Effect of supplementing sheep receiving poor quality roughage with non-protein nitrogen and fermentable energy

By

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Contents

Declaration

I, Dala Du Plessis, hereby declare that the work done in this dissertation is my own original work and that it has not previously been used partially or as a whole at any University for the attainment of any degree.

Dala Du Plessis **September 2013**

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Summary

The effect of supplementing sheep receiving poor quality roughage with non-protein nitrogen and fermentable energy

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This research was conducted in order to enable primary producers to maximize the use of cheap roughage sources while still maintaining body weight during dry winter months when the crude protein (CP) content of roughage sources are at a minimum. The data obtained from this study will give an economic advantage when formulating supplements to be used during this time of the year.

The aim of this study was to determine the optimum level of non-protein-nitrogen (NPN) and fermentable metabolizable energy (FME) to increase microbial protein synthesis, optimize rumen fermentation and increase digestibility of dry matter (DM) and neutral detergent fibre (NDF) in sheep fed on poor quality forages. A metabolic trial was conducted where intake of DM, organic matter (OM), NDF and CP was recorded; rumen volatile fatty acid (VFA) production was recorded as well as rumen pH over the different treatments. Microbial protein synthesis was determined by analysing purine derivatives in the urine. An *in situ* trial was also done to determine changes in ruminal digestibility of DM and NDF on different treatments.

Five treatments were used. Treatment 1 consisted of NPN and FME balanced according to the NRC (2007) requirements for a 50kg whether, and served as a control. Treatment. Treatment 2 consisted 15% less NPN than control but the same amount of FME than control while treatment 3 consisted 15% more NPN than the control but the same amount of FME as the control treatment. Treatment 4 consisted of 15% less FME, but the same amount of NPN, than the control treatment, while treatment 5 consisted of 15% more FME, but the same amount of NPN than the control treatment.

A 5 x 5 Latin square design was used in this study. Five Merino wethers were allowed to adapt to supplements which were infused directly into the rumen at 9:00 and 15:30 every day. After adaptation animals were placed in individual metabolic crates for three and given three day to adapt to crate environment. After the initial three days the sampling period commenced.

Results obtained indicated that treatment had no effect on DM, OM, NDF and water intake but intake of CP was significantly increased for treatment 3 when compared to treatment 2. When intake of DM, OM, NDF and CP, related to metabolic bodyweight ($W^{0.75}$) was calculated, treatment 5 resulted in lower intake of both water and NDF as compared to treatment 4. Differences between levels of FME and NPN in this study was

insufficient to have an influence on DMD, OMD or NDFD however, CP degradability was increased for treatment 3 and treatment 5. Ruminal pH was unaffected by treatment. Increased levels of NH_3-N for treatment 3 when compared to treatment 1 and 2, was observed. Both treatments 2 and 5 resulted significant decreases in rumen NH_3-N. Treatments had no effect on the proportions of VFA produced or on the Acetate to Propionate produced ratio. Treatment 3 caused an improvement in CP an N balance when compared to treatment 1 and 2. Treatment 3, when compared to treatment 1 and 2, lead to an increase in N balance/kg$W^{0.75}$. Treatment 5 caused a higher microbial protein synthesis in contrast to treatment 4. Results from the *in situ* trial showed a decreased a-value (solubility) for the NDF fraction of treatment 3 when compared to treatment 2. The rate of degradability (c) of both DM and NDF was increased for treatment 2 compared with treatment 3. The b, ED and PD values showed no response to treatment.

LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
ADIN	Acid detergent insoluble nitrogen
BW	Bodyweight
Ca	Calcium
CHO	Carbohydrate
CP	Crude protein
DE	Digestible energy
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
DOM	Digestible organic matter
DOMD	Digestible organic matter digested
EU `	European Union
FME	Fermentable metabolizable energy
FOMI	Forage organic matter intake
g	Gram
kg	Kilogram
NPN	Non protein nitrogen
MCP	Microbial crude protein
ME	Metabolizable energy
MJ	Mega joule
ml	millilitre
MP	Metabolizable protein

N	Nitrogen
NDF	Neutral detergent fibre
NDFI	Neutral detergent fibre intake
NH_3-N	Ammonia nitrogen
NRC	National Research Council
OM	Organic matter
OMI	Organic matter intake
P	Phosphorous
RDP	Rumen degradable protein
TDOMI	Total digestible organic matter intake
UDP	Undegradable protein
VFA	Volatile fatty acid
$W^{0.75}$	Metabolic weight

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CHAPTER 1: GENERAL INTRODUCTION

Increasing consumer awareness and demand for food of high quality, traceability and safety standards (Anderson, 2000; Ruegg, 2003), together with the increase in population growth rate globally and especially in developing countries (Steck, 2008), are all forces that place a greater demand on the primary producer, especially those of meat products. These consumer demands are being shaped by the increasing level of education and the abundant amount of readily available information from sources around the world (Anderson, 2000). Issues such as bovine spongiform encephalopathy in the United Kingdom, dioxin contamination of poultry in Belgium (Anderson, 2000) and the recent melamine contamination of both pet and human foods, frequently raises the question of food safety and quality (Baynes & Riviere, 2009).

Antibiotic resistance and concern about antibiotic residues in intensively produced products are currently under the spotlight (Nisha, 2008) and it seems possible that consumer demand will to a certain extent determine the future of these intensive production systems. Even though there are substantial evidence to suggest increased productivity and profitability to the producer when antimicrobials are used as a growth promotant (Callaway *et al.*, 2003), the European Union has nevertheless banned the use of antimicrobials as growth promotants, mostly due to perceived risk and consumer opinion (Miller *et al.*, 2006). In addition, the increasing global trade in animals and animal products will influence the use of antimicrobials (Miller *et al.*, 2006).

Although South Africa produces 85 percent of its meat requirements and the remaining 15 percent is imported from Namibia, Botswana, and Swaziland, (Directorate Agricultural Statistics, 2009) local consumer demand is shaped by these global issues. Thus, even though South African producers are under no obligation to comply with international standards regulating the use of antibiotics, the primary producer in South Africa is increasingly pressured to produce a more natural product in order to comply with both local and international consumer demand.

The structure and size of South African households have undergone dramatic changes in the past decade. The average household size has declined, but the number of households have increased from an estimated 9 059 571 in 1996 to 12 726 000 in 2005 (Population and household projections, 2001-2021, 2007). The estimated population growth for the period 2001 to 2021 is 12.83% (Kruys, 2008). Although this gives a growth rate of less than 1% per year, the South African population is still increasing, placing greater demand on the primary producer with regards to the production of good quality, safe food.

Of the land area of South Africa, 82.4% is used for agricultural enterprises but only 12.15% of this portion is suitable for crop production. The remaining portion can best be utilized by animal production (Nation Master.com, 2012). In 1970 the number of woollen sheep was averaged at around 33 136 000. This number declined to only about 21 994 000 in 2008 (Abstract of Agricultural Statistics, 2009).This decrease can also be seen in the number of animals slaughtered per year which declined from 6 291 000 in the 1975/76 year to 5 812 000 in the 2007/08 year. The amount of mutton produced also declined from 162 000 tonnes in the 1975/1976 year to only about 121 300 tonnes in the 2007/2008 year. The per capita consumption of mutton also decreased from 6.3kg/head in 1975/76 to 3.4kg/head in 2007/08 (Directorate of Agricultural Statistics, 2009).

It is clear that with the increase in human population and concurrent decrease in production figures for mutton in South Africa, primary producers will have to be better equipped to supply good quality and large quantities of consumer acceptable mutton and lamb.

As natural pastures are the cheapest resource available to the primary producer, maximum use must be made of this resource in order to produce meat products in the most profitable way. In order to derive maximum profitability from these natural pastures it is of the utmost importance to supplement only those nutrients which have been shown to be deficient, causing poor animal performance during different seasons (Van Niekerk, 1975). Grazing practices on these pastures should be sustainable and long term planning must take into account possible droughts and disasters, such as the wide spread bush fires in large areas of the Highveld, north eastern parts of the Freestate and Northwest in the spring of 2003 (SAPA, 2003). In order to exploit this cheap source of feed to its full potential in an eco-friendly way, steps should be taken to address the specific nutrient deficiencies and imbalances of the specific pasture on offer. This in turn will ensure optimal animal production during all physiological stages through all seasons, ultimately leading to higher profitability for the producer.

This trial was conducted in order to determine the optimum level of NPN and energy to be used in supplements fed in conjunction with poor quality roughage (CP 2.93%) normally found during the winter months in the high rainfall, mutton producing areas of South Africa. With the feeding of these optimum levels it will be possible to negate the negative effects of the low CP level of the roughage on digestion and ultimately production parameters in sheep grazing these areas. This strategy will enable primary producers to use the cheapest natural resource for optimal animal production in a sustainable system. These optimal levels will lead to an increase in microbial protein production which will in turn lead to increased digestion parameters. If these parameters lead to increased production efficiency of animals it will be a step forward in producing higher, both in quality and quantity, mutton and lamb products in a more profitable way.

CHAPTER 2: LITERATURE REVIEW

Introduction

It was estimated by Van Niekerk (1996) that 85% of the total land area of South Africa was suitable only for use by grazing animals as it was deemed unsuitable for any kind of crop production. Due to erratic and highly seasonal rainfall the quantity and quality of this grazing is highly variable (Poppi & McLennan, 1995; Van Niekerk, 1996). In Southern Africa this is exacerbated by the long term droughts which occur on a cyclical basis (Van Niekerk, 1996). It is estimated (United Nations Climate Change Conference, 2011) that temperatures in the interior of South Africa will rise by 3-4°C by 2050, rainfall patterns will change and these two factors combined will lead to reduced water availability especially in the Western parts of South Africa. These Western parts include the Karoo area, which is the traditional mutton producing area; mutton production from the traditional areas must therefore be reduced to accommodate these climate changes. The higher rainfall areas would then have to compensate for the loss of production from these areas. This can only be achieved by innovative feeding and management practices, such as the optimal supplementary feeding programme during times of key nutrient shortage.

It is well known that nutrition considerably influences wool growth (Reis & Schinckel, 1963). Periods of poor pasture growth or quality is reflected in a reduction in the total fleece growth per animal (Freer & Dove, 2002). The effect of sulphur containing amino acids, most notably cysteine, plays an important role in fibre length and diameter (Reis & Schinckel, 1963; Freer & Dove, 2002). Cysteine arises from several sources, including microbial cysteine entering the intestine, dietary cysteine having escaped ruminal degradation as well as cysteine produced from methionine via the trans-sulphination pathway (Benevanga & Evans, 1983 as cited by Freer & Dove, 2002). The wool growth response to dietary intake reflects a change in the supply of amino acids, energy substrates, vitamins and minerals to the wool follicles (Freer & Dove, 2002). Deficiencies in vitamins may reduce or inhibit fibre growth as several vitamins play an important role in protein synthesis (Freer & Dove, 2002). Mineral deficiencies may cause a reduction in fibre growth and quality of fibre produced (Freer & Dove, 2002). Nutrition plays a marked role in the reproductive performance of sheep as well. For rams, the decrease in sexual behaviour during under feeding is simply a result of the general weakness of the ram (Martin *et al.*, 2004). For ewes under-nutrition may lead to irregular or even arrested oestrous cycles (Lamond *et al.*, 1972). Even though it is well known that ovulation rate in ewes may be increased by flush feeding, it is observed that as little as four days of such supplementary feeding will increase ovulation rate (Martin *et al.*, 2004). Furthermore there is evidence that both over- and underfeeding during the first few weeks after conception may lead to embryonic losses (Martin *et al.*, 2004). During gestation nutrition also plays a vital role in the development of the placenta (Bell, 1984 as cited by Martin *et al.*, 2004). Some other aspects of sheep production is affected by nutrition during gestation: initiation and development of secondary fibre follicles which is a determinant of wool quality in later life. The formation of muscle fibres which could be a determinant of growth and carcass quality, and the differentiation and development of the reproductive system (Martin *et al.*, 2004). It is therefore crucial to determine optimal feeding strategies in times of forage quality restrictions to ensure sufficient production levels.

In both the Southern and Western coastal regions little remains of natural grazing and livestock is dependent on cereal crop residues or improved or established pastures

(Kritzinger, 1987). The principal deficiencies, both for livestock and game, of these areas being that of copper and cobalt (Van Niekerk, 1996).

In the Karoo region, which is a major wool and mutton producing area, grazing consists mostly of highly nutritional shrubs. These shrubs, in contrast to adjoining grassland, maintain their feeding value throughout the year and deficiencies occur mostly during drought periods due to insufficient pasture on offer (Van Niekerk, 1996). Although several trials have shown marginal improvements in live weight gain of lambs when energy is supplemented, protein supplements did not seem to improve live weight gain (Marias *et al.*, 1989; Van Niekerk, 1996; Raats, 1999).

On well managed grazing areas in Limpopo and the Lowveld of Mpumalanga supplementary feeding does not seem to be economically viable (Van Niekerk, 1996).
Large parts of these regions have been converted to game ranching areas as well as a number of private game reserves and as such little remains of the traditional livestock industries. Apart from these livestock industries the Limpopo province mainly produces fruits and vegetables and this is by far the largest contributor to the agricultural sector in this province (Limpopo Tourism Agency, 2011).

Phosphorus deficiency is well documented in the North West and Freestate. A large number of studies have indicated the need for phosphorus supplementation during the rainy season, especially for cattle, in the Armoedsvlakte area of Vryburg (Theiler *et al.*, 1927; De Waal & Koekemoer, 1993, as cited by De Brouwer *et al.*, 2000) De Brouwer *et al.* (2000) also found that supplementation during both winter and summer to be beneficial to mature cows grazing pastures in the Western Highveld region in South Africa. For sheep the need for phosphorus supplementation is less clear although in a study by Fishwick (1978) it was found that the live weight gain was less for unsupplemented sheep than it was for sheep supplemented with a P source. Read *et al.* (1986) also observed that P deficient ewes mobilized more of their body reserved than ewes on a diet containing sufficient P. It was found that P deficient ewes were able to restore much of their body reserved during the non-lactating, non-reproducing period, even so deficient ewes had a lower body mass at the end of the trial tan ewes with sufficient P in the diet (Read *et al.*, 1986). Read *et al.* (1986) also found that P deficiency had no short term effects on reproductive performance but that P sufficient ewes weaned more and heavier lambs from the fourth lambing season onwards.

The sourveld region of South Africa is of particular interest as it is a major wool and mutton producing area. Due to high rainfall, generally exceeding 700mm per annum (Van Niekerk, 1975), un-supplemented animals in this region will typically lose up to 25-30% of their maximum summer body mass during winter (Poppi & McLennan., 1995; Van Niekerk, 1996;). This bodyweight loss will in turn lead to lower calving and lambing percentages, culminating in large financial losses to the primary producer. A vast majority of studies, designed to determine the reason for this winter weight loss in animals grazing poor quality forage, demonstrated a primary nitrogen deficiency (Köster *et al.*, 1996; Olson *et al.*, 1999; Bandyk *et al.*, 2001). Subsequent nitrogen supplementation has resulted in a slower rate of weight loss in both cattle and sheep (Clark & Quin, 1951, as cited by Winks *et al.*, 1970; Von la Chevallerie, 1965, as cited by Winks *et al.*, 1970). It is believed that the results are mainly due to the increased supply of essential nitrogen with a secondary effect due to improved DM and energy intake (Van Niekerk, 1996). A study conducted by Ferrell *et al.* (1999) suggested that energy supplementation when protein is primarily limiting, will stimulate mobilization of body protein. This is most probably due to the negative effect of high levels of readily available carbohydrates on cellulose digestibility (Chappell &

Fontenot, 1968). Pasture intake will subsequently be reduced (Raats, 1999) due to slower rate of digestion in the rumen.

It is generally accepted that there are three main reasons for offering sheep supplementary feeds (Freer & Dove, 2002). In some circumstances supplements are given to negate the negative effect of something that is already present in the diet, such as a high concentration of condensed tannins in browse species (Degen *et al.*, 2000). More often supplements are given to overcome a frank deficiency (Michalk & Saville, 1979; Freer & Dove, 2002), to correct an imbalance of several nutrients in the diet (Michalk & Saville, 1979) or to improve the total nutrient supply to the rumen in order to increase animal performance and thereby economic returns (Kunkle *et al.*, 2000). During periods of drought, which is frequently observed in semi-arid and arid regions, consideration also needs to be given to supplementation when limited quantities of roughage is available (Michalk & Saville, 1979). These situations would require the producer to supply most of the nutritional requirements to animals in the form of maintenance or drought feed (Michalk & Saville, 1979). The objective of supplementary feeding according to Rowe (1986), is to ensure that sheep eat as much forage as possible, yet ingest enough supplementary feed to ensure maintenance or growth. According to Michalk & Saville (1979) the objective of supplementary feeding, during times of adequate forage availability, would be to increase animal production through the supplementation of a single deficient nutrient or the balancing of nutrients when imbalances occur in pasture (Michalk & Saville, 1979). According to Freer & Dove (2002) most grazing situations has three basic outcomes when supplements are given to sheep. Supplementation is the first and most desirable outcome, although rare. This will only occur when the supplement is eaten and pasture intake not reduced. Substitution will occur when large quantities of the supplement are consumed and pasture intake subsequently reduced. Michalk & Saville (1979) stated that expected responses may differ from actual responses to supplementation, due to the substitution of some of the roughage component with supplementary feed, thereby confounding the economic reasoning behind providing supplementation. Krysl & Hess (1993) reported that when increasing amounts of starch are supplemented, the time spent grazing is reduced. This can be disadvantageous in some cases when the reduction in pasture intake is enough to counteract the effects of the supplement. In other cases substitutions can be a desirable effect depending on several factors, including forage quantity, forage quality and production demands (Caton & Dhuyvetter, 1997). Supplementary feeds can also provide other nutrients which will improve the efficiency of feed use (Rowe, 1986). Observations by Ferrell *et al*. (1999) suggested that when intake is low without supplementation, intake response may be expected with supplementation, but if intake is high without supplementation then forage intake response is unlikely. When the intake of a supplement causes an increase in the intake of pasture, complementation is said to take place. This is usually the case when the supplement is given to overcome a frank deficiency. The effectiveness of the supplementation program also depends on the ability to reduce intake variation and meeting the supplement consumption target (Bowman & Sowell, 1997).

Protein supplementation

Live weight gain is dependant mainly on the supply of amino acids and energy yielding substrates delivered to the body tissues, up to the full genetic potential for protein synthesis, which is seldom, if ever, reached by animals grazing natural pasture (Poppi & McLennan, 1995). Nutrient requirements of animals also vary with production level, body weight, genetic potential as well as the environmental conditions in which the animal is kept

(Kunkle *et al.*, 2000). It has been established that during winter, in the sourveld region of South Africa, the most limiting nutrient is nitrogen (Van Niekerk, 1996). Globally this is true for most poor quality forages (Köster *et al.*, 1996; Heldt *et al.*, 1999). It was stated by Bohnert & Cooke (2011) that when forage CP drops below 7 % it is likely that NH_3-N supply to the rumen micro-organisms is inadequate for maximum microbial function. Slyter *et al.* (1979) reported that in ruminal NH_3-N levels drop below 50mg/L microbial protein synthesis will be impaired. It was further reported by Slyter *et al.* (1979) that ruminal NH_3-N levels between 88 mg and 133 mg/L supported optimal microbial protein synthesis. Therefore supplemental N would have to be provided when roughage CP levels drop in order to maintain adequate levels of NH_3-N in the rumen. The minimum levels of ruminal NH_3-N reported by Slyter *et al.* (1979) are supported elsewhere in literature (Boniface *et al.* 1986; Wanapat, 2000). However Roffler & Satter (1975) reported no additional benefit of ruminal NH_3-N levels above 50 mg/L. It would therefore seem as if the critical level of ruminal NH_3-N can be set at 50 mg/L.

Nitrogen supplements are fed either to increase the supply of rumen degradable protein in the rumen for improved fibre digestion (Mathis *et al.*, 2003 as cited by Winks *et al.*, 1970), or to result in an increase in the amount of metabolizable protein (MP) flowing from the rumen to the duodenum (Freer & Dove, 2002). An increased MP supply can result either from increased microbial protein production or an increase in the rumen outflow rate of UDP, but more commonly from a combination of these (Freer & Dove, 2002). This increased nitrogen supply has been shown to increase forage OM intake, and forage DM digestibility as well as improving overall animal performance (Bohnert *et al.*, 2007).

According to Freer & Dove (2002), complementation occurs when protein supplements are fed to sheep grazing these poor quality pastures. This is brought about by the fact that these protein supplements make good a deficiency in rumen degradable protein (RDP). This kind of supplementation supplies N required for microbial fibre fermentation in the rumen. This in turn will increase the rate of digestion of the roughage component (Van Niekerk, 1975; Del Curto *et al*, 1990). Consequently rumen outflow rate will increase, causing a concomitant increase in intake (Van Niekerk, 1975; Pordomingo *et al.*, 1991; Ferrell *et al.*, 1999; Heldt *et al.*, 1999; Freer & Dove, 2002). Most commonly these results are attributed to an increased supply of available N to the rumen micro-organisms, enabling faster growth of the rumen population and increased performance by the host.

When the N supply to the rumen is below optimum the micro-organisms responsible for the fermentation of the fibrous component of the diet are adversely affected, digestion of feed, passage rate and consequently intake will be impaired (Van Niekerk, 1975). As a result the grazing animal will also suffer a lack of energy, this secondary lack of energy plays an important economic role as it is more expensive to meet the energy requirement of the grazing animal than it is to meet the comparatively small protein requirement (Van Niekerk, 1975). The impact of a supplement on the utilization of poor quality forage will depend on the composition of the supplement as well as the amount of supplement taken in by the animals (Heldt *et al.*, 1999a). During a study done by Heldt *et al.* (1999a), it was shown that supplements with a positive effect on intake and digestion of low-quality forages will be those with a high concentration of RDP. Olson *et al.* (1999) also stated that intake and digestion of poor quality forages by beef steers usually increase when supplemental RDP is fed. It was found that supplemental RDP supplied to Dohne Merino wethers enhanced rumen fermentation and forage intake (Notle *et al.*, 2003).

Urea is the most widely used non-protein nitrogen source and is rapidly degraded to ammonia in the rumen (Freer & Dove, 2002; Stanton & Whittier, 2011). Provided that the initial ammonia concentration in the rumen is below optimum (McDonald *et al.*, 2002) and that there is a readily fermentable carbohydrate source (Annison *et al.*, 1954; Stanton & Whittier, 2011), ammonia can efficiently be incorporated into rumen microbial protein (Freer & Dove, 2002; McDonald *et al*, 2002). It has been stated by Stanton & Whittier (2011) that continuous intake of urea leads to improved utilization, as opposed to periodic intake of urea supplements, which could lead to overconsumption of the supplement on days when the supplement is provided. This will lead to excretion of excess urea on some days and a shortage of available N in the rumen to facilitate optimal microbial protein production. However in a study by Currier *et al.* (2004), in which animal performance were measured for animals receiving daily urea supplements as opposed to animals receiving supplements every second day, it was found that there was no difference in animal performance for animals supplemented daily as opposed to every second day. Amino acid supply from microbial protein is similar to that of natural proteins frequently given to animals as a supplement (Stanton & Whittier, 2011). Responses by grazing sheep to urea supplementation are more variable when compared to cattle (Freer & Dove, 2002). This is due to the fact that sheep graze more selectively and this may result in the consumption of a higher quality diet, which may contain sufficient RDP to support good rumen fermentation, even though the average N content of the roughage is below optimum (Freer & Dove, 2002). There is also cause for concern due to large between-sheep variation in urea intake, causing some animals to consume toxic doses and others showing no response to supplementation (Freer & Dove, 2002). Other sources of NPN include biuret, isobutylidene, hydrazine and ammonium salts (McDonald *et al*, 2002; Currier *et al.*, 2004).

Urea is less expensive per unit of nitrogen than natural protein sources both from animal and plant origin (Bohnert & Cooke, 2011). Use of the protein sources from animal origin are currently in the spotlight worldwide due to concerns regarding the safety of these products. Many countries have already banned the use of animal proteins as a protein source to other animals (Freer & Dove, 2002). Plant proteins include grain legumes, pulses, oilseeds and oilseed meals (Freer & Dove, 2002; McDonald *et al.*, 2002). These plant protein differ in lipid content, amount of, starch, non-starch-polysaccharide and protein present. The rumen degradability of these proteins are also dependant on the degree of processing, in particular grinding and heat processing (Freer & Dove, 2002; McDonald *et al.*, 2002). Animal response to these supplements will depend on animal requirement for ME, RDP and UDP as well as their interaction with nutrients provided by other dietary sources (Freer & Dove, 2002).

Energy supplementation

Energy supplementation is normally given when the grazing cannot meet the energy requirements for production (Caton & Dhuyvetter, 1997). These energy demands are dependent upon the level of production and the energy expenditure during grazing. Subsequently energy supplementation may alter the overall energy requirement of grazing ruminants through changes in grazing behaviour or changes in the partitioning of nutrients towards maintenance or production (Canton & Dhuyvetter, 1997). If grazing time is decreased due to supplementation the energy requirement for grazing will also be decreased. As energy from concentrates are used more effectively than energy from forages for both maintenance and weight gain functions when supplemental energy increases, the efficiency of energy utilization must also increase (NRC, 1984).

Limited quantities of supplemental grain may have little or no effect on forage intake when fed at quantities below 0.25% of BW (Matejovsky & Sanson, 1995). According to Caton & Dhuyvetter (1997) for sheep especially, intake will be stimulated by supplementing with low levels, 7.8% of DM intake, of cereal grain. However when higher levels of maize, greater than 23% of total DM intake, was supplemented, forage intake was reduced. Pordomingo *et al.* (1991) conducted a study to determine the effect of different levels of energy supplementation when fed to steers grazing good to medium quality forage. Whole shelled maize was fed at levels of 0, 0.2, 0.4 and 0.6% of BW at 09:00 each day. Analyses of oesophageal collected samples indicated that a ruminal N deficiency was unlikely as total available N as % of OM averaged between 1.64 and 1.24. In this study forage OM intake declined linearly with increasing amounts of whole-shelled maize fed. Despite the reduction in fermentable organic matter intake (FOMI) when maize was fed at 0.4 or 0.6% of BW, the digestible OM intake by steers in these treatments was equal to those on control treatment. This could have resulted from the combined effect of substitution and decreased forage digestibility. Total OM intake was not affected by supplemental maize, due to substitution effects at higher levels (0.4 and 0.6% of BW) of maize supplementation. In this study numerically greater digestible OMI was achieved when supplemental maize was fed at 0.2% of BW. It was stated by Pordomingo *et al.* (1991) that limited quantities of supplemental grain, on a diet where N is not limiting, stimulated OM digestibility and passage rate thereby increasing digesta flow and allowing greater forage intake. These limited quantities of supplemental grain in the presence of adequate N, provide energy to rumen microbes for the production of microbial protein. This leads to increases in rumen populations of microbes, which enhances forage digestibility. In other studies (Henning, 1980 cited by Caton & Dhuyvetter, 1997; Matejovsky & Sanson, 1995) it was found that low levels of maize supplementation increased forage intake but that at increasing levels of maize supplementation, greater than 23% of DMI, forage intake was reduced, due to detrimental effects on forage digestibility as this is a favourable environment for amylolytic bacteria. If the supplement consists of readily digestible fibre rather than grains the effect on forage intake is less negative. Due to lower levels of starch in these fibres, the ruminal pH was less affected and rumen microbial population remained mostly fibrolytic (Caton & Dhyvetter, 1997).

Feeding supplements containing high levels of cereal grains or cereal grains as such often decreases the fermentation of low-quality forage by grazing animals due to the high starch content of these grains (Sanson *et al.*, 1990; Caton & Dhuyvetter, 1997; Heldt *et al.*, 1999a). Low forage intakes with high supplemental carbohydrate (CHO) suggest that the amount of supplemental CHO may affect the potential of the supplemental protein to impact forage intake (Heldt *et al.*, 1999a). This may be due to the reduced availability of N for use by the fibrolytic bacteria due to increased utilization of N by amylolytic bacteria (Heldt *et al.*, 1999a) as well as the reduction of ruminal pH (Mould & Ørskov, 1983 as cited by Caton & Dhuyvetter, 1997). However, when limited quantities of supplemental grain are fed to grazing animals where N is not limiting, there may be no effect on forage intake, total digestible energy (DE) intake may be increased, and OM digestion and passage rate may be improved (Pordomingo *et al*, 1991). Studies aiming to evaluate readily digestible fibre sources as energy supplements yielded different responses than studies conducted with high carbohydrate sources due to lower levels of starch within these fibres. Therefore changes in ruminal pH and carbohydrate effects are not as pronounced (Caton & Dhuyvetter, 1997).

According to Caton & Dhuyvetter (1997) energy supplementation has little to no effect on rate of digestion. In the study of Heldt *et al.* (1999a), high CHO treatments had lower NDF digestion than the low CHO treatments. This indicates that supplemental CHO or the

relative balance between RDP and CHO is an important factor in determining effects on fibre digestion. Variable results have been achieved in studies with energy supplementation, some having found either no effect on total tract digestibility, or increased total tract digestibility (Krysl *et al.* 1989 as cited by Pordomingo *et al.* 1991; DelCurto *et al.*, 1990; Freeman *et al.*, 1992; Matejovsky & Sanson, 1995 as cited by Canton & Dhuyvetter, 1997).

In the experiment of Pordomingo *et al.* (1991), ruminal pH was not affected by supplemented maize. The rumen pH values ranged from 6.0 to 6.4 which is quite typical for steers grazing summer blue gama rangeland in New Mexico (Krysl *et al.*, 1987 as cited by Pordomingo *et al.*, 1991). Ruminal NH_3-N concentrations decreased with increased supplemental energy fed. This is to be expected, if more OM is fermented in the rumen, assimilation of N into microbial protein would be stimulated and protein flow to the intestines should be increased. Daily fluctuations in NH_3-N were minimized by decreased levels of supplemental maize. Ruminal NH_3-N concentrations ranged from 3.8 to 12.4 mg/dL of ruminal fluid. Ruminal volatile fatty acid concentrations were not affected by supplemental maize.

Ørskov (1982) as well as Mould (1983) as cited by Caton & Dhuyvetter (1997) reported that a ruminal pH below 6.2 would inhibit the action of the cellulolytic bacteria in the rumen, thereby indicating that depressions in ruminal pH due to grain supplementation could be responsible for reduced forage digestibility. Russell *et al.*, (1979) indicated that cellulolytic bacteria will diminish at pH ranges between 5.7 and 6.2 and soluble carbohydrate fermenting bacteria will persist until ruminal pH reaches 4.6 – 4.9. Church (1979) as cited by Caton & Dhuyvetter (1997) stated that when fed foraged based diets, ruminal pH varies between 6.2 and 6.8, while the ruminal pH ranged between 5.8 and 6.6 when concentrate based diets were fed. Sanson *et al.* (1990) stated that it seems as if energy supplementation with cereal grains could reduce ruminal pH levels.

According to Heldt *et al.* (1999a) the result of carbohydrate supplementation when animals are grazing low-quality forage seems to depend on the following factors: source of supplemental carbohydrate, amount of supplemental carbohydrate and the amount of supplemental rumen degradable protein. Horn & McCollum (1987) as cited by Canton & Dhuyvetter (1997) suggested that energy supplementation would only have a marginal effect on forage utilization if the amount supplemented was not higher than 30g/kg of metabolic weight ($BW^{0.75}$) which amount to roughly 0.7% of body weight.

Combined energy and protein supplementation

In a study done by DelCurto *et al.* (1990) it was reported that increased supplemental energy reduced intake of low-quality forage when the supplemental CP was 11.5% or below. In contrast, intake of low-quality forage was unaffected when supplemental energy was provided in conjunction with high levels of supplemental CP (>20%) (Sanson *et al.*, 1990). Sanson *et al.* (1990) reported that effects of supplements containing combinations of oil meals and grains have not been consistent. However, according to Heldt *et al.* (1999a) the ability to offer increasing amounts of carbohydrates in a supplement without negative effects on forage intake and digestion seems dependant on the amount of supplemental rumen degradable protein as well as the source of carbohydrate, The most positive results being obtained with either glucose or readily digestible fibre. This may occur due to glucose being a fundamental substrate for most fibrolytic and amylolytic microbes.

Amylolytic microbes have a competitive advantage when utilizing starch as an energy source. This would result in less N for use by fibrolytic bacteria due to increased utilization of N by amylolytic bacteria during rapid fermentation of supplemental starch (Heldt *et al.*, 1999a), leading to lower numbers of fibrolytic microbes in the rumen population with a consequential reduction in forage digestibility.

In an experiment by Heldt *et al.* (1999b) where beef steers were fed a CHO source of either, starch, glucose, fructose or sucrose at 0.30% of body weight/day, together with a RDP source at 0.031% of BW/d, forage OM intake was not affected but NDF digestion decreased. This negative effect on fibre digestion may have been due to the depletion of ruminally available N by amylolytic bacteria, thereby resulting in less available N for fibrolytic bacteria (Heldt *et al.*, 1999b). Heldt *et al.* (1999b) stated that ideally, when a supplement is fed to cattle grazing low-quality forage, the supplement should have the capacity to exert positive effects on forage utilization. Russell (1998) however suggested that the excess of readily fermentable CHO, together with inadequate ruminally available N may have a direct inhibitory effect on certain ruminal microbes and may even be toxic. Sanson *et al.* (1990) fed steers either no supplement, a protein supplement, a protein with low level of maize (0.26% of BW) supplement, and a protein with high level of maize (0.52% of BW) supplement on a basal diet of poor quality hay. In the study of Sanson *et al.* (1990) it was found that animals which received no supplement had higher forage intakes than animals fed a high level of maize in the supplement, whereas the total DM intake was on average the same for protein alone and protein with a high level of maize supplementation but total DM intake was increased when protein with a low level of maize was fed. Digestible DM intake was depressed by 18% when a high level of maize supplement was fed in comparison with a protein supplement only. However organic matter digestibility increased for both treatments containing a high and a low level of maize. The NDF digestion was quadratically decreased as level of maize increased but no effect on cellulose digestion was observed. Forage DM and OM digestion was not affected by treatment. This data suggests that if protein is adequate in the diet, the high levels of maize supplementation will depress forage intake. The quadratic effect observed indicates that forage digestibility is not affected by low levels of maize supplementation. These results also indicate that even though there seems to be no interaction between protein and energy in the supplement, supplementing animals grazing low-quality forage with maize will depress forage intake. In another study done by Heldt *et al* (1999b) steers were fed a CHO source consisting of either, starch, glucose, fructose or sucrose and the supplemental RDP was increased to 0.122% of BW/d. In this experiment FOMI, as well as total OMI increased for all supplements with no differences between CHO sources. All CHO sources resulted in increased OM digestibility, but OMD for starch was lower than for sugars. Sucrose led to lower OMD than monosaccharaides. Supplementation also led to higher NDF digestion when compared to non-supplemented animals. When starch was used as a CHO source the NDFD was lower than for supplements containing sugars.

In the experiment of Heldt *et al.* (1999a) supplementation did not affect ruminal pH, but did increase rumen NH_3-N concentration. Supplementation caused an increase in rumen NH_3-N when compared to no supplement, but the level of maize did not affect the level of rumen NH_3-N (Sanson *et al.*, 1990). These levels of rumen NH_3-N were above the recommended levels for maximum microbial growth (Satter & Slyter, 1974 as cited by Sanson *et al.*, 1990). Supplementation with maize depressed rumen pH at 1, 3, 5, and 7 hours after feeding, this suggest that fermentation of readily available carbohydrates increased as level of maize in the diet increased (Sanson *et al.*, 1990). In the study of Heldt *et al.* (1999b) ruminal pH was decreased, ranging from 6.1-6.6 but at times falling below 6, but ruminal

NH_3-N concentration was increased as well as a significant increase in the level of total rumen organic acids.

Olson *et al*. (1999) conducted a study in which the effect of various levels of supplemental RDP and starch on forage utilization and ruminal function of steers consuming poor quality tall-grass prairie hay were evaluated. Supplements were designed to contain one of three levels of ruminally degradable starch, 0, 0.15 and 0.3% of initial body weight, and one of four levels of RDP at 0.03, 0.06, 0.09 and 0.12% of initial bodyweight. These supplements were administered intraruminally in a dry form once daily at 07h30. The starch source demonstrated rapid solubilisation in ruminal fluid *in vitro*, and was assumed to be completely ruminally degradable. The starch grits provided a relatively pure source of starch as it was devoid of ash, NDF and N. A significant positive effect on intake was noted as the level of supplemental RDP increased. Total and forage OM intake increased linearly with increasing level of RDP, as did intakes of NDF and DOM. The addition of starch to the supplement linearly decreased the intake of forage and total OM, NDF and DOM. This suggests that even at low levels the effect of ruminally degradable starch was to decrease the intake of low quality forage. Olson *et al.* (1999) also stated that the absence of interactive effects of supplemental starch and RDP on forage intake indicated that the negative effects of starch on low quality forage intake could not be fully overcome by the addition of supplemental RDP within the feeding levels used in that particular study. The digestion of DM, OM and NDF increased linearly with the increase in the amount of RDP but decreased linearly with increase in the amount of starch, although digestion of these components did not differ from the negative control. The improvement in digestion in response to RDP supplementation were most likely brought about by alleviating deficiencies in N-containing compounds, as an increase in the supply of NH_3-N facilitated microbial fermentation (Olson *et al.* 1999). In this study improvements in diet digestion were caused primarily by the strong effect of RDP on forage fibre digestion. Digestion of NDF, OM and DM were significantly depressed on the treatment with the lowest level of RDP and highest level of starch. Supplementation decreased the average ruminal pH, for both starch and RDP, indicating increased ruminal fermentation activity. The ruminal NH_3-N concentration was higher for supplemented than non-supplemented steers. The NH_3-N concentration was linearly decreased with starch supplementation, but increased quadratically with RDP supplementation. The greatest increase in response to RDP occurred between 0.09 and 0.12% of BW levels. Total rumen VFA concentration was increased greatly by supplementation, illustrating the ability of supplementation to increase fermentative activity. With increasing RDP supplementation, the total rumen VFA concentration increased linearly, but the addition of starch had no effect on total rumen VFA concentration. Ruminal proportions of acetate and propionate were similar between supplemented and non-supplemented steers. As supplemented starch increased, the molar percentage of acetate in the rumen decreased, and the molar percentage of propionate increased linearly This may reflect changes in the microbial population. In this study supplemented and non-supplemented steers had similar ruminal proportions of butyrate (Olson *et al.*, 1999)

The specific aim of this study was to determine the optimum level of fermentable energy and non-protein nitrogen (NPN) that results in increased NDF digestibility and intake of poor quality roughages fed to sheep, as well as the optimum level of fermentable energy and NPN that optimizes rumen fermentation in sheep fed on poor quality forages. In addition the optimum level of readily fermentable energy and NPN that maximises microbial protein synthesis was to be determined.

The following hypotheses were formulated:

H_0: There is no optimum level of fermentable energy and NPN that results in an increased rate of NDF degradability and intake of poor quality pasture fed to sheep.

H_1: There is an optimum level of fermentable energy and NPN that will result in an increased rate of NDF degradability and intake of poor quality roughage by sheep.

H_0: There is no optimum level of fermentable energy and NPN that will optimise rumen fermentation in sheep fed poor quality roughage .

H_1: There is an optimum level of fermentable energy and NPN that will optimise rumen fermentation in sheep fed poor quality roughage.

H_0: There is no optimum level of fermentable energy and NPN that will maximise microbial protein synthesis in sheep fed poor quality roughage.

H_1: There is an optimum level of fermentable energy and NPN that will maximise microbial protein synthesis in sheep fed poor quality roughage.

CHAPTER 3: MATERIALS AND METHODS

This experiment was approved by The Animal Use and Care Committee of the University of Pretoria (Ec021-08). The experiment was conducted on the Hatfield Experimental Farm of the University of Pretoria.

Animals

A 5x5 Latin Square design was used to determine the effects of different levels of FME to NPN on the digestion and microbial protein synthesis of sheep receiving poor quality roughage. Five ruminally cannulated Merino type wethers, age 32 months ± 6, with average bodyweight 50 kg ±2.4 were used in this trial. Animals were treated for internal parasites before the start of the trial, and during the experimental period according to the FAMACHA method (Barth *et al.* 1996) as required. Hooves were trimmed before the onset of the trial and throughout the experimental period as required. Any sickness was treated immediately.

Animals were allowed a 10 day adaptation period on each new treatment, followed by an 8 day data collection period. Monitoring of rumen pH prior to commencing the trial period showed that a 10 day adaptation period provided sufficient time for rumen pH to stabilize between treatments. Animals were placed in metabolic crates three days prior to commencing data collection. This allowed animals to adapt to the crate environment before data collection. During this time faecal bags were attached but left open, bags were closed on commencement of the data collection period. Fresh water as well as the basal roughage (*Eragrostis curvula)* was available at all times. After the data collection period wethers were assigned to a different treatment.

Preliminary intake trial.

An intake trial was conducted prior to the start of the main trial in order to determine the expected average intake of the poor quality roughage (Table 1) as well as the amount of urea and starch required to meet the maintenance requirements of a 50 kg wether (NRC, 2007). Six wethers with average bodyweight 50 kg was placed in metabolic crates for 8 days. Hay was provided at 1838g (110% of ad lib intake) per animal per day and refusals weighed back. Daily hay intake and refusals were recorded in Table 2. Fresh water was available *ad lib*. Together with the hay, a commercial winter lick (Table 3), Voermol Winter lick – Premix 450 was provided, for which the intake was also recorded. This supplement was fed due to the poor quality of the roughage and fears of rumen stasis in the trial animals existed.

Table 1 Analyses of poor quality roughage (hay) on DM basis

	DM	Ash	CP	NDF	ADF	ADIN
g/100g	94.00	3.79	2.927	81.9	46.7	29.4g/100g CP

Table 2 Results of intake trial

Sheep no	Body Weight kg	Hay fed g/d	Orts g/d	Hay Intake g/d	Lick fed g/day	Orts g/day	Lick intake g/day
05-11	51	1838	638	1200	367	258	108
05-9	50	1838	869	969	367	227	140
06-05	48	1838	500	1338	367	232	135
06-3	49	1838	475	1363	367	137	230
05-3	53	1838	819	1019	367	205	147
D2-1	50	1838	656	1181	367	192	175
Average	**50.1**	**1838**	**659**	**1178**	**367**	**208**	**156**

Table 3 Specifications of commercial lick (Voermol Winter Lick- Premix 450)

Nutrient	Quantity g/kg
Protein	450
% protein ex NPN	94%
Urea	131.2
Ca	12
P	2.4

Table 4: Premix specifications (Feedtek)

Nutrient	Daily intake g/head
Calcium	2.00
Phosphorus	1.50
Sodium	0.7
Chloride	0.6
Potassium	5.7
Magnesium	1.1
Sulphur	0.007
	mg/head
Cobalt	0.11
Copper	4.0
Iodine	0.8
Iron	8.0
Manganese	17.45
Selenium	0.04
Zinc	30.0
Total intake of premix (g/head/day)	**24.08**

Experimental diets

During the experimental period a vitamin and mineral supplement containing no nitrogen or energy sources (Feedtek) was supplied with the treatments (Table 4). The composition of the premix was based on NRC (2007) requirements for a 50 kg wether. Trace mineral content of the poor quality roughage was not taken into account. The suggested intake, by

Feedtek formulators, of 24.08 g/head/day of the vitamin and mineral supplement was divided in two equal parts and infused directly into the rumen together with the treatment. The maintenance requirement for CP of a 50 kg wether is 69 g/d (NRC, 2007). The CP concentration of the hay is 3.18% on a DM basis. When intake is based on the results of the preliminary intake trial, hay will supply only 34.48 g of the required 69 g CP per day. However the ADIN portion of the CP is 29.4 g/100g CP, this portion is completely unavailable to the animal, therefore the roughage will only supply 24.34 g CP/day. To make up the deficit supplemental protein will have to supply 44.66 g CP/day. This amount of CP was used to determine the amount of NPN required in the experimental diets.

If 1kg of urea has a CP value of 2900 g/kg and urea had a DM content of 99.68% (internal lab analyses) then:

44.66/2.9 = 15.4g of urea will be required to fulfil maintenance requirement of a 50kg wether (NRC, 2007)

To determine the amount of urea required on an "as is" basis

15.4/0.9968 = 15.5g of urea for control as well as treatments 4 and 5.

For treatment 2, with 15% less CP (from NPN)

44.66 -15% = 37.96g CP

37.96/2.9 = 13.1g urea (DM basis)

13.1g/0.9968 = 13.13g urea in supplement

For experimental diet 3, with 15% more CP from NPN

44.66 + 15% = 51.36/2.9 = 17.7g urea (DM basis)

17.7/0.9968 = 17.8g urea in the supplement

If the amount of true protein in MCP is taken as 75% and the digestibility as 85% (McDonald *et al.*, 2002), then

44.66/ (0.75 x 0.85) = 70.1 g MCP

To fulfil the daily maintenance requirement, of a 50 kg whether, for CP rumen micro-organisms have to produce 70.1g of MCP per day. In order to produce 9g of MCP the microbes require 1MJ of FME (McDonald *et al.*, 2002)

70.1/9 = 7.8 MJ of FME required per day

The digestible organic matter per kg of DM for the poor quality roughage was determined as 39.12% using *in vitro* digestibility techniques (personal laboratory analyses). In order to calculate the ME value of the roughage the following equation was used:

ME (MJ/kg DM) = 0.016 DOMD McDonald *et al.*, 2002
= 0.016 x391.2

= 6.3 MJ/kg DM

Determination of the amount of starch required in the supplement was done as per the following example: When assuming that the FME value of a feed source is 90.7% of the ME value (McDonald *et al.*, 2002) then the FME value of the roughage will be 5.6 MJ/kg DM. Since the average intake of the roughage is 1.038 kg DM the FME intake will be 6.1 MJ/day. The deficit of 1.9 MJ/day will be made up by supplementing corn starch.

It was assumed that starch has an FME value of 15.99MJ/g DM (Robertson P.H., 2009 personal communication PHRobinson@ucdavis.edu)The DM concentration of the starch was determined as 87.01% (personal laboratory analyses). Therefore:

1.7/0.01599 = 106 g DM starch required

106/0.8701 = 121.8 g of starch in control diet as well as treatments 2 and 3.

Treatment 4, with 15% less FME than control

1.45/0.01599 = 90.37g DM starch
90.37/0.8701 = 103.9g starch in the supplement for treatment 4.

Treatment 5, with 15% more FME than control diet

1.96/0.01599 = 122.6g DM starch required
122.6/ 0.8701 = 141g of starch in the supplement for experimental diet 5.

The five experimental supplements are described in Table 5. The experimental supplements were divided into two equal portions and infused directly into the rumen twice daily at 9:00 and 15:30. The sulphur requirement of a 50 kg wether was also taken into account and included in the treatment (NRC, 2007). The control diet was formulated to meet the maintenance requirement of a 50 kg wether as described by the NRC (2007) for both energy and CP. Treatment 2 contained the same amount of FME as the control but a CP level 15 % lower than the control. Treatment 3 contained the same amount of FME as the control but a CP level 15 % higher than the control diet. Treatment 4 contained the same level of CP than the control but the FME level 15 % lower than the control. Treatment 5 contained the same level of CP than the control but a FME level 15 % higher than the control.

Table 5 Composition of experimental diets (composition on "as is" basis)

Experimental diet	Urea (g)	Starch (g)	Sulphur (g)
1) Control	15.5	121.8	1.8
2) 15% less CP (from NPN) than control	13.13	121.8	1.8
3) 15% more CP (from NPN) than control	17.8	121.8	1.8
4) 15% less FME than control	15.5	103.9	1.8
5) 15% more FME than control	15.5	141.0	1.8

Determination of intake and total tract digestion of DM, OM, CP and NDF

E. curvula hay was milled through a hammermill with a 3cm sieve and offered at 150% of the average intake of the previous two days. During the data collection period intake was determined by weighing feed before being fed in the mornings and afternoons and weighing back the orts before giving fresh feed. Total daily DM intake was recorded for each animal individually. Representative samples of both feed and orts were taken each day for each animal. Feed samples were pooled as all animals were given feed from the same bag, feed being well mixed before each feeding. The five pooled samples were analysed for DM (AOAC 934.01, 2000), OM (AOAC 942.05, 2000), CP (AOAC 968.06, 2000) and NDF (Robertson & Van Soest, 1981) content. Total faecal collections were done during the data collection period, daily representative samples were collected for each individual animal and pooled for each animal during each experimental period.. Faecal collection was done in faecal bags which were used to ensure collection of all faeces voided in order to separate urine and faecal samples. The faecal samples were analysed for DM (AOAC 934.01, 2000), OM (AOAC 942.05, 2000), CP (AOAC 968.06, 2000) and NDF (Robertson & Van Soest, 1981). Results of the feed, orts and faecal samples were used to determine total tract digestion of DM, CP and NDF. For OM intake and digestion the amount of OM supplied by the supplements has been taken into account. An ash value of 0.8% on DM basis has been used for determination of the OM content of the starch in the supplement (K. Botha 2014, personal communication, kbotha@nutrigenics.co.za).

Monitoring of N balance

Daily N intake was determined by analyses of feed samples, total intake and collection and analyses of orts. Nitrogen excretion was determined by calculation of faecal and urinary nitrogen excretion (AOAC 968.06, 2000) with conversion factors for endogenous N. Results obtained from these analyses were used to determine daily nitrogen retention.

Monitoring of rumen fermentation

Rumen fluid samples were collected, by suction strainer through the rumen canullae, over a period of 4 days within the data collection period, with a time shift of three hours every day. This was done in order to obtain a representative 24hr sample. After collection, the samples were preserved with 4 ml of a 25 % H_3PO_4 solution per 20 ml rumen fluid for determination VFA (Webb, 1994) and 5 ml of a 50 % H_2SO_4 solution per 20 ml for determination of NH_3-N (Broderick & Kang, 1980). Daily samples were pooled for each animal during each treatment and subsamples of 50 ml were frozen at -20°C as soon as possible after collection.

Determination of microbial protein synthesis:

Total urine collection was done for 5 days during the data collection period (Chen & Gomes, 1995) in stainless steel pans mounted under the metabolic crates. Urine was collected in containers with 40 ml H_2SO_4 in order to ensure that the final pH of the urine remained below 3 (Chen & Gomes, 1995). This was done in order to prevent bacterial destruction of purines in the urine. Tap water was added to obtain a constant final weight of 4 kg (Chen & Gomes, 1995). This ensured that the final volume of the diluted urine

was the same for each animal every day. Sub-samples of 50 ml of the diluted urine was taken daily and pooled for each animal during each period. Samples were labelled and stored at -20°C until further analyses. Urine was analysed for purine derivatives as an indicator of microbial protein synthesis (Faulkner & King, 1982). High performance liquid chromatography was used for analyses of purine.

Determination of ruminal DM and NDF degradability

At the end of the data collection period a 3 day *in situ* trial was conducted in order to determine the DM and NDF disappearance of the poor quality roughage across experimental supplements. Samples were ground through a 2 mm sieve and 5 g of hay was placed in Dacron bags. Dacron bags were incubated in the rumen for 0; 2; 4; 6; 8; 16; 24; 48 and 72 hours (NRC, 1984). Bag retrieval was done as described by Cruywagen (2006), using opaque nylon stockings as a receptacle. Dacron bags were placed in the receptacle and knots separated individual bags. The receptacle was then fastened to the rumen cannula plug. This ensured easier bag retrieval while allowing only the bag to be removed, to be exposed to air. After retrieval bags were washed in running water until water remained clear. After washing, bags were frozen at -20°C until removal of the last bag at 72h. After defrosting overnight, the bags were dried at 60°C for 24 hours. The residue was analysed for DM and NDF (Robertson & Van Soest, 1981).

Statistical analyses

The Proc GLM model (SAS, 2006) for a Latin Square design was used to do analysis of variance on the raw data from the laboratory.
The statistical model used for Latin square designs are as follows:

$$yi\,jk = \mu + Ti + Pj + Ak + ei\,jk$$

Where *yi jk* = the observation for each variable measured,
μ = the mean,
Ti = treatment effects,
Pj = period effects,
Ak = animal effects and
ei jk = the error.

The Fisher test was used to determine the significance of the difference ($P<0.05$) between means (Samuels & Witmer, 2003). Least square means and standard errors were calculated. The NDF and DM disappearance was analysed using the model of Ørskov & McDonald (1979).

In this study only treatments with one variable and one constant was compared, therefore the control and treatments 2 and 3 were compared and the control and treatments 4 and 5. This is done in order to remove confounding effects when experimental diets with more than 1 variable are compared.

CHAPTER 4: RESULTS AND DISCUSSION

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It has been shown by a number of studies that supplements, both protein and energy, has the potential to increase OM intake (Sanson *et al.*, 1990; Köster *et al.*, 1996; Migwi, *et al.*, 2006) as well as CP intake due to the CP supplied by the supplement as well as increases due to increased DM intake. Therefore OM intake was recorded and statistically analyzed to determine the optimum level of CP and FME. Results are shown in Table 6.

Table 6 The effect of experimental diet on water intake, organic matter intake (OMI), crude protein intake, and neutral detergent fibre (NDF) intake.

Experimental diet	Water intake (ml/day)	OM intake (g/day)	CP intake (g/day)	NDF intake (g/day)
1(Control)	3108	894	56[abc]	672
2(NPN -15%)	2765	993	53[b]	756
3(NPN +15%)	2978	909	67[c]	668
1(Control)	3108	894	56	62
4(FME -15%)	3310	941	59	722
5(FME +15%)	2549	919	60	654
Mean	2942.12	931	59	694
SE	267.8	44.94	3.2	34.96

[ab] Column means with the same superscript do not have significant differences (P>0.05)

Statistically significant differences were found between treatments two and three for daily CP intake. This is to be expected as treatment 2 had a 15% lower CP level than the control diet (treatment 1) and treatment 3 had a 15% higher CP level than the control treatment. No statistically significant differences were found between treatments regarding daily OM intake. The reason for this lack of response may be due to the fact that the difference between levels used in this study was not large enough to elicit a statistically significant response. This is in contrast to other studies where it was found that DM and OM intake increase significantly when animals were supplemented with N (Sanson *et al.*, 1990; Freeman *et al.*, 1992; Olson *et al.*, 1999; Dixon *et al.*, 2003). Cheema *et al.* (1991) also found that OMI as well as water intake were increased by protein supplementation.

Several studies have shown that energy supplementation can increase DMI as well as OMI (Phillips *et al.*, 1995; Migwi *et al.*, 2006). This in turn led to increased CP intake in some studies (Migwi *et al.*, 2006). The lack of such results in the present study could be attributed to the small difference in the levels fed during this study.

In a study by Rokomato *et al.* (2006) it was found that energy supplementation had no effect on water intake. This is consistent with the finding of this study where energy had no significant influence on water intake when it was not related to metabolic weight. However when water intake was related to metabolic weight differences between treatment 4 and 5 were significant

Values for water intake, OM, and NDF intake per kg metabolic bodyweight were calculated to allow more accurate comparison of intake data between treatments. Results are given in Table 7.

Table 7 The effect of treatment on water intake per kg metabolic bodyweight, organic matter intake per kg metabolic bodyweight (OMI/kg $W^{0.75}$) and neutral detergent fibre per kg metabolic bodyweight (NDFI/ kg $W^{0.75}$)

Experimental diet	Water intake (ml)/ kg $W^{0.75}$	OMI (g)/ kg $W^{0.75}$	NDFI (g)/ kg $W^{0.75}$
1(Control)	66	44	37
2(NPN - 15%)	61	50	43
3(NPN +15%)	63	45	38
1(Control)	66^{ab}	44	37
4(FME- 15%)	70^{a}	47	40
5(FME+15%)	53^{b}	44	36
Mean	63	46	39
SE	5.59	2.34	1.93

[ab] Column means with the same superscript do not have significant differences (P>0.05)

For this study, OMI/kg $W^{0.75}$ was less than intakes observed by Dixon *et al* (2003) for sheep fed low quality roughage together with isonitrogenous supplements consisting of either a grain-urea mixture, safflower meal or linseed meal.
Water intake/kg $W^{0.75}$ was influenced by treatment. Sheep receiving 15% less FME than maintenance requirement consuming more water/kg $W^{0.75}$ than sheep receiving 15% more FME than maintenance requirement. Devanda (1976), as cited by Godwin & Williams (1984), stated that free water intake as well as urine volume would increase with the addition of urea to sheep diets. In the study of Godwin & Williams (1984) where wethers were infused intraruminally with urea solution containing 0, 5, 10, 15.6 or 20.6g N/day, urine osmolality decreased. This occurred despite increased urea concentration and total osmolar excretion. Godwin & Williams (1984) concluded that increasing urea excretion increased kidney loss of water per unit osmole. In the diet containing 15% less FME than the control, the utilization of urea in the rumen will be less efficient than when maintenance levels of FME is fed, due to less energy being available for microbial protein production. This decreased efficiency of microbial urea utilization would result in more urea being excreted through the kidneys. It is therefore possible that in order to maintain the osmotic balance sheep consumed more water/kg $W^{0.75}$.

The findings of this study are in contrast to those of Nianogo *et al.* (1999) who found that DM, OM and NDF per kg metabolic weight was higher for diets with a high N level. The lack of significant results in this study may be due to the fact that the highest protein level fed was only 15% above the maintenance requirement of a 50kg wether. Higher levels may be needed to elicit a response. However, the findings of this study is in accordance with the findings of Rokomato *et al.* (2006) who fed thirty 4-5 month old lambs concentrate mixtures with varying levels of protein and energy. The five treatments used were: high protein with high energy, high protein with medium energy, high protein with low energy, medium protein with medium energy and low protein with medium energy. In the study of Rokomato *et al.* (2006) varying levels of protein and energy in supplements did not have an effect on DMI/kg $W^{0.75}$ except on the treatment with low protein and medium energy.

When energy supplementation was provided an increase in the digestibility of OM was observed in the study of Pordomingo *et al.* (1991). The increase found in the study of Pordomingo *et al.* (1991) may however be due to substitution effects, as described by Dove & Freer (2002). It was stated that the relative balance between carbohydrates and N in a supplement will determine the effect on NDF digestibility (Heldt *et al.*, 1999a). The digestibility of OM, NDF and CP of the present study is given in Table 8.

Table 8 Effect of experimental diet on organic matter digestibility (OMD), neutral detergent fibre digestibility (NDFD) and crude protein (CP) digestibility

Experimental diet	OM Digestibility	NDF Digestibility	CP Digestibility
1(Control)	0.54	0.55	0.42[a]
2(NPN-15%)	0.59	0.59	0.35[a]
3(NPN+15%)	0.60	0.6	0.57[b]
1(Control)	0.54	0.55	0.42
4(FME-15%)	0.58	0.6	0.45
5(FME+15%)	0.59	0.58	0.48
Mean	0.57	0.58	0.45
SE	0.02	0.024	0.037

[ab] Column means with the same superscript do not have significant differences (P>0.05)

Values for both DMD and OMD in the present study revealed no significant differences. This is in accordance with findings by Köster *et al.* (2002) where steers were fed supplements with different levels of urea (0, 20 and 40%) as part of the RDP in the supplements. The total N levels of these diets varied from 0.4% to 0.8%. Results from the present study is also in accordance with the study done by DelCurto *et al.* (1990) who fed the following levels of N: 1.92%, 4.48%, 6.56% as well as a non-supplemented control, to steers receiving poor quality roughage with a CP value of 2.6%. Differences were found for DMD between supplemented and non-supplemented animals but no difference was found between treatments (DelCurto *et al.*, 1990). No differences were found between supplemented and non-supplemented regarding NDFD (DelCurto *et al.* 1990). The findings are also in accordance with those of Nolte *et al.* (2003), who fed Dohne Merino

wethers a basal diet of wheat straw with supplemental quantities of RDP of 0, 40, 80, 120 and 160 g/day. Effects of treatment on total tract digestion of OM was found to be minimal. The findings of the present study however deviates from findings by Olson *et al.* (1999) who fed steers a poor quality roughage (CP 4.9%) with supplements containing different amounts of starch, 0; 0.15 and 0.3% of initial bodyweight, as well as differing levels of RDP, 0.03; 0.06; 0.09 and 0.12% of initial bodyweight. It was found that increased RDP would increase OMD and addition of starch would reduce OMD (Olson *et al.* 1999).

Even though differences between treatments were not significant, a tendency could be seen for treatment 3 with 15% higher NPN when compared to control, to increase OMD. This is in accordance with Köster *et al.* (1996) who found an increase in OMD and NDFD up to 180g of supplemental RDP after which additional RDP showed only moderate effects on OMD and NDFD. The tendency seen in the present study is also in accordance with findings by Martin *et al.* (1981) who fed wethers a supplement of 0, 5 or 10g of urea, together with 60 or 180g of molasses as an energy source when given free access to poor quality roughage. Increasing urea level at the same level of molasses had a tendency to increase OMD but differences were not significant. In the experiment by Martin *et al.* (1981) it was found that a higher energy level had a tendency to increase OMD at the same level of urea. This is in contrast to findings of the present study where lower levels of FME tended to increase digestibility of OM and NDF.

Values of NDF digestibility of the present study vary much more than for DMD and OMD. Even so, no significant differences could be found between the effects of experimental diets, again in accordance with results observed by Köster *et al.* (2002). Significant differences were found with regards to CP digestibility. Differences were found between control and treatment 3, with treatment 3 having a much higher CP digestibility. This would be expected, as CP was supplemented at a level of 15% higher than control. This additional 15% CP provided by treatment 3 was made up entirely of urea. Therefore the difference in digestibility may have been due to the difference in potential digestibility between forage CP and urea CP. The same difference was found between treatments 2 and 3. This was to be expected as there is a 30% difference between the N level of treatment 2 and 3. The N in treatment 2 was mostly from forage origin. Due to the high level of ADIN of the basal forage, the digestibility of N in the forage will be lower. If there is a higher proportion of soluble N in the diet, CP digestibility will consequently be increased. These findings of increased CP digestibility with higher N concentration of supplement is in accordance with data by Ortigues *et al.* (1988) who fed 12 cross bred wethers a basal diet of fescue hay (7% CP) with four treatments, no supplement, urea supplement, urea plus molasses and urea plus maize supplements. In the study of Ortigues *et al.* (1988) it was found that total diet N digestibility increased when urea was included in supplements. In the review by Holter & Reid (1958) it was also stated that CP digestibility increased with increased CP level of the diet.

Daily average pH and rumen NH_3-N were recorded. Statistical results were based on the daily average for each treatment and given in Table 9.

Table 9 Effect of experimental diet on average daily rumen ammonia N, pH and acetic acid: propionic acid

Experimental diet	NH_3- N (mg/1000ml)	pH	Acetate: propionate
1 (Control)	84[a]	6.58	0.185
2 (NPN -15%)	76[b]	6.59	0.187
3 (NPN +15%)	114[c]	6.58	0.200
1 (Control)	84	6.58	0.185
4 (FME -15%)	94	6.59	0.193
5 (FME +15%)	79	6.59	0.200
Mean	90	6.59	0.193
SE	0.86	0.032	0.0075

[ab] Column means with the same superscript do not have significant differences (P>0.05)

Even though ruminal pH was relatively constant across treatments, in accordance with findings of Ortigues *et al.* (1988) and Heldt *et al.* (1999b), significant differences in Rumen NH_3-N were found. Treatment 3 with +15%NPN had the highest NH_3-N value while treatment 2 with -15%NPN had the lowest level of NH_3-N. This is to be expected when the amount of N in the experimental diets are compared. These findings are in accordance with those of Köster *et al.* (1997) who fed steers low-quality forage together with isonitrogenous supplements varying in urea content from 0, 25, 50, 75 and 100%. Increase in urea content caused increased levels of ruminal NH_3-N. Results of the present study are also in accordance with those of Shain *et al.* (1998) who fed steers a diet of dry rolled maize with urea levels of 0, 0.88, 1.34 and 1.96% of DM. Ruminal NH_3-N concentrations were increased linearly with increase in urea level. Nolte *et al.* (2003) found a linear increase in ruminal NH_3-N levels as RDP level in the supplement increased. Slyter *et al.* (1979) conducted a study to determine the minimum required rumen NH_3-N concentration to maximize microbial growth. Steers were fed a basal diet of 70% concentrate and 30% forage and infused daily with urea solutions containing, 0, 37, 110 or 130g of urea for the first experiment. Levels of urea were adjusted to 18, 65, 120, 140g per animal per day for the second experiment. Slyter *et al.* (1979) concluded that rumen NH_3-N became limiting to microbial population growth below 50mg/L. These findings are supported by Boniface *et al.* (1986) as well as Wanapat (2000). Slyter *et al.* (1979) further reported that microbial growth was maximized at rumen NH_3-N levels between 88mg and 133mg/L. However in the review by Roffler & Satter (1975) it was found that increasing the rumen NH_3-N above 50mg/L had no benefit regarding microbial protein synthesis. In the present study none of the experimental diets resulted in ruminal NH_3-N concentrations below 50mg/L. This could explain the lack of response to different treatments, as all diets were able to provide NH_3-N concentrations promoting optimal microbial protein synthesis (Slyter *et al.* 1979). Is can also be seen that the control diet, which was set according to maintenance requirements (NRC, 2007), resulted in a ruminal NH3-M concentration well above the 50mg/L NH_3-N required for optimal microbial

protein synthesis (Roffler & Satter, 1975; Slyter *et al.*, 1979; Boniface *et al.*, 1986). No significant differences were found for treatments with varying levels of FME, when ruminal NH_3-N are considered. This is in accordance with the findings of Migwi *et al.* (2006) who found that energy had no influence on the ruminal NH_3-N concentration when animals were fed a urea treated mixture of wheaten chaff and barley straw as a basal ration. Infusion with a sucrose solution was done into the rumen or abomasum or both routes. No difference was found in ruminal NH_3-N concentration for infused vs. non-infused animals.

The ratio between the concentration of acetic acid and propionic acid produced, seemed unaffected by experimental diet as no significant differences between diets were found. These findings are in accordance with findings by Köster *et al*. (2002) who found no effect on concentration of acetic acid: propionic acid produced when steers were fed a basal diet of poor quality roughage with supplements of which varying levels of urea (0, 20, 40%) was supplied as supplemental RDP. The same was found by Olson *et al.* (1999) who fed steers poor quality hay with supplements with starch levels of 0, 0.15, and 0.3% of initial bodyweight, as well as DRP levels of 0.03, 0.06, 0.09 and 0.12% of initial bodyweight. Migwi *et al.* (2006) found that supplementation with readily fermentable energy sources increased the acetate: propionate ratio when animals were fed a basal ration of a urea treated mixture of wheaten chaff and barley straw and were infused with a sucrose solution into the rumen, abomasum or both routes. The lack of response in this study may be attributed to the fact that differences between levels of FME were not large enough to elicit a response.

Analyses of VFA production was done to determine the proportional differences between the main VFA concentrations in the rumen and results are given in Table 10.

Table 10 Effect of experimental diet on proportions of volatile fatty acid (VFA) concentration

Treatment	Acetic acid	Propionic acid	Iso-Butyric	Butyric acid	Valeric acid
1 (Control)	78.49	14.50	0.46	5.59	0.56
2 (NPN -15%)	78.64	14.65	0.46	5.81	0.44
3 (NPN +15%)	77.14	15.21	0.45	6.65	0.55
1 (Control)	78.49	14.50	0.46	5.59	0.56
4(FME -15%)	78.95	15.23	0.41	4.90	0.49
5 (FME +15%)	77.60	15.43	0.65	5.57	0.59
Mean	**78.17**	**15.00**	**0.49**	**5.71**	**0.52**
SE	**0.68**	**0.49**	**0.09**	**0.51**	**0.054**

Experimental diet had no significant effect on the proportions of VFA concentrations in the rumen. The same was found in the study done by Ortigues *et al.* (1988) where supplementation had only slight impact on VFA concentrations. From these results it can be inferred that digestion of the basal diet followed the pattern for roughage based diets

(Ortigues *et al.*, 1988) and that the levels used for supplementation in this trial was not large enough to significantly alter VFA concentrations in the rumen, and therefore able to maintain ruminal microbial population relatively stable. There was a tendency for treatment 3 and 5, to lead to higher levels of propionic acid. This is in accordance with findings of Ortigues *et al.* (1988) who fed wethers a control diet of hay alone, or supplements consisting of 0.9% urea, 1% urea plus 6.5% molasses or 1% urea plus 5.2% maize. Ortigues *et al.* (1988) found that both urea and CHO supplementation increased propionic acid proportions in the rumen. Treatment 5 tended to cause an increase in the proportion of iso-butyric acid, this is in accordance with the study of Ortigues *et al.* (1988) who found that higher level of CHO in the supplement tended to increase the proportion of iso-butyric acid produced.

Nitrogen balance for all animals across all treatments were determined using the equation adapted from Morgan & Whittemore, (unpublished) as cited by McDonald *et al.* (2002). Daily intake of N was calculated as the feed N and daily output was calculated as the sum of fecal and urinary N concentration. Average daily N intake was calculated as follows:

- Feed given (DM) x CP value of feed – Orts (DM) x CP value of orts
  = Daily CP intake from feed.
  (2098.508 x 3.18%) – (1246.509 x 3.69%) = 20.734g CP from feed

- Amount of urea in supplement x N value of urea = Daily N from supplement
  15.5g x 2.9 = 44.95g CP from supplement

- Average daily N intake = N form feed + N from supplement
  20.734 + 44.95 = 65.684g CP intake

Average daily N output was calculated as follows:

- Average daily faecal weight x CP % of faeces
  421.43g x 7.269% = 30.634g N from faeces

- Average daily urinary output x CP % of urine
  14780 mL x 0.0875 = 12.93 g CP output from urine

- Average daily N output = N form faeces + N from urine
  30.63 + 12.93 = 43.56 g CP output.

- Therefore: N in – N out
  65.684 – 43.56 = 22.124 g CP

In order to determine the N balance, the value obtained for the CP balances was divided by the factor 6.25. Results of statistical analyses are given in Table 11.

Table 11 Effect of experimental diet on nitrogen balance and nitrogen balance/kg metabolic weight

Experimental diet	N Balance(g/day)	N Balance (mg N/kg $W^{0.75}$)
1 (Control)	3.11[a]	174.33[a]
2 (NPN -15%)	2.70[a]	152.83[b]
3 (NPN +15%)	4.94[b]	289.03[c]
1 (Control)	3.11	174.33
4 (FME -15%)	4.27	231.22
5 (FME +15%)	3.06	208.6
Mean	**4.08**	**211.20**
SE	**0.54**	**24.14**

[ab] Column means with the same superscript do not have significant differences (P>0.05)

Statistical analyses of the data revealed that treatment 3 had a significantly higher N balance than both control and treatment 2. The difference between treatment 2 and 3 is to be expected as treatment 3 contained a NPN level 30% higher than that of treatment 2. The higher N balance could also be related to the rumen NH_3-N level of treatment 3 being well above that of treatment 2. Therefore it is possible that more N was available for synthesis of microbial protein and increased efficiency of N recycling via saliva, leading to higher N utilization by the animal.
Both treatment 4 and 5 had no significant differences when compared to the control. In the review of Johnson (1976) it was indicated that high energy rations will support greater N balance than rations with lower energy levels. The same was found by Fluharty *et al.* (1999) who fed sheep either a lucern or concentrate diet with or without added ionophores. Sheep on the all concentrated diet showed higher N balance than sheep fed the lucern diet. It is possible that the difference in levels of FME used in this study was not sufficient to cause a higher N balance even though it would seem as if both treatment 4 and 5 tended to have higher N balance than control treatment.

All the values for N balance obtained was higher than reported by some authors in literature for sheep on urea based supplements (Ammerman *et al.*, 1972; Bird, 1974; Chikagwa-Malunga *et al.*, 2000; Currier *et al.*, 2004), but corresponds to findings by Marini *et al.* (2004) who fed sheep a pelleted diet with N concentrations of 15.6, 28.7 and 40.5 g/kg DM, and reported N balance values of 1.5, 5.1 and 4.4 gN/day respectively. The higher N balance values found in the present study may be due to the fact that concentrations of ruminal NH_3-N was above the 50 mg/L required for optimal microbial protein synthesis (Roffler & Satter, 1975; Slyter *et al.*, 1979, Boniface *et al.*, 1986; Wanapat, 2000). Enough N was therefore available for utilization by microbes and no N deficiency was found.

Statistical analyses regarding N balance/kg $BW^{0.75}$ revealed that treatment 3 had a higher N balance/kg $BW^{0.75}$ when compared to treatment 1 and 2. This is most likely due to the higher percentage of NPN supplied in this treatment when compared to control and the diet with -15% NPN.
The lack of significant differences between experimental diets where varying levels of FME were fed leads to the assumption that differences in the energy values of these diets were not large enough to cause an increased N balance (Johnson, 1976; Fluharty *et al.* 1999). From the lack of results it would seem as if N balance per kg $W^{0.75}$ is not solely dependent on energy level of the diet but that several other factors may play a role in increasing N balance/kg$W^{0.75}$.

The microbial protein production for each sheep during each treatment was determined by using the calculations of Chen & Gomes (1995) using the purine derivatives in the urine collected during the sampling period. The results are given in Table 12.

Table 12 Effect of experimental diet on Microbial protein synthesis

Experimental diet	Microbial protein synthesis(g/day)
1(Control)	13.52
2(NPN -15%)	13.80
3(NPN +15%)	9.54
1(Control)	13.52
4(FME -15%)	7.07
5(FME +15%)	14.39
Mean	**11.66**
SE	**2.11**

From the results in Table 12 it can be concluded that levels of NPN and FME used in experimental diet in this study were not sufficient to create expected differences in microbial protein synthesis. This could be due to the fact that all experimental diets led to ruminal NH_3-N concentrations above 50mg/L. the method used to determine microbial protein synthesis (Chen & Gomes, 1995) is an indirect method and therefore opportunities exist for miscalculation of data. Differences in N balance found were not reflected in the microbial protein synthesis. This could be due to higher levels of NH_3-N in treatment 3 in which N was retained. Other studies in literature have found a correlation between DOMI and microbial protein synthesis in the rumen (Cole *et al.* 1976; Chen *et al.*, 1992). The lack of significant response regarding microbial protein synthesis in this study may be due to the small differences in levels of FME used in this study as well as the fact that forage OMI (Table 6) and OMD (Table 7) was unaffected by levels of FME and NPN used in this study. Although not significant, a tendency for increased microbial protein production for sheep on treatment 5 was observed. This may be due to higher amounts of FME available to microbes for assimilation of NH_3-N into microbial protein. Panjaitan (2008)

found that if RDP supply is sufficient, other nutrients such as fatty acids, nucleic acids, vitamins, minerals and true protein is required to maximize efficiency of microbial protein synthesis.

For the *in situ* trial no differences were found between the soluble fraction (a), insoluble potentially degradable fraction (b), potential degradability (PD), or effective degradability (ED) of DM between experimental diets. Results are given in Table 13.

However, a difference was found in the rate of degradability (c) between treatment 2 (NPN -15%) and treatment 3 (NPN+15%) with treatment 3 having a higher rate of degradability than treatment 2. These results are in accordance with findings by Elizalde *et al.* (1999) who determined rumen degradability parameters for steers fed lucern hay at different stages of harvesting, resulting in differing levels of N in the diets. In the study of Elizalde *et al.* (1999) rate of degradability was increased by higher levels of N in the diet. The increased rate of degradability for experimental diet 3 may be due to increased microbial fermentation activity even though no significant increase in OMD was found (Table 8).

Table 13 Effect of experimental diet on ruminal DM degradability parameters

	a Value	b Value	c Value	ED Value	PD Value
1 (Control)	4.83	36.62	0.013[ab]	24.1	41.46
2 (NPN -15%)	5.61	46.56	0.007[a]	25.88	52.17
3 (NPN +15%)	3.07	36.00	0.017[b]	24.85	43.07
1 (Control)	4.83	36.62	0.013	24.1	41.46
4 (FME -15%)	5.9	51.27	0.008	26.79	57.17
5 (FME+ 15%)	7.09	35.46	0.010	24.35	42.55

[ab] Column means with the same superscript do not have significant differences (P>0.05)

The results from the present study regarding DM disappearance, is in accordance with those of Gilbery *et al.* (2006) who fed steers a basal diet of poor quality forage (CP 3.25%) supplemented with varying levels of maize distillers solubles at 0, 5, 10 and 15%. No difference was found in DM disappearance across different supplemental levels. The same was found by Bargo *et al.* (2001) regarding protein supplementation. In cows grazing winter oats supplemented with either low protein sunflower meal, high protein sunflower meal, or high protein feather meal, no difference was found in DM disappearance from the rumen between different supplements.

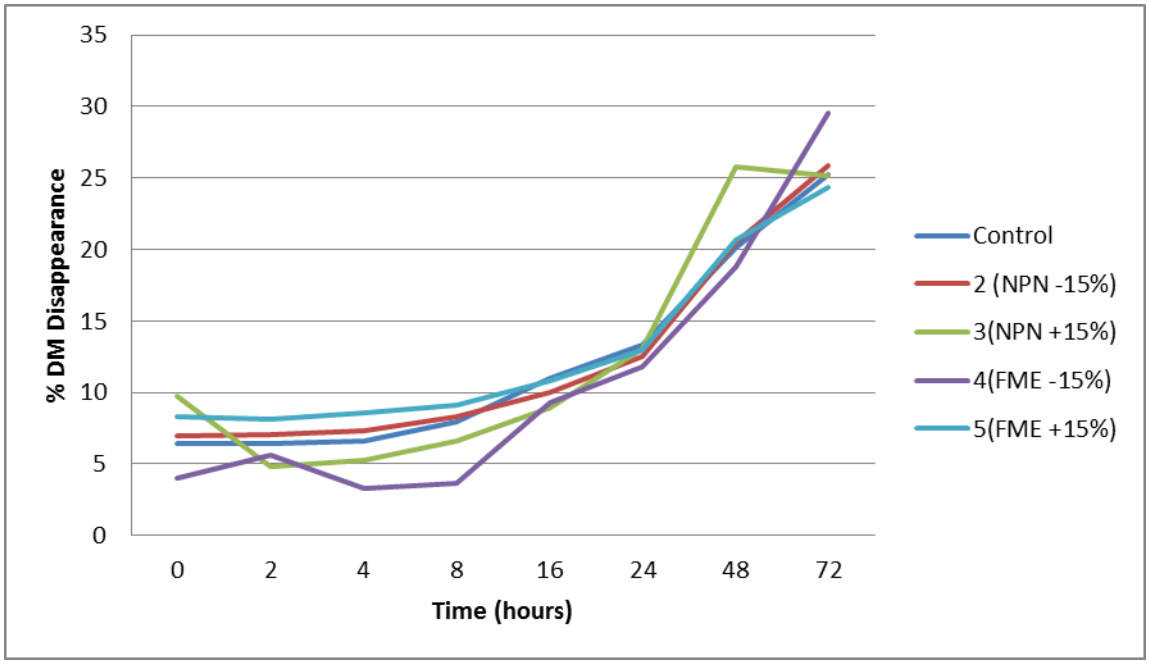

Figure 1 Effect of experimental diet on forage dry matter disappearance over time

From the data represented in Figure 1 it is clear that a lag time was experienced by sheep on treatment 4. This could have been caused by the lack of sufficient FME available to microbes for rapid assimilation of N into microbial protein and subsequent increase in microbial population, as there was a tendency for treatment 4 to support lower microbial protein synthesis. In the review by Varga (1986) it was stated that multiple factors may affect lag time in vivo, under which both microbial count as well as the ionic composition of the rumen fluid is mentioned. Treatment 4 had a markedly higher water intake/kg $W^{0.75}$, which could have led to subsequent changes in the osmotic balance of the rumen fluid.

For *in situ* NDF disappearance no difference was found in the insoluble potentially degradable fraction (b), effective degradability (ED) or potentially degradability (PD). Results are shown in Table 14. Differences were found in the soluble fraction (a) between treatments 2 and 3. As well as the rate of degradability between treatments 2 and 3, with treatment 3 having a much lower solubility but a higher rate of degradability. This is in contrast to the findings of García *et al*. (1995) who supplemented grazing sheep with barley grain with or without urea, and found that supplementation had no influence on the rate of degradation or the effective degradability. The lower solubility of treatment 3 cannot be explained and it most probably due to experimental error in the laboratory analyses. The increased rate of degradation may be due to the fact that rumen microbial activity was increased due to high levels of NH_3-N in the rumen (Table 9). Even though no effect was seen on total tract NDF digestibility (Table 8) where a trend for higher digestibility was observed for NDF but differences between treatments were not significant.

No differences were found when experimental diets with varying levels of FME was compared. This is in contrast to a study done by De Visser *et al.* (1998) who fed lactating cows either early cut or late cut grass silage, with or without 4kg/day of supplemental flaked maize starch. Rate of NDF degradation was decreased with increase in supplemental starch.

De Visser *et al.* (1998) concluded that OM degradability was reduced by supplemental starch and that the extent of the decrease was related to NDF maturity of the forage. The lack of difference between experimental diets in the present study may be due to the fact that differences in levels used were not sufficient to elicit a response in DM as well as NDF degradability.

Table 14 Effect of experimental diet on ruminal neutral detergent fibre degradability parameters

	a Value	b Value	c Value	ED Value	PD Value
1 (Control)	4.16[ab]	37.29	0.016[ab]	23.77	41.46
2 (NPN -15%)	5.07[a]	47.91	0.011[a]	26.51	52.97
3 (NPN +15%)	1.9[b]	34.51	0.022[b]	24.96	36.40
1 (Control)	4.16	37.29	0.016	23.77	41.46
4 (FME -15%)	5.06	52.69	0.012	32.21	57.75
5 (FME+ 15%)	6.18	41.30	0.010	25.65	47.49

[ab] Column means with the same superscript do not have significant differences (P>0.05)

Results for NDF disappearance in this study corresponds with those of Boucher *et al.* (2007) who supplemented the basal silage plus concentrate diet of lactating cows with 0, 0.3, 0.6, 0.9% urea in diet DM. No effect was found on NDF disappearance between treatments. The lack of response may be due to the fact that all experimental diets resulted in ruminal NH_3-N levels above 50mg/L. Slyter *et al.* (1979) found the minimum concentration of ruminal NH_3-N, below which microbial population growth was restricted, to be 50mg/L. Result are given in Figure 2.

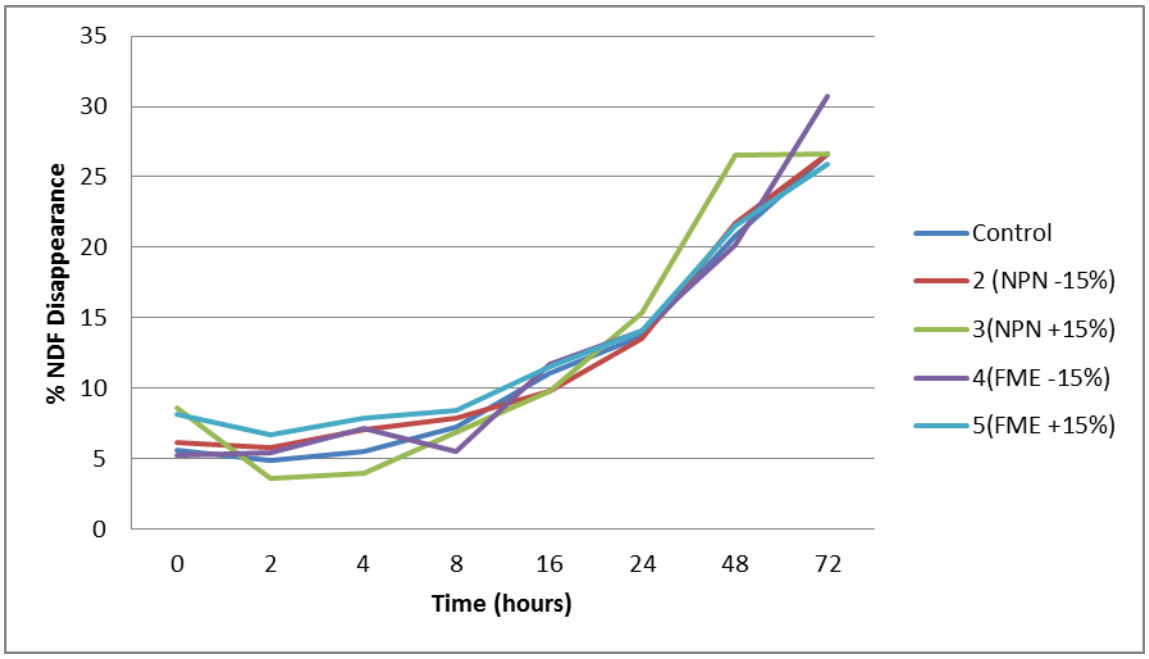

Figure 2 Effect of experimental diet on neutral detergent fibre disappearance over time

CHAPTER 5: CONCLUSION

From the results of this study regarding DM, OM and water intake it can be suggested that the levels of NPN and FME used, had no influence on intakes. No benefit resulted from feeding supplements with higher NPN or FME than maintenance requirements as stated by the NRC (2007). It is interesting to note that supplements with levels of NPN and FME below the maintenance requirement (NRC, 2007) did not have a negative influence on intake of DM, OM and water. It can be suggested that should levels of FME and N in commercial supplements be correctly balanced, performance may be maintained at the same level at lower levels of FME and N, as when maintenance levels (NRC, 2007) are used.

Intake of CP was influenced by higher levels of NPN in the supplement as would be expected. However the increase in CP intake did not lead to an increase of DM and OM intake above that of maintenance. It would therefore seem that maintenance requirements can be met by feeding maintenance levels and that no additional benefit is derived from feeding slightly higher levels of NPN together with the maintenance requirement for FME. It can therefore be concluded that the increase in CP intake when +15% NPN was fed did not result in any benefit regarding OMI and NDFI , even though N balance as well as ruminal NH_3-N was increased for this treatment. The higher N balance can be seen as an advantage as the higher N levels retained in the body is available for meeting maintenance requirements. In this case N deficiency for sufficient microbial protein synthesis will be less likely. The lack of increased intake of DM and OM may be due to the fact that microbial protein synthesized was not influenced significantly by the higher NPN level.

When intake in relation to metabolic weight was investigated, water intake per $kgBW^{0.75}$ was significantly higher for treatment with decreased amounts of FME as compared to treatments with increased amounts of FME for isonitrogenous treatments. When water intake related to metabolic weight was considered for treatments with varying levels of CP, no effect could be found. The NDF intake per $kgBW^{0.75}$ was not influenced by treatment when isonitrogenous or isoenergetic treatments were compared. There seems to be no benefit in supplying animals with the levels used in this study above that considered as maintenance by the NRC (2007).

Levels of FME and NPN in this study were not sufficient to have an influence on OM digestibility or NDF digestibility above that which is needed for maintenance. CP digestibility was however impacted by the different supplements with treatments containing higher amounts of NPN having a higher CP digestibility. Treatments with higher FME did not have a higher CP digestibility than treatments with lower levels of FME. Increased energy supply to rumen microbes therefore seemed to have no influence on digestibility. It can be concluded that at the levels used in this study, only an increase in N concentration at a certain FME level could lead to increased CP digestibility. However, the increase in CP digestibility as well as the increase in N balance at a higher level of NPN and at a certain level of FME, is the only benefit derived from the higher levels of NPN used in this study. The anticipated increase in OMD and NDFD did not result for the levels used in this particular study.

Ruminal NH_3-N was significantly influenced by higher levels of NPN. This is to be expected as urea is rapidly broken down to NH_3-N in the rumen environment. When

higher levels of FME was supplied, the NH_3-N was reduced, although only numerically and not significantly. All experimental diets in this study led to ruminal NH_3-N levels above 50mg/L, which was found by Slyter *et al.* (1979) to be the level below which microbial population growth was reduced. It can be assumed that all levels of supplementation used in this study were able to provide adequate rumen NH_3-N levels for microbial population growth and the further increase in NPN supplementation in this study, above the control level did not result in any additional benefit.

Treatment had no effect on proportions of VFA's produced. It would therefore seem the higher amount of FME above maintenance, and even at 15% less than maintenance FME, supplied in this study had no influence on rumen microbial population. This is possibly due to the fact that rumen NH_3 levels for all experimental diets used in this study resulted in rumen NH_3-N levels supporting optimal microbial protein synthesis. It would appear that if maintenance requirements for NPN and FME as well as vitamins and minerals are met, no additional benefit is derived by higher levels of FME or NPN. Rather if supplements are well balanced with regards to levels of both NPN, FME and other relevant nutrients, levels slightly below maintenance, are unlikely to have a negative influence on animal performance.

The N balance was improved for treatments receiving more NPN for levels used in this study. FME had no influence on N balance whether at a higher or lower level than maintenance, for the levels used in the present study. It would seem as if increased N balance can be obtained by simply increasing the N fraction of a supplement. However the increase in N balance did not lead to increased digestibility of OM or NDF, or to increased microbial protein synthesis. Higher levels of NPN may have a cost implication in commercial situations and the decision to increase NPN levels of supplements will be an economic one.

In situ DM and NDF disappearance was not influenced by levels of NPN and FME used in this study. Rate of degradation was increased by higher levels of NPN, but this did not lead to increases in disappearance of DM and NDF. It can be concluded that in commercial situations using higher levels, such as those used in this study, of both NPN and FME will not lead to an increase in animal performance or gain for the mutton producer.

CHAPTER 6: CRITICAL EVALUATION

Some aspects of this trial were flawed from the start and experimental procedures could have been different in order to obtain more reliable results.

More research should have been done on the method of administering the supplements into the rumen before commencing the experimental period. At the start the supplements were infused via inflexible plastic tubing inserted into the cannula. This method led to prolonged infusion times as well as to minor spillage of supplements. At a later stage it was decided to measure the supplements into rumen degradable paper bag and to insert the bags containing the supplement directly into the rumen. The troublesome infusion of treatments experienced at the start of the experimental period, could have caused unreliable responses in data obtained.

Levels of N in the blood should have been recorded as well in order to give a clearer picture on the N balance of the animals, as well as the changes in circulating levels of N between different treatments.

If rumen pH change over time after administration of supplements had been recorded an indication of the fermentation rate could have been obtained as well as an indication of peak times of fibre fermentation. The possible occurrence of pH below 6.2, at which fibre fermentation would be hampered could have been detected (Russell *et al.*, 1999).

In situ experiments were done with one animal with duplicate sample bags, as opposed to the NRC (1984) method of two animals with one replication per animal. This was done because only one animal was on a specific treatment during each replication. To overcome this, an additional *in situ* trial could have been conducted after the five replications when more animals were available for a single treatment.

The sulphur content of the forage should have been analysed prior to the trial to determine a deficiency. Should a deficiency have been detected, the sulphur fraction should have been included in the vitamin and mineral premix supplied to the animals together with the supplements, as the weighing of such small quantities was troublesome.

Initially daily urine samples were collected for each animal on each treatment (Chen & Gomes, 1995). This would have enabled monitoring of daily variation in purine derivative excretion. Due to financial restraints as well as problems with analytic capability it was decided to pool these samples for each animal across treatments.

Experimental animals were not optimal as rumen cannulas tended to leak and great care had to be taken to ensure that ruminal fluid did not contaminated urine samples. Cannulas were bound with bandages, cotton wool and gauze, in order to eliminate the leaking of ruminal fluid. Investigation could have been done to do sampling through the suction strainer technique as described by Raun and Burroughs (1962), as this would have eliminated the frequent opening of cannulas.

No animal performance measurements were done in this study, should the data be used in the formulation of commercial supplements, studies regarding animal performance on various treatments would have to be done in addition to the trial already conducted.

The supplemental levels in this study were based on a percentage value, either above or below maintenance. It might have been worthwhile to consider basing treatment levels on a percentage of bodyweight as this was done more frequently by other researchers (Olson *et al.*, 1999). However concern regarding rumen stasis existed should treatments below maintenance not be supportive of such low levels of supplementation. This study did not contain a negative control receiving no supplement as it has already been demonstrated that animals grazing poor quality roughage will lose up to 30% of live weight. Further fears regarding the health and viability of animals receiving poor quality roughage with no supplement, prevented the inclusion of a negative control in this particular study.

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