17.036 = 28.73g
CP supplemented:	Maintenance requirement for CP for a 60kg sheep is 79g/d (NRC, 2001) Thus, CP supplemented = 79-28.73 = 50.266g/d
Urea:	99.86% DM basis 290% CP (DM basis)
Optigen® II:	99% DM basis 256% CP (as is)
100% Urea:	290g CP in 100g urea 50.266g CP in 17.33g urea (DM basis) As fed = 17.33/99.68% = 17.39g Urea/sheep/day
75% Urea:	50.266g * 75% = 37.70g 290g CP in 100g urea 37.70g CP in 13g urea As fed = 13/99.68% = 13.02g urea/sheep/day
50% Urea:	50.266*50% = 25.133g 290g CP in 100g urea 25.133g CP in 8.67g urea As fed = 8.67/99.68% = 8.70g urea/sheep/day
25% Urea:	50.266*25% = 12.57g 290g CP in 100g urea 12.57 g CP in 4.33 g urea As fed = 4.33/99.68% = 4.34g urea/sheep/day
100% Optigen® II:	256g CP in 100g Optigen® II (as is basis) 258.59 g CP in 100g Optigen® II (DM basis) 50.266 g CP in 19.44g Optigen® II (DM basis) As fed = 18.8/99% = 19.63g Optigen® II/sheep/day

75% Optigen®II: 50.266*75% = 37.70g
258.59g CP in 100g Optigen®II
37.70g CP in 14.73g Optigen®II
As fed = 14.73/99%
= 14.88g Optigen®II/sheep/day

50% Optigen®II: 50.266*50% = 25.133g
258.59g CP in 100g Optigen®II
25.133g CP in 9.72g Optigen®II
As fed = 9.72/99%
= 9.82g Optigen®II/sheep/day

25% Optigen® II: 50.266*25% = 12.57g
258.59g CP in 100g Optigen® II
12.57g CP in 4.86g Optigen® II
As fed = 4.86/99%
= 4.91g Optigen®II/sheep/day

ME roughage: IVOMD = 35.8g/100g (McDonald *et al.*, 2002)
ME (MJ/kg DM) = 0.016 DOMD
= 0.016 * 358g/kg
= 5.728 MJ/kg DM
FME = ME-MEfat
= 5.728MJ/kg DM

FME required: But, the true protein and digestibility of MCP is 0.75 and 0.85 (McDonald *et al.*, 2002)
For maintenance = 50.266/(0.75*0.85)
= 78.84g MCP/day must be synthesized

Production of 9g MCP requires 1MJ of FME (McDonald *et al.*, 2002)
FME required = 78.84/9
= 8.76 MJ ME/day

Starch: 87.01% DM
15.99 MJ/kg DM

5.728MJ FME in 1000g of roughage
5.90 MJ FME in 1030.80 g of roughage

Starch supplement: 8.76 5.9 = 2.86MJ/day
15.99 MJ in 1000g of starch
2.86 MJ in 178.86g of starch (DM basis)
As is basis = 178.86/87.01%
= 205.56 g/d/sheep

TABLE 3.3 Treatments (g/day) on ¨as fed ´basis

Treatment	Optigen® II	Urea	Starch	Premix
100% Urea	-	17.39	205.56	24.08
75% Urea : 25% Optigen® II	4.91	13.02	205.56	24.08
50% Urea : 50% Optigen® II	9.82	8.70	205.56	24.08
25% Urea : 75% Optigen® II	14.88	4.34	205.56	24.08
100% Optigen® II	19.63	-	205.56	24.08

TABLE 3.4 Mineral specifications of the premix

Macro minerals	Inclusion (g/head/day)	Trace minerals	Inclusion (mg/head/day)
Calcium	2.00	Cobalt	0.11
Phosphorus	1.50	Copper	4.00
Sodium	0.70	Iodine	0.80
Chloride	0.60	Iron	8.00
Potassium	5.70	Manganese	17.45
Magnesium	1.10	Selenium	0.04
Sulphur	0.0070	Zinc	30.0

TABLE 3.5 Nutrient composition of the different treatments (g/kg DM)

	*Treatments				
Nutrients	1	2	3	4	5
CP	229.3	228.88	229.90	230.30	228.50
ME(MJ/kg)	14.95	14.92	14.87	14.83	14.79
Ca	9.08	9.06	9.04	9.01	9.00
P	6.82	6.81	6.78	6.77	6.76

*1 = 100% Urea; 2 = 75% Urea : 25% Optigen® II; 3 = 50% Urea : 50% Optigen® II; 4 = 25% Urea : 75% Optigen® II; 5 = 100% Optigen® II

3.4 Sample collection

The trial ran for 90 days consisting of 5 x 18 day periods. Each experimental period lasted for 18 days, of which 10 days were for adaptation and 8 days for sample collection. Faecal bags were fitted to five wethers and these wethers were placed individually into metabolic cages on day 8 to minimize stress. Rumen pH was measured twice daily before supplemental infusion (08:00 and 16:00) during the adaptation period. A digestibility trial, rumen fermentation trial and an *in situ* trial were done during the 8 day collection period. Roughage intake, faecal, urinary and ruminal fluid collections were done during the 8 day collection period. All the feed, orts, faecal, urine and rumen fluid samples collected during each collection period were immediately preserved if

necessary and frozen for further analysis in the laboratory (A.O.A.C., 2000). The collection and preservation of the different samples are explained in the undermentioned paragraphs.

For the digestibility trial feed intake and faecal output were measured and collected. Roughage intake was determined daily for each individual animal by recording the weight of the roughage offered and the orts. The roughage that was offered was adjusted throughout each collection period to ensure that there was enough feed available at all times. Feed and orts samples were taken for each individual during the 5 collection periods to obtain a representative sample of 5 samples for each treatment (Köster *et al.*, 1996, Olson *et al.*, 1999). Feed and orts samples were frozen at -10°C for further analysis in the laboratory. Faecal weight was recorded for each individual during the morning and afternoon feeding. A representative sample was taken daily for each individual, mixed and pooled over the 6 day collection period (Köster *et al.*, 1996, Olson *et al.*, 1999). Two separate sub-samples of faeces were taken and frozen at -10°C. One sub-sample was used for dry matter (DM) analysis, dried at 105°C and the other sub-sample to be dried at 60°C for nitrogen (N) and neutral detergent fibre (NDF) analysis. The wethers were weighed before and after each experimental period. Feed, orts and faecal (dried at 60°C) samples were grounded through a 1mm sieve before it was analysed. Total tract digestibility for DM, organic matter (OM), CP and NDF was determined by using feed and faecal samples. The total tract digestibility of a roughage for different parameters is determined by giving animals a known amount of roughage and measuring the amount of roughage been left (orts) together with the faeces output. The feed, orts and faecal samples were then dried and analysed for DM, OM, CP and NDF concentrations. The digestibility coefficient of each parameter, was caluculated by using the following equation on a DM basis (McDonald *et al.*, 2002):

(Feed component - Orts - Faecal component)/Feed component

Rumen fluid samples were collected for the determination of rumen fermentation by measuring volatile fatty acid (VFA) concentration, rumen ammonia-nitrogen (NH_3-N) concentration and rumen pH. Rumen fluid samples were collected over 24h at 3 hour intervals during the first day of each collection period, using a suction strainer. Eight samples of rumen fluid were pooled and sampled for pH, NH_3-N and VFA determination over 24h at 03:00, 06:00, 09:00, 12:00, 15:00, 18:00, 21:00 and 24:00. The pH was measured immediately after collection with a pH meter. The pH meter was calibrated with standards and rinsed with distilled water after each measurement. For the analysis of NH_3-N, 5ml of 50% H_2SO_4 preservative was added to

30ml of rumen fluid (Broderick & Kang, 1980) and 4ml of 25% H_3PO_4 preservative was added to 20ml of ruminal fluid for VFA analysis (Webb, 1994). The NH_3-N and VFA samples were pooled separately over the 2 day collection period to obtain a representative sample for each individual during each collection period. Five rumen fluid samples per individual were also collected at 0, 1, 2, 3 and 4 hours after supplemental infusion for the analysis of rumen NH_3-N during each collection period. Samples were preserved and frozen at -10°C for further analysis in the laboratory.

An *in situ* trial was conducted during each collection period to determine the NDF and DM disappearance of the *Eragrostis curvula* hay being fed. Representative samples of the hay were milled through a 2mm sieve and 5g was weighed into Dacron bags. The Dacron bags were incubated in duplicate in the rumen for 0, 2, 4, 8, 16, 24, 48 and 72 hours per sheep (16 Dacron bags per sheep). Two bags were taken out at each time interval, washed until the water was clear and frozen at -10°C. After all the bags were taken out and washed at 72 hours, the Dacron bags were defrosted and dried at 60°C (NRC, 2001). The residues were analysed for NDF and DM.

Urine samples were collected for the determination of rumen microbial protein (MCP) synthesis by analysing it for purine derivatives (Chen & Gomes, 1992) and creatinine. The urine of each individual animal was collected using a urine pan. The urine of each individual was preserved with H_2SO_4, the amount of H_2SO_4 depended on the amount of urine collected and the pH of the urine each day. After each daily collection the pH of the urine was measured to obtain a pH of 3 by adding H_2SO_4. After a pH of 3 was obtained, the urine was diluted with water up to 4000ml. A sub-sample (50ml) was then taken from each individual animal's diluted urine, to obtain a representative sample for each day (Chen & Gomes, 1992). The urine samples of the 6 day collection period were pooled to obtain 5 representative samples for the 5 treatments.

3.5 Parameters and analytical methods

3.5.1 Dry matter and organic matter

The DM concentration of feed, orts, faecal and *in sacco* samples was calculated as recommended by procedure 934.01 A.O.A.C. (2000). The OM concentration of the feed, orts and faeces was calculated by using the ash concentration of the samples.

3.5.2 Calcium and phosphorous

The calcium concentration of the feed was analysed. The calcium concentration was determined with a Perkin Elmer atomic absorption spectrophotometer according to Giron (1973). Phosphorous was analysed according to the photometric method (procedure 965.17 AOAC,2000).

3.5.3 Crude protein and nitrogen

The nitrogen concentration of feed, orts, faecal and urinary samples was determined by using the Leco FP-428 according to the Leco instrumental manual (procedure 968.06 AOAC, 2000). The final nitrogen concentration was displayed as percentage nitrogen. The crude protein concentration was calculated by multiplying the nitrogen percentage with 6.25.

3.5.4 Purine derivatives and creatinine

Urine samples were analysed for purine derivatives according to Chen & Gomes (1992) by high-performance liquid chromatography. Creatinine in the urine samples was analysed by the use of a Quantichrom creatinine assay kit (Biocom biotech, 161 Roedolf street, Clubview East, Pretoria, 0157) and a spectrophotometer. These two parameters were used to calculate rumen MCP.

3.5.5 Neutral detergent fibre and acid detergent lignin (ADL)

The NDF concentration of the feed, orts, faecal and *in sacco* samples was determined by the filter bag technique using the ANKOM technology method 9. The ADL of the feed samples was determined according to Goering & Van Soest (1970).

3.5.6 Rumen VFA, rumen NH_3-N and rumen pH

A modified technique of Webb (1994) was used to determine the VFA concentration. The rumen NH_3-N was determined by the method according to Broderick & Kang (1980). The rumen pH was measured with a pH meter (Minilab IFSET pH metre noble IQ 120) immediately after each individual sample was drawn.

3.5.7 Derived parameters

The parameters in section 3.5.1 to 3.5.6 were used for the calculation of the following:

3.5.7.1 DM, OM, NDF and nitrogen

- Dry matter intake (DMI)
- Organic matter intake (OMI)
- Digestible organic matter intake (DOMI)
- DM digestibility
- OM digestibility
- NDF digestibility
- Nitrogen intake
- Apparent nitrogen digestibility
- Nitrogen balance
- DM disappearance
- NDF disappearance

3.5.7.2 Rumen fluid samples

- Pooled VFA concentration
- Diurnal and pooled rumen NH_3-N concentration
- Diurnal and average rumen pH

3.5.7.3 Purine derivatives and creatinine

- Rumen microbial protein (MCP)

3.6 Statistical analysis

Data obtained from the laboratory was subjected to an analysis of variance using the Proc GLM model (Statistical Analysis System., 2006) for a Latin square design model. The statistical model used for a Latin square design is:

$y_{ijk} = \mu + T_i + P_j + A_k + \varepsilon_{ijk}$

Where y_{ijk} is the observation for each variable measured, μ is the mean, T_i the treatment effects, P_j the period effects, A_k the animal effects and ε_{ijk} the error. The Fisher test was used to determine the significance of the difference ($P<0.05$) between means (Samuels, 1989). Least square means and standard errors were calculated. The model of Orskov & McDonald (1979) was used for statistical analysis of the NDF and DM disappearance rate.

Chapter 4

Results and discussion

4.1 Roughage quality

The *Eragrostis curvula* fed to the sheep throughout the experiment, was of poor quality as illustrated in Table 4.1. The CP concentration of the roughage ranged between 3.29% to 3.60%, therefore far below the CP requirements of a 60kg sheep at maintenance level. At maintenance a 60kg sheep requires 79g/kg CP (DM) (7.9% CP). The CP, Ca and P concentrations of the hay were very low, indicating that it was a poor quality hay (NRC, 2001).

TABLE 4.1 Quality of the roughage consumed by sheep under various treatments on a dry matter basis

	*Treatments				
Parameters (%) (DM basis)	1	2	3	4	5
DM	93.85	94.67	94.95	94.70	93.73
CP	3.60	3.29	3.58	3.33	3.45
NDF	84.62	83.03	83.02	83.31	83.44
ADL	10.25	11.12	9.11	10.40	10.16
Ash	3.71	3.90	4.05	3.47	3.89
Ca	0.23	0.21	0.22	0.23	0.23
P	0.09	0.09	0.09	0.09	0.1

*1 = 100% Urea; 2 = 75% Urea : 25% Optigen® II; 3 = 50% Urea : 50% Optigen® II; 4 = 25% Urea : 75% Optigen® II; 5 = 100% Optigen® II

The NDF concentration was high, ranging from 83.02% to 84.62%. Meissner & Paulsmeier (1995) suggested that the intake of forages with a NDF concentration higher than 55-60%, will not supply sufficient energy to the ruminant to sustain optimum production, due to the slow fermentation rate and long retention times. With the relatively high ADL concentration, it could

be expected that the NDF and DM digestibility of the hay will be low. Acid detergent lignin is negatively correlated to DM and NDF digestibilities (Jung *et al.*, 1997).

4.2 Intake

Significant differences were observed between treatments for all the intake variables studied (See Table 4.2). Treatment 4 (25% Urea : 75% Optigen® II) was significantly higher than Treatments 1 (100% Urea), 3 (50% Urea : 50% Optigen® II) and 5 (100% Optigen® II) for DMI and OMI. The NDF intake of Treatment 4 was significantly higher than Treatment 3 and 5. The DOMI of Treatment 4 was also significantly higher than Treatment 2 and 5. Treatment 4 was also significantly higher than Treatments 1 and 5, when intake was measured on kg $W^{0.75}$ basis for DMI and OMI.

TABLE 4.2 Effect of slow and rapid release rumen N on intake, DMI, OMI and DOMI (DM basis)

	*Treatments					
Parameters	1	2	3	4	5	**SE
Intake (g/day)						
DMI	916.67^{b}	941.83^{ab}	927.53^{b}	1008.17^{a}	888.92^{b}	24.51
OMI	882.67^{b}	905.11^{ab}	889.96^{b}	973.15^{a}	854.42^{b}	23.45
NDF	777.86^{ab}	781.44^{ab}	768.47^{b}	840.96^{a}	747.21^{b}	20.63
DOMI	450.52^{ab}	438.74^{b}	453.66^{ab}	498.26^{a}	427.90^{b}	15.71
Intake (g/kg$W^{0.75}$)						
DMI	42.66^{b}	44.62^{ab}	43.67^{ab}	46.96^{a}	41.97^{b}	1.22
OMI	41.08^{b}	42.88^{ab}	41.91^{ab}	45.33^{a}	40.34^{b}	1.17
NDF	36.20^{ab}	37.01^{ab}	36.17^{ab}	39.16^{a}	35.27^{b}	1.03
DOMI	20.95^{ab}	20.75^{b}	21.30^{ab}	23.21^{a}	20.15^{b}	0.76

^{a,b,c} Means within a row with different superscripts differ significantly ($P<0.05$)
*1 = 100% Urea; 2 = 75% Urea : 25% Optigen® II; 3 = 50% Urea : 50% Optigen® II; 4 = 25% Urea : 75% Optigen® II; 5 = 100% Optigen® II
**SE = standard deviation

Wahrmund *et al.* (2007) observed no significant difference in DMI between the control, urea and Optigen® II treatments in a study where the authors evaluated Optigen®II as a NPN source for beef cows. The authors ascribed the insignificance in DMI to the fact that the roughage (Bahiagrass) used, had a CP value of 6.77%, which nearly meets the requirements of a non-pregnant cow. However in this study, the CP concentration (3.29-3.60%) of the roughage was far less than the level required by a 60 kg sheep (7.9% DM) for maintenance (NRC, 2001). In contrast to the results of Wahrmund *et al.* (2007), Ribeiro *et al.* (2011) reported significant differences in

terms of DMI, OMI, CP intake and NDF intake between treatments of urea, urea/SRU and SRU. Most of the differences were found between treatments containing SRU (SRU and urea/SRU) and urea, with those containing SRU having the highest intakes. The intakes of both SRU and urea/SRU were similar on a kg/day and g/kg$W^{0.75}$ basis. Ribeiro *et al.* (2011) concluded that hay DMI of the SRU treatments have been higher than the other treatments due to the sufficient provision of nitrogen for rumen fermentation by the SRU treatments and that the low hay intake levels associated with the urea treatments, were due to the high urea inclusion levels in the diet. High levels of urea in the diet are associated with decreased dry matter intake by ruminants. Urea levels higher than 2% (20g/kg) in the feed, depresses dry matter intake, regardless of whether it is included in the feed or infused into the rumen (Wilson *et al.*, 1975). A reduced intake with addition of urea in diets may be due to its bitter taste or physiological mechanisms, including increased rumen and blood ammonia concentrations (Wilson *et al.*, 1975; Grovum & Chapman, 1988). Ribeiro *et al.* (2011) had urea levels more than 20g/kg dietary DM and therefore it might be the reason for the low DMI with the urea treatment. In the current study the 100% urea treatment had similar intake levels than the 100% Optigen® II. The urea levels in this study were below 2% of dietary DM for all treatments. The urea levels of Treatment 1, containing the highest amount of urea (17.39g urea/day), was only 1.4% of the dietary DM. Thus, no effect on intake were observed due to too high levels of urea.

The tendency of 25% Urea:75% Optigen® II to have a higher intake, may be due to improved fermentation by the synchrony of N release from urea with the fast fermentation of fermentable energy and the slower release of N from Optigen® II with the slow fermentation of fibre.

4.3 Digestibility

The only difference ($P<0.05$) observed between treatments in terms of digestibility (See Table 4.3) was that of N. The 100% Optigen® II treatment had a significantly lower apparent N digestibility when compared to the other treatments. No significant differences ($P>0.05$) were observed for DMD, OMD and NDF digestibility.

The low apparent N digestibility observed with 100% Optigen® II (Treatment 5) might be ascribed to the slow release of ammonia (Fig 4.2) in the rumen from Optigen® II, or it might be due to asynchrony between the available FME and N from Optigen®II to the rumen microbes.

TABLE 4.3 Effect of slow and rapid release rumen N on roughage digestibility (DM basis)

Parameters (%)	*Treatments 1	2	3	4	5	**SE
DMD	46.33	43.79	45.80	47.05	45.64	1.14
OMD	49.18	46.17	48.47	49.63	48.42	1.16
NDF digestibility	57.58	54.15	55.70	56.88	56.91	1.21
Apparent N digestibility	91.23^{a}	89.29^{a}	92.52^{a}	91.96^{a}	83.75^{b}	1.11

^{a,b,c} Means within a row with different superscripts differ significantly (P<0.05)
*1 = 100% Urea; 2 = 75% Urea : 25% Optigen® II; 3 = 50% Urea : 50% Optigen® II; 4 = 25% Urea : 75% Optigen® II; 5 = 100% Optigen® II
** SE = standard deviation

Consequently, some of the Optigen® II might have left the rumen without being hydrolysed or incorporated into rumen microbial protein. According to these findings, it could be suggested that postruminal N concentration will increase, and therefore increased faecal N levels could be expected when Optigen® II is used to substitute urea completely in a supplement (Taylor-Edwards *et al.*, 2009a). The results of this study were similar to those of Taylor-Edwards *et al.* (2009a), where the authors evaluated the effects of urea and slow release urea on the N balance of beef steers. The authors found no significant differences (P>0.05) for DMD, OMD, NDF and ADF digestibility, however the apparent N digestibility of the polymer coated SRU (manufactured by Agri-Nutrients Technology Group, Petersburg, VA) was significantly lower than the urea treatment, and the faecal N excretion of the SRU was significantly higher than that of the urea treatment. In a study previously done by Löest *et al.* (2001) with biuret as a slow releasing NPN source, the authors observed that biuret had a lower apparent CP digestibility than urea or a urea/biuret combination. The authors suggested that some biuret was not hydrolysed in the rumen and passed through to the small intestine, thus providing less N for the rumen microbial growth and microbial protein synthesis.

Ribeiro *et al.* (2011) found no difference between urea and Optigen® II for DM, OM, NDF digestibility and apparent N digestibility. These authors ascribed the inability to improve digestibility to rumen fill due to a higher intake with N inclusion, which improves rumen microbial growth and therefore decreasing digestion time. Dry matter intake and dry matter digestibility are dependant on the NDF concentration and NDF digestibility of a feed, because of the variability in digestibility of fibre (Mertens, 2009). According to Mertens (2009), one of the most important factors affecting DMD is the NDF concentration and NDF digestibility of a feed. The author suggested that if the NDF digestibility of a forage could be improved, the DMD would improve if the NDF concentration does not increase. However, NDF digestibility is negatively

correlated to the NDF concentration of forage. As the plant matures, lower DMD digestibilities could be expected due to an increase in NDF concentration and a decrease in NDF digestibility (Mertens, 2009). Mertens (2009) also suggested that NDF digestibility might have an influence on intake due to the reduction of the filling effect of the NDF concentration. Dry matter intake of a forage could be improved if the NDF digestibility of a forage is increased. The increased NDF digestibility could result in quicker digestion of cell walls, therefore reducing the volume of the forage in the rumen and increasing the passage rate (Mertens, 2009). According to the suggestions of Mertens (2009) and the results observed in Table 4.3, it could be suggested to the fact that there were no significant differences ($P>0.05$) between the DM, OM and NDF digestibilities, so these digestibility variables couldn't have any negative effect on the intake variables as been reported in Table 4.2.

4.4 Rumen fermentation

4.4.1 Rumen pH

The average rumen pH was relatively constant (6.28 - 6.34) between the treatments with no differences ($P>0.05$) (See Table 4.4). In contrast, Wahrmund *et al.* (2007) reported that rumen pH tended to be affected by urea source, where Optigen® II tended to have a lower pH than the control and urea treatments. In line with this study, Taylor-Edwards *et al.* (2009b) found no differences between urea and slow release urea (Agri-nutrients technology group, Petersburg,VA) treatments in terms of rumen pH.

The average pH was similar among treatments over 24 hours, with significant differences ($P<0.05$) observed at 06:00 and 21:00. Significant differences were recorded for treatment*time interactions for all treatments indicating that the time of day might have had an influence on the pH levels. A general increase in rumen pH was observed before the morning feeding at 08:00 (21:00-06:00) as can be seen in Fig. 4.1. This may be due to increased rumination during night time, roughage intake in the early mornings (Bae *et al.*, 1979) and N influx into the rumen when nitrogen concentrations were low. Intake of roughage stimulates saliva production and rumination and therefore, an increase in pH may occur due to the buffering capacity of saliva (Maekawa *et al.*, 2002). Roughage tends to have a more stable pH as a result of a slow fermentation and digestion rate, due to its high fibre/low energy content (Chapaval *et al.*, 2008; Ribeiro *et al.*, 2011). A decline in rumen pH was observed after 12:00. Nevertheless, it was

TABLE 4.4 Effect of slow and rapid release rumen N on rumen pH

Parameters	*Treatments 1	2	3	4	5	**SE
Average pH	6.29	6.28	6.29	6.33	6.34	0.04
24h pH						
03:00	6.34_{2}	6.16_{2}	6.34_{23}	6.30_{2}	6.32_{2}	0.1
06:00	6.60^{a}_{1}	6.44^{b}_{1}	6.52^{ab}_{12}	6.56^{ab}_{1}	6.52^{ab}_{12}	0.05
09:00	6.58_{1}	6.56_{1}	6.46_{12}	6.58_{1}	6.44_{123}	0.05
12:00	6.48_{12}	6.40_{1}	6.58_{1}	6.44_{12}	6.60_{1}	0.08
15:00	6.26_{23}	6.16_{2}	6.12_{3}	6.26_{23}	6.24_{3}	0.07
18:00	6.32_{23}	6.38_{12}	6.34_{23}	6.42_{2}	6.32_{2}	0.05
21:00	5.7^{b}_{4}	6.02^{a}_{2}	6.06^{a}_{3}	5.94^{ab}_{4}	6.04^{a}_{4}	0.08
24:00	6.1_{3}	6.12_{2}	5.92_{3}	6.10_{34}	6.24_{3}	0.12

^{a,b,c} Means within a row with different superscripts differ significantly (P<0.05)
$_{1,2,3}$ Means within a column with different subscripts differ significantly (P<0.05)
*1 = 100% Urea; 2 = 75% Urea : 25% Optigen® II; 3 = 50% Urea : 50% Optigen® II; 4 = 25% Urea : 75% Optigen® II; 5 = 100% Optigen® II
** SE = standard deviation

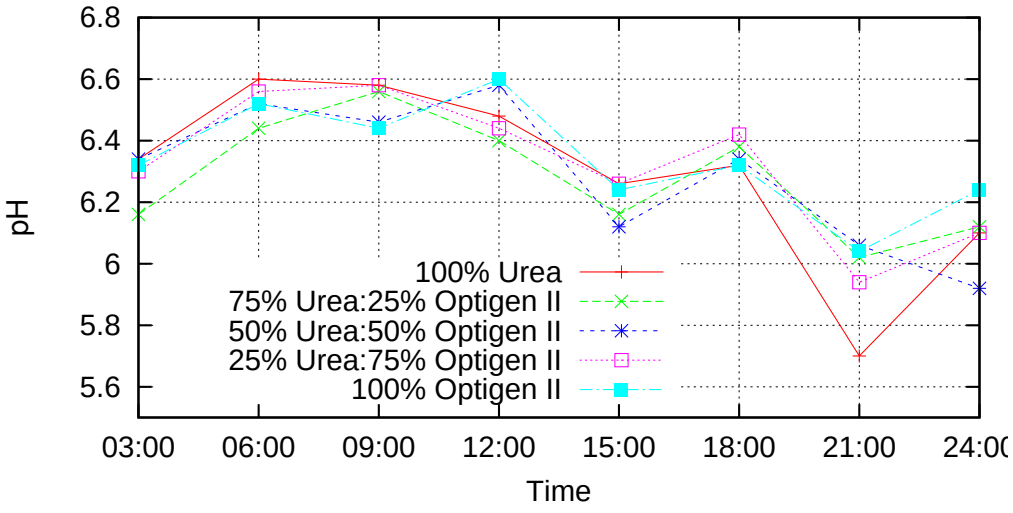

FIGURE 4.1 Effect of slow and rapid release rumen N on rumen pH over 24 hours

never below 6.0-6.1 to have a negative influence on fibre digestion (Mould *et al.*, 1983). A decrease in rumen NH_3-N due to an increased utilization of rumen NH_3-N by rumen microbes for microbial growth and microbial protein synthesis, could be the reason for the decline in rumen pH after 12:00. A slight increase in pH was observed from 15:00 until 18:00. The increase in pH followed after 15:00 could be due to the afternoon feeding taking place at 16:00, resulting in increased saliva production during intake. Another overall decrease in rumen pH was observed after 18:00, with the lowest pH value (5.7) observed at 21:00 for 100% Urea. The reason for this decline in rumen pH might be due to a combination of decreased forage intake and the high

availability of fermentable energy from starch degradation by the micro-organisms in the rumen.

According to the results of a study done by Ribeiro *et al.* (2011) where the authors studied the effects of a slow release polymer-coated urea or urea, on the digestibility and rumen parameters of cattle fed a low quality hay (29.4g CP/kg), the urea treatment had the highest pH levels 1-2 hours after feeding. Optigen® II; a combination of Optigen® II and urea had lower pH values than urea. Therefore, confirming the results of Wahrmund *et al.* (2007) who also found that the urea treatment had higher rumen pH levels than the Optigen®II treatment, and indicating that urea undergoes a faster rate of hydrolysis, thus increasing the rumen pH. In the current study there were no definite tendencies indicating that Optigen® II and a combination of Optigen® II/Urea had lower pH levels than urea. Irrespective of the references mentioned, no differences were found in this study, with no possible explaination.

According to Mould *et al.* (1983) fibre digestion and cellulolytic activity are inhibited when the rumen pH falls below 6.0-6.1. Therefore, with average rumen pH values of 6.28-6.34 in this study, no negative effects on fibre digestion could be attributed to the pH levels in the rumen.

4.4.2 Rumen NH_3-N concentration

No differences ($P>0.05$) were observed between treatments in terms of average rumen NH_3-N concentrations (See Table 4.5). Significant differences were observed between treatments at 2h, 3h and 4h after feeding. Treatments with higher levels of urea differed significantly from treatments containing high levels of Optigen® II. Treatments containing higher concentrations of Optigen® II (75%Optigen® II:25%Urea and 100% Optigen® II) had lower ($P<0.05$) rumen NH_3-N concentrations 2-4 hours after feeding. According to the study of Ribeiro *et al.* (2011), urea and SRU/urea treatments had higher NH_3-N concentrations than SRU alone. The SRU treatment had a continuous higher concentration of rumen NH_3-N over 6 hours after feeding and continuous maximum concentrations of rumen NH_3-N up to 4 hours after feeding. However, the urea treatment peaked at 2 hours (after infusion) and oscillated more over the 24 hour sampling period than the SRU treatment. The authors also found no difference between rumen NH_3-N concentrations before infusions between the NPN supplements, with rumen NH_3-N concentrations below 5mg/dL. In this study rumen NH_3-N concentration was only measured until 4 hours after feeding. Higher concentrations of NH_3-N (See Table 4.5) were observed for treatments containing higher levels of Optigen® II (75%Optigen® II:25%Urea and 100% Optigen®

II) just before feeding (0 hours), with rumen NH_3-N concentrations (2-5mg/dL) similar to those of Ribeiro *et al.* (2011). The higher rumen NH_3-N concentration could be due to a slower N release from Optigen® II.

TABLE 4.5 Effect of slow and rapid release rumen N on rumen NH_3-N concentration (mg/100ml)

	*Treatments					
Parameters (mg/100ml)	1	2	3	4	5	**SE
Average rumen NH_3-N	5.84	4.93	5.00	5.40	4.55	0.55
Rumen NH_3-N after infusion						
0h	2.88_{2}	4.22_{3}	3.12_{2}	3.56_{2}	3.95_{3}	0.50
1h	3.49_{2}	3.80_{3}	4.63_{2}	3.94_{2}	4.53_{3}	0.49
2h	7.68^{a}_{1}	7.00^{ab}_{2}	8.06^{a}_{1}	6.71^{ab}_{1}	4.77^{b}_{23}	0.74
3h	8.85^{ab}_{1}	10.03^{a}_{1}	8.57^{ab}_{1}	7.96^{ab}_{1}	7.45^{b}_{1}	0.65
4h	9.64^{a}_{1}	9.82^{a}_{12}	8.59^{ab}_{1}	8.59^{ab}_{1}	7.52^{b}_{12}	0.40

^{a,b,c} Means within a row with different superscripts differ significantly (P<0.05)
$_{1,2,3}$ Means within a column with different subscripts differ significantly (P<0.05)
*1 = 100% Urea; 2 = 75% Urea : 25% Optigen® II; 3 = 50% Urea : 50% Optigen® II; 4 = 25% Urea : 75% Optigen® II; 5 = 100% Optigen® II
** SE = standard deviation

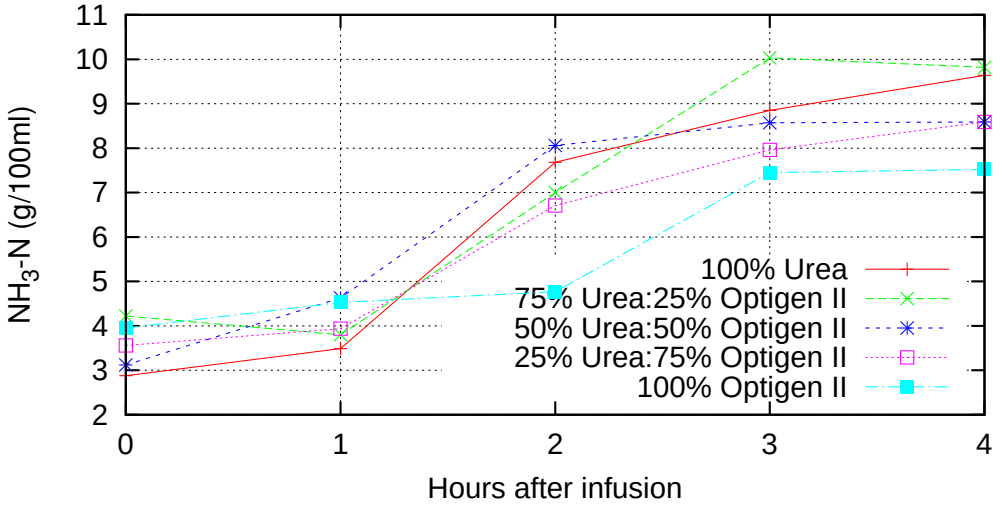

FIGURE 4.2 Effect of slow and rapid release rumen N on rumen NH_3-N concentration from 0 hours to 4 hours after infusion

Significant differences were recorded for treatment*time interactions for all treatments as presented in Table 4.5. The rumen NH_3-N concentration at 2 hours after feeding was significantly higher than at 1 hour after feeding for all treatments except for the 100% Optigen®II treatment. Therefore indicating that the rumen NH_3-N concentration only started to increase significantly at 1 hour after feeding for treatments 1, 2, 3 and 4. The rumen NH_3-N concentration of the

100% Optigen®II treatment started to increase significantly between 2 and 3 hours after feeding, suggesting that 100%Optigen®II indeed has a slower ammonia release rate than the other treatments containing urea. As illustrated in Fig. 4.2 it seems that there was a lag in NH_3-N release in the rumen up to 1 hour after feeding for the treatments containing urea and up to 2 hours for the 100% Optigen® II. According to Varga (1987), lag time is the time when digestion of a feed is limited by rumen micro-organisms due to various factors such as pH, ingested air, temperature, type of substrate, the maturity of the forage and the nutrients required by rumen microbes that might influence the adhesion of rumen micro-organisms to the specific substrate and therefore digestion. The pH level in the rumen could affect the adhesion of rumen microbes to the feed (Varga, 1987). Varga (1987) stated that a pH of 5.8 in the rumen resulted in a decrease of rumen NH_3-N concentration, NDF digestibility and attached microbes. Having a look at the present study, the pH could not have had an influence on either the rumen NH_3-N concentration neither on NDF digestibility, due to the fact that the pH was never below 5.8 for long durations. In the present study the lag phase in rumen NH_3-N concentration might be due to the fact that supplements infused into the rumen were given via a brown paper bag, and thus could have delayed the solubilization of the urea and Optigen®II. With a lag of NH_3-N over the first hour after feeding it could be expected that NDF disappearance will be negatively affected during the first few hours after intake, due to the lack of sufficient nutrients (rumen NH_3-N) for the fibre digesting rumen microbes for adhesion to the poor quality *Eragrostis curvula* hay (Varga, 1987).

The NH_3-N concentrations of the treatments containing higher levels of Optigen® II (75% Optigen® II:25%Urea and 100% Optigen® II) increased at a slower rate than those containing a higher percentage urea (See Fig. 4.2). The higher NH_3-N concentration observed for treatments containing Optigen® II before feeding could have been due to a continuous slower rate of release and a lower concentration of rumen NH_3-N concentration in the rumen after feeding. A slower rate of release may not be the only reason why the NH_3-N concentrations were lower for 75%Optigen® II:25%Urea and 100% Optigen® II, but it might have been due to improved utilization by rumen bacteria. Continuous release of NH_3-N should improve fibre digestion and the utilization of the diet due to the prolonged availability of nitrogen to the rumen bacteria (Wahrmund *et al.*, 2007). In the study of Wahrmund *et al.* (2007), Optigen® II tended to have a lower blood urea nitrogen (BUN) level than urea, indicating that rumen nitrogen utilization was improved with Optigen® II, due to improved synchrony between nitrogen release and available FME. In the present study the average rumen NH_3-N concentrations were between 4.55 and 5.84 mg/dL for the different treatments. Thus, it can be concluded that enough NPN was provided to

optimize rumen microbial growth (Satter & Slyter, 1974). Rumen NH_3-N of 5mg/dL might be enough for an animal at maintenance, but high producing animals or animals consuming highly digestible diets, might need higher concentrations of rumen NH_3-N for microbial growth to meet their nitrogen requirements for growth and milk production (Taylor-Edwards *et al.*, 2009b).

Similar results for rumen NH_3-N concentrations were observed by Taylor-Edwards *et al.* (2009b), when steers were fed a corn silage diet: a ground corn-based vitamin and mineral supplement mixed with urea or SRU (manufactured by Agri-Nutrients Technology Group, Petersburg, VA). The authors found a decrease in ruminal NH_3-N concentrations due to a slower ammonia release rate from the SRU. According to Taylor-Edwards *et al.* (2009b), urea increased the average rumen NH_3-N concentrations by 58% relative to a slow release N source. A 25% increase in rumen NH_3-N concentration at 2 hours was seen after feeding, and a maximal increase in rumen NH_3-N concentration of 147% at 8 hours was observed for urea compared to SRU. Therefore, concluding that a slow release N source has a slower release rate in the rumen than urea, thus reducing the rumen NH_3-N concentrations which could prevent ammonia toxicity. In the present study urea increased the rumen NH_3-N concentration by 61% at 2 hours after feeding relative to Optigen®II. At 4 hours after feeding there was only a 28% difference in rumen NH_3-N concentration between urea and Optigen®II. The average rumen NH_3-N concentration of urea was 22% higher than Optigen®II (See Table 4.5).

The lowest rumen NH_3-N concentration was observed at 0 hours (just before feeding), with 100% urea having the lowest (2.88 mg/dL) concentration. However, it was still above the limiting concentration (2mg NH_3-N/dL) suggested by Satter & Slyter (1974), for microbial growth and digestion to fulfill in the maintenance requirements of sheep. Therefore, according to the results, rumen NH_3-N concentrations as reported in this study, should not have had a negative effect on microbial growth and digestion, and should supply enough microbial protein for maintenance requirements. The ability of a ruminant to ensure N influx into the rumen when N levels in the diet are low, may be a reason why complete depletion of NH_3-N in the rumen didn't occur and therefore providing enough N for microbial growth, even before feeding (Rihani *et al.*, 1993).

4.4.3 Volatile fatty acid concentration

The only differences (P<0.05) observed between total and individual VFA that were affected by treatment, were the concentrations of butyrate and isobutyrate (See Table 4.6). The treatment containing 100% urea differed significantly from the other treatments, containing the lowest butyrate concentration (4.85%) and it was significantly lower than the other treatments. In previous studies, butyrate concentrations tended to increase with fermentable energy and RDP supplementation (Olson *et al.*, 1999; Heldt *et al.*, 1999; Golombeski *et al.*, 2006). According to Olson *et al.* (1999), butyrate concentration increased with higher levels of supplemental energy when steers were fed prairie hay (4.9% CP), casein and starch. Heldt *et al.* (1999) observed that non-starch supplements resulted in higher butyrate concentrations than starch supplements when beef steers were fed prairie hay (5.7%), different levels of casein and different carbohydrate sources (starch, glucose, fibre). Both these authors concluded that the higher butyrate concentrations of non-starch supplements were due to higher glucose levels observed with non-starch supplements. Therefore, according to both Olson *et al.* (1999) and Heldt *et al.* (1999), it was the supplemental energy that was responsible for the increased butyrate concentration in the rumen and not the RDP source.

No differences (P>0.05) were observed among treatments for the acetate:propionate ratio and the other individual VFA. According to the results of a study done by Taylor-Edwards *et al.* (2009b), analysing the effects of a slow-release N source on ruminal digesta, the authors concluded that substituting urea with a slow release N source or 100% urea, rarely has an effect on other ruminal fermentation concentrations than rumen NH_3-N concentration. According to the results of this study and of previous studies (Taylor-Edwards *et al.*, 2009b), VFA concentration was not affected differently by urea and/or a slow release N source.

The VFA results in this study were similar to those observed by Currier *et al.* (2004b), where the authors studied the effects on ruminal fermentation when ruminants consuming a low quality hay were supplemented with urea or biuret. According to Currier *et al.* (2004b), no differences between urea and biuret treatments were found in terms of VFA concentration. The authors also concluded that high concentrations of acetate are characteristic of high forage diets. Acetate production increased with increasing levels of fibre in the diet. According to Currier *et al.* (2004b) an increase in VFA concentration can only be expected when a natural protein is provided in the diet. Natural proteins contain branched chain amino acids which are the precursors

TABLE 4.6 Effect of slow and rapid release rumen N on volatile fatty acid production in the rumen

Parameters	*Treatments					**SE
	1	2	3	4	5	
Total VFA (mmol/L)	87.50	82.82	84.35	82.01	85.49	1.96
Acetate:Propionate	4.44	4.64	4.59	4.89	4.86	0.22
Acetate (%)	76.76	76.50	76.66	77.26	76.00	0.69
Propionate (%)	17.56	17.04	16.92	16.01	16.43	0.67
Butyrate (%)	4.85^{b}	5.46^{a}	5.50^{a}	5.81^{a}	5.60^{a}	0.20
Isobutyrate (%)	0.38^{b}	0.48^{a}	0.43^{ab}	0.42^{ab}	0.45^{ab}	0.03
Valerate (%)	0.45	0.51	0.49	0.50	0.52	0.04

^{a,b,c} Means within a row with different superscripts differ significantly (P<0.05)
*1 = 100% Urea; 2 = 75% Urea : 25% Optigen® II; 3 = 50% Urea : 50% Optigen® II; 4 = 25% Urea : 75% Optigen® II; 5 = 100% Optigen® II
** SE = standard deviation

for branched chain VFA (isobutyrate and valerate). In this study a limited amount of natural protein was present due to the low CP value of the *Eragrostis curvula*, therefore limiting VFA production.

4.5 DM and NDF disappearance from the rumen

According to the results presented in Table 4.7 for the NDF disappearance of the *Eragrastis curvula* from Dacron bags in the rumen, there were no significant differences (P>0.05) among treatments for the soluble fraction (a), insoluble potentially degradable fraction (b), degradation rate (c) and effective degradability (ED). Treatment 2 also had a significantly (P<0.05) higher soluble fraction for DM disappearance of the *Eragrostis curvula* than Treatment 4. Treatment 4 had the highest effective degradability for both DM disappearance and NDF disappearance, although it was non significant (P>0.05).

Likewise, in a similar study done by Ribeiro *et al.* (2011), no differences were found between the control (only *Brachiaria humidicola* hay), urea (urea + hay), urea/SRU (hay + combination of urea and SRU) and SRU (hay + SRU) treatments for the insoluble potentially degradable fraction (b), degradation rate (c) and effective degradability (ED) of both the DM and NDF disappearances of the poor quality hay containing 2.49% CP on a DM basis. According to Balcells *et al.* (1993), an increase in effective degradability of a nitrogen deficient forage could be expected if nitrogen is additionally added into the feed to overcome the nitrogen deficiency.

TABLE 4.7 Effect of slow and rapid release rumen N on DM and NDF disappearance of a poor quality roughage

Parameters (%)	*Treatments 1	2	3	4	5	***SE
**DM disappearance						
a	5.86^{ab}	6.94^{a}	5.70^{ab}	5.26^{b}	6.30^{ab}	0.41
b	67.91	70.74	57.78	69.86	59.09	9.73
c	0.012	0.014	0.014	0.015	0.011	0.004
ED	27.14	27.24	27.97	28.14	26.46	1.11
**NDF disappearance						
a	2.52	3.16	2.07	1.93	2.82	0.45
b	64.46	57.66	68.97	68.51	67.70	8.99
c	0.014	0.016	0.013	0.014	0.011	0.003
ED	25.81	25.07	27.33	27.00	25.12	1.24

^{a,b,c} Means within a row with different superscripts differ significantly (P<0.05)
*1 = 100% Urea; 2 = 75% Urea : 25% Optigen® II; 3 = 50% Urea : 50% Optigen® II; 4 = 25% Urea : 75% Optigen® II; 5 = 100% Optigen® II
**A: soluble fraction; B: insoluble potentially degradable fraction; C: degradation rate; ED: effective degradability
*** SE = standard deviation

However, in the current study a lower effective degradability (Table 4.7) was observed for both DM and NDF than in the study of Ribeiro *et al.* (2011).

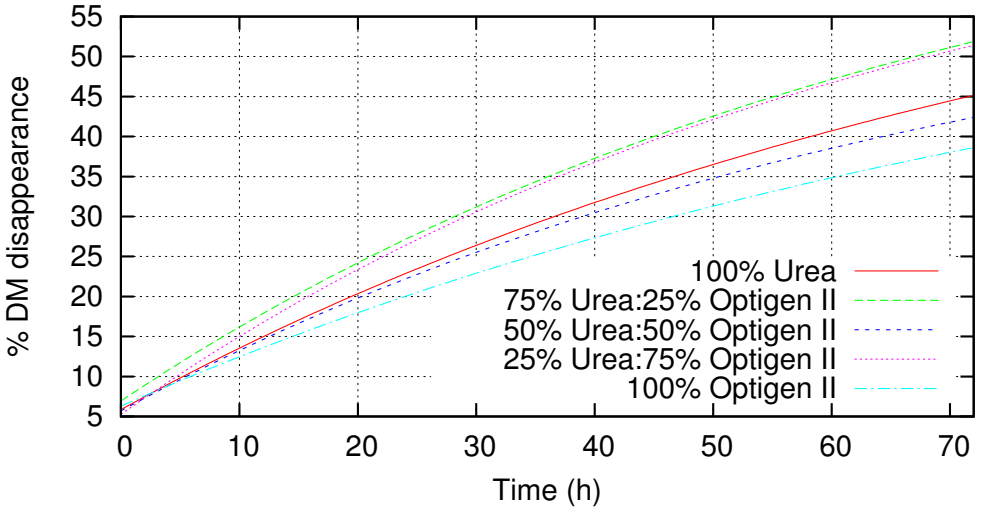

FIGURE 4.3 Effect of slow and rapid release rumen N on DM disappearance of *Eragrostis curvula* over 72 hours

According to Detmann *et al.* (2009), NDF degradation is optimized at 8mg/dL rumen NH_3-N and NDF intake at 15mg/dL rumen NH_3-N. This might explain why no significant differences were observed between treatments for NDF disappearance in this study. Although, the average rumen NH_3-N was below 8mg/dL for all the treatments over a 24 hour period, after 3 hours of

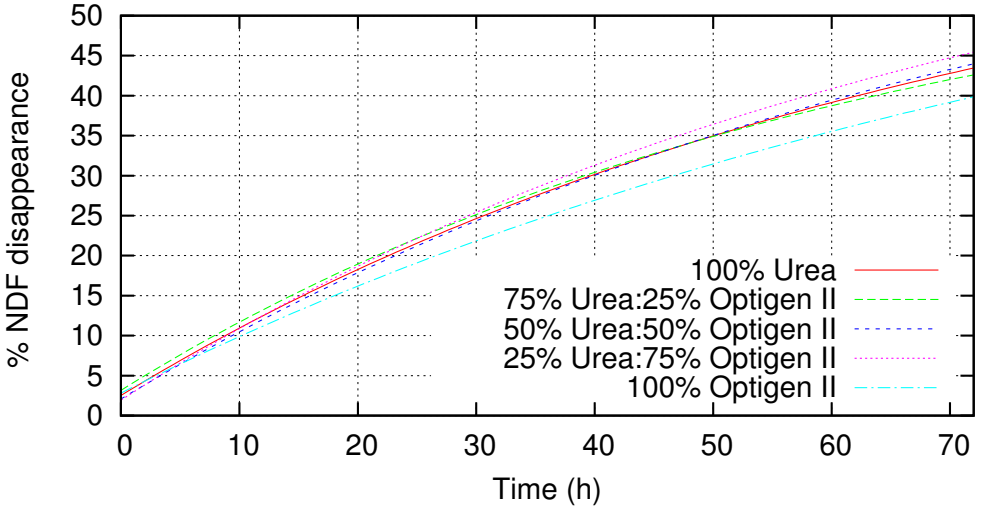

FIGURE 4.4 Effect of slow and rapid release rumen N on NDF disappearance of *Eragrostis curvula* over 72 hours

infusion (See Fig 4.2) the rumen NH_3-N concentrations increased above 8mg/dL which is ideal for fibrolytic enzymes for the optimization of NDF degradation. Therefore, it was possible that the NDF degradation was optimized for at least 1 hour. It seems that no lag time was present with the DM and NDF disappearance of the *Eragrostis curvula* (See Fig 4.3 and 4.4) as was expected due to the lag time in rumen NH_3-N release. Ribeiro *et al.* (2011) reported that there was no lag time for DM and NDF disappearance of the roughage for both treatments (urea and SRU) 3 to 6 hours after incubation in this study. It is suggested that the degradability of DM and NDF is not only dependent on the rumen NH_3-N concentration, but also on the fermentability of the diet and the chemical and physical structures of the diet (Boucher *et al.*, 2007).

4.6 Nitrogen intake and excretion

Nitrogen intake of Treatment 1 and 3 was significantly higher than Treatment 2 and 5 (See Table 4.8). However, Treatment 2 and 5 have significantly higher N-excretion levels ($P<0.05$) than Treatment 3, with no differences in urinary N excretion between treatments. With Treatment 2 and 5 having the lowest N intake and the highest N excretion, it seems that less N was available for microbial growth and digestion. Therefore, it could possibly explain the significantly lower intake levels of *Eragrostis curvula* of Treatment 5 (See Table 4.2). The high N-excretion of Treatment 2 and 5 could be due to the lower apparent N digestibilities (See Table 4.3), although

not significant, observed in this study for these two specific treatments. The N balance of Treatment 3 differed significantly (P<0.05) from Treatments 2 and 5. Treatment 2 and 5 also differed significantly (P<0.05) from each other, with Treatment 5 having the lowest N balance. The significant higher N-balance of Treatment 3 could be explained by the significant higher N-intake and significant lower N-excretion compared to Treatment 2 and 5. The significant lower N-balance of Treatment 5 might be due to the lowest DMI (non significant), the high N-excretion due to the lowest significant apparent N digestibility and the lowest significant N-intake obtained for this specific treatment.

TABLE 4.8 Effect of slow and rapid release rumen N on nitrogen intake and excretion

	*Treatments					
Parameters	1	2	3	4	5	**SE
N-intake (g/day)	13.55^{a}	12.95^{bc}	13.65^{a}	13.32^{ab}	12.76^{c}	0.16
N-excretion (g/day)	6.60^{ab}	7.13^{a}	6.12^{b}	6.95^{ab}	7.26^{a}	0.31
Faecal N (g/day)	5.61^{ab}	5.92^{ab}	5.49^{b}	6.00^{a}	5.93^{ab}	0.16
Urinary N (g/day)	0.99	1.21	0.62	0.95	1.33	0.25
N-balance	6.96^{ab}	5.81^{b}	7.53^{a}	6.37^{abc}	5.5^{c}	0.38

^{a,b,c} Means within a row with different superscripts differ significantly (P<0.05)
*1 = 100% Urea; 2 = 75% Urea : 25% Optigen® II; 3 = 50% Urea : 50% Optigen® II; 4 = 25% Urea : 75% Optigen® II; 5 = 100% Optigen® II
** SE = standard deviation

The results of this study in terms of faecal N was different from those of Taylor-Edwards *et al.* (2009a), where the treatment containing SRU had a significantly higher faecal N excretion than the urea treatment, and no differences were found between urea and SRU treatments for N retention. The authors explained this occurrence by suggesting that the higher N excretion with the SRU treatment could be due to the coating that might obstruct full release of urea from the SRU source in the rumen, resulting in the passage of SRU through the digestive tract. Instead, Galo *et al.* (2003) found that the treatment containing no SRU had a significantly higher faecal excretion than those containing SRU. The authors postulated that this could have been due to damage that occurred to the coating of the SRU during mixing, resulting in a faster urea release rate. Galo *et al.* (2003) also found no significant differences between treatments for N balance. In a study done by Bourg *et al.* (2009), the authors found that faecal- and urinary N excretion were not different between urea and Optigen® II treatments. No differences were observed between N intake and absorption between treatments. The only tendencies that occurred were that higher levels of Optigen® II increased faecal N excretion. Bourg *et al.* (2009) suggested that Optigen® II can replace urea due to no major differences in N intake, N excretion and absorption.

According to the results found in this study it seems that treatments containing higher levels of Optigen® II have higher N-excretion (faecal and urinary) values (Table 4.8), which suggests that N released from Optigen® II may not have been captured in MCP (Galo *et al.*, 2003). Therefore, it could be suggested that Optigen®II might have a slower release rate of urea in the rumen that resulted in N loss for MCP synthesis. Faecal N was the most prevalent route for N excretion in this study, suggesting that the rumen NH_3-N concentrations did not exceed the ability of the rumen microbes to utilize the available rumen NH_3-N. Urinary N can become the prevalent route of excretion in the case where protein deamination is present in the body or when accumulation of NH_3-N in the rumen occurs due to the inability of rumen microbes to utilize all the available rumen NH_3-N, that might result in high blood urea levels (Galo *et al.*, 2003).

4.7 Microbial nitrogen

The concept of urinary purine derivatives (PD) as an indication of rumen microbial N supply, is based on the assumption that exogenous purines are produced by rumen micro-organisms through degradation and absorption of microbial nucleic acids. Therefore, urinary PD excretions are positively correlated to microbial nitrogen flow from the rumen and are a good indication of rumen microbial N synthesis (Chen & Gomes, 1992; Chen *et al.*, 1995; Moorby *et al.*, 2006). Purine derivatives were analysed with two methods to minimize possibilities of not having representative samples of the urine for the determination of rumen microbial N synthesis. The total PD method of Chen & Gomes (1992) is the preferred method for the prediction of rumen microbial N synthesis. However, high standard errors were observed with this method (See Table 4.10). By using the spot urine method (Chen *et al.*, 1995) as a control measurement, PD values were more or less the same between treatments, and the standard errors were smaller (See Table 4.9). The spot urine method is based on the assumption that creatinine (C) excretion in urine is a function of metabolic weight and is excreted at a constant rate (Chen *et al.*, 1995; Moorby *et al.*, 2006; Cetinkaya *et al.*, 2006). The PD:C ratio is usually used as an estimation of MCP production due to the independence of creatinine to the volume of daily urine (Chen *et al.*, 1995). Chen *et al.* (1995) found that the PD:C ratio has diurnal stability and is highly correlated to the PD excretion, therefore it could be used as an alternative for microbial N supply estimation.

In a study where Chen *et al.* (1995) used the urine spot method, the authors observed that DMI

and PD excretion were positively correlated. The diets with the highest DMI had the highest PD excretion. These authors also observed that DMI/$W^{0.75}$ positively affected the efficiency of rumen microbial N supply. In this study Treatment 4 had the highest significant ($p<0.05$) DMI but it did not differ significantly from the other treatments in terms of PD excretion for both the spot urine method and the total PD method. Chen *et al.* (1995) also suggested that a higher DMI/$W^{0.75}$ could improve the efficiency of microbial N supply due to the shorter retention time of feed in the rumen. Instead, Treatment 4 in the present study with the highest DMI/$W^{0.75}$ value (See Table 4.2), has the lowest significant rumen microbial N supply efficiency of 19.8g MCN/kg DOMI according to the spot urine method.

TABLE 4.9 Effect of slow and rapid release rumen N on purine derivatives and rumen microbial nitrogen synthesis according to Chen *et al.* (1995).

	*Treatments					
Parameters	1	2	3	4	5	**SE
Spot PD excretion (mmol/day)	11.38	11.24	11.23	11.41	11.23	0.06
Spot MCN (g/day)	9.76	9.62	9.62	9.77	9.62	0.06
gMCN/gDOMI	0.0222^{ab}	0.0220^{ab}	0.0224^{ab}	0.0198^{b}	0.0232^{a}	0.001

^{a,b,c} Means within a row with different superscripts differ significantly ($P<0.05$)
*1 = 100% Urea; 2 = 75% Urea : 25% Optigen® II; 3 = 50% Urea : 50% Optigen® II; 4 = 25% Urea : 75% Optigen® II; 5 = 100% Optigen® II
** SE = standard deviation

The concentrations observed with the total PD method for PD excretion, MCN, gMCN/g DOMI as well as the standard errors were higher compared to that of the spot urine method (Table 4.10). No differences ($P>0.05$) were observed with the use of the total purine derivative method regarding purine absorption, purine excretion, microbial nitrogen production or the efficiency of microbial supply (gMCN/gDOMI) among different treatments; as can be seen in Table 4.10 (Chen & Gomes, 1992). The results of the total PD method was similar to those found by Galo *et al.* (2003). The same authors found no significant differences for MCP and for the efficiency of microbial supply (gMCP/gDOMI) between treatments containing urea and/or SRU, when dairy cattle was fed diets containing either urea or a combination of urea and Optigen 1200. Analysis with the spot urine method resulted in no differences ($P>0.05$) for PD excretion, nor for microbial nitrogen supply as presented in Table 4.9. However, a significant difference was observed between Treatments 4 and 5 for the efficiency of microbial supply (gMCN/gDOMI). The MCN concentrations of all five treatments obtained by the spot urine method in the current study are just below the protein maintenance requirements of a 59kg sheep (NRC, 2001). The results of the spot urine method regarding microbial nitrogen synthesis are similar to those of

TABLE 4.10 Effect of slow and rapid release rumen N on purine derivatives and microbial nitrogen synthesis according to Chen & Gomes (1992)

	*Treatments					
Parameters	1	2	3	4	5	**SE
PD excretion (mmol/day)	23.03	20.06	18.86	21.17	21.24	1.28
PD absorption (mmol/day)	27.40	24.52	22.43	25.19	25.28	1.52
MCN (g/day)	39.94	41.70	33.18	33.88	44.36	3.94
gMCN/gDOMI	0.0441	0.0418	0.0349	0.0375	0.0444	0.003

[a,b,c] Means within a row with different superscripts differ significantly (P<0.05)
*1 = 100% Urea; 2 = 75% Urea : 25% Optigen® II; 3 = 50% Urea : 50% Optigen® II; 4 = 25% Urea : 75% Optigen® II; 5 = 100% Optigen® II
** SE = standard deviation

Ramos *et al.* (2009). Ramos *et al.* (2009) fed sheep diets varying in concentrate to forage ratios and different types of forage. The authors found that diets containing high levels of forage had a microbial nitrogen supply of 9.45g/day and an efficiency of microbial nitrogen of 17.9g/kg-DOMI. Yu *et al.* (2001) reported similar values for microbial nitrogen than Ramos *et al.* (2009). Yu *et al.* (2001) studied the effect of various dietary proteins on microbial protein synthesis. The control diet in Yu *et al.* (2001)'s diet consisted out of oats straw and lucerne. The authors recorded microbial nitrogen concentrations of 9.98g/day for the control diet. With the creatinine being corrected for metabolic weight it seems that urea can be completely substituted by Optigen®II without having any negative effects on MCN production.

Comparing the results of the two methods and considering results found by other authors, it seems that the spot urine method in this study was more accurate. The MCN results obtained with the total PD method were far too high for a sheep on maintenance, therefore a error might have occurred during calculations or analysis of the urine.

Chapter 5

Conclusion

Sheep production in South Africa is mostly based on extensive grazing systems. The quality and quantity of natural veld are often the limiting factor for optimum production. To overcome these deficiencies, animals have to be supplemented.

Urea is commonly used as a N source in supplements, but due to its high solubility it is hydrolysed at a faster rate in the rumen than what the rumen microbes can utilize. Therefore, it is assumed that some of the rumen NH_3-N from urea is lost for microbial protein synthesis and rumen fermentation. Taking this into account, some technologies have been developed to slow the release rate of urea in order to meet the rumen microbes requirements and to match the available N with the slow release of energy from poor quality roughages. Optigen®II is one of the NPN sources developed to give a sustained and slow even release of N over 24 hours, with the aim of meeting the rumen microbes requirements and improving rumen microbial protein synthesis and rumen fermentation.

This study showed that higher intakes were recorded when a combination of urea and Optigen®II (25%urea : 75% Optigen® II) was infused into the rumen of the wethers, compared to the other treatments, suggesting a possible synchronization between N released and fermentable energy available in the rumen. In terms of intake, no differences were found between 100% urea and 100% Optigen®II, suggesting that urea can be substituted with Optigen® II without having any negative effects on intake.

A lower apparent N digestibility was observed for 100% Optigen®II compared to the other

treatments in this study, suggesting that Optigen®II had a slower rumen NH_3-N release or that asynchrony might have occurred between the available FME and N. It is possible that some of the Optigen®II might have flushed out of the rumen without being utilized. The higher total N excretion observed for the 100% Optigen®II treatment might have been caused by the significant low apparent N digestibility, suggesting that some of the N might have not been utilised for MCP synthesis. Although a higher total N excretion and lower apparent N digestibility was observed for 100% Optigen®II, no differences were observed for MCN between treatments. Therefore, suggesting that the efficiency of utilisation of the 100% Optigen®II treatment was higher than the other treatments containing urea. The efficiency of MCN supply was also higher for 100% Optigen®II compared to the other treatments, suggesting that Optigen®II MCN was more effectively utilized by rumen microbes for microbial protein synthesis. The N-balance of the 100% Optigen®II treatment was lower compared to the other treatments. This could be ascribed to the lower N intake, low DMI intake (non significant) and the high N excretion.

The average rumen pH and VFA concentration were not influenced by the type of NPN source or the ratio in which urea and Optigen®II were infused in the rumen. Treatment*time interactions were observed for pH, suggesting that the time of day has an influence on the pH level. The only difference observed with the VFA was in terms of the butyrate concentration, where 100% urea had a lower concentration compared to the other treatments. No significant differences were observed for average rumen NH_3-N concentrations between treatments, although 100% Optigen® II had the lowest concentration. Treatment*time interactions were observed for all treatments. The rumen NH_3-N concentration for all the treatments except for the 100% Optigen®II treatment increased significantly after 1 hour of infusion. The NH_3-N concentration of 100% Optigen®II only increased significantly after 2 hours of infusion, suggesting that treatments containing higher levels of Optigen®II has a slower rumen NH_3-N release rate.

In terms of DM and NDF disappearance in the rumen from Dacron bags, no differences were observed between treatments. With no significant differences for both average rumen NH_3-N and *in situ* degradability of DM and NDF of the forage, it could be suggested that urea can be substituted with Optigen® II or a combination of the two may be used as a NPN supplement, without having any negative effects on rumen NH_3-N concentration and NDF rumen degradability.

By considering all the results recorded in this study, it could be suggested that urea can be substituted by Optigen®II as a NPN source without having negative effects on intake, digestibility, rumen fermentation and microbial protein synthesis. The main advantage of substituting feed grade urea with Optigen®II would be the reduction in potential hazards, such as ammonia toxicity. However, from an economical perspective, Optigen®II, is on a R/kg N (R 22.68/kg Optigen®II) basis more expensive than feed grade urea (R 7.00/kg urea) with a lower concentration of N (41g N/100g Optigen®II vs 46g N/100g urea).

Chapter 6

Critical evaluation

6.1 Experimental diets

In the current study, 5 different treatments consisting out of different proportions of slow and rapid release NPN sources were compared with each other. A suggestion that could be made, in relation to this study, is to include a control treatment that does not contain any of the two NPN sources. By doing this, one will be able to see if any of these two NPN source does have a significant influence in terms of roughage intake, roughage digestibility, rumen fermentation and microbial protein synthesis from the control treatment. This additional treatment would require an extra wether, that would result in the use of a 6x6 Latin square design instead of a 5x5. But without a NPN source providing rumen NH_3-N to the rumen microbes on a poor quality roughage, rumen stasis might occur which will lead to reduced DM intake and starvation. Therefore, it might be impossible to include a control diet in a study like this, where NPN sources are the only N source to the animal.

6.2 Sample collection

The shortcoming in this study regarding sample collection, is that we have only measured rumen ammonia nitrogen concentrations up to 4 hours after feeding. To obtain a more satisfactory answer on the release of rumen NH_3-N in the rumen, measurements have to be made until a decline in rumen NH_3-N concentrations are observed. Thus, I would suggest that the rumen

NH_3-N should be collected in hourly intervals from the morning feeding until the afternoon feeding.

6.3 Solubility of urea and Optigen®II

An indication of the rumen NH_3-N release rate from urea and Optigen®II in the rumen was derived from the rumen NH_3-N concentrations recorded over 4 hours after feeding. However, according to the results recorded for rumen NH_3-N, a lag time was observed within 1 hour after feeding for the treatments containing urea, which might be incorrect due to the fact that urea is highly soluble. This lag time observed might have been due to asynchrony between the availability of FME and rumen NH_3-N from the urea. Therefore, a suggestion would be to record the solubilities of urea and Optigen®II in the rumen by using the *in sacco* method. However, it might be difficult to measure the disappearance of urea with the use of Dacron bags due to its high solubility.

6.4 NDF and DM disappearance

In this study the NDF and DM disappearance graph did not level out before 72 hours. Suggesting that the roughage in the Dacron bags was still undergoing rapid degradation after 72 hours. It is however, highly unlikely for roughage to degrade at such a rate after 72 hours. This error might be due to erroneous calculations or methods used during the incubation of the Dacron bags.

6.5 Recommendations

For a study like this one, where two products are compared, it will be favourable if a production study could be conducted to determine animal performance. Based on an intake and digestibility trial it seems that it might not be economically efficient to use the one above the other as a supplement. This suggestion could be proved by conducting a production study.

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