THE DEGRADABLE PROTEIN REQUIREMENTS OF BEEF CATTLE CONSUMING WINTER FORAGE HAY FROM THE PURE GRASSVELD TYPE

by

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Preface

This Dissertation is presented in three related articles, augmented by a general introduction and conclusion. Although care has been taken to avoid repetition, some repetition was inevitable especially with relevance to the last two chapters on the efficient replacement ratio of true protein by urea.

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I hereby declare that the dissertation hereby presented for the degree MSc., at the University of the Free State, is my independent work and has not been previously presented by me for a degree at another university. I further more cede copyright of the dissertation in favour of the University of the Free State

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POTCHEFSTROOM
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CONTENTS

The degradable protein requirements of beef cattle consuming winter forage hay from the pure grassveld type

Chapter 4: The influence of different levels of urea in rumen degradable protein supplements on the performance of beef cows grazing natural winter grassveld

List of abbreviations

DM	- Dry matter
ADF	- Acid detergent fibre
GE	- Gross energy
NDF	- Neutral detergent fibre
ad lib	- free access
N	- Nitrogen
S	- Sulphur
NPN	- Non protein nitrogen
CP	- Crude protein
MP	- Microbial protein
RDP	- Rumen degradable protein
RDPI	- Rumen degradable protein intake
RUP	- Rumen undegradable protein
DIP	- Digestible intake protein
NSC	- Non-structural carbohydrate
SC	- Structural carbohydrate
OM	- Organic matter
DOM	- Digestible organic matter
DOMI	- Digestible organic matter intake
DE	- Digestible energy
ME	- Metabolisable energy
MEI	- Metabolisable energy intake
$BW^{0.75}$	- Metabolic body weight
SD	- Standard deviation
^{o}C	- degrees celsius
g	- gram
g/d	- gram per day
kg	- kilogram
MJ	- megajoule
NH_3	- Ammonia

NH_3N	- Ammonia nitrogen
VFA	- Volatile fatty acids
BCVFA	- Branched chain volatile fatty acids
BCS	- Body condition score

CHAPTER 1

General Introduction

Grazing animals depend on native pastures (veld) as the main feed source or energy source. This natural feed varies however in quality and quantity as a result of seasonal changes as well as regional influences. Rainfall is the major driving force affecting dry matter (DM) production of veld and therefore, animal performance (De Waal, 1994).

Low quality roughage is the predominant energy source available to grazing animals for a considerable time of the year. These feeds are characterised by their high fibre and low crude protein contents that are poorly digested and have low metabolisability (Mawuenyegah *et al.* 1997). Protein deficiency reduces feed intake in ruminants by limiting the rate of microbial growth and the digestion of organic matter in the rumen and hence the clearance of digesta from the rumen (Redman *et al.,* 1980; Hunter & Siebert, 1987; DelCurto *et al.,* 1990; Mawuenyegah *et al.*, 1997). To optimise the utilisation of these forages and maintain acceptable animal performance, it is essential to enhance intake and digestion through provision of supplemental protein. Nitrogen (N) is generally considered to be the first limiting nutrient for ruminants grazing low quality forages (Köster *et al.*, 1996; Mawuenyegah *et al.*, 1997; Nolte & Ferreira, 2005).

The chemical constituents of herbage can be divided into cell wall constituents mainly cellulose, hemi-cellulose, pectin and lignin and the cell contents. The availability or digestibility of these constituents varies from the complete digestible sugars to the largely indigestible lignin. The digestibility of cellulose or hemi-cellulose is influenced by the degree of lignification, duration of fermentation and their digestibility potential. The efficiency of herbage as a nutrient source for the herbivore is a function of its content of non-structural constituent and extent to which the potential nutrients of the cell wall can be released during fermentation (Jones & Wilson, 1987).

Environmental factors such as temperature, water availability and light have an effect on growth and digestibility of forage. At high temperatures less digestible carbohydrates are stored in the plants and more fibre which is less digestible is produced, resulting in decreased digestibility of the plant due to an increase in cell wall content. Cloudy, low light conditions tend to produce roughage that is less digestible than roughages produced in abundant light (Jones & Wilson, 1987; Ferreira, 1999). Another important factor controlling nutritive value is the age of the plant tissue. The dry matter digestibility and protein content of plants both decrease with increasing age whereas the lignin content increases (t'Mannetjie, 1984).

Herbivores are able to derive a considerable and often a major proportion of their energy needs from the complex polysaccharides of the cell wall during fermentation by microbial organism action in the rumen. This energy is additional to that derived from non-structural carbohydrates, proteins and lipids all of which are highly digestible. The end products of fermentation are mainly volatile fatty acids and these are used as an energy source. The proportion of various acids formed is influenced by herbage composition and affects the efficiency of utilisation of energy for maintenance and production. Potential digestibility may not be realised if the diet is deficient in essential nutrients such as N and sulphur (S) required for efficient microbial fermentation (Jones & Wilson, 1987).

One of the main factors that limit consumption of low quality forage by ruminants is N availability in the rumen (Jones & Wilson, 1987; Hunter & Siebert, 1987; DelCurto *et al.,* 1990; Mawuenyegah *et al*., 1997; Basurto-Gutierrez *et al*., 2003; Nolte & Ferreira, 2005). When N requirements are met, microbial growth will be enhanced as well as rumen fermentation. This will enhance extensive fermentation of cellulose and hemi-cellulose. Hume *et al.* (1970) found strong relationships between increasing N intakes and cellulose digestibility, intake of low quality roughage and body mass gain in growing animals. Supplemental protein has also been reported to improve maintenance of mature cow mass and body condition during the winter grazing period (DelCurto *et al*., 1990). Non-protein nitrogen (NPN) sources can be an inexpensive way to overcome N deficiency. However the rapid release of N decreases the efficiency of its utilization by bacteria and could possibly lead to ammonia toxicity. In contrast true protein sources are degraded slower in the rumen compared to NPN, extending N availability for longer periods. True protein sources improve protein production by supplying amino acids, peptides and branched chain amino acids. Additionally, transfer of urea from blood into the gastro-intestinal tract is an important mechanism to save N and to maintain microbial fermentation for cattle consuming low quality forages (Petersen *et al.,* 1985; Firkins *et al.,* 1986; Basurto-Gutierrez *et al*., 2003).

Generally positive responses to protein supplementation are expected with forages containing less than 6-8% crude protein (CP) (DelCurto *et al.,* 1990). Hume *et al.* (1970) and Jones & Wilson (1987) concluded that production of protein in the rumen was limited by factors other than energy and N. The efficiency of utilisation of dietary N was maximal when dietary N:S ratio is about 10:1, therefore S must be considered as a possible limiting factor. Under most dietary conditions, rumen micro-organisms are the major source of the protein that is available to the ruminant animal and the N status of the host is controlled by the yield of microbial protein (MP) from the rumen (Cotta & Russels, 1982; Sniffen & Robinson, 1987; Hume, 1970).

CP measures both true protein and NPN. Protein for ruminants is divided into two types namely the rumen degradable protein (RDP) and rumen undegradable protein (RUP). RUP or by-pass protein is the protein which escapes digestion in the rumen and is digested in the lower alimentary tract. It is effective in improving livestock performance (especially during growth, late pregnancy and lactation) as it is catabolised in the lower tract to form amino acids which are then absorbed and incorporated into muscle, milk or wool. RDP on the other hand, must be reformed into microbial protein (MP) to be of nutritional value. RDP is fermented in the rumen and is broken down to amino acids, peptides and ammonia which serve as nutrients for the rumen microbes. Peptides and amino acids can be directly incorporated into MP (Nolan *et al.*, 1976), which increases the efficiency of MP production as well as production rate (Ferreira, 1999; Nolte, 2000; Jacobs, 2005). Macrae & Lobley (1986) concluded that in most situations the quantities of MP plus RUP reaching the duodenum have a greater influence on productivity than any aspect of protein quality.

The ruminant receives 40-80% of its daily amino acid requirement from microbial protein flowing to the small intestine (Sniffen & Robinson, 1987; Hume *et al.,* 1970). The growth rate of rumen microbes is greatly affected by the availability of ammonia, peptides and amino acids. There is variation in the form of N required by different types of microorganisms. The organisms splitting non-structural carbohydrate (NSC) i.e. starch pectin, sugars, etc. are able to utilise peptide N and ammonia whereas those splitting structural carbohydrates (SC) i.e. cellulose and hemicellulose, are unable to use amino N and have to rely on ammonia as their source of N (Russel *et al.,* 1992; McDonald *et al.,* 2002; Nolte & Ferreira, 2005). Another source of N to the rumen microbes is NPN, primarily urea which only provides ammonia to the rumen microbes. Although 80% of rumen bacteria and protozoa can grow with ammonia as their sole source of N, peptides and amino acids can be directly incorporated into MP, with a resultant benefit in rumen microbial growth efficiency (Baldwin & Allison, 1983; Nolte, 2000). Bryant & Robinson (1962) cited by Jacobs (2005) stated that 82% of rumen bacteria can grow with ammonia as their sole N source, 25% would grow unless ammonia was present and 56% could utilise either ammonia or amino acids. The microbial protein synthesised in the rumen may be protozoal or bacterial, the relative proportions depends upon the conditions within the organ. Low rumen pH tends to reduce protozoal activity and stimulate that of certain bacteria. The digestibility of bacterial protein is lower (about 0.75) as compared to that of protozoa (about 0.90), however the latter constitutes 5-15% of the microbial protein flow and its influence on the overall digestibility will be small (McDonald *et al.*, 2002).

Voluntary intake of forages is controlled by physical constraints, primary rumen fill and the removal of digesta from the rumen. Clearance of the digesta occurs by process of digestion and passage to the post-ruminal tract, which is a function of fermentation rate and rate of outflow from the rumen (Meissner *et al.,* 1995). The rate of digestion of plant cell wall by the rumen microbes will be depressed if the supply of N particularly in the form of ammonia, amino acids and peptides arising from ingested plant material or from endogenous recycling into the rumen is sub-optimal for microbial requirements (Wilson & Kennedy, 1996; Redman *et al.*, 1980; McDonald *et al.,* 2002). Another contributing factor for the clearance of digesta is particle size reduction, which occurs through ingestive chewing, ruminative chewing and passage through the reticulo-rumen. There is a relationship between duration of eating and the energy required to grind the dried forage which indicates that highly fibrous forages require more chewing effort for the formation of a bolus suitable for swallowing, and chewing time increases with grass maturity (Wilson & Kennedy, 1996). Supplementing low quality roughage with N has a positive effect on rumination characteristics by increasing DM intake and DM digestibility (Hannah *et al.,* 1991; Mathis *et al.,* 2000; Köster *et al.,* 1996; McCollum & Galyean, 1985). Supplementing readily available N seems to be more crucial than providing energy only, indicating that microbial growth is more dependent on dietary N than energy sources (Cronjé, 1990; Heldt *et al.,* 1999; DelCurto *et al.,* 1990). This confirmed that energy is not the first limiting nutrient in low quality roughages and CP supplementation improves the animal's energy status (DelCurto *et al.,* 1990). A voluntary reduced rumen clearance may be observed under marginal conditions when feed is scarce, a ruminant prolongs the residence time of the digesta in the rumen in order to maximize the digestive recovery of nutrients (Mawuenyegah *et al.*, 1997).

Glucose plays a vital role in the metabolism of all mammals. Ruminants absorb little or no glucose directly from the gastro intestinal tract and therefore rely almost entirely on gluconeogenesis to satisfy the requirement (Macrae & Lobley, 1986). In situations where glucose supply is deficient, a certain amount of dietary protein may be diverted to glucose production reducing the amount of amino acids available for protein deposition. Cronjé (1990) suggested that a low ratio of glucose to acetate may not only limit the efficiency of energy utilisation but, also the efficiency of protein synthesis if amino acids are used for gluconeogenesis.

Supplementation of forages by grains to increase overall energy intake and availability to the animal resulted in a depressed digestibility and intake of the forages, while energy intake increased marginally or not at all (Van Niekerk & Jacobs, 1985; Meissner *et al.,* 1991; Heldt *et al.,* 1999). The problem is associated with a depression in fibre or cell wall degradation in the rumen both in rate and extent. Meissner *et al.,* (1991) stated that adding 10-15% readily fermentable carbohydrate can impair fibre digestion, although severe depressions are usually associated with more than 30% of DM intake. They concluded that the amount of cell wall content is a distinguishing factor, because results suggest that impairment of fibre digestion and intake may be expected for forage with neutral detergent fibre (NDF) content below 55-60%. This could be due to fibrolytic digesters being less active and multiplying less vigorously while the competition between them and proliferating amylolytic bacteria is less severe. For forages with NDF content above 55-60% digestion of cell wall was slow disregarding energy supplementation. This could be because the structural components are well developed and the lignification becomes a significant factor while the fibrolytic micro-organisms are prevented from gaining access to the fermentable tissues of the plant material (Van Niekerk & Jacobs, 1985; Meissner *et al.,* 1991; Meissner *et al.,* 1995; Henning *et al.,* 1993). A low ruminal pH generally decreases the rate of fibre digestion, hence rumen digesta above pH 6 has been used as an index of ruminal fibre digestion (Owens & Goetsch, 1986; Meissner *et al.*, 1991). Reduced cellulose digestion may be due to the reduced prevalence or activity of cellulolytic species.

Livestock supplementation under range condition can be costly especially when supplements are used inefficiently. Generally RDP is considered to be the dietary component that is “the first limiting” for the utilization of low-quality forage deficient in N. Therefore, providing supplements with adequate amounts of RDP to ruminants fed low quality forage commonly promotes increased forage intake and flow of nutrients to the small intestine (Redman *et al.,* 1980; Hunter & Siebert, 1987; DelCurto *et al.,* 1990; Köster *et al.*, 1996; Hannah *et al.,* 1991; McCollum & Galyean, 1985; Nolte & Ferreira, 2005). Because protein supplementation can be costly, it is important to identify the amount of RDP required to maximize digestible organic matter intake (DOMI) and duodenal protein flow (Köster *et al.*, 1996).

Urea and biuret contain N concentration that is 5 to 7 fold that of commonly used plant protein such as soyabean meal and cottonseed meal (Köster, 1995). These NPN sources can be an inexpensive way to overcome N deficiency. Urea is very unpalatable and will have a lower stimulatory effect on voluntary intake as compared to true proteins but, in practice it is possible to disguise the unpalatability with other components of supplementary diet if the urea level is not too high (Nolte, 2000). NPN will have no benefit to ruminants unless it is converted in the rumen to ammonia and used for microbial synthesis thus, it is essential to be used only in supplements when the conditions are favourable for conversion (Köster, 1995). However the rapid release of N decreases the efficiency of N utilization by bacteria and could possibly lead to ammonia toxicity as compared to true protein sources which are degraded slower in the rumen extending N availability for longer periods (Petersen *et al.,* 1985; Firkins *et al.,* 1986; Basurto-Gutierrez *et al.*, 2003).

Köster (1995) did a study to determine the amount of RDP to maximize DOMI in beef cows consuming low quality forage. According to the results, the mature non-pregnant beef cows required 4g total RDP/kg $BW^{0.75}$ to maximise DOMI. This value is ±70% higher than the amount suggested as a minimum requirement by the farm feed act (Act 36/1947) in South Africa and 30-50% higher than that commonly recommended (Köster, 1997). Results from this study are currently used as guideline to formulate protein supplements more accurately for beef cattle consuming low quality roughage. Accordingly it was found by Köster (1995) that urea could provide up to 50-70% of the supplemental RDP intake, the rest should be provided as true protein that can be achieved with the use of an oilcake meal. In contrast Jacobs (2005) found with South

Africa's winter pasture hay that urea can be used as the only degradable protein source to supply 4g total RDP/kg $BW^{0.75}$. Factors like physiological stage of the animal and roughage type (physical size and chemical composition) could probably influence the results. It is therefore of utmost importance to know if the recommendations made by Köster (1995) which are based on low quality roughage produced in the USA are applicable to low quality roughages in sub-tropical regions. Therefore the RDP requirement of beef cattle at different physiological stages consuming different types of low quality roughage needs further investigations.

The purpose of this study was to determine the supplemental RDP requirement and optimum ratio of supplemented NPN RDP (urea) to natural RDP to maximise DOMI in pregnant beef cows consuming low quality roughage produced from the pure grassveld type in South Africa.

In Chapter 2, the supplemental RDP requirement of pregnant beef cows consuming winter grass hay (from pure grassveld type) was determined using natural protein supplement (calcium caseinate) at different inclusion levels. The optimal level of NPN inclusion in RDP supplements was investigated in Chapter 3 where different amounts of natural protein were replaced by NPN. In Chapter 4, substituting natural RDP from cotton seed oilcake with urea in RDP supplements on the performance of pregnant beef cows grazing natural winter grassveld was evaluated.

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CHAPTER 2

The rumen degradable protein requirements of beef cows consuming winter grassveld hay

2.1 Introduction

Tons of low quality forages from dry natural pastures, grain and crop by-products are available as feed source for grazing animals. The ruminant animal has a digestive system that enables it to utilise the relatively unavailable energy from cellulose, hemi-cellulose and pectin in the fibre component of these forages. Pregnant beef cows consuming low quality forages especially during winter in South Africa are subjected to nutrient deficiencies. One of the main factors limiting utilisation of low quality forage by ruminants is nitrogen (N) availability in the rumen. The N deficiency results in reduced feed intake by limiting the rate of microbial growth and digestion of organic matter (OM) in the rumen and hence slow clearance of digesta in the tract (Köster *et al.*, 1996; Basurto-Gutierrez *et al.*, 2003; Freeman *et al*., 1992; Lintzenich *et al.,* 1995; DelCurto *et al.,* 1990). Lack of N sources for microbes can be overcome with supplements of protein or non-protein nitrogen (NPN) compounds that are degradable in the rumen (Hunter & Siebert, 1987; Mawuenyegah *et al.*, 1997).

The growth rate of rumen micro-organisms is greatly affected by the availability of ammonia, peptides and amino acids. Starch and sugar degrading bacteria require peptides and amino acids for optimal growth while cellulolytic bacteria use ammonia as primary N source (McDonald *et al.*, 2002; Sniffen & Robinson, 1987). Ammonia is acknowledged to be the sole source of N for 80% of the rumen microbes but the amino acids and peptides play an important role in the N supply (Baldwin & Allison, 1983; Cotta & Russel, 1982).

Considering the high cost of protein supplementation, it is therefore essential to determine the amount of rumen degradable protein (RDP) required to increase the digestibility and intake of low quality roughages in order to optimise animal performance. According to Köster's (1995) findings the mature non-pregnant beef cow requires 4g total RDP/kg $BW^{0.75}$ to maximise digestible organic matter intake (DOMI) from low-quality tall-grass prairie forage. It is however important to verify whether the recommendations made by Köster are applicable in sub-tropical regions. Furthermore Köster (1995) used non-pregnant cows and it is important to determine the RDP requirements and energy intake of pregnant beef cows consuming low quality forage during winter in South Africa.

The purpose of this study was to determine the supplemental RDP (calcium caseinate) requirements to maximise DOMI and metabolisable energy intake (MEI) in pregnant beef cows consuming low quality hay from the Northern Variation of the *Cymbopogon-Themeda* pasture type (pure grassveld) in South Africa.

2.2 Materials and Methods

2.2.1 Animals

Thirty five pregnant Afrikaner x Simmentaler crossbred cows (average initial live weight of 517.08 kg; SD ± 53.06) were randomly allocated to five treatments. The cows were fasted (feed and water) overnight, before weighed at the beginning and the end of the trial. Animals were fed in individual pens and had free access to clean water. The trial period consisted of a 14-day adaptation, a 21-day intake study and a digestibility study which comprised of the last 7 days of the intake study (total 35 days).

2.2.2 Diet

The dormant winter pasture hay of the Northern Variation of the *Cymbopogon-Themeda* (no. 48b) pasture type (pure grassveld) was cut, baled and stored in a shed. According to Acocks (1988) the Northern Variation of Cymbopogon-Themeda grassland type merges easily into the western variation of the Bankenveld. According to Mucina & Rutherford (2006) the trial area falls in the Carlentonville Dolomite Grassland (Gh 15) in the (34) Rockey Highveld Grassland (Low & Rebello, 1996) which is characterised by summer rainfall between 650 to 750 mm per year, temperatures vary between -12°C and 39°C, with an average of 16°C and severe frequent frost occurring in winter. The dominating grass species are: *Eragrostis chloromelas, Heteropogon contortus, Setaria sphacelata, Themeda triandra, Cymbopogon pospischilii, Elionurus mutucus, Eragrostis curvula, Eustachys paspaloides, Panicum coloratum, Aristida congesta* and *Cynodon dactylon.*

The hay was offered at 130% of the previous five day average consumption per animal. Based on previous research (DelCurto *et al.*, 1990; Hannah *et al.,* 1991; Köster, 1995), one of the major responses to protein supplementation is increased forage intake thus it was decided to allow cows *ad lib* consumption of forage and to measure responses to supplementation rather than to restrict forage intake to the level of unsupplemented cows. The lick and hay were fed twice daily at 08:00 and 14:00. Experimental treatments provided the following supplemental RDP levels/cow/day from casein: (1) control, 0g (2) 180g (3) 360g (4) 540g and (5) 720g. The RDP supplementation in the form of calcium caseinate (90% CP on dry matter basis and 100% rumen degradable - Köster, 1995) was thoroughly mixed with a 500g molasses based concentrate (Table 1), divided into two equal portions and offered first before the hay. The mineral premix comprised of the following macro- and micro minerals (1.50% Ca, 1.0% P, 1.95% Na, 2.39% K, 4.41% Cl, 0.54% S, 0.39% Mg, 3.43 ppm Co, 205.99 ppm Cu, 657.91 ppm Mn, 2.0 ppm Se, 619.12 ppm Zn, 1109.78 ppm Fe, 10.0 ppm I) to prevent mineral deficiencies.

Table 1: Physical composition of molasses based concentrate

Raw material	**%**
Mineral premix	0.5
Monocalcium phosphate	4.18
Salt	5.0
Begasse	21.0
Molasses	69.32

2.2.3 Sampling

Representative feed samples were collected daily at both feeding times. During the intake study orts were collected each morning, weighed and a sample was taken per cow for each feeding time. During the digestibility study, faecal samples were collected each morning and a representative sample of 10% was taken per cow. The faecals were dried at 50°C for 96 hours, weighed and pooled for each cow to have a representative sample. The composite feed, orts and faecal samples were weighed and milled through a 1mm sieve. The representative sample obtained by the quartering method was stored for later analysis.

The day after the end of digestibility study (day 36), approximately 35ml of rumen fluid was obtained from each animal three hours after the initiation of morning feeding. A vacuum pump and plastic rumen tube were used to extract the samples. The samples were strained through four layers of cheese cloth and pH was immediately determined using a portable meter.

2.2.4 Laboratory analysis

The chemical composition of feed, orts and faeces was determined according to the methods prescribed by the AOAC (1995).

Samples were dried at 100°C in a convection oven to a constant mass in order to determine dry matter (DM) content. The OM content was determined by incinerating samples in a muffle furnace at 500°C for 8 hours. Kjeldahl N and neutral detergent fibre (NDF) were determined according to the Van Soest *et al.*, (1991) methods. Gross energy (GE) was determined by means of an adiabatic bomb calorimeter.

2.2.5 Statistical analyses

The SAS (1994) program was used to analyse the data using PROC ANOVA. A complete randomized design was used. Treatment means that were found to be significantly different ($P < 0.05$) were further subjected to multiple comparison test using Tukey's test.

Furthermore treatment sum of squares were partitioned into linear, quadratic and cubic effects of RDP level with orthogonal polynomials and R^2 were calculated. In addition RDP intake (RDPI) required for maximum DOMI was determined using a single slope, broken-line (Robbins, 1986) with the NLIN procedure of SAS (1994). The same procedures were followed to determine the RDPI required for maximum MEI.

2.3 Results and Discussion

2.3.1 Chemical composition

The quality of the natural winter pasture hay used in this study was characterised by a low crude protein (CP) of 2.26% and a high NDF content of 73.94% (Table 2). The degradability of protein in the *Cymbopogon-Themeda* grass hay as determined by Jacobs (2005) was 67.5%. Research has indicated that microbial growth in the rumen of animals consuming a low protein diet may be restricted by the inadequate supply of ammonia, peptides and amino acids resulting in reduced rate of cellulose digestion (Redman *et al.*, 1980; McDonald *et al.,* 2002; Köster *et al.,* 1996). The protein supplement used during the study was casein and it is believed to increase the available pool of amino acids and peptides in the rumen (Redman *et al.*, 1980). Miner *et al.* (1990) noted that microbial yield may be enhanced by the availability of growth limiting organic acids supplied by an RDP source that degrades slowly.

Table 2: Chemical composition (on dry matter basis) of grass and supplements

Item	Grass	Molasses based concentrate + RDP (casein)				
		Supplemental rumen degradable protein				
		0 g	180 g	360 g	540 g	720 g
Dry matter %	92.84	91.48	84.46	85.60	87.05	87.76
Organic matter %	90.82	87.82	89.90	90.70	92.27	92.62
Crude Protein %	2.26	4.04	31.97	37.58	53.25	59.71
Neutral Detergent Fibre %	73.94	23.22	16.22	13.62	10.45	3.79

Generally CP less than 6-8% in the basal forage is considered to be the threshold value as far as digestion is concerned, since protein supplementation seems to have little benefit on digestion of medium to high quality roughages (Clanton & Zimmerman, 1970). Supplementary protein has shown to improve utilisation of low quality roughages in many studies (Ammerman *et al.,* 1972; Stokes *et al.,* 1988, Guthrie & Wagner, 1988; McCollum & Galyean, 1985; Heldt *et al.,* 1999). Meissner *et al.* (1991) stated that digestion of cell wall constituents is slow or depressed when NDF content was above 55-60%, as was the case in the present study. The rate of digestion and retention time in the rumen are the major determinants of voluntary intake of poor quality roughages.

2.3.2 Digestibility study

2.3.2.1 Intake

The effect of increasing the level of supplemental RDP on the digestibility of the diet by pregnant beef cows is illustrated in Table 3. Protein supplementation during the study resulted in a statistical significant increase (P < 0.01) in grass dry matter intake (DMI), total DMI and total organic matter intake (OMI). This increased intake with higher levels of RDP supplementation could reduce the digestibility of the diet. McDonald *et al.* (2002) stated that an increase in food intake causes a faster rate of passage of digesta. Accordingly the digesta is then exposed to the action of digestive enzyme for a shorter period and there may be a reduction in its digestibility especially for the slowly digestible cell wall constituents. In the present study the NDF content of the grass hay which represents cell wall components was as high 74%. Therefore a higher intake level could have a detrimental influence on the digestibility of low quality grass hay

2.3.2.2 Digestibility coefficients

No statistical significant (P > 0.05) influence of RDP level on the apparent digestibility of DM, OM, NDF and GE could be detected. This is unexpected as RDP supplementation is related to microbial growth and a subsequent increase in digestion of low quality forage. As already discussed, an increase in voluntary intake of low quality roughage as a result of CP supplementation enhances the faster rate of passage of digesta which in turn reduces the time available for microbial digestion, especially for the slowly digestible cell wall constituents (Scolljegerdes *et al*., 2004; Hannah *et al.,* 1991; McDonald *et al*., 2002; Koster *et al*., 1996). Hence there is a counteracting force of passage rate and digestion (Badyk *et al*., 2001). The significant (P < 0.0001) higher total DMI with an increasing RDP level could contribute to these findings. These results are consistent with the findings of Nolte (2000) who found that OM digestibility was not affected by increasing levels of supplemental RDP.

In contrast with DM, OM, NDF and GE the apparent digestibility of CP increased in a linear and quadratic manner (P = 0.0001) with a higher RDP level in the diet. This could be attributed to the corresponding increasing digestible CP content of the experimental diets with higher RDP inclusion. A negative protein digestibility (-5.8%) was recorded when no RDP was supplemented and there was a significant increase (46.9%) at 180g RDP/d. These results were similar to the findings of Church & Santos (1981) and Köster *et al.*, (1996) who observed negative N digestibility when wheat straw and low quality tall-grass prairie forage was fed to cattle respectively without protein supplementation. McDonald *et al.*, (1981) mentioned that the apparent digestibility of CP is particularly dependant upon the proportion of protein in the feed. The reason for this is that the metabolic faecal N represents a constant tax upon dietary N. If a diet contains a low CP level, as was the case for the 0% supplemental RDP a negative digestibility coefficient could occur (McDonald *et al.*, 1981).

2.3.2.3 Digestible nutrients

From Table 3 it is evident that the digestible nutrient content of various diets were related to the apparent digestibility results. Accordingly a statistical non-significant ($P > 0.05$) increase in metabolisable energy (ME) content occurred with an increased RDP intake (RDPI) level. The energy intake would be the most important criteria of the effect of different RDPI levels on the utilisation of the low quality roughage fed in this study.

Table 3: Effect of increasing level of supplemental rumen degradable protein on digestibility of low quality grass hay

Item	Supplemental rumen degradable protein					Significance	Significance of contrasts[1]			
	0g	180g	360g	540g	720g	P	L	Q	C	CV[2]
Grass DMI (kg/cow/day)	4.57[a]	6.61[b]	7.67[b]	7.14[b]	7.31[b]	0.0002	0.0001	0.0027	0.2280	17.321
							0.3075*	0.1724*	0.0245*	
Supplemental DMI (kg/cow/day)	0.46[a]	0.59[b]	0.77[c]	0.96[d]	1.14[e]	0.0001	0.0001	0.0001	0.0001	0.000
							0.9964*	0.0027*	0.0008*	
Total DMI (kg/cow/day)	5.03[a]	7.21[b]	8.44[b]	8.10[b]	8.74[b]	0.0001	0.0001	0.0051	0.1469	14.484
							0.4927*	0.1099*	0.0266*	
Total OMI (kg/cow/day)	4.54[a]	6.51[b]	7.65[b]	7.35[b]	7.69[b]	0.0001	0.0001	0.0034	0.2501	15.496
							0.4358*	0.1356*	0.0184*	
			Apparent digestibility coefficients (%)							
Dry matter	57.60	61.23	62.15	61.28	61.54	0.8026	0.3821	0.4272	0.6717	12.320
							0.0249*	0.0205*	0.0058*	
Organic matter	62.07	65.65	66.08	65.50	65.64	0.8028	0.3987	0.4210	0.6387	10.510
							0.0232*	0.0210*	0.0071*	
Crude protein	-5.83[a]	46.88[b]	52.16[bc]	67.19[bc]	72.51[c]	0.0001	0.0001	0.0001	0.0275	29.253
							0.6707*	0.1106*	0.0305*	
Neutral detergent fibre	61.56	66.26	65.32	64.19	63.17	0.8002	0.9006	0.2868	0.5311	11.837
							0.0005*	0.0372*	0.0127*	

Item	Supplemental rumen degradable protein					Significance	Significance of contrasts[1]			
	0g	180g	360g	540g	720g	P	L	Q	C	CV[2]
Gross energy	58.99	62.95	63.48	65.24	63.69	0.5144	0.1577	0.3141	0.9884	10.743
							0.0630*	0.0314*	0.0000*	
Apparent digestible nutrients (%)										
Digestible OM	56.02	59.34	59.89	59.47	59.68	0.7492	0.3182	0.4155	0.6477	10.455
							0.0323*	0.0214*	0.0067*	
Digestible protein	-0.14[a]	2.27[b]	2.93[b]	5.68[c]	7.41[d]	0.0001	0.0001	0.4732	0.3904	19.230
							0.9227*	0.0010*	0.0014*	
Digestible NDF	42.04	45.07	44.31	42.43	40.38	0.4940	0.3551	0.1436	0.5720	12.396
							0.0264*	0.0674*	0.0098*	
Metabolizable energy (MJ/kg)[3]	8.21	8.87	8.98	9.05	9.14	0.4480	0.1003	0.4163	0.6335	11.348
							0.0850*	0.0201*	0.0069*	

Row means with different superscripts differ significantly

[1]L = linear, Q = quadratic, C = cubic
[2]CV = Coefficient of variance
[3]Metabolisable energy = Digestible energy X 0.8 (McDonald *et al.*, 2002)
* = R^2

DMI = Dry matter intake

OMI = Organic matter intake

CPI = Crude protein intake

2.3.3 Intake study

The effect of increasing RDP levels on forage intake by beef cows is presented in Table 4. Although supplemental RDPI levels were predetermined, the actual intakes were not attained precisely due to the laboratory analysis results of both lick DM and lick CP content (Table 2). The grass DMI, total DMI, total OMI, DOMI and ME intake during the current study increased in a linear and quadratic manner ($P \leq 0.05$) with increasing proportions of supplemental RDP. The linear regression displayed a moderate prediction of DOMI/kg $BW^{0.75}$ (R^2 =0.45) and ME/kg $BW^{0.75}$ (R^2 =0.50) from RDP intake. However according to the multiple comparison test the largest increase ($P = 0.0001$) in the daily energy intake of beef cows occurred with a 2.80g RDPI/kg $BW^{0.75}$ (189g supplemental RDP/cow/day). This confirms that N is a limiting nutrient in the utilisation of low quality roughages. Köster *et al.,* (1996) and Nolte (2000) observed similar intake responses in intake parameters with increasing proportions of supplemental RDP (casein) provided to beef cows consuming low quality prairie hay (1.9% CP) and wheat straw (3.2% CP) fed to sheep, respectively. Köster *et al.* (1996) and Scott & Hibberd (1990) noted that the diminishing responses highlights that the potential to stimulate intake via digestible intake protein (DIP) is limited. In the present study a diminishing and statistically non-significant ($P > 0.05$) response in daily energy intake was observed with more than 2.80g RDPI/kg $BW^{0.75}$. The limits are probably set largely by characteristics of the forage being consumed (inherent fermentability and protein availability) and the animal's nutrient requirement (Mathis *et al.,* 2000).

A significant linear increase ($P \leq 0.0006$) in the grass DMI as percentage of body mass and total DMI (grass + lick) as a percentage of body mass was observed. The grass DMI as percentage of body mass increased significantly with 3.77g daily RDP/kg $BW^{0.75}$ (406g total RDPI/cow/day), and was however less than the 1.7% and ± 2.4% recorded by Köster (1995) and Jacobs (2005) respectively. Although not statistically different from 1.3%, the highest figure recorded in the present study was a grass DMI of 1.5% of body mass. The experimental cows used in the present study were in late gestation and this could probably explain the lower grass DMI as percentage of body mass. According to Forbes (1986), a decrease in food intake which is often seen at oestrus and during late pregnancy is probably due to the high levels of circulating oestrogen, although progesterone acts to protect the animal against this for most of the pregnancy period. Furthermore there might also be effects of competition for abdominal space which could affect intake during late pregnancy.

Table 4: Effect of increasing level of supplemental rumen degradable protein on intake in beef cows consuming low quality grass hay

Item	Supplemental rumen degradable protein					Significance	Significance of contrasts[1]			
	0g	180g	360g	540g	720g	P	L	Q	C	CV[2]
Grass DMI (kg/cow/day)	4.98[a]	6.63[b]	7.41[b]	6.97[b]	7.15[b]	0.0004	0.0003	0.0048	0.2108	14.598
							0.2836*	0.1601*	0.0283*	
Grass DMI as % of body mass	0.96[a]	1.33[ab]	1.45[b]	1.41[b]	1.54[b]	0.0043	0.0006	0.1082	0.2270	20.224
							0.3000*	0.0559*	0.0310*	
Supplemental DMI (kg/cow/day)	0.46[a]	0.59[b]	0.77[c]	0.96[d]	1.14[e]	0.0001	0.0001	0.0001	0.0001	0.000
							0.9964*	0.0027*	0.0008*	
Total DMI (kg/cow/day)	5.44[a]	7.22[b]	8.19[b]	7.93[b]	8.29[b]	0.0001	0.0001	0.0059	0.2260	13.055
							0.4297*	0.1226*	0.0213*	
Total DMI as % of body mass	1.05[a]	1.44[ab]	1.60[b]	1.61[b]	1.78[b]	0.0006	0.0001	0.1557	0.2462	18..950
							0.4076*	0.0374*	0.0247*	
Total OMI (kg/cow/day)	4.54[a]	6.51[b]	7.65[b]	7.35[b]	7.69[b]	0.0001	0.0001	0.0034	0.2501	15.496
							0.4358*	0.1356*	0.0184*	
Digestible OMI (kg/cow/day)	3.05[a]	4.28[b]	4.90[b]	4.72[b]	4.95[b]	0.0001	0.0001	0.0015	0.1374	12.974
							0.4627*	0.1455*	0.0277*	
Digestible OMI (g/kg $BW^{0.75}$)	28.03[a]	40.44[b]	45.45[b]	45.07[b]	49.30[b]	0.0001	0.0001	0.0411	0.1724	17.024
							0.4515*	0.0683*	0.0293*	
CPI (g/cow/day)	128.64[a]	352.42[b]	470.80[c]	673.09[d]	850.59[e]	0.0001	0.0001	0.8503	0.0447	6.501
							0.9800*	0.0000*	0.0020*	

Item	Supplemental rumen degradable protein					Significance	Significance of contrasts[1]			
	0g	180g	360g	540g	720g	P	L	Q	C	CV[2]
DCPI (g/cow/day)	-5.78[a]	158.93[b]	244.55[c]	457.77[d]	617.66[e]	0.0001	0.0001	0.0896	0.6536	16.135
							0.9488*	0.0039*	0.0003*	
RDPI grass (g/day)	80.99[a]	107.84[b]	116.73[b]	107.10[ab]	110.55[a]	0.0048	0.0070	0.0103	0.1342	16.127
							0.1724*	0.1542*	0.0487*	
RDPI lick (g/day)	18.53[a]	189.01[b]	289.52[c]	509.90[d]	681.22[e]	0.0001	0.0001	0.0001	0.0001	1.650
							0.9893*	0.0039*	0.0002*	
Total RDPI (g/day)	99.51[a]	296.86[b]	406.25[c]	618.14[d]	791.77[e]	0.0001	0.0001	0.0265	0.0185	3.771
							0.9890*	0.0007*	0.0008*	
RDPI (g/kg $BW^{0.75}$)	0.91[a]	2.80[b]	3.77[c]	5.90[d]	7.85[e]	0.0001	0.0001	0.0568	0.1948	10.989
							0.9577*	0.0041*	0.0018*	
ME intake (MJ/cow/day)	44.66[a]	64.04[b]	73.47[b]	71.74[b]	75.79[b]	0.0001	0.0001	0.0016	0.1338	12.951
							0.5117*	0.1307*	0.0259*	
ME intake (MJ/kg $BW^{0.75}$)	0.41[a]	0.60[b]	0.68[b]	0.69[b]	0.75[b]	0.0001	0.0001	0.0417	0.1635	17.019
							0.4981*	0.0620*	0.0279*	

Row means with different superscripts differ significantly

[1]L = linear, Q = quadratic, C = cubic
[2]CV = Coefficient of variance
* = R^2
DMI = Dry matter intake, OMI = Organic matter intake, CPI = Crude protein intake, DCPI = Digestible crude protein intake, RDPI = Rumen degradable protein intake, ME - Metabolizable energy = Digestible energy X 0.8 (McDonald *et al.*, 2002)

The single slope, broken-line model suggested by Köster *et al.* (1996) was also used in the current study to estimate the RDP requirement since it yields lower estimates than the polynomial regression procedure (Baker, 1986). Generally the quadratic regression procedure yields larger values because it predicts requirements where maximum response is obtained. Considering the high cost of protein supplementation and the reduced magnitude of incremental improvements in DOMI as maximum response is approached, the single slope broken-line model seems to be the more cost effective approach (Baker, 1986; Robbins, 1986). Furthermore the single slope broken-line model (Figure 1) predicted DOMI/kg $BW^{0.75}$ from RDPI/kg $BW^{0.75}$ with a higher accuracy (R^2 =0.45) than the quadratic regression procedure (R^2 = 0.07). According to this model 4.03g daily RDPI/kg $BW^{0.75}$ was required to maximise DOMI of pregnant beef cows consuming winter grass hay from the pure grassveld type.

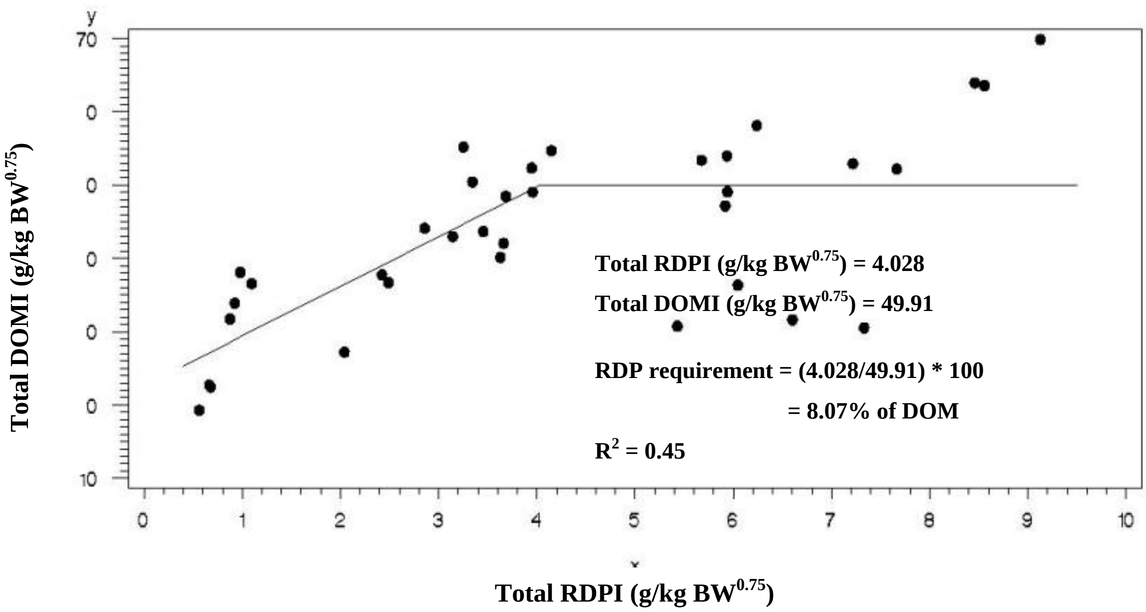

Figure 1: **Daily rumen degradable protein intake (RDPI) required to maximise digestible organic matter intake (DOMI) of pregnant beef cows using a single slope broken-line model**

Expression of the required RDP as a percentage of DOM is essential as the RDP required amount to maximize DOMI will vary with the inherent digestibility of the forage (Köster, 1995; Mathis *et al.*, 2000). According to the single slope broken-line model (Figure 1), it was estimated that the

8.07% RDP of DOM would be required to maximise total DOMI (49.91g DOMI/kg $BW^{0.75}$) of pregnant beef cows consuming the low quality hay fed in the present study (4.03g total RDPI/kg $BW^{0.75}$). This corresponds to some extent with the predictions of Köster *et al.* (1996) who found that 11.1% of DOM (4.01g total RDPI/kg $BW^{0.75}$) would be required to maximise total DOMI (36.15g/kg $BW^{0.75}$) of low quality prairie hay (1.9% CP) fed to non pregnant beef cows. In contrast Van der Merwe (2010 – unpublished data) found that pregnant beef cows consuming winter grass hay from the False grassveld type (sour veld in the eastern parts of South Africa – 4.91% CP) required 9.36% RDP of DOM (3.63g total RDP/kg $BW^{0.75}$) to maximise total DOMI (38.66 g/kg $BW^{0.75}$). The 8.07% RDP of DOM in the present trial is almost equal to the value of 9.36% found by Van der Merwe (2010 – unpublished data). In a study by Nolte *et al.* (2003) the single slope broken-line model predicted the total daily DOMI for sheep fed wheat straw (3.2% CP) as 27.01g/kg $BW^{0.75}$ with an associated total RDP requirement of 11.6% of DOM (3.30g/kg $BW^{0.75}$). The differences in the results could be attributed to differences in forage quality, the type and physiological status of the animal. Wilson & Kennedy (1996) mentioned that physical characteristics of the fibre particles such as tissue origin, shape, buoyancy and specific gravity could play a role by affecting comminution, digesta load, digestive weakening and ease of passage.

The digestibility of forage influences the availability of CP to the microbial population and host. Forage digestibility as well as CP content must be considered when predicting intake responses to supplemental protein (DelCurto *et al*., 1990). Köster (1995) stated that once DIP requirements are met, any additional DIP would result in wastage of N which would narrow the cost:benefit ratio. Excess DIP can result in excessive ruminal ammonia concentration that will be absorbed through the rumen wall, converted to urea in the liver and excreted in the urine (McDonald *et al.,* 2002). Besides wasting expensive N, the additional ammonia load may also increase the energetic cost associated with ammonia detoxification in the liver (Köster *et al.,* 1996).

The RDP requirements to maximise MEI of pregnant beef cows according to the single slope broken-line model (R^2 =0.50) is shown in Figure 2. This model revealed that 3.94g total daily RDPI/kg $BW^{0.75}$ (297g supplemental RDP/500kg pregnant cow) was required to maximise the MEI from winter grassveld hay to 0.72 MJ/kg $BW^{0.75}$. The latter is equivalent to 76.13 MJ/cow/day and this corresponds to the NRC (1984) requirements of 76.55 MJ/cow/day for mature (500 kg) pregnant (in the last trimester) beef cows. According to the single slope broken-line model a moderate relationship (R^2 =50) existed between RDPI and MEI. This means that more animals should probably be used to improve the accuracy of prediction. The inclusion of more animals in experimental trials is however often a problem to accommodate.

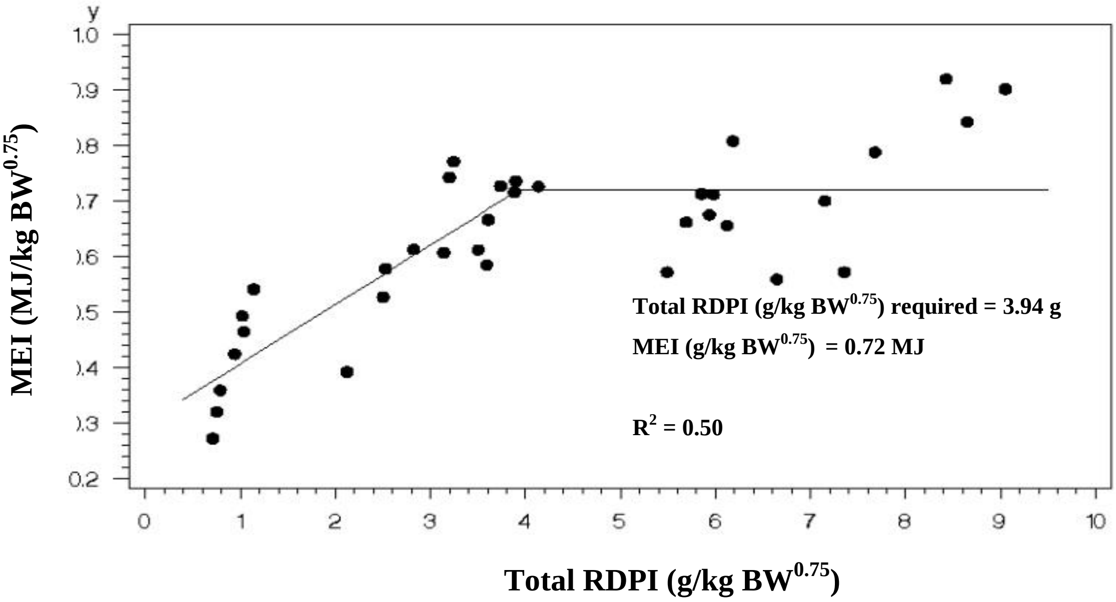

Figure 2: Daily rumen degradable protein intake (RDPI) required to maximise metabolisable energy intake (MEI) of pregnant beef cows using a single slope broken-line model

2.3.4 Body mass changes

The body mass changes of the cows during the experimental period are illustrated in Table 5. The pregnant cows (last trimester of gestation) in all the treatments experienced a loss of body mass. Adaptation to the pens and individual feeding could have also contributed to the loss in body mass. The body mass loss decreased linearly and quadratically with rising amounts of RDP and seems to be minimised at 3.77g daily RDPI /kg $BW^{0.75}$ and was significantly (P = 0.0021) less than the control group. DelCurto *et al.* (1990) stated that protein supplementation during gestation minimised body mass loss in mature beef cows grazing tall-grass prairie. The response to supplemental protein appeared to be dependant on the cow's physiological status. Hollingsworth-Jenkins *et al.*, (1996) established from their study that the protein requirements of gestating beef cows grazing native range can be met with a highly rumen degradable protein source without inclusion of bypass protein and/or energy sources. The trial period in the present study was relatively short to make reliable observations on body mass changes.

Table 5: Effect of increasing levels of supplemental rumen degradable protein on the body mass changes of beef cows consuming low quality grass hay

	Supplemental rumen degradable protein					Significance	Significance of contrasts[1]			
Item	**0g**	**180g**	**360g**	**540g**	**720g**	**P**	**L**	**Q**	**C**	**CV**
Initial mass (kg)	550.57	526.00	516.00	507.14	485.71	0.2258	0.0222	0.9195	0.6627	9.969
							0.1614*	0.0003*	0.0054*	
Final mass (kg)	500.57	499.43	500.29	480.86	466.14	0.6482	0.1680	0.5221	0.9653	10.579
							0.0614*	0.0129*	0.0001*	
Mass change (kg)	-50.00[a]	-26.57[ab]	-15.71[b]	-26.29[b]	-19.57[b]	0.0021	0.0021	0.0158	0.1100	-54.893
							0.2204*	0.1267*	0.0526*	
Final mass as % of initial mass	90.93[a]	94.89[ab]	96.90[b]	94.90[ab]	95.91[b]	0.0141	0.0117	0.0319	0.1901	3.285
							0.1603*	0.1128*	0.0400*	

Row means with different superscripts differ significantly

[1]L = linear, Q = quadratic, C = cubic
[2]CV = Coefficient of variance
* = R^2

2.3.5 Rumen fluid pH

Erfle *et al.* (1982) observed that rumen pH affects microbial growth rate and microbial protein efficiency. The pH of ruminants consuming predominantly forage diet is near neutrality (± 7) and this is confirmed by the results in Table 6. It is also clear that supplemental RDP did not influence the rumen pH significantly. This suggests that pH did not limit the activity of cellulolytic bacteria in the rumen.

Table 6: Effect of increasing level of supplemental rumen degradable protein on rumen fluid pH

	Supplemental rumen degradable protein					Significance	Significance of contrasts[1]			
Item	0g	180g	360g	540g	720g	P	L	Q	C	CV
pH	7.00	7.05	7.04	7.07	6.93	0.7842	0.6562	0.2870	0.7263	2.859
							0.0076*	0.0443*	0.0047*	

[1]L = linear, Q = quadratic, C = cubic
[2]CV = Coefficient of variance
* = R^2

Conclusions

According to the multiple comparison test a statistical significant increase in DOMI and MEI from the low quality hay occurred when the daily RDPI of pregnant cows was increased up to 2.80g/kg $BW^{0.75}$ (2.97g total RDPI/500kg cow/day). Thereafter a non-significant and diminishing increase in energy intake occurred. However, a significant increase in grass DMI and decrease in body mass loss were observed with a 3.77g daily RDPI/kg $BW^{0.75}$ (406g total RDPI/500kg cow/day). These findings were supported by predictions with the broken-line model that 3.94g total daily RDPI/kg $BW^{0.75}$ (417g total RDPI/500kg pregnant cow/day) is needed to maximise MEI (76MJ ME/500kg cow/day) from winter forage hay of the Northern variation of the *Cymbopogon-Themeda* pasture type (pure grassveld). This means that 8% RDP of DOM is needed to maximise energy intake and supply in the requirements of beef cows during the last trimester of gestation.

Casein as a RDP source was used in the present study. Therefore, in an effort to reduce supplementary cost, it is however important to investigate the potential to substitute amino acid N with non protein N (urea) in RDP supplements for pregnant beef cows consuming the low quality forage used in the present study.

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CHAPTER 3

Effect of substituting casein with urea in rumen degradable protein supplements for beef cattle consuming winter grassveld hay

3.1 Introduction

Rumen microbes are determinant of rate of carbohydrate digestion and protein reaching the lower digestive tract thus their growth rates and yields are important for both energy utilisation and protein production (Maeng & Baldwin, 1976). Ruminal micro-organisms derive most of their energy from the fermentation of carbohydrates and they are categorised according to the type of carbohydrate they ferment (Russell *et al.,* 1992).

The bacteria degrading non-structural carbohydrate (NSC) i.e. starch pectin, sugars, etc. are able to utilise ammonia (NH_3), peptides or amino acids as a nitrogen (N) source and can produce NH_3 while those fermenting structural carbohydrate (SC) i.e. cellulose and hemi-cellulose can only utilise NH_3 as a N source (Russel *et al.*, 1992; McDonald *et al.,* 2002, Nolte & Ferreira, 2005, Maeng & Baldwin, 1976). Since NH_3 is acknowledged as the primary N source of cellulolytic bacteria (Cotta & Russell, 1982; McDonald *et al.,* 2002; Russel *et al.*, 1992), it can be supplied by relatively inexpensive sources of non-protein nitrogen (NPN) such as urea because protein is the most expensive ingredient in ruminant supplements.

Ammonia is acknowledged to be the sole source of N for 80% of the rumen microbes but the amino acids and peptides play an important role in the N supply (Baldwin & Allison, 1983, Cotta & Russel, 1982). In this regard, stimulating rumen microbial growth by urea supplementation has financial benefits in terms of cost of protein but may be inferior to natural protein in terms of animal performance especially used as the sole source of dietary N (Forero *et al.*, 1980; Rush *et al.,* 1976; Clanton, 1978, Williams *et al,* 1963; Maeng & Baldwin, 1976). The latter is because urea is rapidly hydrolysed and much of the NH_3 produced in excess of available energy supplied by especially low quality forage, is lost and animal performance is often lower than desired (Rush *et al.,* 1976; Williams *et al,* 1963; Basurto-Gutierrez *et al*, 2003). Research results of Forero *et al.* (1980) indicated that factors other than a continuous supply of NH_3 might be limiting to the use of urea with low quality forage. Certain nutritional factors such as preformed amino acids, branched chain volatile fatty acids (BCVFA), minerals or vitamins which are absent in urea may be necessary for efficient bacterial utilisation of urea (McDonald *et al.,* 1988; Forero *et al.*, 1980). The results of Köster (1995) suggested that between 50% and 75% of supplemental rumen degradable protein (RDP) could be provided from urea, without compromising forage intake and digestion. However, Jacobs (2005) found that the replacement of a natural supplement RDP source such as cotton seed oilcake with urea did not influence the digestibility and energy intake of steers on low quality roughage. Factors like forage type, RDP source and physiological stage of cattle could influence the results.

In Chapter 2 the optimal levels of RDP intake was identified to maximise the energy intake of beef cattle consuming low quality winter grass hay. A true 100% RDP source namely calcium caseinate was used. NPN sources like urea is however less expensive. Therefore it is important to determine the optimal level of NPN inclusion in RDP supplements to support ruminal fermentation and promote microbial synthesis that will ensure an optimal energy intake of beef cattle on low quality forages. The objective of the current trial was to determine the effect of increasing proportions of supplemental N from urea, in degradable protein supplements containing true protein on digestion and energy intake of pregnant beef cows consuming low quality winter grass hay from the Northern Variation of the *Cymbopogon-Themeda* pasture type (pure grassveld) in South Africa.

3.2 Materials and Methods

3.2.1 Animals

Thirty five pregnant Afrikaner x Simmentaler crossbred cows (average initial live weight of 466.17kg, SD± 36.79) were randomly allocated to five treatments. Animals were housed in individual pens. The trial period consisted of a 14-day adaptation period, a 21-day intake study and a digestibility study during the last 7 days of the intake study (total 35 days). The cows were fasted (feed and water) overnight, and weighed at the beginning and end of each trial.

3.2.2 Diet

The dormant winter pasture hay of the Northern Variation of the *Cymbopogon-Themeda* (no. 48b) pasture type (pure grassveld) as described in Chapter 2 was cut, baled and stored in a shed. Cows had *ad libitum* access to this low quality hay and clean water. The hay was offered at 130% of the previous five day average consumption per animal (DelCurto *et al*., 1990; Hannah *et al.,* 1991; Köster, 1995).

The hay was fed twice daily at 08:00 and 14:00 while the RDP supplement was fed in the morning before the hay. The supplemental RDP was from urea and calcium caseinate (100% degradable). Supplements were formulated to supply the 4.028g daily RDP intake/kg $BW^{0.75}$ as determined from the previous trial (Chapter 2) to maximize digestible organic matter intake (DOMI) of pregnant beef cows. The RDP in the form of calcium caseinate and/or urea was thoroughly mixed with a 300g molasses based concentrate to ensure the desirable RDP intake. The physical composition of the supplements is outlined in Table 1. The composition of the molasses based concentrate was 0.5% mineral premix, 4.18% monocalcium phosphate, 5.0% salt, 21.0% begasse and 69.32% molasses. The mineral premix comprised of the following macro- and micro minerals namely 1.50% Ca, 1.0% P, 1.95% Na, 2.39% K, 4.41 Cl, 0.54% S, 0.39 Mg, 3.43 Co, 205.99 ppm Cu, 657.91 ppm Mn, 2.0 ppm Se, 619.12 ppm Zn, 1109.78 ppm Fe, 10.0 ppm I. Feed grade sulphur was added in the premix to maintain a ratio of 10 N to 1 S.

Table 1: Physical composition of the rumen degradable protein supplements

Item	**Supplemental rumen degradable protein from urea**				
	0%	**25%**	**50%**	**75%**	**100%**
Ingredients (As is g/day)					
Molasses basis	300	300	300	300	300
Casein	214	162	106	53	0
Urea	0	17	33	50	73

3.2.3 Sampling

During the intake and digestibility study representative feed samples were collected daily at both feeding times. Orts were weighed each morning and a representative sample was taken for each cow. During the digestibility study faecals were collected each morning, weighed and a representative sample of 10% was taken per cow. The faecal samples were dried at 50°C for 96 hours, weighed and pooled for each cow to obtain a representative sample. The composite feed, faecal and ort samples were weighed and milled through a 1mm sieve. A representative sample was obtained by means of the quartering method and stored for later analysis.

At the end of the digestibility study (i.e. day 36), approximately 35ml of rumen fluid was obtained from each animal three hours after the commencement of the morning feeding. A vacuum pump and plastic rumen tube were used to extract the samples. The samples were strained through four layers of cheese cloth and pH was immediately determined using a portable meter. For determination of the concentration and molar proportions of individual volatile fatty acids (VFA), 10ml of each sample was frozen and stored. The remaining 25ml was acidified with a few drops of concentrated sulphuric acid to achieve a pH of approximately 2. Samples were then frozen and stored for later ammonium-nitrogen (AN) analysis.

3.2.4 Laboratory analysis

The chemical composition of feed, orts and faeces was determined according to the methods prescribed by the AOAC (1995).

Samples were dried at 100°C in a convection oven to a constant mass in order to determine dry matter (DM) content. The organic matter (OM) content was determined by incinerating samples in a muffle furnace at 500°C for 8 hours. Kjeldahl N, acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined according to the Van Soest *et al.*, (1991) methods. Gross energy was determined by means of an adiabatic bomb calorimeter. Rumen fluid volatile fatty acid (VFA) contents were determined by gas chromatography. Ammonia N (NH_3N) concentration was determined by a spectrophotometer.

3.2.5 Statistical analyses

The SAS (1994) program was used to analyse the data using PROC ANOVA. A complete randomized design was used. Treatment means that were found to be significantly different ($P < 0.05$) were further subjected to multiple comparison test using Tukey's test.

Furthermore treatment sum of squares were partitioned into linear, quadratic and cubic effects of urea levels with orthogonal polynomial and R^2 were calculated.

3.3 Results and Discussions

3.3.1 Chemical composition

Due to annual climatic variations and year to year variations of forage quality, the grass hay was analysed annually to confirm its quality. The grass hay used during this study in 2006 (Table 2) was characterised by a higher crude protein (CP) of 5.22% and almost similar NDF of 71.76% as compared to the 2.26% CP and 73.94% NDF of the 2004 hay (Chapter 2).

Table 2 Chemical composition of the grass hay and supplements (on dry matter basis)

Item	Grass	Supplemental rumen degradable protein from urea				
		0%	25%	50%	75%	100%
Dry matter %	92.30	85.72	84.27	82.78	81.15	81.27
Organic matter %	94.24	87.54	87.60	86.79	87.31	85.94
Crude Protein %	5.22	40.46	39.02	42.67	41.90	41.12
Neutral Detergent Fibre %	71.76	10.91	11.85	12.69	14.22	15.21
Acid detergent fibre %	45.18	7.06	8.10	8.61	9.58	11.79

The supplements were formulated to be equivalent in chemical composition. However, according to Table 2 the fibre content increased as casein was replaced by urea in the supplement. This was probably due to an increased percentage molasses in the supplements with increasing urea levels (Table 1). These higher fibre levels could influence the digestibility of the supplements detrimentally.

3.3.2 Digestibility study

The effect of substituting casein with urea in RDP supplements on the digestibility of low quality roughage by pregnant beef cows is outlined in Table 3.

3.3.2.1 Intake

No statistical difference ($P > 0.05$) in grass and total DM intake (DMI) of cows in the various treatments occurred during the digestibility study. Therefore differences in intake could not be a factor influencing digestibility coefficients.

3.3.2.2 Digestibility coefficients

No statistical significant ($P > 0.05$) influence of increasing proportions of urea (as a supplemental RDP) on the apparent digestibility of DM, OM, NDF, ADF and GE could be detected (Table 3). Accordingly no significant ($P > 0.05$) linear, quadratic or cubic relationship between urea level and digestibility coefficients occurred. Similarly Jacobs (2005) observed no significant results in digestibility and intake when urea was used to replace cotton oilcake in RDP supplements for steers consuming the same type of winter grass hay. In addition Petersen *et al.,* (1985) reported no treatment differences for rumen organic matter digestibility (OMD) and abomasal OM flow in steers fed mature native forage (5.1% CP) and a supplement where urea was substituting soybean meal on an isonitrogenous basis. The absence of response in digestibility was verified by Egan & Doyle (1985) who stated that intraruminal urea supplementation did not result in clear evidence of a change in either digestibility of OM or the rate of digestion of cell wall constituents. This is in contrast to Kropp *et al.,* (1977) who reported a decrease in rumen OMD of weathered range grass (5% CP) when urea substituted soybean meal in a CP supplement. Nolte (2000) observed that increasing the proportion of N from urea on isonitrogenous basis in RDP supplements fed to sheep consuming wheat straw tended to decrease the total tract OM and NDF digestibilities ($P \leq 0.10$). Köster *et al.* (1997) replaced casein with urea and found a constant positive response for OM and NDF digestibility up to 75% N from urea, where after they observed a negative response. The variation in results of different studies could probably be attributed to factors like animal type and its physiological stage, the degradable protein source supplied and the forage type.

Table 3: Effect of increasing proportions of supplemental nitrogen from urea on the digestibility of low quality grass hay

Item	Supplemental rumen degradable protein from urea					Significance	Significance of contrasts[1]			
	0%	25%	50%	75%	100%	P	L	Q	C	CV
Grass DMI (kg/cow/day)	8.10	7.80	7.33	8.66	9.36	0.0758	0.0448 0.1111*	0.0569 0.0994*	0.7794 0.0020*	16.463
Supplemental DMI (kg/cow/day)	0.44[a]	0.40[b]	0.36[c]	0.33[d]	0.30[e]	0.0001	0.0001 0.9925*	0.0001 0.0055*	0.0001 0.0020*	0.000
Total DMI (kg/cow/day)	8.54	8.20	7.69	8.99	9.67	0.0975	0.0701 0.0913*	0.0548 0.1034*	0.7884 0.0019*	15.757
Total OMI (kg/cow/day)	8.06	7.76	7.26	8.48	9.13	0.0957	0.0695 0.0915*	0.0540 0.1039*	0.8062 0.0016*	15.673
Apparent digestibility coefficients (%)										
Dry matter	55.83	55.67	52.41	57.13	57.21	0.7456	0.6355 0.0072*	0.4234 0.0206*	0.8627 0.0010*	13.269
Organic matter	59.77	59.73	57.01	60.72	60.91	0.8201	0.6852 0.0053*	0.4710 0.0169*	0.9178 0.0003*	11.199
Crude protein	40.74	40.71	34.90	39.60	34.47	0.6819	0.3048 0.0337*	0.9827 0.0000*	0.7588 0.0030*	28.730
Neutral detergent fibre	60.40	60.59	57.90	61.57	61.57	0.8585	0.6928 0.0051*	0.5483 0.0118*	0.9234 0.0003*	11.511
Acid detergent fibre	57.45	57.59	54.77	59.10	59.12	0.7681	0.5659 0.0106*	0.4882 0.0155*	0.8718 0.0008*	12.096

Item	Supplemental rumen degradable protein from urea					Significance	Significance of contrasts[1]			
	0%	25%	50%	75%	100%	P	L	Q	C	CV
Gross energy	55.28	55.16	51.59	56.26	56.18	0.7601	0.7459	0.4330	0.8849	13.509
							0.0034*	0.0198*	0.0007*	
Apparent digestible nutrients (%)										
Digestible OM	56.44	56.51	53.84	57.32	57.53	0.8179	0.6915	0.4741	0.9444	11.138
							0.0051*	0.0167*	0.0002*	
Digestible protein	2.87	2.77	2.38	2.70	2.16	0.2971	0.0805	0.8724	0.4987	26.835
							0.0931*	0.0007*	0.0133*	
Digestible NDF	41.22	41.63	39.59	43.18	43.01	0.6472	0.3872	0.5240	0.8235	11.742
							0.0237*	0.0128*	0.0016*	
Digestible ADF	24.71	24.96	23.77	26.27	26.09	0.5603	0.2868	0.5275	0.7442	12.494
							0.0356*	0.0124*	0.0033*	
Metabolizable energy (MJ/kg)[3]	7.77	7.78	7.20	7.86	7.84	0.7484	0.8626	0.4346	0.9438	13.660
							0.0011*	0.0084*	0.0002*	

Row means with different superscripts differ significantly

[1]L = linear, Q = quadratic, C = cubic
[2]CV = Coefficient of variance
[3]Metabolisable energy = Digestible energy X 0.8 (McDonald *et al.,* 2002)

* = R^2

DMI = Dry matter intake

OMI = Organic matter intake

NDF = Neutral detergent fibre

ADF = Acid detergent fibre

The possibility also exists that unknown factors could influence the results and warrant further investigation. In this regard Rush *et al.* (1976) and Williams *et al.* (1963) mentioned that urea is readily hydrolysed by rumen bacteria to carbon dioxide and ammonia, and if there are sufficient carbo-skeletons (α-keto acids) which primarily arise from dietary carbohydrate, the liberated ammonia can be used to synthesize bacterial protein. However if there is a shortage of c-skeletons and the rumen pH is correct, the NH_3 will enter the rumen epithelium and be absorbed into the body (Williams *et al.,* 1963; Oh *et al.,* 1969). Chalmers *et al.* (1976) as cited by Nolte (2000) noted that a rumen pH < 7 is not conducive for passive absorption of NH_3 from the rumen into the bloodstream. These factors could have contributed to the different results reported in the available literature where different forage types and supplements (carbo-skeletons) were used.

3.3.2.3 Digestible nutrients

The influence of supplemental RDP levels from urea on apparent digestible nutrients was in accordance with the digestibility coefficients results (Table 3).

3.3.3 Intake study

From the results in Table 4 it is evident that there was a linear increase in grass DMI (P = 0.0355) at increasing levels of urea, with the highest intake realised when urea was used as a sole source of N. The accuracy of prediction (R^2 =0.04) was however low. This finding was supported by non significant difference (P > 0.05) in grass DMI when using multiple comparison test. The latter test revealed however statistical differences (P < 0.05) in DOMI and metabolisable energy intake (MEI) of pregnant beef cows. It seems however that only the 50% supplemental RDP from urea treatment showed statistically significant lower DOMI and MEI values. The lower values of the 50% supplemental RDP is difficult to explain. On the other hand DOMI and MEI increased in a linear and quadratic manner (P < 0.05) with the increasing proportion of urea although the accuracy of their prediction was low. Therefore, it seems that urea can supply all the supplemental N to beef cows consuming low quality hay. Similarly Jacobs (2005) reported no significant difference in grass DMI, total DMI and DOMI when substituting the degradable protein in oilcake with that of urea in supplements for steers consuming the same forage type.

Table 4: Effect of increasing proportions of supplemental nitrogen from urea on the intake of cows consuming low quality grass hay

Item	Supplemental rumen degradable protein from urea					Significance	Significance of contrasts[1]			
	0%	25%	50%	75%	100%	P	L	Q	C	CV[2]
Grass DMI (kg/cow/day)	8.23[ab]	8.32[ab]	7.70[a]	8.73[ab]	9.33[b]	0.0526	0.0355	0.0648	0.8120	11.659
							0.1195*	0.0905*	0.0014*	
Grass DMI as % of body mass	1.69	1.78	1.74	1.94	1.96	0.2281	0.0328	0.8021	0.8681	13.943
							0.1392*	0.0018*	0.0008*	
Supplemental DMI (kg/cow/day)	0.44[a]	0.40[b]	0.36[c]	0.33[d]	0.30[e]	0.0001	0.0001	0.0001	0.0001	0.000
							0.9925*	0.0055*	0.0020*	
Total DMI (kg/cow/day)	8.67[ab]	8.67[ab]	8.06[a]	9.05[ab]	9.63[b]	0.0822	0.0658	0.0622	0.8719	11.409
							0.0930*	0.0957*	0.0007*	
Total DMI as % of body mass	1.94	1.87	1.83	2.01	2.02	0.3926	0.2480	0.2286	0.4499	11.338
							0.0405*	0.0441*	0.0171*	
Total OMI (kg/cow/day)	8.06	7.76	7.26	8.48	9.13	0.0957	0.0695	0.0540	0.8062	15.673
							0.0915*	0.1039*	0.0016*	
Digestible OMI (kg/cow/day)	4.96[ab]	4.70[ab]	4.34[a]	5.19[ab]	5.54[b]	0.0050	0.0207	0.0047	0.5610	11.388
							0.1230*	0.1921*	0.0071*	

Item	Supplemental rumen degradable protein from urea					Significance	Significance of contrasts[1]			
	0%	25%	50%	75%	100%	P	L	Q	C	CV[2]
Digestible OMI (g/kg $BW^{0.75}$)	50.90[ab]	47.00[ab]	45.04[a]	53.03[ab]	54.28[b]	0.0125	0.0566	0.0096	0.1706	10.728
							0.0859*	0.1676*	0.0431*	
CPI (g/cow/day)	598.64	557.13	523.66	610.55	606.66	0.1462	0.4350	0.0694	0.2691	12.677
							0.0167*	0.0949*	0.0339*	
DCPI (g/cow/day)	245.51	231.43	187.28	242.77	216.81	0.6559	0.6351	0.5096	0.5968	35.765
							0.0071*	0.0137*	0.0088*	
ME intake (MJ/cow/day)	68.34[ab]	63.99[ab]	58.08[a]	71.18[b]	75.52[b]	0.0023	0.0258	0.0022	0.4391	11.405
							0.1073*	0.2184*	0.0120*	
ME intake (MJ/kg $BW^{0.75}$)	0.70[ab]	0.64[ab]	0.60[a]	0.73[b]	0.74[b]	0.0048	0.0653	0.0050	0.1229	10.605
							0.2566*	0.1892*	0.0518*	

Row means with different superscripts differ significantly

[1]L = linear, Q = quadratic, C = cubic
[2]CV = Coefficient of variance
* = R^2

DMI = Dry matter intake

OMI = Organic matter intake

CPI = Crude protein intake

DCPI = Digestible crude protein intake

ME - Metabolizable energy = Digestible energy X 0.8 (McDonald *et al.*, 2002)

In contrast Köster *et al.* (1997) reported a trend for DOMI to exhibit a decrease when supplemental N from urea exceeded 50%. A significant decrease in DOMI ($P < 0.03$) was however observed when supplemental N from urea exceeded 75%. Accordingly Maeng & Baldwin (1976) found that the substitution of small amounts of amino acids with urea on an isonitrogenous basis stimulated microbial growth in an in vitro system and an optimum ratio of amino acid-N to urea was 25 to 75. Nolte (2000) in a study with sheep fed wheat straw, observed the highest DOMI for the 25% urea N treatment after which there was a significant linear decrease in total OM intake and DOMI ($P \leq 0.02$) with increasing urea inclusion levels.

Erfle *et al.*, (1982) stated that the supplementation of a low protein diet with urea stimulated the total microbial numbers in the rumen but, it was not effective as an intact protein for certain microbial species. Although most species of ruminal bacteria can use ammonia as a sole N source, evidence indicates that the presence of pre-formed amino acids can substantially improve ruminal microbial yields (Maeng & Baldwin, 1976; Hume, 1970; Kang-Meznarich *et al.*, 1981). Furthermore BCVFA are important growth factors for fybrolytic bacteria (Lientzenich *et al.*, 1995). In contrast, other studies cited by Köster (1995) namely Huhtanen & Elliot, (1956); Cline *et al.*, (1966) and McCollum *et al.*, (1987) have failed to demonstrate improved fiber digestion when BCVFA were added to low protein feeds. In addition Oh *et al.* (1969) indicated that rumen micro-organisms appear to be capable of synthesizing their essential cellular components utilising urea as the sole source of N. The results of the present study seem to be in accordance with these latter findings.

The grass DMI as a percentage of body mass (Table 4) increased linearly ($P = 0.0328$) with the increasing proportion of urea. The highest value of 1.96% was realised with 100% urea level. This was higher than the 1.54% obtained in Chapter 2. The former value was also higher than the 1.7% recorded by Köster *et al.* (1997) and below the 2.4% observed by Jacobs (2005). The highest value recorded in the present study for total DMI as percentage of body mass was 2.02% at 100% urea inclusion level (Table 4) but there was no significant difference among the treatments. Factors such as temperature, animal type, physiological stage of the experimental animals and grass type could probably explain the different results among various studies regarding grass intake as percentage of body mass.

3.3.4 Body mass changes

It seems from Table 5 that the cows tend to loose body mass (maximum 5.5%) during the experimental period. Adaptation to the pens and individual feeding could have also contributed to the loss in body mass. A linear increase in mass loss (P = 0.0023) was observed with increasing level of supplemented urea, and the highest mass loss occurred where 100% of the supplemental degradable protein was from urea. These results were however not supported by the intake results. Furthermore these mass changes were recorded over a short period of time and should be interpreted with caution.

3.3.5 Rumen characteristics

The main end products of carbohydrate metabolism by rumen micro-organisms are VFA (acetic, propionic and butyric acid), carbon dioxide and methane. Additional fatty acids are formed generally in small quantities by deamination of amino acids in the rumen, these are iso-butyric from valine, valeric from praline, etc. (McDonald *et al.,* 1988). VFA arising from ruminal fermentation is the primary energy source for the animal (Russel *et al.,* 1992). The proportion of various acids produced is influenced by the forage composition and it affects the efficiency of energy utilisation for maintenance and production (Jones & Wilson, 1987). The predominant acid is acetic and roughage diets high in cellulose give rise to high molar percentage of acetic acid. When the proportion of concentrates in the diet increases then propionic acid increases while acetic decreases (McDonald *et al.,* 1988). Acetate and butyrate are used efficiently by fattening animals but cannot contribute to the glucose supply. Propionate can be used for gluconeogenesis but a high ratio of propionate to acetate in the rumen is associated with a reduction in milk fat (Russell *et al.*, 1992). Potential digestibility may not be realised if the diet is deficient in essential nutrients such as N and sulphur required for efficient microbial fermentation (Jones & Wilson, 1987). Köster (1995) stated that branched chain amino acids serve as precursors to the BCVFA. The considerable increase in the latter in cows supplemented with true protein seems to be directly linked to the provision of readily available precursors in the digestible intake protein (Erfle *et al.,* 1982).

Table 5: The effect of increasing proportions of supplemental nitrogen from urea on body mass changes of beef cows

Item	Supplemental rumen degradable protein from urea					Significance	Significance of contrasts[1]			
	0%	25%	50%	75%	100%	P	L	Q	C	CV
Initial mass (kg)	451.29	471.43	452.00	466.57	489.57	0.3073	0.1174	0.4565	0.2892	7.985
							0.0742*	0.0162*	0.0332*	
Final mass (kg)	446.43	457.14	437.29	439.43	464.00	0.6154	0.6962	0.3495	0.2399	8.240
							0.0048*	0.0276*	0.0439*	
Mass change (kg)	-4.86[a]	-14.29[ab]	-14.71[ab]	-25.57[ab]	-27.14[b]	0.0273	0.0023	0.6070	0.7605	-78.558
							0.2611*	0.0063*	0.0022*	
Final mass as % of initial mass	99.07[a]	96.93[ab]	96.68[ab]	94.72[ab]	94.20[b]	0.0251	0.0022	0.4511	0.7446	2.965
							0.2610*	0.0136*	0.0025*	

Row means with different superscripts differ significantly

[1]L = linear, Q = quadratic, C = cubic
[2]CV = Coefficient of variance
* = R^2

From the results in Table 6 it seems that the molar proportion of acetic, propionic and butyric acid was not affected ($P > 0.05$) by the substitution of urea, while the molar percentage of iso-butyric acid and iso-valeric acid significantly decreased ($P < 0.0001$) with increasing supplemental degradable protein from urea. In contrast Köster *et al.* (1997) observed ammonia N (NH_3N) and acetate to increase linearly ($P \leq 0.02$) as the percentage of supplemental N from urea increased. They also outlined that propionate was not affected by treatment while molar percentage of butyrate, isobutyrate, valerate and isovalerate decreased ($P \leq 0.05$) with increasing proportions of N from urea. In the present study NH_3N also increased linearly ($P = 0.0426$) with increasing supplemental N from urea but, the accuracy of prediction was however very low ($R^2 = 0.1253$). Jacobs (2005) found a significant increase ($P < 0.0252$) in the concentration of propionate when the supplemental degradable protein from urea increased up to 75%. According to the NH_3N and main short chain fatty acids (acetic acid, propionic acid and butyric acid) content in the rumen fluid of cows in the present study, increasing levels of supplemental degradable protein derived from urea did not influence rumen fermentation and microbial production.

The ruminal pH was not affected by an increasing proportion ($P > 0.05$) of supplemental N from urea (Table 6). In vitro studies indicated that the efficiency of microbial protein synthesis can decline significantly at pH < 6.0 (Russell *et al.*, 1992). Ruminal pH varied from 6.6 to 6.9 in the various treatments and should have been sufficient to support adequate fibre digestion. Compared to the present study, Jacobs (2005) and Nolte (2000) reported similar findings with steers and sheep respectively for both ammonia N and pH while Köster *et al.* (1997) reported similar results with beef cattle for ruminal pH but NH_3N increased linearly with an increasing urea content in the supplement.

Table 6: Effect of increasing proportions of supplemental N from urea on rumen fluids characteristics of beef cows consuming low quality grass hay

	Supplemental rumen degradable protein from urea					Significance	Significance of contrasts[1]			
Item	**0%**	**25%**	**50%**	**75%**	**100%**	**P**	**L**	**Q**	**C**	**CV**
pH	6.58	6.86	6.81	6.72	6.77	0.6232	0.5618	0.3279	0.2623	5.165
							0.0105*	0.0303*	0.0399*	
					(mMol/l)					
NH_3N	8.36	10.36	9.98	10.32	11.75	0.2425	0.0426	0.9114	0.2837	26.240
							0.1253*	0.0004*	0.0333*	
Acetic acid	43.12	41.54	43.87	40.67	45.22	0.4709	0.5834	0.3527	0.5293	11.756
							0.0091*	0.0265*	0.0120*	
Propionic acid	8.72	8.58	8.95	9.04	8.44	0.8409	0.9367	0.4509	0.3769	12.856
							0.0002*	0.0186*	0.0256*	
Iso-butyric acid	0.62[a]	0.50[ab]	0.41[bc]	0.30[cd]	0.22[d]	0.0001	0.0001	0.7206	0.9967	25.419
							0.6849*	0.0014*	0.0000	
Butyric acid	4.90	4.90	4.91	5.01	5.16	0.9594	0.4907	0.7226	0.9708	15.470
							0.0159*	0.0042*	0.0000	
Iso-valeric acid	0.84[a]	0.70[ab]	0.44[bc]	0.42[bc]	0.20[c]	0.0001	0.0001	0.7321	0.7158	37.666
							0.5831*	0.0016*	0.0018*	

Row means with different superscripts differ significantly

[1]L = linear, Q = quadratic, C = cubic
[2]CV = Coefficient of variance
* = R^2
NH_3N = Ammonia nitrogen

To enhance microbial protein synthesis and reduced wastage, when urea is used it should be given with a source of readily available energy (McDonald *et al.,* 1988). If the rate of protein degradation exceeds that of carbohydrate fermentation, large quantities of N can be lost as NH_3 and if the rate of carbohydrate fermentation exceeds that of protein degradation microbial protein production can decrease (Russel *et al.,* 1992). The entry of readily available carbohydrate into the rumen will also bring a rapid fall in pH then reduce likelihood of urea toxicity (McDonald *et al.,* 1988).

Satter & Slyter (1974) and Slyter *et al.* (1979) indicated that a concentration of 20 to 50mg NH_3N/l is enough to support maximum growth rate of rumen bacteria and excessive high levels of NH_3 up to 800mg NH_3N/l did not inhibit microbial growth and nothing was also gained from excessive supplementation. Therefore, the NH_3N concentration in the present study appeared to be adequate for maximum microbial protein synthesis for all treatments. Although a high but not significant NH_3N concentration in the study was observed with the highest urea levels, the lack of treatment effect on rumen digestibility and intake parameters indicate that NH_3N was probably not limiting microbial fermentation.

3.4 Conclusions

It seems from the intake and digestibility results that the RDP requirements of pregnant beef cattle consuming low quality grass hay from the Northern Variation of the *Cymbopogon-Themeda* pasture type (pure grassveld) can be supplied solely by urea. This was supported by the short chain fatty acid and NH_3N content of rumen fluid, indicating that rumen fermentation and microbial production were not detrimentally influenced by supplying all the supplemental RDP from urea.

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CHAPTER 4

The influence of different levels of urea in rumen degradable protein supplements on the performance of beef cows grazing natural winter grassveld

4.1 Introduction

Reproduction and growth are the two most important factors in a beef production system. Optimal nutrition and management practices ensure maximum fertility and sufficient milk production to produce a healthy, well grown weaner. For ideal condition score during spring, the cow must at least maintain her body reserves during winter period otherwise higher levels of nutrition will be needed to allow maximal reproduction. However, it is more economical to maintain body mass and condition during winter than to regain it in spring.

Winter supplementation of protein may improve reproductive efficiency and calf weaning weights (DelCurto *et al.,* 1990). Increased production appears to be mediated through increased forage intake which has been correlated with increases in forage digestibility, rate of digestion and digesta flow (Köster, 1995; DelCurto *et al.,* 1990). There has been a long standing interest in the potential of non-protein nitrogen (NPN) as a substitute for true protein in winter supplements, as true protein is one of the costly components is supplements. Like true protein, urea based supplements stimulate intake and digestibility (Swingle *et al.*, 1977; Pate *et al.,* 1990) of low quality forages. However, livestock performance with NPN based supplements was generally inferior when compared to the performance of animals receiving true protein (Forero *et al.*, 1980; Rush *et al.,* 1976; Clanton, 1978, Williams *et al.,* 1963; Maeng & Baldwin, 1976).

Köster (1995) results suggested that between 50% and 75% of supplemental rumen degradable protein (RDP) could be provided from urea without compromising forage intake and digestion. On the other hand from digestibility and intake results of the previous study (Chapter 3), it was concluded that the RDP requirements of beef cattle consuming low quality grass hay can be supplied solely by urea. This was similar to Jacobs' (2005) findings where cotton seed oilcake was replaced with urea in RDP supplements for steers. In these studies low quality forage hay was fed intensively to cattle. The possibility exist that these results are not applicable for grazing animals because they would select that of higher nutritional value than the hay they are given. Considering the contrary results in literature and the fact that different types of low quality forage hay was intensively fed to various categories of beef cattle that could influence their selection behaviour, it is of utmost importance to investigate this aspect with grazing pregnant beef cows. Therefore, the objective of the study was to evaluate the effect of substituting natural RDP from cotton seed oilcake with urea in RDP supplements on the performance of pregnant beef cows grazing natural winter grass from the Northern Variation of the *Cymbopogon-Themeda* pasture type (pure grassveld).

4.2 Materials and Methods

4.2.1 Animals

Fifty six pregnant Afrikaner x Simmentaler crossbred cows were used at the initial stage of the trial. The cows were randomly divided into two groups of 28 cows each and allocated to one of the two treatments. The trial was executed over four consecutive winter periods from 2003 to 2006. The experimental cows were randomly allocated to the different treatments each year. Due to the replacement (culling and selection), mortalities and stock theft the number of cows varied between 18 and 28 per treatment during different years. The trial started each year in May and ended in November and a protein lick was provided during this period. At the end of the trial period the two groups of cows were combined and managed together receiving a 50:50 dicalcium-phosphate (DCP): salt (NaCl) lick on summer veld. The cows were mated during the beginning of December each year for a period of 63 days (3 heat cycles). One bull per group was used and in an effort to avoid bull effect, the bulls were rotated between the groups on a monthly basis.

Cows were weighed at the start of the trial and every 28 days thereafter. Fasted body mass (BM) was determined after the cows had been left overnight without food and water (approximately 16 hours). Cows were also compared in terms of condition score (scale 1 to 5, 1=extremely emaciated and 5=very fat) (Van Niekerk & Louw, 1990) at the start (May), end of the lick phase (November) and end of autumn (April) before the start of winter lick phase the following year. The latter was taken as the final body condition score (BCS), since it reflected the lick carry over effect and cows had recovered from calving season while the November BCS was not a good reflection of the lick effect because it was taken during the calving season. Average daily gain (ADG) was determined from April to April the following year. Pregnancy was determined by rectal palpation during May annually. Reproductive parameters determined were percentages achieved in conception, calving and weaning. Measurements on the calves included birth mass, average daily gain at day of age (ADA), weaning mass and 205 day corrected weaning mass (CWM).

4.2.2 Diet

The cows grazed the dormant winter pasture of the Northern Variation of the Cymbopogon-Themeda (no. 48b) pasture type (pure grassveld) as described in Chapter 2. The treatments were as follows: 1) lick comprised of the supplemental RDP from urea as a sole source (100% NPN) and 2) lick comprised 50% of RDP from urea and the remaining 50% from cottonseed oilcake. The supplemental RDP was from urea (100% degradable) and cotton seed oilcake (78.24% degradable). The degradability values of cotton seed oilcake and winter pasture hay were determined by Jacobs (2005) and were used to formulate the RDP supplements. The cows in the two treatments were rotated between the camps on a monthly basis to cancel possible camp effects. The supplements were formulated to supply in the daily RDP requirements of cows (4.03g RDP/kg $BW^{0.75}$) as found in Chapter 2. Supplements were provided every second day during 2003 and every third day during the remaining years of the trial (2004, 2005 and 2006), due to varying number of cows per treatment each year. The cows had free access to clean water. The physical and chemical composition of the licks and mineral premix are outlined in Table 1 and Table 2 respectively.

Table 1: Physical and chemical composition of the licks[1]

Raw material (%)	50% urea	100% urea
Physical composition		
Urea feed	4.35	7.83
Salt	5.0	5.0
Monocalcium phosphate	2.65	4.21
Begasse	11.0	22.71
Molasses	40.0	60.0
Cotton oilcake	36.75	---
Mineral premix	0.25	0.25
Chemical composition (%)		
Crude protein	28.22	25.39
Degradable protein	25.0	25.0
Undegradable protein	3.22	0.39
Metabolizable energy (MJ/kg)	9.13	8.49
Non protein nitrogen	2.00	3.60
Non protein nitrogen equivalent urea	12.5	22.48

1. Commercial feed formulation

Table 2: The composition of the mineral mix in the lick treatments

Macro minerals (%)	Ca	P	Na	K	Cl	S	Mg
50% urea	1.01	1.0	1.96	1.91	3.78	0.44	0.42
100% urea	1.42	1.0	1.94	2.07	4.21	0.47	0.35
Micro minerals (ppm)	**Co**	**Cu**	**Mn**	**Se**	**Zn**	**Fe**	**I**
50% urea	2.00	109.94	339.0	1.11	333.55	622.44	5.00
100% urea	2.32	105.73	357.39	1.00	317.69	1027.87	5.00

1. Commercial feed formulation

4.2.3 Statistical analysis

The data were analysed as a 2x4 factorial design (effect of 2 NPN levels and 4 years). Data was subjected to PROC ANOVA using the General Linear Models (GLM) procedures of SAS (1995). The differences between means were separated using the Tukey's studentized range (HSD) test.

4.3 Results and Discussions

4.3.1 Cow performance

From the results in Table 3, it is evident that increasing the proportion of supplemental RDP from urea did not have any effect ($P = 0.9938$) on the end live mass of the cows. A lick effect was detected on the end BCS at 5% level ($P = 0.0218$), with the group receiving 100% urea lick in a better condition for the first two years and the group receiving 50% urea lick slightly ($P < 0.05$) better during the third year. The latter performance resulted in lick x year interaction ($P = 0.0196$). According to the results in Table 4, there was no statistical significant ($P > 0.05$) difference in the live mass of the cows except during October 2003, May 2005 and September 2006. The difference in favour of the group receiving the 50% urea lick during October 2003 and September 2006, probably resulted from the cows that conceived earlier during the mating season. Although not statistically significant, the ADG of the group receiving 100% urea lick was better than that of 50% urea lick except during 2005. The performance of the latter could have been influenced by the number of non pregnant cows (8 from a total of 18) in the 100% urea receiving group.

In a study where cows consuming low quality forage were supplemented with molasses-cottonseed meal-urea, molasses-urea or molasses, Pate *et al.* (1990) found that the supplementation treatments did not affect cow live weight or condition score during any period of the year. Köster (1997) on the contrary found that the magnitude of responses observed for body weight and body condition change was not great however the trend indicated a decline in performance at the highest level of urea inclusion.

Table 3: The influence of different levels of supplemental rumen degradable protein from urea on the performance of beef cows grazing winter grassveld

Item		*Year*					*Significance (P)*			
	Lick	2003	2004	2005	2006	Mean	Lick	Year	Interaction	CV[6]
Initial live mass	**A**[1]	515.48	530.90	529.22	494.94	517.64	0.8752	0.6584	0.3831	10.8
May (kg)	**B**[2]	519.61	518.00	515.89	522.69	519.05				
	Mean	517.55	524.45	522.56	508.82					
Initial BCS	**A**	2.61	3.25	2.53		2.80	0.3258	0.0001	0.9196	13.0
May (1 to 5)	**B**	2.71	3.31	2.57		2.86				
	Mean	2.66^{a}	3.28^{b}	2.55^{a}						
End live mass	**A**	549.70	529.40	492.78	577.29	537.29	0.9938	0.0001	0.4549	12.1
April (kg)	**B**	551.68	502.67	514.44	537.21	537.21				
	Mean	550.69^{a}	516.04^{ab}	503.61^{b}	578.68^{a}					
End BCS[3]	**A**	3.39^{a1}	3.42^{a1}	2.53^{b1}		3.11	0.0218	0.0001	0.0196	14.2
April (1 to 5)	**B**	2.53^{ab2}	2.14^{a2}	2.61^{b1}		2.43				
	Mean	2.96	2.78	2.57						
Wean mass	**A**	248.27^{a}	247.22^{a}	214.83^{b}	199.88^{b}	227.55	0.8536	0.0011	0.0226	12.7
May (kg)	**B**	231.33^{a}	231.38^{a}	222.16^{a}	229.50^{a}	228.59				
	Mean	239.80	239.30	218.50	214.69					
CWM[4]	**A**	258.00	242.33	203.94	202.00	226.57	0.8000	0.0001	0.2100	14.7
	B	250.50	231.88	204.54	224.63	227.89				
	Mean	254.25^{a}	237.10^{b}	204.24^{c}	213.32^{cd}					
ADA[5]	**A**	1058.52	1010.44	841.81	815.38	931.53	0.7500	0.0001	0.1895	15.4
	B	1019.33	959.38	839.71	914.75	933.29				
	Mean	1038.93^{a}	984.91^{a}	840.76^{b}	865.07^{b}					

abcd - Row means with different superscript differ significantly ($P < 0.05$)

12 – Columns means with different superscripts differ significantly ($P < 0.05$)

1. A – 100% RDP from urea
2. B – 50% RDP from urea
3. BCS – Body condition score
4. CWM – Corrected weaning mass
5. ADA – Average daily from birth to wean
6. CV – Coefficient of variance

Table 4: The live mass (kg) of cows receiving supplements with different levels of rumen degradable protein from urea

	2003			2004		
	100% urea	50% urea	[1]P	100% urea	50% urea	[1]P
April	516.57	517.71	0.9319	530.90	523.16	0.6715
May	524.07	538.64	0.3543	551.80	543.79	0.6874
June	550.89	551.68	0.9569	540.30	523.05	0.3383
July	549.04	544.82	0.7643	543.40	554.68	0.5253
August	550.25	557.29	0.6237	578.90	595.89	0.4071
September	561.32	579.39	0.2101	562.50	583.68	0.4236
October	518.36[a]	569.14[b]	0.0009	497.80	539.79	0.1443
November	467.57	486.86	0.1136	478.95	481.26	0.9122
April	549.70	550.43	0.9549	529.40	502.67	0.2746
April to April ADG[2]	101.74	97.36	0.8564	-4.47	-45.66	0.4207
	2005			**2006**		
	100% urea	**50% urea**	**[1]P**	**100% urea**	**50% urea**	**[1]P**
April	530.11	515.22	0.4846	495.88	518.13	0.2831
May	538.11[a]	490.39[b]	0.0215	494.94	522.69	0.1560
June	529.22	515.89	0.5106	495.53	532.06	0.1046
July	526.44	502.33	0.1907	483.06[a]	527.00[b]	0.0440
August	525.11	508.56	0.3797	482.41	510.38	0.1774
September	546.44	546.39	0.9976	501.53[a]	547.75[b]	0.0415
October	497.72	491.00	0.7675	483.59	544.13	0.0768
November	478.22	466.00	0.5466	529.76	547.50	0.2808
April	492.78	514.44	0.2925	577.29	580.06	0.9199
April to April ADG[2]	-111.78[a]	-2.33[b]	0.0394	242.30	184.34	0.5393

Row means with different superscripts differ significantly ($P < 0.05$)

1. P = Level of significance

2. ADG = Average daily gain

4.3.2 Calf performance

From the results in Table 3, it is evident that urea levels did not significantly ($P > 0.05$) influence weaning mass, CWM and ADA of calves. These results were similar to those reported by Rush, *et al.* (1976) and Köster (1995) who reported that the birth mass of calves, calf ADA and weaning mass were not affected by the level of urea fed to their dams before calving.

The lack of lick treatment effects during the current trial, is in support of the results of the previous trial (Chapter 3) where the sole supply of RDP requirements of beef cattle consuming low quality grass by urea did not influence forage intake and digestion. Similarly Oh *et al.* (1969) reported urea can be used as the only RDP for the following reasons: 1) nitrogen (N) was the most limiting nutrient in the low quality grasses, 2) ammonia (NH_3) from urea, meets the requirement of rumen bacteria to synthesize nitrogenous compounds such as protein and nucleic acids, 3) bacteria are capable of synthesizing essential cellular components from the low quality grasses. In contrast, previous research demonstrated the failure of NPN to be as effective as natural protein in supporting maintenance of beef cows consuming low quality winter forage (William *et al.,* 1963; Nelson & Waller, 1962; Forero *et al.*, 1980; Rush *et al.,* 1976). Some researchers suggested that the replacement of true protein by 100% urea N may result in a depression in microbial production and digestion due to limitations in bacterial growth factors such as peptides, amino acids and branched chain volatile fatty acids (Hume, 1970; Redman *et al,* 1980; McDonald *et al.,* 1988; Forero *et al.*, 1980). However, the results of Oh *et al.* (1969) argued that since microbial protein was similar between treatments (supplementation with urea, urea + volatile fatty acids (VFA), caproic acid, casein), rumen bacteria other than the cellulolytic species might be capable of synthesizing the branched chain amino acids from carbohydrates in the feed. The cellulolytic bacteria which require the branched chain fatty acids to synthesize the branched chain amino acids might have obtained those essential carbon skeletons from the branched chain amino acids in the protein of the non-cellulolytic bacteria (Oh *et al.*, 1969).

Proteolytic and deaminative enzymes produced by rumen microorganisms degrade dietary protein to VFA's, peptides, amino acids and NH_3 (Kang-Meznarich & Broderick, 1981). Since NH_3 is acknowledged as the primary N source of cellulolytic bacteria (Cotta & Russell, 1982; McDonald *et al.,* 2002; Russel *et al.*, 1992), it can be supplied by relatively inexpensive sources of NPN such as urea because protein is the most expensive ingredient in ruminant supplements. Bryant & Robinson (1962) cited by Nolte (2000) stated that 82% of rumen bacteria can grow with NH_3 as the sole source of N, 25% would not grow unless NH_3 was present and 56% could utilise either NH_3 or amino acids.

Conclusions

The lack of significant lick treatment effect on live mass, BCS and performance of the calves suggests that urea can be used as a sole RDP source in supplements of beef cows grazing low quality winter pasture from the Northern variation of the Cymbopogon-Themeda type (pure grassveld).

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General conclusions

Grazing animals depend on the available low quality forages from the natural veld, grain and crop by-products as their main feed source or energy source for a considerable period of the year. These high fibrous roughages with low crude protein are poorly digested and cannot be efficiently utilised by ruminants. Since nitrogen (N) is generally considered as the first limiting nutrient for ruminants grazing low quality forages, it is essential to enhance their intake and digestion through provision of supplemental rumen degradable protein (RDP).

Considering the high costs of protein supplementation, it is important to determine the amount of RDP required to increase the intake and digestibility of low quality forages so as to ensure optimal animal performance. From the results of the present study, 4.03g total RDP intake/kg $BW^{0.75}$ or 8% RDP of DOM was needed to maximise energy intake and supply the requirements of beef cows during their third trimester of gestation. The total RDP requirements to maximise energy intake of beef cattle consuming low quality forage was remarkably similar to those reported by Köster (1995). In contrast Van der Merwe (2010 – unpublished data) found that pregnant beef cows consuming winter grass hay from the False grassveld pasture type needed 3.68g total RDP intake/kg $BW^{0.75}$ to maximise energy intake. The differences in the total RDP required can be influenced by the differences in forage quality and the type of animal as well as its physiological status. In this regard the digestible fibre content of the forage is therefore of utmost importance since there is a positive relationship between the digestible fibre content and RDP requirements of beef cattle consuming low quality forage. This relationship is accommodated by expressing the required RDP as percentage of DOM. The 8% RDP of DOM value in the present study is almost equal to the 9% reported by Van der Merwe (2010 – unpublished data). This is however lower than 11% RDP of DOM reported by Köster (1995).

In an effort to reduce supplementary cost, the potential to substitute amino acid N (casein) with non protein N (urea) in supplemental RDP was investigated. The substitution level with urea was from 0 to 100%. The intake and digestibility results confirmed that urea can be used as a sole source of the supplemental RDP required by beef cows consuming low quality forage. This was supported by the short chain fatty acids and ammonia N content in the rumen fluid indicating that rumen fermentation and microbial production were not detrimentally influenced by supplying RDP from urea.

It could be argued that intensive feeding of hay would not be representative of veld grazing, because the grazing animals would usually select plant material of higher nutritional value than the hay they are fed. The results regarding the level of urea in RDP supplements were however supported by the grazing trial. The grazing cow performance results confirmed that urea can be used as the only source of supplemental RDP without influencing changes in live mass, condition score and weaning performance of the calves. It could be further argued that the benefit of selection under grazing circumstances will however decline from west to east in South Africa because of less variation in different grass species. This selection benefit will be at it lowest during winter especially in frost prone areas e.g. the highveld and eastern areas of South Africa. The experimental hay in this study was obtained from an area where less selection occurs. In these areas natural veld quality declines almost to that of a monoculture low quality roughage.

Abstract

A trial was conducted to determine the total rumen degradable protein intake (RDPI) required to maximise the digestible organic matter intake (DOMI) of beef cows consuming low quality grass hay from the Northern variation of *Cymbopogon-Themeda* pasture type (pure grassveld).

Thirty five pregnant Afrikaner x Simmentaler crossbred cows (±517.08kg, SD 53.06) were randomly allocated to 5 treatments. Treatments provided the following RDP levels/cow/day 0g, 180g, 360g, 540g and 720g. A RDP source, calcium caseinate (90% crude protein (CP) on dry matter basis and 100% rumen degradable) was used and mixed with molasses based concentrate. The cows had *ad lib* access to low quality grass hay (2.26% CP, 73.94% neutral detergent fibre). The trial period consisted of 14 days adaptation, 21 days intake study and 7 days digestibility study. No statistical significant ($P > 0.05$) influence of RDP level on the apparent digestibility of dry matter (DM), organic matter (OM) and neutral detergent fibre (NDF) was detected. The grass DM intake (DMI), DOMI and metabolisable energy intake (MEI) increased in a linear and quadratic manner ($P < 0.05$) with increasing levels of supplemental RDP. The single broken-line model predicted DOMI/kg $BW^{0.75}$ with higher accuracy ($R^2 = 0.45$) than the quadratic regression procedure ($R^2 = 0.07$). According to this model 4.03g daily RDPI/kg $BW^{0.75}$ or 8.07% RDP of DOM was required to maximise DOMI of pregnant beef cows consuming winter grassveld hay.

In a second trial the potential to substitute true protein with urea was investigated. Urea replaced 0%, 25%, 50%, 75% and 100% of the natural supplemental RDP. The same procedure as described in the first trial was followed. The increasing proportion of urea did not significantly ($P > 0.05$) influence the apparent digestibility of DM, OM and NDF. There was a linear increase in grass DMI ($P = 0.0355$) at increasing levels of urea, with the highest intake observed when urea was used as a sole source of nitrogen (N). DOMI and MEI increased in both linear and a quadratic manner ($P < 0.05$) with increasing levels of urea. The molar proportions of acetic, propionic and butyric acid were not affected ($P < 0.05$) by the substitution of urea, while the molar percentages of iso-butyric and iso-valeric acid were significantly decreased ($P < 0.0001$) with increasing urea levels. Ammonia N increased linearly ($P = 0.0426$) while the ruminal pH was not affected ($P > 0.05$) by increasing the proportion of urea. It seems that urea can be the sole RDP source in supplements for pregnant beef cows consuming the low quality grass hay.

In the third trial, the influence of replacing natural protein with urea on the performance of beef cows grazing natural winter grassveld was investigated. Pregnant Afrikaner x Simmentaler crossbred cows were randomly allocated to the two treatments. The number of cows per treatment varied between 18 and 28 each year. The trial was executed over four consecutive winter periods from 2003 to 2006. The treatment licks comprised of: 1) 100% supplemental RDP from urea and 2) 50% supplemental RDP from urea and 50% from cottonseed oilcake. Lick provision was controlled to ensure the total RDPI as recommended in the first trial. Increasing the proportion of supplemental RDP from urea did not have a significant ($P = 0.9938$) effect on the end live mass of the cows. The urea levels did not significantly influence ($P > 0.05$) weaning mass, corrected weaning mass and average daily gain of the calves. The lack of significant lick treatment effect on live mass, body condition score and performance of the calves suggests that urea can be used as a sole source of RDP.

Key words: Beef cattle, rumen degradable protein, intake, roughage, urea, digestibility.

Uittreksel

‘n Proef is onderneem om te bepaal wat die totale rumendegradeerbare proteïen- (RDP) behoefte van vleisbeeskoeie is om droë organiese materiaalinname (DOMI) van swak gehalte hooi van die Noordelike Variasie van *Cymbopogon-Themeda*-veld te maksimaliseer. Vyf-en-dertig dragtige Afrikaner X Simmentaler kruiskoeie (±517.08kg, SA 53.06) is ewekansig aan vyf behandelings toegedeel. Proefbehandelings het 0, 180, 360, 540 en 720 g.koei^{-1}.dag^{-1} aanvullende RDP voorsien. ‘n RDP bron, kalsium kaseïenaat (90% ruproteïen op ‘n droëmateriaal-basis en 100% rumendegradeerbaar) is gebruik en met ‘n molasse-basis konsentraat vermeng. Die koeie het ad lib. toegang tot swak gehalte winterveldgrashooi gehad (2.26% ruproteïen en 73.94% neutraal onoplosbare vesel). Die proeftydperk het uit 14 dae aanpassing, 21 dae inname en 7 dae verteerbaarheidstudie bestaan. Daar was geen statisties betekenisvolle invloed ($P > 0.05$) van RDP aanvullingspeil op die skynbare verteerbaarheid van droë materiaal (DM), organiese materiaal (OM) en neutraal onoplosbare vesel (NDF) gemeet nie. Die DM-inname (DMI), DOMI en metaboliseerbare enegie inname (MEI) het op lineêre en kwadratiese wyses ($P < 0.05$) toegeneem met toenemende RDP aanvullingspeile. Die enkele gebroke-lyn model het DOMI/kg BW$^{0.75}$ vanaf RDPI/kg BW$^{0.75}$ met meer akkuraatheid ($R^2 = 0.45$) as die kwadratiese regressie prosedure ($R^2 = 0.07$) beskryf. Volgens die model benodig koeie wat winter grasveldhooi benut 4.03g RDPI/kg BW$^{0.75}$ of 8.07% RDP van DOMI om inname te maksimaliseer.

‘n Tweede proef het die moonlikheid om ware proteïen met ureum te vervang ondersoek. Ureum het 0, 25, 50, 75 en 100% van die ware proteïen-aanvulling vervang. Dieselfde prosedures soos beskryf vir die eerste proef is gevolg. Toenemende ureumpeile het nie betekenisvolle invloede ($P > 0.05$) op die skynbare vertaarbaarheid van DM, OM, en NDF uitgeoefen nie. Daar was ‘n lineêre toename in gras DMI ($P = 0.0355$) met toenemende ureumpeile en die hoogste inname is behaal teen die vlak waar ureum al die aanvullende stikstof (N) voorsien het. DOMI en MEI het op lineêre en kwadratiese wyse toegeneem ($P < 0.05$) met toenemende ureumpeile. Die molêre verhoudings van asyn-, propion en bottersuur is nie deur die vervanging van ware proteïen met ureum beïnvloed nie ($P < 0.05$) terwyl die molêre verhoudings van iso-botter- en iso-valeriensuur betekenisvol ($P < 0.0001$) deur toenemende ureumpeile verlaag is. Ammoniak N het liniêre toegeneem ($P = 0.0426$) terwyl rumen pH nie deur toenemende ureumpeile beïnvloed is nie

($P > 0.05$). Dit blyk dat ureum die enigste bron van RDP in aanvullings vir dragtige vleisbeeskoeie wat swak gehalte hooi benut kan wees.

In die derde proef is die invloed van die vervanging van natuurlike proteïen met ureum op die prestasie van vleisbeeskoeie op wintergrasveld ondersoek. Dragtige Afrikaner X Simmentaler kruiskoeie is ewekansig aan twee behandelings toegedeel. Die getal koeie het tussen 18 en 28 gewissel tussen behandelings gedurende 2003 tot 2006. Die behandelings het bestaan uit 100% en 50% aanvullende RDP vanaf ureum. Aanvulling is beheerd voorsien om 'n RDPI soos deur die eerste proef bevind te voorsien. Die toenemende verhouding aanvullende RDP vanaf ureum het geen betekenisvolle verskil op die eindmassa van die koeie gehad nie ($P = 0.9938$). Die vlak van ureum insluiting het nie 'n betekenisvolle ($P > 0.05$) invloed op die speenmassa, gekorrigeerde speenmassa en gemiddelde daaglikse toename van die kalwers uitgeoefen nie. Die gebrek aan betekenisvolle behandelingseffek op lewende gewig, kondisietelling en kalfprestasie dui daarop dat ureum as enigste bron van RDP gebruik kan word.

Sleutelwoorde: Vleisbeeste, rumen degradeerbare proteïen, inname, ruvoer, ureum, verteerbaarheid.