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Effects of intermittent feed supplementation on certain rumen parameters and intake of sheep fed a medium quality roughage

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DECLARATION

I, Marli van Eeden declare that this dissertation, for the degree Msc. (Agric) Animal Science Animal Nutrition at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Marli van Eeden

SIGNATURE: ……

DATE :

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I herewith thank Lindeque Du Toit for all the help and kindness he has bestowed upon me. I specially want to thank Herman Mynhardt for assisting in the calculations pertaining to the microbial protein production. My family and friends who stood behind me in difficult times, I appreciate and thank you for your time and love. Roelf Coertze, thank you for the hard work on my statistics and always making time for me.

Last but not least, I want to thank my promoter, Prof W.A van Niekerk for giving me the opportunity to form part of his legacy.

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LIST OF ABBREVIATIONS

AA – Amino acids
ADF – Acid detergent fibre
ADG – Average daily gain
ATP – Adenosine triphosphate
$BM^{0.75}$ – Metabolic body weight
BW – Body weight
CNS – Central nervous system
CO_2 – Carbon dioxide
CP – Crude protein
CPS – Critical particle size
DE – Digestible energy
DIP – Degradable intake protein
DMI – Dry matter intake
DOM – Digestible organic matter
ED – Effective degradability
H_2SO_4 – Sulfuric acid
H_3PO_4 – Phosphoric acid
Kg – Kilogram
LAG – Discrete lag
ME – Metabolisable energy
Mg/l – Milligram per liter
MJ – Mega joule
ml – Milliliter
Mmol/d – Millimol per day
Mmol/l – Millimol per liter
MPR – Microbial passage rate
MRT – Mean retention time
MTD – Minimal total discomfort
N – Nitrogen
NDF – Neutral detergent fibre
NDFI – Neutral detergent fibre intake
NE – Netto energy
NGGR – Non glucogenic to glucogenic ratio
NH_3 - Ammonia
NH_3-N – Ammonia nitrogen
NH_4 – ammonium
Nmic – Intestinal flow of microbial nitrogen
NMICR – Relative production of microbial nitrogen in the rumen
NPN – Non-protein nitrogen
OM – Organic matter
RAN – Rumen ammonia nitrogen
RDP – Rumen degradable protein
RFC – Readily fermentable carbohydrates
RNA – Ribonucleic acid
RNB – Relative nitrogen balance in the rumen
ROO – Reticulo omasal orifice
RR – Reticulo rumen
RUP – Rumen undegradable protein
SA – South Africa
UIP – Undegradable intake protein
VFA(s) – Volatile fatty acid(s)

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SUMMARY

EFFECTS OF INTERMITTENT FEED SUPPLEMENTATION ON CERTAIN RUMEN PARAMETERS AND INTAKE OF SHEEP FED A MEDIUM QUALITY ROUGHAGE

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This work aimed to estimate the effect of intermittent feeding of a supplement to sheep on certain rumen parameters, intake and total tract apparent digestibility as well as nitrogen retention under typical practical conditions where sheep received a fixed amount of supplement. This study focused on the time effect per week rather than the amount of supplement given per week. The reason for evaluating the time effect in term of intermittent supplementation rather than the amount given is because, under typical farming conditions sheep most probably receive a limited amount of supplements, either on a daily basis or every 2nd or 3rd day or once a week basis. The intake and total apparent tract digestibility of DM, OM, NDF, ADF and CP as well as the measurement of rumen pH, RAN, VFA's, microbial protein production and nitrogen balance were assessed in a 4x4 Latin square experiment. Four Merino wethers (52 ± 1kg BW) received medium quality *Eragrostis curvula* hay (Weeping love-grass) as the basal diet and were supplemented with Voermol production lick at 250g per sheep every day, 250g per sheep every 2nd day, 250g per sheep every 3rd day or 250g per sheep once a week. The CP contents in the diet ranged from 6.67 g of DM/kg $BW^{0.75}$ to 9.57 g of DM/kg $BW^{0.75}$. It was observed that there was no difference between the treatments in terms of DM, OM, NDF and ADF intake as well as for the total tract apparent digestibility. The rumen pH showed no difference between treatments and was within range (6.3 to 6.8) normally sufficient to support adequate NDF and ADF digestion, while there was a tendency for the NGGR and acetic- to propionic-acid ratio to increase as the supplementation frequency decreased. There was also a tendency for N retention and RAN to increase when the supplementation frequency increased, although the average RAN

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concentrations of the sheep receiving supplementation everyday, every 2nd day, every 3rd day and once a week, fell beneath the required concentration (8mg/100ml) needed for efficient microbial fermentation. Supplementing sheep every 2nd day, every 3rd day and once a week resulted in a negative efficiency of nitrogen utilization, while supplementing everyday had a positive efficiency of nitrogen utilization.

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CHAPTER 1

GENERAL INTRODUCTION AND MOTIVATION

Feeding livestock has become a challenge due to climate changes and the increase in feedstuff prices. Alternative feed recourses are used in situations where ruminant animals are unable to attain sufficient high quality forage to support production (Salem, 2010). The ever-growing concern around growth promoting technology being introduced into animal production systems with the aim of improving the cost-efficiency for animal growth, has encouraged scientists to look for other alternatives, not only pertaining to feedstuffs but also including modified supplementary and feeding practices that increase efficiency to some extent and also improves the environmental impact. Arid to semi-arid regions struggle to perpetuate year round feed recourses and with the cost increase relating to agriculture with regards to transport and petroleum, the livestock sector is being threatened (Salem, 2010).

Sheep farming is practiced throughout the whole of South Africa but is predominantly more focused in the arid areas like the Northern Cape, Western Cape, Eastern Cape, Free state and Mpumalanga (DAFF, 2012). All 9 provinces together represent an estimated total of 24.6 million sheep, the Eastern Cape with approximately 29%, Northern Cape with 25%, Free State with 20%, Western Cape with 12% and Mpumalanga with 7% of the sheep population (Meissner *et al.*, 2013). The remaining 14% of the sheep population is distributed across Kwazulu-Natal, North-West, Limpopo and Gauteng (DAFF, 2012). The average gross production value from 2002 to 2012 was around R 3.4 billion per annum and the gross value of mutton production increased continuously during that time. In South Africa the demand for mutton is increasing as the human population keeps growing and the economical status of the average person improves. South Africa is currently a net importer of mutton products and this is exaggerated by the recent increase in predation and stock theft, leading to a decrease in sheep numbers (DAFF, 2012; Meissner *et al.*, 2013). The balance between demand and supply determines the inflation of mutton, as seen in 2002 where the price on the farm was R 15.22/kg and increased tremendously to R 40.48/kg in 2011 (DAFF, 2012).

The main sheep production areas in South Africa consist of sweetveld and are known for their low abundance but high quality of vegetation (DAFF, 2012). Along the coastal areas of Vredendal, Citrusdal, Capetown, Bredasdorp, George, Port Elizabeth and all the way east to Umtata the vegetation resembles that of mixed- and sourveld, which is of high abundance and low quality (Tainton, 1999). In all these different veld types, supplementation would be used in accordance to the goals that the farmer has set for his/ her enterprise. Supplementation is any form of feedstuff given to animals with

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the goal of alleviating a deficiency and also has the added benefit of improving feed utilization while being used as a production enhancer (Doyle, 1987). The arid areas of South Africa primarily make use of extensive farming systems and thus when supplementing sheep on an extensive system, the questions most frequently on the minds of many are whether infrequent supplementation holds any disadvantage towards production and what duration of time sheep can go without supplementation while maximizing cost efficiency. Thus the main objectives of this dissertation were to examine the effect that intermittent supplementation of a production lick has on rumen fermentation and feed intake of sheep based on an extensive system.

Little experimental work has been focused on the duration of feeding intervals until recently. In a study conducted by McIlvain and Shoop (1962) with Hereford steers supplemented with cottonseed cake either daily or every 3rd day, it was noted that the winter and summer gain of the two groups of animals were not significantly different. The size and complexity of the rumen in cattle may have played a key roll in the outcome of this study, as it takes approximately 4 to 7 days for residues to pass from the rumen. In South Africa a study was conducted by Meaker and Liebenberg (1984) with Afrikaner x Sussex cattle, where one group received a protein supplement *ad libitum* and the other group received the protein supplement from the Monday to the Thursday (4 days) and then had no lick from the Friday to the Sunday (3 days). The results showed no signs of over consumption from the groups of cattle receiving intermittent supplementation, although there was a slight weight difference between the cattle, favoring the *ad libitum* feeding. The intermittent supplementation did show a cost saving facet and had no detrimental effect on the cattle, in fact the calves had an improved corrected 205-day weaning mass and the cows had better reconception rates (Meaker and Liebenberg, 1984).

Brundyn *et al.* (2005) conducted a study to determine what the effect of the frequency of supplementary feeding would have on the production of SA mutton Merino ewes during late pregnancy and early lactation, while grazing on wheat stubble. Two groups of ewes received 200 g production lick per ewe daily, two groups received 400 g production lick per ewe every 2nd day, two groups 600 g production lick per ewe every 3rd day and two control groups received no production lick. The results indicated that there were no significant differences between the body weights of the groups that received supplementation every day and every 2nd day. The lambing percentage was higher for the ewes receiving supplementation every day and every 3rd day, as compared to the groups being supplemented every 2nd day. The aforementioned data showed that on an economical point of view sheep could be supplemented every 3rd day, as this will also decrease labour and transportation costs. Taking previous research studies into account brings us to the aim and purpose of this study that elaborates on the idea of supplementing sheep intermittently and is predominantly focused on what happens in the rumen when receiving a production lick either at 250g per sheep every day, 250g

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per sheep every 2nd day, 250g per sheep every 3rd day or 250g per sheep once a week. This current study differs from those studies done by McIlvain and Shoop (1962), Meaker and Liebenberg (1984) and Brundyn *et al.* (2005), as mentioned previously. In the current study the weekly amount changes between treatments, whereas McIlvain and Shoop (1962), Meaker and Liebenberg (1984) and Brundyn *et al.* (2005) increased the amount of supplement given when the frequency of supplementation decreased. The reason why, in the current study, it was decided not to increase the amount of supplement given when the frequency of supplementation decreased per treatment was based on what happens in practice. When supplementing intermittently farmers don't usually increase the amount of supplement given in order to add up to a recommended amount per week and this is the reason why this current study was conducted differently to previous studies done.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Animal production and growth, whether it is in correspondence to monogastric or ruminant nutrition, start with the intake of feedstuffs (Weston and Poppi, 1987; Romney and Gill, 2000). This topic seems relatively straightforward in everyday conversation but when looking at intake on a scientific and more detailed frame of reference it becomes more intricate as an amalgamation of factors influence the outcome thereof (Poppi *et al.*, 1994). The two-phase hypotheses and optimization theory is generally used to describe and explain certain aspects of intake in relation to animal response (Garnsworthy and Cole, 1990; Poppi *et al.*, 1994; Forbes, 2005). Ruminant animals not only have physical constraints like rumen fill, but also have digestive and metabolic constraints where the former is in connection with the microbial population and the latter with nutrient supply and supplementation practices (Garnsworthy and Cole, 1990; Forbes, 2005).

The interrelationship between microbial action, degradation and digestibility form part of a complex array of metabolic reactions, which in turn are influenced by the type and quantity of forage, as well as supplementation (Akin, 1986; Chilibroste *et al.*, 2005). There are two other supplementary responses other than supplementation itself namely, complementation and substitution, which is prompt by the nutritional characteristics of the pasture and the production status of the animal (Dove, 2002). When looking at the aforementioned principles it can be comprehended that everything forms an integrated system that functions together in order to sustain animal production (Ellis *et al.*, 1999). Investigating rumen function on grounds of fermentation and kinetics reveals an interesting insight

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into how different feedstuffs and supplementary practices influence nutrient availability, uptake and distribution (Owens and Goetsch, 1986; Ulyatt *et al.*, 1986; Ellis *et al.*, 1999; Kennedy, 2005; Theodorou and France, 2005) . The most spectacular phenomenon found in ruminant physiology is the animals' capability of forming a symbiotic relationship with microbes that are harbored in the rumen (Hungate, 1966; Weston, 2002).

2.2 INTAKE

Many biotic and/or abiotic factors prevent ruminants from satisfying their nutritional requirements. Voluntary intake consists out of two facets, one being short term that covers individual meals and its patterns and secondly, long term that involves intake over a period of at least 24hours (Forbes, 2005). The remaining question is whether meals are initiated at random while controlling its size or weather the interval between meals is controlled by the size of the previous meal. In general it has been concluded that feeding behavior is predominantly determined by satiety rather than hunger mechanisms (Forbes, 2005).

Intake models and predictions are based on two quantitative theories

1. Two- phase hypotheses*:* Ruminants eat until their energy requirements are met unless a constraining factor like rumen fill or heat dissipation prevents them. This hypotheses is based on the relationship between feed intake and feed composition (Garnsworthy and Cole, 1990; Forbes, 2005).
2. Optimization hypotheses: An amalgamation of factors affect intake simultaneously and this leads to a process of optimization rather than the elimination underlying feed intake (Ketelaars and Tolkamp, 1992a; Ketelaars and Tolkamp, 1992b; Romney and Gill, 2000).

Closer investigation has shown that the intake of highly fibrous feedstuffs are positively correlated to digestible energy concentration up to 12MJ DE/kg DM, while beyond this energy level the relationship is an inverse of each other. The aforementioned relationship can be explained in terms of the limitation that fibre digestion has on intake when microbial fermentation is not optimal due to an under supply of energy (Forbes, 2005). Poppi *et al.* (1994) had a more complex approach and proposed metabolic constraints like genetic limits to protein deposition and ATP degradation. Here the idea was that a build-up of AA due to an oversupply and low ability to utilize protein will constrain feed intake and the second speculation was that a protein deficiency would cause an ATP accumulation and this would be an indication of an inefficient metabolism. The overall trend seen with

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intake is that it is positively correlated to body weight although animals that are overweight have a lower intake compared to their leaner counterparts (Weston and Poppi, 1987; Forbes, 2005).

Optimization theories

These theories are aimed at the cost-benefit relationship in controlling feed intake.

Efficiency of oxygen utilization for NE production

Emmans and Kyriazakis (1995) disproved the theory that animals eat to obtain maximum yield of NE per unit of oxygen consumed, thus eating for benefit while avoiding the negative aspects of eating (Ketelaars and Tolkamp, 1992a; Ketelaars and Tolkamp, 1992b; Romney and Gill, 2000). Further research done by Whittemore *et al.* (2001) supports these findings that optimization is not the main determinant of intake but is controlled by requirements unless other factors intervened (Forbes, 2005).

Minimal total 'discomfort'

With either an excess or deficiency of nutrients and energy the animal will be driven to re-address any metabolic imbalance and reduce discomfort (Garnsworthy and Cole, 1990). Discomfort can be generated by factors other than nutrients, like the inability to dissipate heat due to environmental factors and eating constraints (Romney and Gill, 2000; Forbes, 2005). To express the mismatch between supply and demand a calculation can be made at different intake levels to find the minimum total discomfort (MTD) (Forbes, 2005).

Calculations:

1. *MTD regarding nutrients* (Forbes, 2005)

A sheep eats 1kg of forage per day and needs 0.25 kg CP/day but the forage only contains 0.10kg CP/kg DM.

$$CP\ intake/day = CP\ needed - (CP\ in\ forage \times intake)$$

$$MTD = \frac{Actual\ CP\ intake}{Needed\ CP\ intake}$$

$$CP\ intake/day = 0.25 - (0.10 \times 1) = 0.15\ kg\ CP/day$$

$$MTD = \frac{0.15}{0.25} = 0.6$$

$$= 0.6^2$$

$$= 0.36$$

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To remove a negative value obtained and to highlight the large deviations the square root of the discomfort is used.

2. *MTD regarding time* (Forbes, 2005)

A sheep eats 0.015 kg DM/minute and needs to consume 1 kg DM/day

$$DM\ intake/day = DM\ intake\ per\ min \times 60min$$

$$MTD = \frac{Needed\ DM\ intake - Actual\ DM\ intake}{Needed\ DM\ intake}$$

$$DM\ intake/day = 0.015kg \times 60min$$

$$MTD = \frac{1kg - 0.9\ kg}{1kg}$$

$$= 0.1^2$$

$$= 0.01$$

Intake, time of grazing and feed properties can be manipulated to minimize animal discomfort, as there is no single mechanism that controls intake and many signals act in unison to regulate intake and diet selection (Romney and Gill, 2000). Gut fill restricts intake up to a breakpoint in digestibility, after this point the animals energy requirements control intake as the relationship between intake and digestibility becomes negative (Weston, 2002).

Figure 2.2.1 describes the relationship between ME, CP and NDF, intake. When digestibility decreased and nutrient requirements were restricted due to rumen fill and the time spent eating, the total relative discomfort increased, as Weston (2002) also pointed out previously (Forbes, 2005).

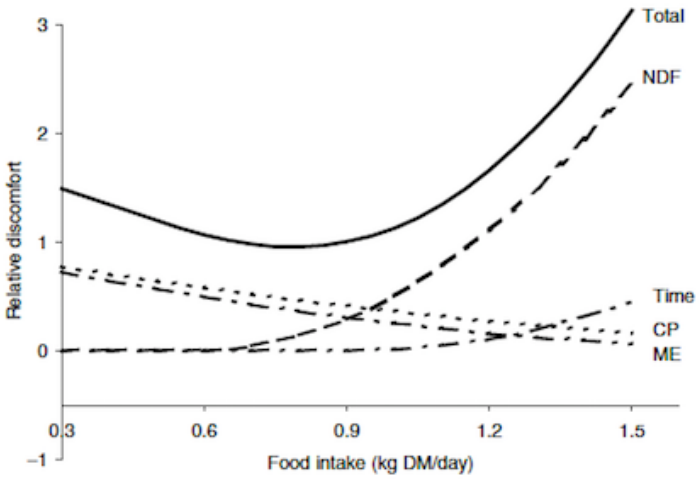

Figure 2.2.1 Relative discomfort relating to ME, CP, NDF and the time spent eating with total discomfort for a range of daily intake (Forbes, 2005)

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The MTD model should be linked to a model of rumen and animal metabolism to predict intake. It is seen in Figure 2.2.1, that when the NDF intake increases the discomfort level also increases. This phenomenon can be explained in terms of rumen fill, as the total discomfort level decrease when ME and CP intake increase because the nutrient requirements are being met. Ruminant production in regards to genetic potential may be constrained by the following factors, namely: the spatial contribution of biomass in the pasture as well as forage properties and environmental aspects (Weston, 2002). The reasons for the aforementioned factors are based on the principal that the most common constraint to intake on a pasture base system is the accusation of forage. It is the bite rate, bite size and bite mass as well as the time spent eating that influenced forage intake because the larger the bite mass became, the lower the bite frequency and time spent eating will need to be, thus leading to a greater overall intake and intake rate (Minson, 1990; Weston, 2002). Studies related to intake and digestion will benefit from examining the underlying mechanisms involved in nutrient utilization. For example, when feeding less frequently the intake of ruminants were influenced due to the timing of nutrient supply and release (Hosking, 1987). The reason why high intake rates were required for maximum production was because ruminants lost a varying amount of energy through faeces, heat dissipation, rumination and metabolic processes (Weston, 2002).

Figure 2.2.2 illustrates the major determining factors influencing intake and how negative and positive feedback mechanisms are involved (Weston *et al.,* 2002).

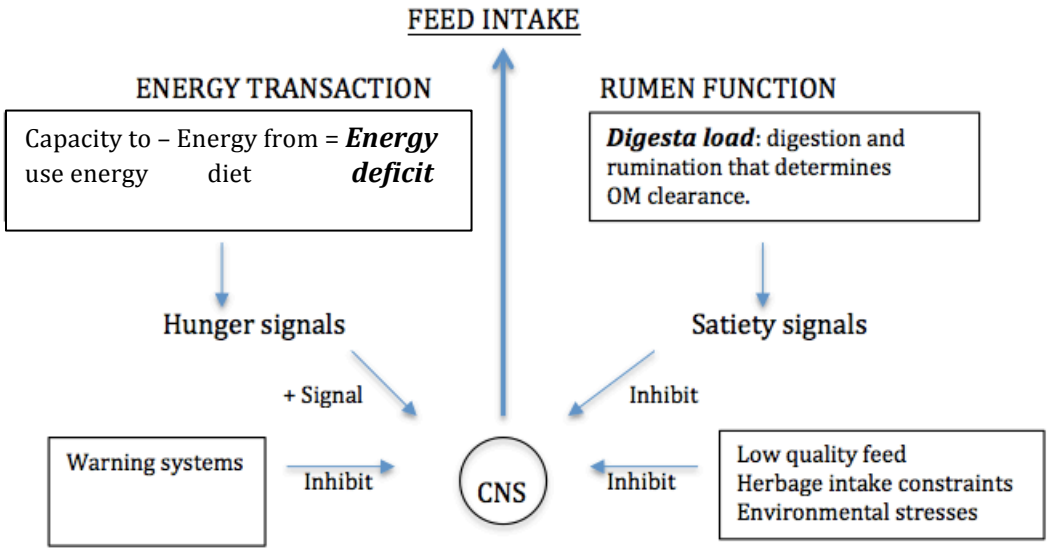

Figure 2.2.2 Schematic representation of the main regulatory mechanisms pertaining to pasture intake (CNS, central nervous system). Adapted from Weston (2002)

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Forage intake and microbial action

The characteristics of forage are diverse in its properties and contribution towards being an energy and protein source for ruminants. For forages to be of use to ruminants it has to be attacked, degraded and biologically converted to usable nutrients by the microbial population found in the rumen (Akin, 1986). Rumen protozoa and bacteria rapidly break down starch, where fungi usually break down the bundle sheath cells that protect the starch. The microbial population in the rumen utilize sources of branch chain fatty acids like, 2-methyl-butyrate, isobutyrate and isovalerate in addition to ammonia for maximum fibre digestion (Akin, 1986).

Depending on environmental factors and forage species the microbial population and diversity also changed and adapted to the diet composition of the animal for optimal performance (Theodorou and France, 2005). Hungate started researching microbes back in the 1940s and to date there are more than 200 species of rumen bacteria; over 100 species of protozoa and at least 12 species of fungi known (Hungate, 1966). The complex interrelationships between microbes are fascinating, as they complement each other in their cross-feeding interactions, although competition and predation also exist (Akin, 1986).

Protozoa

This class of rumen microflora with concentrations ranging from 10^5 – 10^6/ml increases the surface area of plant cells for microbial enzyme attack, but only account for 28% of the fibre digestion capability (Akin, 1986; Theodorou and France, 2005). Entodiniomorph protozoa is speculated to have cellulase and hemicellulase and is capable of engulfing plant cell walls as well as utilizing insoluble proteins associated with particulate matter, while rumen flagellates and holotrichs utilize soluble protein internally as well as protein associated with particulate matter (Akin, 1986; Nolan and Dobos, 2005). *Epidinium ecuadatum* on the other hand is especially known for their cell wall digestion, as it has hemicellulolytic and xylanolytic activity (Nolan and Dobos, 2005). Protozoa is mainly responsible for the digestion of protein-rich sources as well as degrading bacteria and excreting ammonia as end product. Protozoa obtaining most of their nitrogen through phagocytosis of other microorganisms and thus contribute greatly to the recycling of microbial cells in the rumen, although eliminating protozoa from the rumen can increase protein outflow rate and improve production (Nolan and Dobos, 2005).

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Bacteria

Bacteria is the dominant group of microbes found in the rumen involved in fibre digestion with concentrations ranging from 10^9 - 10^{10}/ml (Theodorou and France, 2005). The main determining factors for the proliferation and growth of bacteria are the growth factors being supplied and the combination thereof. Van Gylswyk (1970) found that the first limiting factor in the utilization of a low quality hay is the low nitrogen availability, which results in a less desirable rumen environment pertaining to the microbial population and as a consequence, the daily intake decreases. When low quality forage or hay is supplemented with a protein source like urea, there is an increase in the digestibility of cellulose and hemicellulose, although the count of cellulolytic and total culturable bacteria did not markedly increase. The lack of an increased bacterial count could be explained by an increased rate of passage out of the rumen (Van Gylswyk, 1970). One of the reasons that fibre digestion increased was that the predominant type op cellulolytic flora changes from one of high cellulolytic butyrivibrios that proliferate on hay fed diets to ruminococci that proliferate more on urea-supplemented hay diets. Branch chain volatile fatty acids derived from protein are required for the growth of several species of cellulose-digesting bacteria although, the balance and growth of bacterial species in the rumen is determined more by the level of nutrients rather than the differences in nutritional requirements (Van Gylswyk, 1970). The main cellulose digesting species are: *Fibrobacter succinogens, Ruminococcus albus, Ruminococcus flavefaciens and Eubacterium cellulosolvens.* These bacteria adhere closely to cell walls as their complex array of enzymes break down plant tissue, while *Butyrivibrio fibrisolvens* and *Bacteroides ruminicola* together with other bacteria digest hemicellulose (Akin, 1986). High starch diets require more amylolytic bacteria such as, *Bacteroides amylopilus, Selenomonas ruminatium* and *Streptococcus bovi*s as well as the soluble carbohydrate utilizers, like *Megasphaera elsdenii*, while proteolytic bacteria include *B. amylophilus, B. fibrisolves, B. ruminicola* and the *Butyrivibrios* families (Nolan and Dobos, 2005).

Fungi

This group of microbes is known as motile zoospores that invade cell wall tissues by thallus and rhizoids. The primary role of fungi is to colonize plant cell walls and increase the accessibility of other microbes (Theodorou and France, 2005). *Neocallimastix frontalis, Piromonas communis* and *Sphaeromonas communis* are some examples of fungi that have a complete complement of enzymes that enable the total degradation of available structure carbohydrates in the plant cell walls, and together with methanogens the activity of cellulose digestion is enhanced (Akin, 1986). A unique feature of fungi is that they have the ability to weaken lignified plant tissue and have high proteolytic

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activity, but make out less than 8% of the microbial biomass and are found in concentrations of 10^3 – 10^5/ml (Nolan and Dobos, 2005).

2.3 SUPPLEMENTATION

In rangeland conditions ruminants will require supplementation during certain times of the year, as the nutrient supply from grasslands do not always supply sufficient energy and nutrients for animal demand and optimal production, especially across different environments (Van Niekerk, 1975; Garnsworthy and Cole, 1990; Dove, 2002). Supplementation can be any feedstuff that is added in addition to a basal diet that enhances the diet of the animals nutritionally, improves the digestibility and metabolisability as well as any other deficiency and therefore improving overall animal performance. Seasonality and unfavorable years has an immense impact on the profitability of any livestock enterprise, as the cost of extra supplementation influences the income generated, while increases in the efficiency and method of supplementary feeding will prove to be of economical benefit. (Dove, 2002). Supplements usually, but not always, increase ADG but knowing what supplementation to provide in different situations can be advantageous to production. The effect that supplementation has on intake can be described in terms of forage quality and quantity as well as the associative effects it has on voluntary intake and the availability of energy and other nutrients (Moore *et al.*, 1999). Higher rates of supplementation can substitute the basal diet at a rate that is influenced by the nutritional characteristics and composition of the forage and the supplement (Faverdin *et al.*, 1995; Moore *et al.*, 1999).

The rate at which roughage intake decreases with an increase in supplement intake (substitution rate), reflects the effects of the supplement on the fractional rates of digestion and the outflow of digesta from the rumen (Dixon and Stockdale, 1999). High starch content in the supplement and a low potential for digestion and physical breakdown of the roughage are factors that are expected to increase substitution rate. It is suggested that the voluntary intake of concentrates are limited by the ruminal by-products of digestion, rather than by rumen fill, rumen load or by the energy requirements. When supplementation is fed in addition to a forage diet, the amount of digesta in the rumen including the dry matter load is altered and the rate at which cell wall constituents are digested may either increase or decrease depending on the supplementation response (Van Niekerk, 1975; Dove, 2002). The passage rate has a direct effect on digestion, pH and ammonia concentrations in the reticulo-rumen, which has an effect on microbial protein production and thus the amount of energy and amino acids available at tissue level (Doyle *et al.*, 1988; Faverdin *et al.*, 1995). Overall the economical impact of feeding any type of supplementation is not only affected by the feedstuff itself but also the practical consideration thereof, as the adaptation period required and the frequency of

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feeding as well as the variation between supplement intake, play an important role in cost to benefit ratio and decision-making (Van Niekerk, 1975; Dove, 2002).

Supplementation response

Grazing systems are comprised of quantitative, qualitative and morphological aspects that form part of the interlinking relationship between pasture and the grazing ruminant (Chilibroste *et al.*, 2005). Supplementation that by definition enhances the nutritional value and overcome nutritional deficiencies can have a positive (supplementary and/or complimentary) effect or a negative (substitution) effect.

There are at least three reasons why we offer ruminants supplements. In certain situations and circumstances one would like to add supplementation to the diet to negate the effect of something that is already present in the diet (Dove, 2002). As an example supplementing polyethylene glycol to diets of sheep containing high tannin concentrations especially when grazing and browsing *Acacia* spp., can dramatically overcome the negative effect tannins has on intake, protein degradation and availability that influences overall performance (Faverdin *et al.*, 1995; Dove, 2002). In most of the cases supplementation is primarily given to overcome deficiencies or to improve total nutrient supply (Faverdin *et al.*, 1995). The complexity of explaining all aspects in terms of the specific nutrients that are responsible for a supplementary response, narrows the discussion down to the main dependent variable namely, feed intake. The understanding of supplementary feeding on grazing systems is further complicated by the fact that we only know three basic things: the amount and quality of the supplement given, the production aspects of the herd and the amount and quality of the pasture on offer (Faverdin *et al.*, 1995).

Interaction between herbage and supplement intake

Three basic outcomes of ruminants being supplemented on grazing systems (Faverdin *et al.*, 1995):

1. *Substitution:* This phenomenon occurs when most to all of the supplements are eaten and as a result reduces forage intake. This is the least desired outcome as this will increase input costs regarding supplementation but in certain situations it could be desired as a sparing effect on the pasture.
2. *Supplementation:* This is beneficial and desired, as the intake of forage is not reduced, although this rarely happens.
3. *Complementation:* This is when the supplement increases forage intake through improving the nutritional value of the diet by correcting deficiencies and/or enhances digestibility and

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nutrient supply at tissue level. This will be the most efficient outcome on an economical point of view, as natural forage are cheap commodities and make out the majority of a grazing animals diet.

Supplementation and complementation

Complementation is mostly found when there are no assumed deficiencies of essential nutrients and is the most sought after response, as the maximum economical benefit of supplementation is only realized when you can identify those nutrient deficiencies that limit animal performance (Van Niekerk, 1975; Dove, 2002). When grazing poor quality pastures with low nitrogen availability, the provision of supplementation with readily available nitrogen and protein sources will increase the rate of digestion, as the microbial population will in response be in a more favorable ecological state. This will in turn increase the passage rate of the roughage component and in response increase forage intake, as rumen fill will no longer be the primary limiting factor. The mean retention time in the rumen, which is in close proximity with passage rate, can be explained in terms of the following equation: MRT=1/breakdown rate. At a certain point with an increase of the supplementation rate, the relationship between supplement and pasture intake will become substitution rather than complementation and this suggests that rumen microbial requirements have been met and that other constraints on intake are operational (Faverdin *et al.*, 1995; Dove, 2002). Example, when lambs grazing on mature pasture were supplemented with oat-grain and sunflower meal, pasture intake increased. When doubling the amount of supplements fed, both pasture and total intake increased although pasture intake was now less than the supplement intake (Freer *et al.*, 1988).

Substitution

This topic is still actively researched, as it is complex and difficult to extend results obtained from pen studies to grazing situations. Substitution rate (coefficient) is determined by dividing the amount of decreased forage intake (e.g. 150g) by the amount of supplement intake (e.g. 300g). This will give you a substitution rate of 50% (150g/300g = 50%), which means that the ruminant gives up 0.5kg of forage for 1kg of supplement (Romney and Gill, 2000). When supplementation has no effect on forage intake then the substitution coefficient is zero, gaining full benefit and advantage from feeding the supplement (Hagos and Melaku, 2009). There are a few factors that will determine the substitution rate between forage and supplementation, although it still remains difficult to quantify the expected responses (Romney and Gill, 2000; Hagos and Melaku, 2009).

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The following factors are of importance:

1. Substitution will on average be greater when the quantity and quality of the pasture is high, although it can still occur when forage is sparse and of low quality. The reason for this being that there are almost always exceptions to most of the rules pertaining to the outcome and expectations when working with biological systems, and thus other determining factors like grazing behavior and methods of supplementation, play an important role (Minson, 1990; Dixon and Stockdale, 1999). Good quality grasses will have a reasonably high digestibility, thus the amount of forage needed to meet the animals' nutrient requirements will be smaller; as a consequence the supplements on offer will be consumed at greater rates because supplements are easily assessable and requires less energy expenditure than grazing. The interaction between herbage and supplementation is not as simple, as associative effect can take place where the nature of the supplement can either suppress or enhance herbage intake (Dixon and Stockdale, 1999). High starch supplements (e.g. wheat) shift the rumen microbial ecology more in the direction of amylolytic organisms than cellulolytic organisms and this in theory slows down cell wall digestion and the passage rate of digesta from the rumen, which in turn decreases the overall intake (Minson, 1990; Dixon and Stockdale, 1999).
2. Substitution will usually be greater when high quality and greater amount of supplements are fed (Dove, 2002).
3. Physiological differences will play an important role in the substitution rate, as it will be less in situations where animals are in high nutritional demand and require the most of all available recourses. As an example, lactating animals will have a lower substitution rate than early pregnant animals because the physiological status and demand for energy differ between these two stages (Minson, 1990; Dixon and Stockdale, 1999; Dove, 2002).

Types of supplementation

Energy supplementation (high carbohydrates)

Cereal grains are the primary energy rich feedstuffs due to its high readily digestible carbohydrates in the form of starch. It is cheap in terms of cost per MJ ME but expensive per kg crude protein. Energy supplementation can also act as an increaser of microbial protein production through an increased capture of nitrogen sources (rumen ammonia, NPN) by the microbial population. Protein to energy ratio is important, as high energy diets with low protein values can be negative in regards to production and pasture intake. In this scenario the proliferation of amylolytic bacteria in the rumen increases at the expense of cellulolytic bacteria and lactic acid utilizing bacteria, this leads to a lower digestibility and in response a reduced passage rate (Romney and Gill, 2000).

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Protein supplementation

Protein supplements are given mainly for two reasons, one being to increase the amount of RDP and RUP and secondly to improve microbial protein leaving the rumen. One of the most widely used NPN sources is urea, but in all instances in relation to protein sources, it is important to have a balanced energy to protein ratio for the maximum benefit (Van Niekerk, 1975). Protein sources of plant origin include grain legumes, pulses, oilseeds and oilseed meals that can improve forage intake up to 14-17% due to an increase in rumen microbial proliferation and the influence of body energy reserve mobilization (Van Niekerk, 1975; Romney and Gill, 2000). Protein supplementation is the most expensive component of a balanced diet especially in the winter months, when protein is the main limiting nutrient (Romney and Gill, 2000).

Mineral supplementation

Minerals as a group should not be overlooked. The reason being that it is essential for life, as complicated biochemical reactions are dependent on minerals to sustain growth, production, osmotic balance and structural roles in all animals (White, 1996). Mineral deficiencies have been reported in grazing animals, although these deficiencies are mainly due to interactions between the minerals themselves. Major deficiencies or an oversupply of minerals can lead to clinical illness of animals, while marginal deficiencies are rarely noticed. It is mainly the economical benefit relative to cost that determines whether mineral supplementation will be implemented, although animal health and production also play key roles in decision making (White, 1996).

Table 2.4.1 Essential mineral elements for animals (McDowell, 1996; White, 1996)

Major elements (Macrominerals)	**Trace elements (Microminerals)**	
Calcium[a]	Cobalt[a]	Vanadium[b]
Phosphorus[a]	Copper[a]	Boron[b]
Magnesium[a]	Iron[a]	Lithium[b]
Potassium[a]	Iodine[a]	Lead[b]
Sodium[a]	Selenium[a]	Fluorine[b]
Sulfur[a]	Zinc[a]	Cadmium[b]
	Molybdenum[a]	Tin[b]

[a] Important under grazing conditions
[b] Inconclusive if these are essential

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Conserved forages

Conserved forages are used as supplementation in situations where the natural rangeland is depleted, either due to biotic and abiotic factors. It is difficult to make hay or silage with a nutritional value that can compare to that of a good quality pasture. Hay and silage are not that expensive per ton DM, but the cost per MJ of ME and CP per kg is much higher (Van Niekerk, 1975; White, 1996).

Forage crops

Forage crops are usually not considered as supplements but acts as a fodder supplement during certain times of the year when pasture quality and quantity are limited. These forage crops play an important role in a fodder flow plan, although there is a cost to benefit ratio as well as a cropping penalty that need to be taken into account when one is setting aside a part of land to grow fodder crops, specifically to feed either during winter or summer as part as a fodder flow plan. The improved production during the feeding period of these fodder crops must offset the increased grazing pressure during the rest of the year, as the stocking rate will have a direct effect on the cropping penalty for example, if the stocking rate is low then the cropping penalty will also be of a lower value (Van Niekerk, 1975; White, 1996).

Winter or dry season supplementation

Phosphorus supplements

Dry grassland is a poor source of available energy, protein, carotene and mostly phosphorus. In SA the phosphorus deficient soils are a main cause of botulism and poor performance. Botulism is caused by a neurotoxin produced by any of seven strains of *Clostridium botulinum,* found in soil and/ or animal carcasses. This disease is closely related to drought and nutritional deficiencies (Ibrahim and Shigidi, 2014). Phosphorus deficiency is mostly the determining factor in relation to bolulism, as animals develop pica in an attempt to satisfy their nutritional deficiency. This leads to the typical behavior of chewing foreign objects like, animal carcasses, wooden posts and plastic bottles (Ibrahim and Shigidi, 2014). Research has shown that there is a non-significant increase in live weight gain when supplementing phosphorus in winter months when animals are losing weight. Thus in these conditions phosphorus is not the limiting factor, although it is of importance (Van Niekerk, 1975).

Protein and energy

Energy deficiencies are secondary to protein deficiencies and it is more expensive to meet the grazing animals' energy requirement than meeting the lower protein requirement (Van Niekerk, 1975). There is a lack of control in regards to energy and protein intake and this complicates the

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interpretation of the results. Research has shown that a ratio of 10-13% for dietary total digestible intake protein to total digestible OM will maximize the OM intake of low-medium quality forage (Brown and Pitman, 1991; Köster *et al.*, 1996).

Figure 2.4.1 describes how intake constraints affect substitution rate. The energy and protein concentrations has an effect on the heat production as well as rumen fill, which as a result has an effect on the substitution rate (Dixon and Stockdale, 1999).

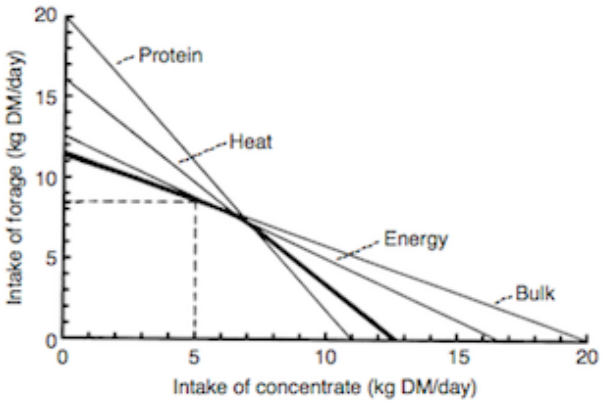

Figure 2.4.1 Diagram representing the potential intake of forage and a concentrate according to calculated limitations of energy, bulk, and heat production (Forbes, 2005). The thick line represents the factor that is limiting intake of forage for any intake of concentrate. For example when concentrate increases from 0 to 5 kg DM/day then forage intake deceases from 11.5 kg to 8.5 kg DM/day thus the substitution rate is 3.0/5.0= 0.60 (Dixon and Stockdale, 1999)

Effect of supplementation on rumen microbial population and pH

Depending on the diet being fed, microbes compete for substrates such as ammonia, peptides and sulphur for their growth. Fibrous components are hydrolyzed much slower than readily digestible carbohydrates; therefore the energy available for microbial growth also becomes more slowly available when feeding high fibre diets. The microbial substrates are utilized preferably by those microorganisms that utilize RFC, but when substrates like nitrogen and sulphur are limiting, the digestion of feedstuffs will be affected (Dixon and Stockdale, 1999). Manipulating the rumen and its microbial population seems like a daunting task when there is no supplementation on offer. This is true to a certain point as energy, protein and mineral supplements aid in alleviating growth-limiting deficiencies on a microbial as well as an animal level (Dixon and Stockdale, 1999; Russell and Strobel, 2005). It is also shown that by feeding supplements in different combinations and concentrations, it is possible to control the outcome of fermentation to some extent and thus the proliferation of different

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microbial species (Russell and Strobel, 2005). Microbial fermentation is one of the many different factors that has an effect on rumen pH, by means of decreasing the pH when the production of VFA's are higher than the absorption thereof (Russell and Strobel, 2005). The rumen pH is not only important in relation to rumen and animal health but has a great effect on the efficiency of fibre digestion (Shriver *et al.,* 1986). As seen in Table 2.4.2, the ideal pH for NDF digestion is between 6.2 and 7, while at a pH lower than 6.2 NDF digestibility is substantially reduced and near completely neglectable at a pH lower than 6, as a washout of cellulytic microbes occur. A second factor that is often overlooked is the feeding frequency. For example, when feeding a mixed forage-concentrate ration once a day, the pH of the rumen decreases for several hours after feeding, while feeding the ration in four portions throughout the day, the rumen pH is maintained at levels more favorable for fibre digestion and microbial attachment (Shriver *et al.*, 1986).

Table 2.4.2 Effects of pH on fermentation (Adapted from Shriver *et al.,* 1986 and Varga, 1987)

	pH[a]			
ITEM	5.8	6.2	6.6	7.0
Ammonia N, mg/100ml	4.2	20.0	25.6	26.7
NDF digestibility, %	8.1	33.1	32.4	33.1
Attached microbes, %	28.8	48.4	40.5	39.3

[a] Linear and quadratic effects ($p<0.01$)

When there is a requirement to decrease rumen fermentation in relation to an improved deliverance of metabolites like protein to the hindgut, treating grains and other readily fermentable feedstuffs with fats, phenols and aldehydes can increase feed efficiency (Garnsworthy and Cole, 1990) . These compounds bind to the protein matrix surrounding starch thus making it less available for rumen microbes and more available to be digested in the hindgut. Other means of decreasing rumen fermentation include heat treatment and extrusion of feedstuffs (Garnsworthy and Cole, 1990). In frame of reference to insalivation and its effect on the functionality and health of the rumen, saliva is an important component that adds to a well-balanced rumen environment and rumination processes. Saliva with both buffering and nitrogen recycling properties, help ruminants compensate for a low protein diet by the recycling of nitrogen as urea through the saliva (Garnsworthy and Cole, 1990; Dixon and Stockdale, 1999).

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2.4 RUMEN FERMENTATION AND KINETICS

Particle dynamics

The fractional passage rate of feed particles from the reticulo-rumen to the reticulo-omasal orifice increased as fibre intake increased, although it was implied that diurnal variation may exist when dealing with rumen kinetics (Owens and Goetsch, 1986). The clearance of digesta is not only affected by digestibility but also by particle dynamics that are defined by the functional specific gravity and size of particulate matter, as well as animal species, physiological and chronological age (Weston, 2002). Factors like palatability, bulk density and rate of digestion determine the amount of large particles as well as the efficiency of size reduction and the resistance to fragmentation, which in turn has the potential to limit the ability of ruminants to satisfy their metabolic capacity to utilize energy. It is important to understand particle dynamics, digestion and passage from the reticulo-rumen, as this predicts microbial protein production and energy available for animal growth and animal production (Kennedy, 2005). The different particle sizes can be described by the size of sieve it passes through (Kennedy, 2005).

Example:

Large particles – Retained on a screen of 1.18 or 1.0 mm aperture.

Medium particles – Passing a 1.18 or 1mm screen and retained on a 0.5 to 0.6 mm aperture.

Small particles - Passing a 0.5 or 0.6 mm screen and retained on a 0.15 to 0.05 mm aperture.

Fine particles - Passing the smallest screen.

Ruminant physiology is unique in that individual feed particles follow a circuitous route in the alimentary tract that is facilitated through rumination, as it is the primary function of facilitating the clearance of digesta from the reticulo-rumen, while the cycle length of feed particles throughout the alimentary tract is largely dependent on the extent of large particle comminution during rumination (Kennedy, 2005). Particle comminution associated with rumination, mastication and insalivation is necessary to aid in the colonization of microbes in the rumen through increasing the surface area of particulate matter (Kennedy, 2005). The duration of rumination can vary between 10 – 12 h/day depending on the amount of feed intake, fibre content and feeding value (Ulyatt *et al.*, 1986; Kennedy, 2005).

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Dynamics of particle flow

Particle flow out of the rumen can be explained in terms of the different pools associated with particle size and its characteristics in accord with particle composition. The different pools in the rumen can be classified as:

Pool A – Liquid portion that flows freely and include very small particles like solubilized protein, AA, minerals and sugars (Owens and Goetsch, 1986).

Pool B – Particles of proper size and density that can exit the reticulo-rumen that is associated with higher forage diets promoting cyllulolytic digestion (Owens and Goetsch, 1986).

Pool C – Particles that are unable to leave the reticulo-rumen at their current state (Owens and Goetsch, 1986).

Before the onset of feeding the volume of pool A was at its greatest and the amount of feed residues are at its lowest. Adding fibre to the diet increases mastication and salivation that increased digestion, but an increase of fluid passage rate in pool A and B will in contrast decreased the time available for digestion. When fluid passage rate increased, the particle fractions in pool A and B was depleted and the particle fractions in pool C increased. Another aspect to take into account is that the total rumen volume was negatively related to passage rate when the actual outflow remained constant, but on overall the production of ruminants increased as feed intake increased because the maintenance costs was diluted due to an increase in passage rate (Owens and Goetsch, 1986).

During lag time, pool A increases as pool B and C are solubilized and broken down (Owens and Goetsch, 1986). Feedstuffs that are used as concentrates are colonized before that of roughage, as a result the microbial nitrogen in pools A, and B may be larger due to the lower mass of pool C in comparison to high fibre diets (Owens and Goetsch, 1986). The available energy for microbial growth will be greater in pool C than pool B and the lowest in pool A, because with each step of fermentation and particle breakdown less energy is available, as the microbes utilize it for growth, thus as fermentation proceeds microbial nitrogen and energy will move from pool C to pool B and finally to pool A (Owens and Goetsch, 1986). There are a few controversies regarding passage rate when supplementing minerals. Some research indicate an increase in ruminal volume but no alteration in osmolarity, while Kellaway *et al.* (1978) found that fractional passage rate was increased (Owens and Goetsch, 1986). The former statement can be explained through the osmotic effect that minerals and salts have on the alteration of rumen kinetics, as mineral supplementation pulls water from particulate matter, leading to an expected increase in passage rate of pool B in fibre based diets and an increase in passage rate of pool A in concentrate based diets (Muller and Kilmer, 1979).

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Microbial colonization and weakening of particles

The rumen is a fermentation vessel that has a redox potential between -300 mV and 135 mV and harbors protozoa, bacteria and fungi all acting together to form the overall microbial biomass (Theodorou and France, 2005). The temperature of the rumen is relatively stable at around 38-42°C, which is determined by fermentation and the metabolism of the animal (Theodorou and France, 2005). The efficiency of microbial synthesis is directly related to the passage rate of particle from the reticulo-rumen while the microbial biomass is determined by the degree of fermentation, as there is a quadratic increase in microbial biomass in the rumen when the particle size of feedstuffs decrease to those retained on screens of 0.15 and 0.08 mm apertures (Weston, 2002). Within 10 min of ingestion fibrolytic bacteria adhere to feed particles and as the size of particles decrease the surface area for microbial attachment increases (Weston, 2002). Microbial digestion do not significantly affect the comminution of particles although fungi have the ability to weaken and disrupt larger particles because they produce a variety of enzymes that degrade cell- wall polysaccharides and disrupt the covalent linkages connecting lignin and hemicellulose (Weston, 2002). A long MRT of particles in the reticulo-rumen improved nutrient utilization, however it also restricted feed intake due to intake being limited by the capacity of the rumen (Lechner-Doll *et al.*, 1991). When intake increased there was only a 20-40% decrease in MRT, although this is not a constant because of rumen volume and species variation. With a shorter MRT microbial fibre digestion in the reticulo-rumen was reduced but in turn increased the hindgut digestibility, although total tract apparent digestibility was still reduced by approximately 2-7% at higher intake levels (Lechner-Doll *et al.*, 1991).

Factors affecting the mean retention time

1. *Particle size*

As particle size increased the probability of clearance from the reticulo-rumen decreased exponentially with the size of the particles (Poppi *et al.*, 1980). Theoretical concepts and dynamic mathematical models have been implemented to differentiate between pools based on particle size (Ulyatt *et al.*, 1986; Lechner-Doll *et al.*, 1991). This has led to the conclusion that a critical particle size (CPS) exists to witch particles should be comminuted before those particles can leave the reticulo-rumen, through the riticulo-omasal orifice (ROO) (Ulyatt *et al.*, 1986; Lechner-Doll *et al.*, 1991). The CPS of sheep and cattle is in the range of 1-2mm and is not really affected by feed intake or quality, although it is suggested that the ROO can pass particles several times larger than the CPS, as a result of end-on delivery (Van Soest, 1982; Ulyatt *et al.*, 1986).

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2. *Breakdown of particles*

The two processes that aid in particle breakdown is mastication and rumination, where rumination is the more important contributor to particle comminution (Owens and Goetsch, 1986; Lechner-Doll *et al.*, 1991). Secondary to these processes are the microbial activity, as microbial fermentation indirectly contributed to particle breakdown (Lechner-Doll *et al.*, 1991). According to Faichney (1986) the MRT of large particles was approximately 12hours and the breakdown rate can be determined by using the reciprocal value of the MRT for example, 1/12 = 0.083, thus 8.3% of the large particles were comminuted per hour (Owens and Goetsch, 1986; Lechner-Doll *et al.*, 1991). In comparison to large particles the MRT of small particles was approximately 18.4hours and this suggests that the CPS is not the only determinant of passage rate, as small particles should theoretically pass out of the rumen at a greater rate than the larger particles. One of the determining factors in relation to the passage of small particles was that with an increase of DM intake, the filter bed entrapped smaller particles, which is known as the *filter-bed effect* (Ulyatt *et al.*, 1986; Lechner-Doll *et al.*, 1991).

3. *Particle density*

The functional density is defined as the sum of all the factors contributing to the effective buoyancy of particles, these factors include: structural components, population of microbial biomass, size and shape of the particles as well as the amount of fluid and gas entrapped in the particles (Lechner-Doll *et al.*, 1991). Large particles, that were more buoyant and had a lower density than that of small particles, were retained longer. This meant that there was a linear relationship between density and MRT, which was negatively correlated (Lechner-Doll *et al.*, 1991).

4. *Distribution of particles in the reticulo-rumen*

The dorsal rumen environment was associated with larger buoyant particles as compared to the ventral ruminal portion where smaller more dense particles accumulated and from there flowed towards the reticulo-rumen (Lechner-Doll *et al.*, 1991). It was in the reticulo-rumen, which was comprised out of the first two compartments of the rumen where over 60% of OM digestion took place (Ulyatt *et al.*, 1986). When looking at the different particle sizes and the stratification thereof, one needs to explain it in terms of a distribution coefficient (D), which is quantified as the stratification of the different particle size pools in the reticulo-rumen.

$$D = A_{pool\,1}/A_{pool\,2} \quad \text{(Kennedy, 2005)}$$

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For example if the amount of medium size particles in the fibre mat (A_{pool2}) is greater than the medium size particle in the ventral digesta (A_{pool1}), then the distribution coefficient will be greater than 1 and this indicates either incomplete mixing, heterogeneity of particle buoyancy or the physical entrapment within the dorsal mat (Kennedy, 2005). The fibre mat acts as a filter bed as mentioned previously and compares to the 'lag-rumination' as described by Ellis *et al.* (1999), where it was shown the small particles moved through the raft with the fluid portion. It was the ratio between the free water and the size of the large particle pool, which were the main determinants of particle movement and thus the stratification in the raft.

5. *Quantitative contributions of particle size and density*

There is an exponential relation between MRT and particle size and a linear relation between MRT and particle density, while the particle size has an inverse relation to particle density and MRT (Poppi *et al.*, 1980; Lechner-Doll *et al.*, 1991). The major contributing factor to variation in MRT was the particle density (59%) rather than the particle size (28%). In terms of particle shape it was the concentrical-type particles that were passed from the rumen faster than cylindrical ribbons, while it was the volume of particles that were probably more important than the weight and mass when it come to feed intake (Owens and Goetsch, 1986).

6. *Separation of particles in the reticulo-rumen*

For particles to leave the reticulo-rumen they have to be present in the ventral reticulum when the ROO opens. The mechanisms involved in concentrating small, highly dens particles in the ventral reticulum included: The entrapment of larger particles in the fibre mat, allowing small particles to pass, and secondly the motility of the reticulum itself where most of the particle separation took place (Van Soest, 1982; Lechner-Doll *et al.*, 1991).

Passage rate and digestion

Passage rate is equal to the actual outflow of particles divided by the pool size and can be combined directly with fractional digestion rate to calculate the magnitude of rumen digestion (Owens and Goetsch, 1986; Weston, 2002). Particle size and liquid flow determines greatly the selective passage of rumen digesta, where most of the small particles including microbial cells associated with the liquid portion that remaines in the rumen for 10-24 hours, whereas the attached microorganism associated with larger particles can remain in the rumen for 2-3 days (Theodorou and France, 2005). The ruminal escape as a portion of flow is equal to the passage rate divided by the sum of the fractional digestibility and passage rate of digestible materials (Owens and Goetsch, 1986).

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Formulas adapted from Weston (2002) and Owens and Goetsch (1986)

$$\text{Passage rate } (h^{-1}) = \frac{\text{Actual rumen outflow } (ml.h^{-1} \text{ or } g.h^{-1})}{\text{Pool size (total ml or g)}}$$

$$\text{Ruminal escape} = \frac{\text{Passage rate } (h^{-1})}{\text{Passage rate } (h^{-1}) + \text{Digestion rate } (h^{-1})}.$$

Studies have shown that a reduction in particle length increased the passage rate from the rumen and decreased the DM and fibre digestibility, although there were many conflicting results in terms of digesta outflow. The method of feeding and the supplementation frequency also influenced the passage rate and digestibility of feedstuffs, as Robles *et al.* (2007) showed. When adapting the feeding schedule so that meals are offered more frequently, it tended to increase ruminal passage rate and the escape of potentially degradable substrates (Robles *et al.*, 2007). Particle size and functional specific gravity were important forage characteristics influencing the passage rate out of the rumen, as Abouheif *et al.* (2012) concluded in their study using twenty four Najdi male lambs where they evaluated the effects of particle length and the frequency of feeding on nutrient digestibility and retention time in the rumen. The conclusion stated that particle length had a significant effect on ruminal retention time, as shown in Table 2.5.1 (Abouheif *et al.*, 2012).

Table 2.5.1 Digesta retention times and nutrient digestibility coefficients (%) of growing lambs fed at different frequencies and with different particle lengths of alfalfa hay (Adapted from Abouheif *et al.*, 2012)

	Particle length			**Feeding frequency**	
	9.5 mm	14 mm	Long Hay	Once/day	Twice/day
Digesta retention times					
Ruminal retention (h)	33.3^{b}	35.1^{b}	40.0^{a}	38.7^{a}	33.8^{b}
Lower tract retention (h)	12.2	12.8	12.9	11.8	13.4
Digestibility coefficients (%)					
DM	77.3	76.8	78.1	76.4	77.3
CP	82.6	83.1	83.7	83.1	83.6
ADF	45.6^{b}	46.1^{ab}	49.8^{a}	47.2	48.4
NDF	48.1^{b}	47.6^{b}	53.4^{a}	51.3^{a}	47.0^{b}

^{a,b} Values bearing different superscripts in the same row differ significantly from each other ($p<0.05$)

The retention time of lambs fed twice daily differed significantly from those fed only once a day, as depicted in Table 2.5.1. With a more frequent feeding schedule the animals increased their water intake and ruminated at more constant intervals throughout the day aiding in particle comminution and an increased passage rate associated with an increase in feed intake and lower substitution rates (Robles *et al.*, 2007; Abouheif *et al.*, 2012). When concentrating on the digestibility in relation to

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passage rate and particle length, the passage rate decreased with an increase in particle size and length leading to an increase in ADF and NDF digestibility, as seen in Table 2.5.1. In addition, the microbial population adapted to the diet being fed for example. When the forage to concentrate ratio decreased there was a decrease in the pH of the rumen, as more lactic acid producing bacteria proliferated leading to a decrease in cellulolytic activity and a depression in ADF and NDF digestibility (Abouheif *et al.*, 2012).

Ramos *et al.* (2009) did a study using six Merino sheep where they received experimental diets with forage: concentrate ratios of 70:30 or 30:70, which were fed either with alfalfa hay or grass hay. The diets were given twice daily at a rate of 56 g of DM/kg $BW^{0.75}$. The apparent rumen digestibility of OM, NDF and ADF decreased in the high concentrate diets, as the pH dropped and the passage rate increased, which is depicted in Table 2.5.2. This decrease in the apparent rumen digestibility and the increase in passage rate resulted in an increase of total tract apparent digestibility, because the lower tract digestion increased in response to the high concentrate diets, as seen in Table 2.5.2 (Ramos *et al.*, 2009).

Table 2.5.2 The effect that forage:concentrate ratios and type of forage has on digestibility and intake in sheep fed diets with forage:concentrate ratios of 70:30 (HF) or 30:70 (HC) and alfalfa hay (A) or grass hay(G)(Adapted from Ramos *et al.*, 2009)

	Diet			
	HFA	HCA	HFG	HCG
Intake				
DM, g/kg of $BW^{0.75}$	53.1	54.9	47.6	48.1
OM, g/d	1,028	1,071	922	943
Total tract apparent digestibility, %				
OM	72.9	75.3	67.8	74.3
NDF	59.3	57.8	59.9	59.7
ADF	56.4	49.8	54.4	50.5
Apparent rumen digestibility, %				
OM	58.9	49.5	58.1	52.0
NDF	61.5	52.1	61.1	54.5
ADF	59.0	45.3	55.9	47.2

Microbial efficiency and rumen kinetics

The microbial efficiency and the dilution rate of the culture medium are positively correlated; decreasing the rumen residence time for microbes will in return reduce the energy expended on the bacterial maintenance (Hespell and Bryant, 1979). The efficiency of microbial growth on a nitrogen

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basis is related to the fluid dilution rate of chemostat studies as shown by the Hespell and Bryant (1979) equation:

Efficiency of microbial protein production (MOEFF)

$$MOEFF = ((m \times D^{-1}) + Y_{max}^{-1})^{-1} \quad \text{.....(Hespell and Bryant, 1979)}$$

m, Maintenance coefficient (0.000451 kg OM/g N.h)

Y_{max} , maximum yield (46.7g N/kg OM fermented)

D, Dilution rate (h^{-1}) for bacterial medium

The dilution rate was calculated by subtracting the rate of bacterial breakdown at steady state from the growth rate of bacteria at steady state. The microbial nitrogen flow generally changed in the same direction as the OM flow, as indicated by *in vivo* studies (Owens and Goetsch, 1986). The microbial population was linked to different stratification levels in the rumen as there were microbes that adhere to the rumen wall and those that associate with either small particles (pool B) or larger rumen solids (pool C). High forage diets harbored larger amount of cellulolytic bacteria in pool B and C, while protozoa were predominantly found in the rumen fluid portion (pool A). Interestingly, protozoa have a low outflow rate indicating that they associated with particles and have a low density, which lowered passage rate (Dehority, 1984).

Microbial passage rate can be calculated through the following equation:

$$MPR = aPR_1 + bPR_p \quad \text{.....(Oldham, 1984)}$$

aPR_L , liquid passage rate, a is the fractional portion of microbes in the liquid pool .

bPR_p , passage rate of particles(solids), b is the fractional portion of microbes in the particle pool.

Microbial protein production and the measurements for the estimation thereof is still a wildly researched topic. The efficiency of microbial protein production varied between different feeding levels and the amount of fermentable metabolizable energy available. Chen *et al.* (1990) developed a new non-invasive method to estimate microbial protein reaching the duodenum, rather than the previous version where fistulated animals and microbial markers were needed. The new and still improving method described and developed by Chen *et al.* (1990) estimated microbial protein production based on purine derivatives (allantoin, hypoxanthine, xanthine and uric acid) measured in the urine. It it assumed that the duodenal nucleic acids are mostly of microbial origin and of limited amount because of the rapid degradation shown by free RNA. In addition, it is also assumed that the purine-based catabolites are most likely recovered in the urine, although many of these assumptions need validation (Perez *et al.*, 1996a; Perez *et al.*, 1996b).

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The following table shows how the VFA and ammonia-nitrogen concentrations in the rumen fluid and purine derivatives found in the urine of ewes, change when given 550g of lucerne hay per day and supplemented with increasing amounts of barley (Perez *et al.*, 1996a).

Table 2.5.3 The volatile fatty acid and ammonia-nitrogen concentrations in the rumen fluid and purine derivatives found in the urine of ewes fed 550g lucerne hay/day and supplemented with increasing amount of barley (Adapted from Perez *et al.*, 1996a)

	Barley supplementation (g/day)			
	0	220	400	550
Ammonia-N (mg/l)	170.7	165.4	177.7	201.8
Total VFA (mmol/l)	74.5	84.1	92.3	100.9
Main VFA proportions (%)				
Acetic acid	76.78	71.91	69.67	65.78
Propionic acid	16.47	15.96	16.87	19.41
Butyric acid	7.25	12.13	13.44	14.81
Urinary excretion (mmol/d)				
Allantion	4.65	7.06	11.62	8.77
Hypoxanthine	0.76	0.91	0.96	1.06
Xanthine	0.14	0.12	0.14	0.09
Uric acid	0.46	0.59	0.59	0.66
Total purine derivatives (mmol/d)	5.88	8.68	13.47	10.50

The reason as described by Perez *et al.* (1996a) for the total purine derivatives being lower when 550g of barley was supplemented per day in comparison to 400g per day, was because of the rumen dilution rate being reduced with the 550g of barley supplemented and also the higher maintenance requirement of the amylolytic bacteria that was associated with a lower efficiency of microbial syntheses due to wasteful turnover of nutrients. Through optimizing microbial protein synthesis, the efficiency of nitrogen utilization was increased and the excretion of urinary nitrogen was reduced (Ramos *et al.*, 2009). As the efficiency of rumen nitrogen utilization increased, the amount of urinary nitrogen and fermentative carbon losses in the form of CO_2 and NH_4 decreased (Chumpawadee *et al.*, 2006).

A synchrony index can be used to describe the efficiency of rumen OM and nitrogen utilization, where an index of 1 represents a perfect synchrony between energy and protein and a value of less than 1 indicates a degree of asynchrony (Nolan and Dobos, 2005). The asynchrony of available energy and protein in the rumen can lead to inefficient microbial growth and high NH_3 absorption (Nolan and Dobos, 2005). Chumpawadee *et al.* (2006) conducted an experiment showing that DM, ADF and OM digestibility increased linearly as the synchrony index increased, although the NDF and CP digestibility were not affected by the increasing synchrony index, the amount of microbial protein production and fermentation still improved (Nolan and Dobos, 2005). Detmann *et al.* (2009) conducted a study using

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Holstein x Zebu heifers provided a basal diet of *Brachiaria decumbens* and supplemented with an increasing amount of nitrogenous compounds. The CP contents in the diets ranged from 51.9 to 136.3g/kg DM. The results observed by Detmann *et al.* (2009) indicated that effective degradability (ED) of NDF and discrete lag (LAG) presented a *linear-response-plateau* according to the RAN concentration. This resulted in a break point in the range of 8mg/100ml for ED and LAG, which represents the maximum estimate and minimum estimate, respectively. The RAN concentration defined to optimize NDF degradation and intake was 8mg/100ml and 15mg/100ml, respectively. The NH_3-N concentration in the rumen decreased as the synchrony index increased and this indicates a more efficient utilization of nitrogen by the microbes to produce microbial protein. The importance of NH_3-N as an end product of proteolysis is realized when one considered that 40-95% of the nitrogen in bacteria is derived from NH_3-N, which in response lead to an increase in the efficiency of microbial fermentation and thus an increase in total volatile fatty acid production (Nolan and Dobos, 2005; Chumpawadee *et al.*, 2006).

Volatile fatty acid production

Microbial fermentation is the life source of all ruminant animals, where the waste products of fermentation are: VFA, methane, ammonia-N and lactic acid. The VFA's are to a greater extent acetate, propionate and butyrate and to a lesser extent valerate, caproate, isobutyrate, isovalerate and 2-methylbutyrate (France and Dijkstra, 2005). A study done by Bergman *et al.* (1965) indicated that inter-conversions took place between VFA's for example, the inter-conversions between propionic acid and acetic acid as well as propionic acid and butyric acid fractions were small, whereas the carbon structure of butyric acid was mostly derived from acetic acid, while a smaller amount of acetic acid carbon came from butyric acid (Bergman *et al.*, 1965; Leng and Leonard, 1965).

Not only carbohydrates acted as microbial substrates, but also a small proportion of lipids and greater concentrations of dietary protein contributed to VFA production, especially when rumen degradable proteins are fed (France and Dijkstra, 2005). Many branch chain VFA's are required for the growth of certain microbes, thus this adds to the complex interrelationship of the rumen micro-flora. It is important to note that the fermentation pattern determined the molar proportion and fractions of VFA that were produced in relation to the microbial population (France and Dijkstra, 2005). Factors that influenced the fermentation patters were frequency of feeding, forage structure, particle size, intake level and the use of chemical additives (France and Dijkstra, 2005). With high forage diets the molar relationship of acetate to propionate and butyrate would be close to 70:20:10, as seen in Table 2.5.3 (France and Dijkstra, 2005). When the pH of the rumen decreased the

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absorption of VFA increased, as most of the VFA's was in the undissociated form, while the efficiency to witch VFA's were used for animal production was determined by the balance between glucogenic (propionate) supply to that of non-glucogenic (acetate and butyrate) supply (France and Dijkstra, 2005; Russell and Strobel, 2005). The fermentation rate reflected the rate of degradation and can be expressed as the non-glucogenic/glucogenic ratio (Chilibroste *et al.*, 2005).

Non-glucogenic to glucogenic ratio

$$NGGR = \frac{(HAc + 2HBu)}{HPr}$$ ……(Chilibroste *et al.*, 2005)

HAc – Acetic acid
HBu – Butyric acid
HPr – Propionic acid
NGGR - non-glucogenic/glucogenic ratio

Higher degradation rates yielded a greater proportion of propionate relative to that of butyrate and acetate. As the ratio increased the digestibility of the fibre fraction decreased proportionally as the overall passage rate increased (Chilibroste *et al.*, 2005). The synchronization of rumen fermentation is important in the hopes of increasing animal production efficiency, as the protein to energy ratio has an immense effect on the proliferation and growth of rumen microbes that directly affects the nutrients available for animal utilization (Chilibroste *et al.*, 2005).

AIM

This work aimed to estimate the effect of intermittent feeding of a supplement to sheep on certain rumen parameters, intake and total tract apparent digestibility as well as nitrogen retention under typical practical conditions where sheep received a fixed amount of supplement. This study focused on the time effect per week rather than the amount of supplement given per week. The reason for evaluating the time effect in term of intermittent supplementation rather than the amount given is because, under typical farming conditions sheep most probably receive a limited amount of supplements, either on a daily basis or every 2nd or 3rd day or once a week basis.

HYPOTHESES

1. ***H_0:*** There is no significant difference between feed intake and total tract apparent digestibility when feeding a fixed amount of production lick daily and feeding a fixed amount of production lick intermittently.

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H_A : There is a significant difference between feed intake and total tract apparent digestibility when feeding a fixed amount of production lick daily and feeding a fixed amount of production lick intermittently.

2. ***H_0 :*** There is no significant difference between VFA concentration, rumen pH, microbial protein production and rumen ammonia nitrogen concentration when feeding a fixed amount of production lick daily and feeding a fixed amount of production lick intermittently.
   H_A : There is a significant difference between VFA production, rumen pH, microbial protein production and rumen ammonia nitrogen concentration when feeding a fixed amount of production lick daily and feeding a fixed amount of production lick intermittently.

CHAPTER 3

MATERIAL AND METHODS

The experiment was conducted on the Hatfield Experimental Farm of the University of Pretoria and all the laboratory analyses were done at Nutrilab: Department of Animal and Wildlife Sciences, University of Pretoria.

3.1 EXPERIMENTAL DESIGN AND ANIMALS

The experimental design used was a 4x4 Latin square as shown in Table 3.1.1, where four Merino wethers each received a different treatment over four different periods. The data collection was based on a seven day (week) period to simulate a practical week supplementation schedule to determine the effect that intermittent supplementation of a production lick would have on rumen fermentation and intake. All animals were treated for internal parasites before and during the trial and their hooves were all trimmed, while other maintenance tasks like wool shaving around the cannulas were implemented to insure that infection and fly attraction was minimized. The Animal Ethics Committee of the University of Pretoria approved this study, with project number EC036-15, on the 20th of April 2015.

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Table 3.1.1 Overview of the experimental design. (Kuehl, 2000)

	Animal number			
Period #	**P1302**	**P1305**	**P1307**	**P1311**
1	A[1]	D	C	B
2	C[3]	B	A	D
3	D[4]	C	B	A
4	B[2]	A	D	C

#Period of 3 weeks (2 weeks adaptation and 1 week data collection)
[1] Treatment A = Supplement given everyday, starting on Monday (7 times)
[2] Treatment B = Supplement given every 2nd day after initial Monday feed (4 times)
[3] Treatment C = Supplement given every 3rd day after initial Monday feed (3 times)
[4] Treatment D = Supplement given once a week (only on Monday)

With every new period that lasted three weeks, all the sheep had the first two out of the three weeks set aside as an adaptation period before the data collection week commenced. The sheep were all placed in individual holding pens during the adaptation period where *Eragrostis curvula* hay (g/day) and water were provided *ad libitum*. Three days prior to the data collection week, the sheep were placed in individual standard metabolic crates according to accepted national and international ethical guidelines and faecal bags were fitted to each of the animals to allow them to adapt.

Preliminary intake trial

The intake trial lasted 14 days and was conducted prior to the start of the main trial. Each individual sheep was fed *Eragrostis curvula* hay *ad libitum* and the orts were weighed back the following day before feeding. The amount (g/day) of *Eragrostis curvula* hay that was offered in the metabolic crates during the experimental trial was 120% of the *ad libitum* intake, as determined by the last seven days of the preliminary intake trial. In the preliminary intake trial each sheep received 250g of the Voermol production lick in addition to *Eragrostis curvula* hay. This was done to prevent rumen stasis and to insure an adequate supply of nutrients. In the adaptation and data collection period each sheep received the Voermol production lick according to their appointed treatments (Table 3.1.2).

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Table 3.1.2 Feeding schedule of the experimental production lick [a] given to the sheep according to the appointed treatments

Period [#]	*Sheep Number*	***Week day***						
		Mon.	**Tues.**	**Wed.**	**Thurs.**	**Fri.**	**Sat.**	**Sun.**
1	P1302	A[1]	A	A	A	A	A	A
	P1305	D[4]	----	----	----	----	----	----
	P1307	C[3]	----	----	C	----	----	C
	P1311	B[2]	----	B	----	B	----	B
2	P1302	C	----	----	C	----	----	C
	P1305	B	----	B	----	B	----	B
	P1307	A	A	A	A	A	A	A
	P1311	D	----	----	----	----	----	----
3	P1302	D	----	----	----	----	----	----
	P1305	C	----	----	C	----	----	C
	P1307	B	----	B	----	B	----	B
	P1311	A	A	A	A	A	A	A
4	P1302	B	----	B	----	B	----	B
	P1305	A	A	A	A	A	A	A
	P1307	D	----	----	----	----	----	----
	P1311	C	----	----	C	----	----	C

[a] 125g of the Voermol production lick was placed directly into the rumen twice a day and remained constant throughout the trial
[#]Period of 3 weeks (2 weeks adaptation and 1 week data collection)
[1] Treatment A = Supplement given everyday, starting on Monday (7 times)
[2] Treatment B = Supplement given every 2nd day after initial Monday feed (4 times)
[3] Treatment C = Supplement given every 3rd day after initial Monday feed (3 times)
[4] Treatment D = Supplement given once a week (only on Monday)

3.2 EXPERIMENTAL DIET

Hammer-milled *Eragrostis curvula* hay (Weeping love-grass) with particle length of 2-3cm was used as the basal diet and fed at 7:00 am every morning. The Voermol production lick was placed into rumen degradable paper bags and inserted directly into the rumen during the adaptation and data collection periods according to the feeding schedule as depicted in Table 3.1.2. Two bags were filled with 125g of production lick per bag and inserted into the rumen twice a day, one bag at 7:00am and the other bag at 17:00pm, which added up to 250g/day per sheep. Voermol advises to supply a minimum of 250g of Voermol production lick per day per sheep.

Table 3.2.1 provides the nutritional properties of the *Eragrostis curvula* hay that was used as the basal diet in this current study. This data was used in the calculations with regards to the determination of intake and total tract apparent digestibility as well as nitrogen balance.

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Table 3.2.1 Chemical analysis of the *Eragrostis curvula* hay

DM %	Ash (g/kg)	CP (g/kg)	ME [1] (MJ/kg)	NDF (g/kg)	ADF (g/kg)	ADIN (g/100g CP)	IVOMD [2] (g/kg)
92.1	33.8	70	7.10	598.9	364.1	14.25	444
100	36.7	76	7.71	650.3	395.3	15.41	482

[1] ME (MJ/kg DM) = 0.016 x IVOMD. (McDonald *et al.,* 2011)
[2] Analyzed according to Tilley and Terry (1963)

In vitro fermentation of *Eragrostis curvula* hay as described by Tilley and Terry (1963) was used to determine the digestibility of the organic matter. The IVOMD (g/kg DM) was in turn used to estimate the ME (MJ/kg DM) of the *Eragrostis curvula* hay.

1. *ME (MJ/kg DM) = 0.016 x IVOMD (g/kg DM)* …… (McDonald *et al.*, 2011)
   = 0.016 x 482
   = 7.71 MJ ME/kg DM

Table 3.2.2 Composition of the Voermol production lick

REG NR V10108 (Act 36/1947)				
		(g/kg)		(mg/kg)
Crude Protein[1]	(Min)	250	Manganese	100
Urea	(Max)	55	Copper	40
Crude Fibre	(Max)	80	Cobalt	0.4
Moisture	(Max)	160	Iron	150
Calcium	(Max)	12	Iodine	2
Phosphorus	(Max)	8	Zink	150
Potassium	(Min)	18	Selenium	0.4
			Vitamin A (IE/kg)	10 000
			Energy (MJ ME/kg)	8
INTAKE: 250 g/sheep/day				

[1] 67.4% in the form of NPN and 2% RUP

Table 3.2.3 Chemical analyses of the Voermol production lick[#]

DM (%)	OM (g/kg)	NDF (g/kg)	ADF (g/kg)	CP (g/kg)	Ash (g/kg)
87.3	733.3	263.3	196.0	259.4	140.1
100	839.6	301.5	224.4	297.0	160.4

[#] This data was used in the calculations with regards to the determination of intake and total tract apparent digestibility as well as nitrogen balance

3.3 DETERMINATION OF INTAKE AND TOTAL TRACT APPARENT DIGESTIBILITY

At the beginning of each experimental period all the sheep were weighed and given *Eragrostis curvula* hay at 120% of the *ad libitum* intake, as determined in the preliminary intake trial. In the preliminary intake trial and in the main experimental trial the intake of each individual sheep was determined by subtracting the remaining orts of each day from the initial amount of feed given per day.

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Total tract apparent digestibility was determined for each of the treatments across all of the collection periods, as described by Osuji *et al.* (1993) and McDonald *et al.* (2011). The total intake of *Eragrostis curvula* hay was recorded for each individual sheep during the adaptation period and the data collection period, while the total faecal collection was only done in the data collection period. The faeces and orts were collected and weighed each morning before all the sheep were fed. The Voermol production lick was taken into account when determining the intake and total tract apparent digestibility as well as nitrogen balance.

The general formula to calculate digestibility coefficients (DC):

$$DC = \frac{\text{Parameter consumed (g/kg DM)} - \text{Parameter in faeces (g/kg DM)}}{\text{Parameter consumed (g/kg DM)}}$$ ……(McDonald *et al.*, 2011)

The composition of the hay expressed in terms of digestible nutrients for example NDF:

Digestible parameter (g/kg DM) = NDF (g/kg DM) in hay x DC…… (McDonald *et al.*, 2011)

Sampling for analysis

For each individual sheep a daily representative grab sample of *Eragrostis curvula* hay was sampled and stored at -20° C in a zip seal bag. The same principle was used for the daily faecal and ort collection, where a representative grab sample of 5-10% of the total amount of faeces and a large enough grab sample of the orts was sampled per sheep and stored at -20° C in a zip seal bag. The hay, orts and faecal grab samples were pooled per treatment across all the collection periods.

Methods used in laboratory analysis

All the samples of *Eragrostis curvula* hay, orts and faeces were analyzed for DM according to AOAC (2000a) procedure 934.01, organic matter according to AOAC (2000b) procedure 942.05, crude protein according to AOAC (2000c) procedure 968.06 using a Leco-Trumac Nitrogen determining apparatus. The ADF and NDF was analyzed according to Robertson and Van Soest (1981) and Goering and Van Soest (1988) respectively. A small portion of the faecal samples were dried at 100°C for 24hours to determine DM (Osuji *et al.*, 1993) and the remaining amount of faecal samples were dried at 60°C for 48hours and used for the overall proximal analysis. The results obtained were used to determine DM, NDF, ADF, OM and CP intake and digestibility, while taking the DM, NDF, ADF, OM and CP supplied by the Voermol production lick into account.

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3.4 MONITORING RUMEN FERMENTATION

Sampling for analysis and the methods used in the laboratory

Rumen fluid samples were collected once a day for each treatment across every data collection period with a time shift of 3hours and 25minutes each day (Table 3.4.1.) representing a 24hour cycle over 7 days. Samples were collected from various parts of the rumen and placed in 4-layers of cheesecloth so that all the rumen fluid could be squeezed out and the remaining material placed back into the rumen.

The pH of the rumen fluid samples were taken prior to being preserved with 4ml of a 25% H_3PO_4 solution per 20ml of rumen fluid for volatile fatty acid determination, as described by Webb (1994) and 5ml of a 50% H_2SO_4 solution per 30ml of rumen fluid for NH_3 – N determination, as described by Broderick and Kang (1980). The daily samples that were taken per treatment across every data collection period were stored separately at -20°C in bottles, so that the day-to-day VFA and NH_3 – N concentration could be measured.

Table 3.4.1 Timetable of the rumen fluid sampling for volatile fatty acid and ammonia nitrogen determination

Collection Period	*Sheep Nr.*	*Week day sampling times*						
		Mon.	**Tues.**	**Wed.**	**Thurs.**	**Fri.**	**Sat.**	**Sun.**
1,2,3 and 4	All the sheep	07:00	10: 25	13: 50	17: 15	20: 40	00: 05	03: 30

3.5 MONITORING NITROGEN BALANCE AND MICROBIAL PROTEIN PRODUCTION

The nitrogen balance per treatment across all the collection periods were determined using the equation adapted from Morgan and Whittemore, (unpublished), as cited by McDonald *et al.* (2002).

Calculations to determine N-Balance:

Daily N intake

1. *Eragrostis curvula* hay

Daily hay N intake = Feed offered (kg DM/day) x N in hay (g/kg DM) – Orts (kg DM/day) x N in orts (g/kg DM)

2. Voermol Production lick

Daily lick N intake = Lick intake (kg DM/day) x N in lick (g/kg DM)

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3. Total N intake

Daily N (g/day) intake = Daily hay N intake + Daily lick N intake

Daily N output

1. Faecal N output

Daily Faecal N (g/kg DM) = Faeces (kg/day) x Faecal N (g/kg DM)

2. Urinary N output

Daily urinary N (g/kg) = Urine (kg/day) x Urinary N (g/kg)

3. Total N output

Daily N (g/day) output = *Daily Faecal N (g/kg DM) + Daily urinary N (g/kg)*

Nitrogen Balance (g) = Total N intake – Total N output

Sampling for analysis

Total urine collection was done for each treatment across every collection period by connecting stainless steel pans under the metabolic crates (Chen and Gomes, 1995). The urine was sampled and the total amount was measured each morning together with the orts and faecal collection. The urine was collected in containers large enough to prevent an overflow, while cheesecloth was used to make sure that no foreign material ended up in the urine samples. To preserve the urine, 100ml of 10% H_2SO_4 was added to the collection containers right before the collection started. The preservation insured that the final urine pH was no more than 3 (the amount of H_2SO_4 added was adjusted as needed). The low pH inhibits bacterial breakdown of purines that would otherwise lead to a loss of nitrogen (Osuji *et al.*, 1993; Chen and Gomes, 1995). A sample size of 50ml was taken per treatment and pooled per sheep across all the collection periods. The urine samples were stored at -20°C in bottles so that urine nitrogen concentration could be measured.

Microbial protein production

After the initial 50ml of urine was sampled for the determination of the nitrogen balance, the remaining volume of urine in the collection containers were diluted with water to make up a final volume of 4liters (Chen and Gomes, 1995). This prevented precipitation and insured that all the samples for each sheep were made up to the same volume so that a 50ml sub sample of diluted urine could be taken daily. Each of the 50ml samples taken per treatment across all the collection periods

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were pooled per sheep and stored at -20°C in bottles so that microbial protein production could be determined.

Method used in the laboratory

The urine samples as well as the *Eragrostis curvula* hay and ort samples were analyzed for CP according to AOAC (2000c) procedure 968.06 using a Leco-Trumac Nitrogen determining apparatus. The CP (g/kg) of the feed, orts, urine and faeces were determined by multiplying the nitrogen concentration (g/kg) obtained by the factor 6.25 (McDonald *et al.*, 2011). Endogenous nitrogen production was assumed to have no significant contribution towards the fecal and urinary CP concentrations and was thus not taken into account. The method described by Chen and Gomes (1995) was used for the determination of microbial protein production, while high performance liquid chromatography was used on the diluted 50ml urine samples to analyze for purine- derivatives.

3.6 STATISTICAL ANALYSIS

The statistical model for a Latin square design:

$$Y_{ij} = \mu + \rho_i + \gamma_j + \tau_k + e_{ij} \quad \text{.....(Kuehl, 2000)}$$

$$i\,,\, j\,,\, k = 1,2,\ldots,t$$

Y_{ij} = Observation on the experimental unit in the i^{th} row and the j^{th} column of the design
μ = the mean
ρ_i = the row (Period) effect
γ_j = the column (Animal) effect
τ_k = the effect of the k^{th} treatment
e_{ij} = are random, independent experimental errors with meal 0 and variance σ^2

An analysis of variance with the GLM model (SAS, 2006) for a Latin square design was used for all the repeated variables like VFA, RAN, purine derivatives, nitrogen balance, microbial protein production, intake, total tract apparent digestibility as well as rumen pH to determine the significant differences between the periods, treatments and sheep. The means and the standard error of the means (SEM) were calculated, while the significance of differences ($P < 0.05$) and tendencies ($P \leq 0.10$) between means were determined using Fischer's test (Samuels and Witmer, 2003).

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CHAPTER 4

RESULTS AND DISCUSSION

4.1 INTAKE AND TOTAL TRACT APPARENT DIGESTIBILITY

Table 4.1.1 Intake and total tract apparent digestibility of wethers receiving *Eragrostis curvula* hay and supplemented intermittently with 250g of Voermol production lick, everyday (A), every 2nd day (B), every 3rd day (C) or once/week (D)

	Treatment				SEM*
	A	B	C	D	
Intake, g/kg DM per BW$^{0.75}$					
Hay, DM	65.6	67.2	62.9	66.5	4.0
Total, OM (hay + lick)	71.4	68.9	63.3	64.2	1.9
NDF	54.0	53.8	49.9	51.7	0.4
ADF	32.1	32.0	29.2	30.4	3.1
CP	9.6^{a}	8.1^{b}	7.4^{b}	6.7^{b}	3.8
Total tract apparent digestibility, %					
DM	48.1	48.6	45.3	43.7	1.4
OM	49.5	49.9	46.5	44.9	1.3
NDF	48.8	50.2	47.5	45.3	2.7
ADF	43.2	45.2	42.7	40.6	1.7
CP	59.9^{a}	56.0^{a}	53.3^{ab}	48.7^{b}	1.7

^{a,b,c} Values bearing different superscripts in the same row differ significantly (P<0.05)
* Standard error of means

When referring to the DM intake per metabolic body weight as depicted in Table 4.1.1, the results indicated that there were no differences between the treatments. There was a tendency for every 2nd day (treatment B) supplementation having a higher intake value. There were no differences between the treatments pertaining to OM intake per metabolic body weight, which included not only hay intake but also the lick intake. The ADF and NDF intake remained fairly constant between the treatments and no differences were indicated. The crude protein intake per metabolic weight showed that treatment A differed from all the other treatments, while treatments B, C and D did not differ from each other. The sheep receiving 250 g of Voermol production lick everyday had a higher CP intake per metabolic body weight in comparison to the rest of the treatments, while treatment D had the lowest CP intake, as expected due to the amount of supplement received. The total tract apparent digestibility of DM and OM followed the same trend and did not differ from each other. The total tract apparent digestibility of CP indicated no difference between treatment A and treatments B and C. In addition there was a difference between treatment D and treatments A and B, while treatment C did not differ from treatment D.

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Discussion

One of the potential reasons why there were no differences between DM, OM, ADF and NDF intake could be explained in terms of the hay quality being of such nutritive value that the addition of the production lick only had an effect on the CP intake (McGuire *et al.*, 2013). It has been suggested that DMI is maximized and will not respond to supplementation when daily NDF intake is roughly higher than 12.5g/kg BW/ day (Mertens, 1985). This supports the findings in the current study, as there were no differences between the treatments in reference to intake due to the NDF intake being higher than 12.5g/kg BW/day for all the treatments. Atkinson *et al.* (2010) conducted a study with Suffolk wether lambs (34.5 ± 2.04 kg BW) that were fed a basal diet of mature crested wheatgrass hay *ad libitum* and one of four supplements: 1) a high RDP supplement provided once daily, 2) high RDP supplement provided every 2nd day, 3) a high RUP supplement provided every 2nd day or 4) a 50:50 mixture of the RDP and RUP supplement, provided every 2nd day. The amount of RDP and RUP given was 0.23 and 0.30% of BW respectively. Supplementing every 2nd day was at twice that of daily supplementation, resulting in all supplements being provided on an isonitrogenous basis of 7.85 g N/day across a 48hour supplementation interval. The results of Atkinson *et al.* (2010) indicated that OM, NDF and ADF intake was not affected by protein degradability or supplementation frequency and that decreasing the frequency of daily supplementation to every 2nd day, had no effect on the total intake, as confirmed by McGuire *et al.* (2013) and Canesin *et al.* (2014), which both did similar studies. McGuire *et al.* (2013) did their studies on wether lambs and steers supplemented with urea and soybean meal, while Canesin *et al.* (2014) did their studies on steers (325 ± 65.7kg BW) supplemented with urea, citrus pulp and cottonseed meal, either once daily, once daily except Saturdays and Sundays or every 2nd day at 10, 14, and 20 g/kg BW per day, respectively. The aforementioned studies support the results obtained in the current study (Table 4.1.1), as daily and every 2nd day supplementation, resulted in intake values that showed no difference. There was a tendency for feed intake to be higher for daily supplementation, whereas the NDF and ADF digestibility tended to be higher when supplementing every 2nd day, as supported by the digestibility studies done by Atkinson *et al.* (2010).

Protein supplementation as infrequent as every 2nd day may improve ruminal digestion by means of stabilizing the rumen environment for microbes, as supported by Bohnert *et al.* (2002), Schauer *et al.* (2005), Cappellozza *et al.* (2013) and McGuire *et al.* (2013). The apparent total tract apparent digestibility of DM, OM, ADF and CP was increased with supplementation but not affected by these individual studies' supplementation frequencies or the nitrogen sources. Bohnert *et al.* (2002) provided low-quality meadow hay to wethers (36 ± 1kg BW) and supplemented: daily, every 3rd or every 6th day with DIP (82% of CP) and UIP (60% of CP) at approximately 0.19% of BW/day (averaged over a 6-day period). Schauer *et al*. (2005) supplemented Angus x Hereford cows either daily (0.91 kg,

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DM basis) or every 6th day (5.45 kg, DM basis) with cottonseed meal (43% CP, DM basis). Cappellozza *et al.* (2013) supplemented steers (464 ±26kg BW) with urea (provided to meet 100% of DIP requirements) or soybean meal (provided on an isonitrogenous basis), either daily or every 2nd day.

Tellier *et al.* (2004) found in support of the results of this study (Table 4.1.1) that the frequency (everyday, every 2nd day or every 3rd day) of supplementing steers (474 ± 30kg BW) with barley-grain-based concentrates had no influence on the digestibility of DM, NDF and ADF. Tellier *et al.* (2004) concluded that concentrates could be fed to steers every 2nd day without any negative effects on intake and digestibility of DM, NDF and ADF, while having positive effects due to a reduction (4%) in energy lost as heat. Brundyn *et al.* (2005) and McGuire *et al.* (2013) concluded according to their respective studies, that providing supplements every 2nd or every 3rd day held no disadvantage in relation to animal production (rumen fermentation and enteric methane production) and reduced labour and transportation costs of up to 50%, which is also supported by Schauer *et al.* (2005) and Canesin *et al.* (2014). It is important to take note that the comparisons made between the current study and that of the cited studies, differ on grounds of the amount of supplement provided on a weekly basis. It is possible to compare the different results due to the feeding frequency pattern that follow the same trend and seem to deliver the same results.

4.2 RUMEN PH AND VOLATILE FATTY ACID CONCENTRATION

Table 4.2.1 represents the results of the pH measured from the rumen samples collected, which represented a 24hour cycle over 7 days.

Table 4.2.1 Rumen pH of wethers receiving *Eragrostis curvula* hay and supplemented intermittently with 250g of Voermol production lick, everyday (A), every 2nd day (B), every 3rd day (C) or once/week (D)

	Treatment				SEM*
	A	B	C	D	
Sampling time					
07:00	7.0_{1}	7.0_{1}	6.9_{1}	7.0_{1}	0.1
10:25	6.7_{12}	6.6_{2}	6.7_{12}	6.6_{25}	0.1
13:50	6.3_{23}	6.4_{2}	6.5_{236}	6.5_{235}	0.1
17:15	6.3_{23}	6.3_{23}	6.2_{346}	6.3_{45}	0.1
20:40	5.8_{4}	5.8_{4}	6.0_{45}	6.0_{4}	0.1
00:05	6.1_{34}	6.0_{34}	6.2_{356}	6.2_{34}	0.1
03:30	6.6_{2}	6.4_{23}	6.5_{26}	6.7_{12}	0.1
Average	6.4	6.4	6.4	6.5	0.1

[1,2,3,4,5,6] Values bearing different subscripts in the same column differ significantly (P<0.05)
* Standard error of mean

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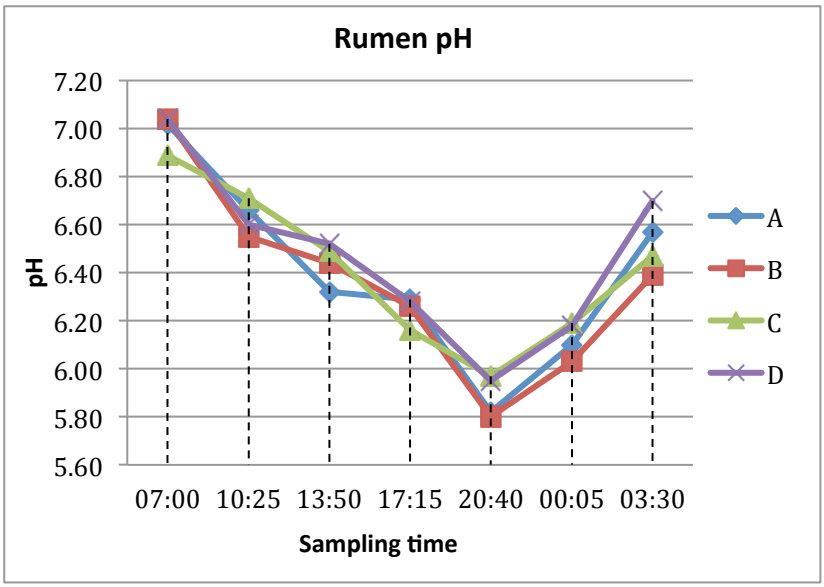

Figure 4.2.1 Rumen pH of wethers receiving *Eragrostis curvula* hay and supplemented intermittently with 250g of Voermol production lick, everyday (A), every 2nd day (B), every 3rd day (C) or once/week (D). The pH measurements represent a 24hour cycle over 7 days

The average rumen pH between treatments did not differ, as depicted in Table 4.2.1. The pH of the rumen was relatively neutral early in the morning and gradually decreased after the 7am feeding and after the 5pm feeding, which resulted in the pH being at its lowest around 20:40pm. The pH gradually started to increase as rumination commenced, but overall the pH remained in the range of 6.2 and 6.8. The ideal pH for NDF digestion is between 6.2 and 7. At a pH lower than 6.2, NDF digestibility is substantially reduced and near completely neglectable at a pH lower than 6 (Shriver *et al.*, 1986).

Discussion

The stability of the rumen relating to feeding frequency and pH is supported by Faichney (1968), Yang and Varga (1989), Atkinson *et al.* (2010) and Canesin *et al.* (2014), as the individual studies indicated that the rumen pH was not affected by supplementation frequencies. There was a sampling and time effect, as observed in the study done by Beaty *et al.* (1994), which supplemented Angus x Hereford steers either daily or every 3rd day. The results obtained in the current study, as depicted in Table 4.2.1 is supported by research done by Cappellozza *et al.* (2013) where protein supplements in the form of urea or soybean meal were placed directly into the rumen of steers at 1.3 g DM/ kg BW for daily supplementation and 2.6 g DM/ kg BW for every 2nd day supplementation. When examining the study done by Beaty *et al.* (1994) where they provided different ratios of protein supplementation in the form of rolled sorghum grain and soybean meal to steers, either daily (1.98 kg supplement) or

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every 3rd day (4.62 kg supplement). It was observed that the interaction between day x sampling time x frequency of supplementation on rumen pH was significant, while showing that the pH remained higher when supplementing every 3rd day in comparison to feeding everyday. This led to the lowest pH reading being measured 4hours after being supplemented. The aforementioned findings are in accord and support the results obtained in the current study where the lowest pH reading of 5.8 was taken between 3 and 4hours after the supplements where administered inta-ruminally, as represented in Figure 4.2.1. When the rumen pH started to drop below 6, cellulytic microbes began to wash out of the rumen and this could have influenced NDF and ADF digestibility negatively (Shriver *et al.*, 1986). The studies that are cited and used to support the findings of the current study differ on grounds of the weekly amount of supplement provided, but the results are comparable in terms of the supplementation frequencies.

Table 4.2.2 refers to the three major VFA's (acetic acid, propionic acid and butyric acid) as a percentage of the total concentration of volatile fatty acids. From these three VFA proportions, the non-glucogenic/glucogenic ratio as well as the acetic/propionic ratio was determined.

Table 4.2.2 Percentage volatile fatty acid concentration of wethers receiving *Eragrostis curvula* hay and supplemented intermittently with 250g of Voermol production lick, everyday (A), every 2nd day (B), every 3rd day (C) or once/week (D)

	Treatment				SEM*
	A	B	C	D	
Percentage VFA, %					
Acetic acid	72.2[a]	72.5[a]	72.1[a]	76.2[b]	0.4
Propionic acid	19.3[a]	18.5[a]	18.9[a]	16.0[b]	0.3
Butyric acid	8.5[b]	9.0[a]	8.9[ab]	7.8[c]	0.2
NGGR#	4.7[a]	4.9[a]	4.8[a]	5.8[b]	0.1
Acetic/Propionic ratio	3.8[a]	3.9[a]	3.8[a]	4.8[b]	0.1

[a,b,c] Values bearing different superscripts in the same row differ significantly (P<0.05)
* Standard error of means
# Non-Glucogenic /Glucogenic Ratio = (Acetic acid + 2Butyric acid)/Propionic acid. (Chilibroste *et al.*, 2005)

It was observed that for both acetic- and propionic-acid, treatment D differed from treatments A, B and C, while treatments A, B and C did not differ from each other. The propionic acid concentration was higher for treatments A, B and C and significantly lower for treatment D, while the acetic acid concentration was lower for treatments A, B and C, and significantly higher for treatment D. In terms of butyric acid, treatment A differed from treatments B and D, while treatment C differed from treatment D. Both the acetic/propionic ratio and non-glucogenic/glucogenic ratio showed that treatment D differed from treatments A, B and C, while treatments A, B and C did not differ from each other. The NGGR and acetic/propionic ratio followed the same trend, which showed that the NGGR as

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well as the acetic/propionic ratio was lower for treatments A, B and C, and significantly higher for treatment D.

Discussion

The propionic acid concentration decreased as the supplementation frequency decreased, while there was a tendency for the acetic acid concentration to increase in response to the decrease in total tract apparent digestibility of DM, OM, NDF, ADF and CP. The efficiency to witch VFA's are used for animal production was determined by the balance between glucogenic (propionate) supply to that of non-glucogenic (acetate and butyrate) supply, as stated by France and Dijkstra (2005). Chilibroste *et al.* (2005) described that higher degradation rates yielded a greater proportion of propionate relative to that of butyrate and acetate. Chilibroste *et al.,* (2005) also described that when the NGGR increased the digestibility of the fibre fraction decreased proportionally as the overall passage rate increased. It was noted that the non-glucogenic/glucogenic ratio as well as the acetic/propionic ratio was lower when supplementing once daily, every 2nd day and every 3rd day in comparison to supplementing only once a week (Table 4.2.2). In Table 4.2.2 it is shown that no differences were found between supplementing once daily, every 2nd day and every 3rd day, while supplementing only once a week indicated a large difference and as a result had below optimal fermentation. The aforementioned results can be linked with the findings of the study done by Brundyn *et al.* (2005), where it was observed that supplementing a production lick at 200 g per ewe daily, 400 g per ewe every 2nd day, 600 g per ewe every 3rd day, held no disadvantage in relation to animal production in terms of weight gain.

Table 4.2.3 sums up the total VFA concentration measurements taken throughout the study and shared a connection with the rumen pH, which added to the evidence of the rumen fermentative processes. Sample collection represented a 24hour cycle over 7 days.

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Table 4.2.3 Total volatile fatty acid concentration (mmol/L) of wethers receiving *Eragrostis curvula* hay and supplemented intermittently with 250g of Voermol production lick, everyday (A), every 2nd day (B), every 3rd day (C) or once/week (D)

	Treatment				SEM*
	A	B	C	D	
Sampling time					
07:00	$108.3^{a}{}_{1}$	$152.0^{b}{}_{12}$	145.6^{ab}	56.9^{c}	17.3
10:25	$148.8^{a}{}_{12}$	$165.4^{a}{}_{1}$	131.8^{a}	57.6^{b}	17.3
13:50	$157.5^{a}{}_{2}$	$161.3^{a}{}_{1}$	114.6^{b}	56.1^{c}	12.9
17:15	$164.1^{a}{}_{2}$	$176.4^{a}{}_{1}$	115.3^{b}	71.2^{c}	14.2
20:40	$184.8^{a}{}_{2}$	$178.2^{a}{}_{1}$	154.6^{a}	66.9^{b}	16.0
00:05	$151.9^{a}{}_{3}$	$102.2^{b}{}_{2}$	145.3^{a}	75.6^{b}	13.4
03:30	$162.9^{a}{}_{32}$	$112.0^{b}{}_{2}$	144.9^{ab}	63.2^{c}	14.2
Average	154.1^{a}	149.6^{a}	136.0^{a}	64.0^{b}	6.0

^{a,b,c} Values bearing different superscripts in the same row differ significantly (P<0.05)
^{1,2} Values bearing different subscripts in the same column differ significantly (P<0.05)
* Standard error of mean

Treatment D differed from treatments A, B and C when examining the average total VFA concentration. Treatment D had an immensely lower VFA concentration in comparison to treatments A,B and C. There was no difference between the average total VFA concentration of treatment A and treatments B and C, as depicted in Table 4.2.3. When comparing the rumen pH (Table 4.2.1) with the volatile fatty acid concentrations (Table 4.2.3) there was a clear and distinct connection between the lowest pH and the highest concentration of total volatile fatty acids. At 20:40pm the highest VFA concentrations directly reflected the lowest pH readings.

Discussion

Atkinson *et al.* (2010) fed wether lambs a basal diet of mature crested wheatgrass hay *ad libitum* and one of four supplements: 1) a high RDP supplement provided once daily, 2) high RDP supplement provided every 2nd days, 3) a high RUP supplement provided every 2nd day or 4) a 50:50 mixture of the RDP and RUP supplement, provided every 2nd day. Canesin *et al.* (2014) did their study on steers (325 ± 65.7kg BW) supplemented with urea, citrus pulp and cottonseed meal, either once daily, once daily except Saturdays and Sundays or every 2nd day at 10, 14, and 20 g/kg BW per day, respectively. In support of the current study, the studies conducted by Atkinson *et al.* (2010) and Canesin *et al.* (2014) indicated that total VFA concentrations were not affected by protein digestibility or supplementation frequency. The research done by Cappellozza *et al.* (2013) where protein supplements in the form of urea or soybean meal were placed directly into the rumen of steers at 1.3 g DM/ kg BW for daily supplementation and 2.6 g DM/ kg BW for supplementing every 2nd day, supports the findings of Atkinson *et al.* (2010) and Canesin *et al.* (2014). The VFA results of Cappellozza *et al.* (2013) support

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the findings of the current study in terms of the molar proportions of acetate, propionate and butyrate that differed, as shown in Table 4.2.2.

The VFA's produced by fermentation was directly proportional to DM, OM, NDF, ADF and CP digestibility, as depicted in Table 4.1.1. The results of the rumen pH, as shown in Table 4.2.1, could be linked to the VFA results. The lowest rumen pH readings were associated with the highest VFA concentrations. The results of the current study indicated that the rumen pH was within range (6.3 to 6.8) normally sufficient to support adequate NDF and ADF digestion. When examining the average VFA (Table 4.2.3) concentrations between the treatments it is seen that when supplementing only once a week resulted in an immensely low VFA concentration of 64.0 mmol/L rumen fluid. When supplementing once daily, every 2nd day and every 3rd day the VFA concentrations were 154.1, 149.6 and 136.0 mmol/L rumen fluid, respectively. The aforementioned findings indicated that although treatments A, B and C did not differ, there was a tendency for the VFA concentrations to increase as the supplementation frequency increased. Based on the very low concentration of VFA for supplementing only once a week, it would not be advised to follow this supplementation regime.

4.3 NITROGEN BALANCE AND MICROBIAL PROTEIN PRODUCTION

Table 4.3.1 Nitrogen balance of wethers receiving *Eragrostis curvula* hay and supplemented intermittently with 250g of Voermol production lick, everyday (A), every 2nd day (B), every 3rd day (C) or once/week (D)

	Treatment				SEM*
	A	B	C	D	
Nitrogen, g N/day					
Intake	29.4[a]	26.6[a]	25.2[ab]	19.9[b]	1.1
Faeces	12.2[a]	12.1[a]	12.1[a]	9.9[b]	0.4
Urine	6.2	4.5	5.3	4.0	0.6
Nitrogen retention	11.0[a]	10.0[ab]	7.9[ab]	6.0[b]	0.8
Proportion of N intake, %					
Faeces	41.2[a]	45.6[a]	48.1[ab]	49.3[b]	1.8
Urine	21.2	16.7	20.5	20.7	2.2
Nitrogen retention	37.6	37.7	31.4	30.0	2.4

[a,b] Values bearing different superscripts in the same row differ significantly (P<0.05)
* Standard error of means

The nitrogen intake of treatments A and B differed from treatment D, while treatments A, B and C did not differ from each other and in addition treatment C showed no difference from treatment D. The nitrogen intake of D was very much lower than that of treatments A, B and C. There was a difference between treatment D and treatments A, B and C pertaining to faecal nitrogen excretion, as

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shown in Table 4.3.1, while treatments A, B, and C did not differ from each other. The faecal excretion was higher for treatments A, B and C, while treatment D was significantly lower. The data revealed that the nitrogen retention measured, as g N/day differed between treatment A and D, while treatments A, B and C did not differ from each other. The fecal excretion expressed as a proportion of N intake, indicated that treatments A and B differed from treatment D, while treatments A, B and C did not differ from each other. In addition, treatment C did not differ from treatment D. The faecal excretion as a proportion of nitrogen intake showed an increase in excretion as the supplementation frequencies decreased from treatment A to D. The nitrogen retention when expressed as a proportion of N intake, showed no difference between the treatments, although there was a tendency for the nitrogen retention to decrease as the supplementation frequencies decreased from treatment A to D.

Discussion

Examining the nitrogen retention as a proportion of N intake (Table 4.3.1) it was observed that there was a tendency for the amount of N retained to decrease when the frequency of supplementation decreased. These findings are supported by the results obtained by Atkinson *et al.* (2010), which did a study with Suffolk wether lambs (34.5 ± 2.04 kg BW) that was fed a basal diet of mature crested wheatgrass hay *ad libitum* and one of four supplements: 1) a high RDP supplement provided once daily, 2) high RDP supplement provided every 2nd day, 3) a high RUP supplement provided every 2nd day or 4) a 50:50 mixture of the RDP and RUP supplement provided every 2nd day. The RDP and RUP were provided at a rate of 0.23 and 0.30% of BW, respectively. Supplementing every 2nd day was at twice that of daily supplementation, resulting in all supplements being provided on an isonitrogenous basis of 7.85 g N/day across a 48hour supplementation interval. Atkinson *et al.* (2010) documented that supplementing every 2nd day reduced the N retention as a percentage of N intake by means of a lower microbial efficiency, but showed an overall increase in the OM apparent tract digestibility. The results of N retention as a percentage of N intake observed by Atkinson *et al.* (2010) differ from the current study (Table 4.3.1), as there was no difference between the treatments for N retention as a proportion if N intake. The reason for this is due to the amount of supplement provided per treatment. The N intake in the current study was greater for daily supplementation and less when supplemented infrequently and thus resulted in the N retained as a proportion of N intake to remain unchanged. The CP apparent digestibility (Table 4.1.1) for daily supplementation tended to be higher due to the total intake of CP being higher for that treatment. McGuire *et al.* (2013) made an interesting discovery that the supplementary frequency when supplementing wether lambs and steers with urea and soybean meal showed no effect on nitrogen retention. McGuire *et al.* (2013) further explained that infrequent nitrogen supplementation to ruminants consuming low quality forage had minimal effects on the total nitrogen retention and the digested nitrogen retained resembled the amount of daily supplementation.

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Bohnert *et al.* (2002) provided low-quality meadow hay to wethers (36 ± 1kg BW) and supplemented: daily, every 3rd or every 6th day with DIP (82% of CP) and UIP (60% of CP) at approximately 0.19% of BW/day (averaged over a 6-day period). The aforementioned study does not support the findings of the current study, as Bohnert *et al.* (2002) observed that there was a tendency for the faecal N concentration to increase, as the supplementation frequency decreased. In support of the current study and in contrast to the study of Bohnert *et al.* (2002), McGuire *et al.* (2013) found that there was a tendency for faecal N excretion to decrease, as the supplementation of urea or soybean meal decreased. When examining the faecal excretion as a proportion of N intake (Table 4.3.1) it was noted that the faecal excretion increased when the supplementation frequency decreased. A possible reason for this can be described by the NGGR (Table 4.2.2) and the utilization of N between the different treatments. Chilibroste *et al.,* (2005) described that when the NGGR increased the digestibility of the fibre fraction decreased proportionally due to the overall increase in passage rate. It was noted in the current study that the non-glucogenic/glucogenic ratio as well as the acetic/propionic ratio was lower when supplementing once daily, every 2nd day and every 3rd day in comparison to supplementing only once a week (Table 4.2.2).

Both Bohnert *et al.* (2002) and McGuire *et al.* (2013) support the findings of the current study in terms of daily N retention, as it was found that the daily N retention had a tendency to decrease when the supplementation frequency decreased (Table 4.3.1). Detmann *et al.* (2014) conducted a study using Holstein x Zebu heifers provided a basal diet of *Brachiaria decumbens* and supplemented with an increasing amount of nitrogenous compounds (casein, albumin, urea, soybean meal or a mixture of these sources). It was indicated that there was a quadratic and negative relationship between the relative production of microbial nitrogen (NMICR) and apparent relative nitrogen balance in the rumen (RNB). This led to the estimated conclusion that when a theoretical state of nitrogen equilibrium (RNB=0) was achieved in the rumen, 62% of the nitrogenous compounds were of microbial origin. The work done by Detmann *et al.* (2014) further indicated that there was no influence on rumen nitrogen balance from dietary DOM and the CP to DOM ratio. In support of the current study, as shown in Table 4.3.1, Detmann *et al.* (2014) indicated that the rumen nitrogen balance increased linearly when the dietary CP increased.

Table 4.3.2 describes the measured RAN concentration as a response to the different treatments. Figure 4.3.1 visually illustrates the measured RAN concentrations and, as seen there were no peaks for treatment D. This is due to the fact that the supplements were provided only once a week.

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Table 4.3.2 Rumen ammonia nitrogen concentration (mg NH_3 – N/100ml rumen fluid) of wethers receiving *Eragrostis curvula* hay and supplemented intermittently with 250g of Voermol production lick, everyday (A), every 2nd day (B), every 3rd day (C) or once/week (D)

	Treatment				SEM*
	A	B	C	D	
Sampling time					
07:00	$9.9^{a}{}_{1}$	$6.7^{b}{}_{1}$	$7.1^{b}{}_{1}$	$6.1^{b}{}_{1}$	0.8
10:25	$8.4^{a}{}_{2}$	$5.5^{b}{}_{12}$	$5.4^{b}{}_{3}$	$5.3^{b}{}_{13}$	0.8
13:50	$5.8^{a}{}_{3}$	$4.3^{ab}{}_{2}$	$3.5^{b}{}_{2}$	$3.2^{b}{}_{2}$	0.8
17:15	$9.2^{a}{}_{12}$	$5.4^{c}{}_{12}$	$6.3^{c}{}_{13}$	$2.9^{b}{}_{2}$	0.8
20:40	$6.1^{a}{}_{3}$	$6.1^{a}{}_{13}$	$3.8^{b}{}_{2}$	$3.0^{b}{}_{2}$	0.8
00:05	4.0_{4}	4.3_{2}	3.2_{2}	3.8_{23}	0.8
03:30	3.9_{4}	4.8_{23}	3.8_{2}	3.4_{2}	0.8
Average	6.8^{a}	5.3^{c}	4.7^{c}	3.9^{b}	0.2

^{a,b,c} Values bearing different superscripts in the same row differ significantly (P<0.05)
^{1,2,3,4} Values bearing different subscripts in the same column differ significantly (P<0.05)
*Standard error of mean

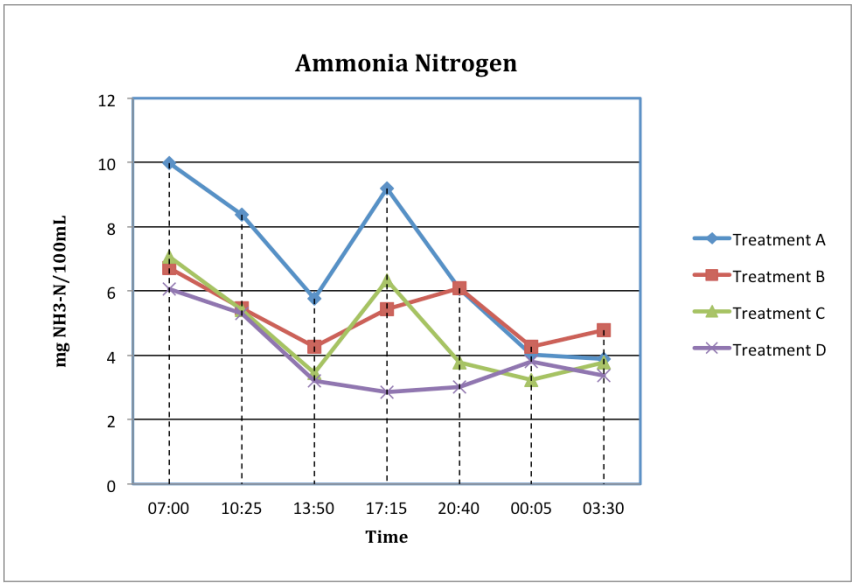

Figure 4.3.1 Rumen ammonia nitrogen (mg NH_3 – N/100ml) of wethers receiving *Eragrostis curvula* hay and supplemented intermittently with 250g of Voermol production lick, everyday (A), every 2nd day (B), every 3rd day (C) or once/week (D)

The RAN concentration, as described in Table 4.3.2 showed that treatment A differed from all the other treatments when comparing it between 7:00am and 10:25am, where after treatment A differed from treatments C and D at 13:30pm and again differed from all the other treatments at 17:15pm. There was no difference between the treatments from 00:05am to 03.30am and the RAN concentration seemed to remain constant between this timeframe, as seen in Table 2.3.2. In comparison with the rumen VFA measurements the results revealed that when the concentration of VFA peaked a few hours after feeding, the RAN concentrations rapidly decreased, while peaking at the time of feeding. When examining the average RAN concentrations, it was noted that there was a

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difference between treatment A and treatments B, C and D. Treatment B and C had no difference between each other, but differed from treatments A and D. The average RAN concentration was higher for treatment A and the lowest for treatment D.

Discussion

Erdman *et al.* (1986) conducted a study using Holstein cows that were fed a TMR and infused with urea N at 0, 33, 67, and 100g/day. In support of the findings of the current study, where the Voermol production lick had 29.7% CP (67.4% NPN and 2% RUP), Erdman *et al.* (1986) indicated that the time of sampling had a significant effect on RAN, as higher values occurred at or near feeding and lower values at 2 to 6hours post feeding. Canesin *et al.* (2014) supplemented steers (325 ± 65.7kg BW) with urea, citrus pulp and cottonseed meal, either once daily, once daily except Saturdays and Sundays or every 2nd day at 10, 14, and 20 g/kg BW per day, respectively. In contrast to the findings of Erdman *et* al., (1986), Canesin *et al.,* (2014) observed that RAN peaked 2hours after supplementation (4.64 mg NH_3–N/100ml) and that the concentration decreased for up to 8hours post-supplementation. Although the study of Canesin *et al.,* (2014) differed from the current study pertaining to the time it took the RAN concentration to peak, it was well in accord with the time it took for the RAN concentration to decrease.

Detmann *et al.* (2009) conducted a study using Holstein x Zebu heifers provided a basal diet of *Brachiaria decumbens* and supplemented with an increasing amount of nitrogenous compounds. The CP contents in the diets ranged from 51.9 to 136.3g/kg DM. The results observed by Detmann *et al.* (2009) indicated that effective degradability (ED) of NDF and discrete lag (LAG) presented a *linear-response-plateau* according to the RAN concentration. This resulted in a break point in the range of 8mg/100ml for ED and LAG, which represents the maximum estimate and minimum estimate respectively, as depicted in Figures 4.3.2 and 4.3.4. The RAN concentration defined to optimize NDF degradation and intake was 8mg/100ml and 15mg/100ml, respectively. It was concluded that the difference between these estimates appeared to be due to a better adequacy of the microbial protein to metabolizable energy ratio (Detmann *et al.*, 2009). In the current study it was observed that the RAN concentrations of the sheep receiving supplementation every 2nd day, every 3rd day and once a week fell beneath the required RAN concentration (8mg/100ml) needed for efficient microbial fermentation (Table 4.3.2). Supplementing everyday fell beneath the required concentration, between 10:25am and 17:15pm as well as from 20:40pm onwards. The findings of the current study relating to the average RAN concentration indicated that the sheep receiving supplementation everyday had the

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highest RAN concentration, while there was a tendency for the RAN concentration to decrease as the amount of CP intake decreased.

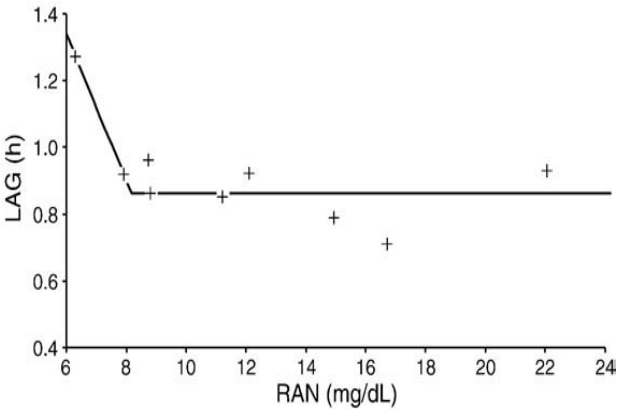

Figure 4.3.2 Relationship between discrete lag (LAG) and rumen ammonia nitrogen (RAN) concentration ($\hat{Y}$ = 2.6660 + 0.2210X, ∀ X ≤ 8.1672; $\hat{Y}$ = 0.8601, ∀ X > 8.1672; R^2 = 0.7642) (n = 50; + = least square means of treatments). (Detmann *et al.,* 2009)

Figure 4.3.3 illustrates that NDF intake had a quadratic profile as a function of RAN concentration, and that the critical point for maximum response was obtained at 15.17 mg NH_3 – N/100ml. Figure 4.3.4 illustrates that the ED of NDF had a *linear-response-plateau* pattern as a function of RAN concentration and that the ED of NDF increased until the RAN concentration reached 8.00 mg/100ml, after which the ED estimates became unchangeable (Detmann *et al.*, 2009). Figure 4.3.5 illustrates the quadratic pattern between intestinal flow of microbial nitrogen (Nmic) and RAN concentration, where the critical point was reached at 14.52 mg NH_3 –N/100ml.

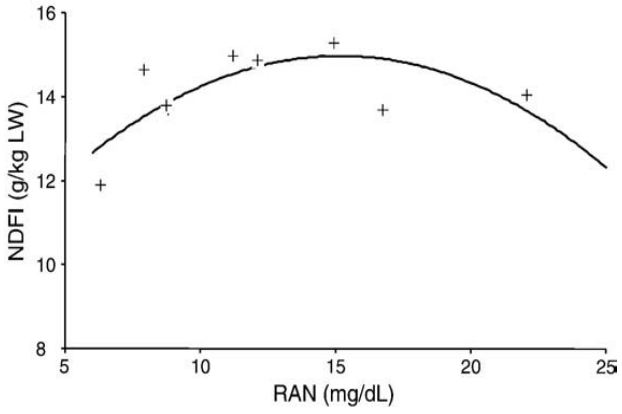

Figure 4.3.3 Relationship between neutral detergent fibre intake (NDFI) and rumen ammonia nitrogen (RAN) concentration ($\hat{Y}$ = 8.6387 + 0.8353X – 0.027525X^2; R^2 = 0.5087) (n = 50; + = least square means of treatments), (Detmann *et al.*, 2009)

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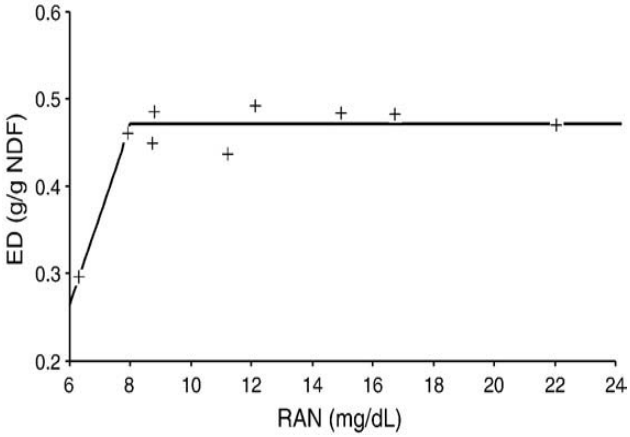

Figure 4.3.4 Relationship between effective degradability (ED) of neutral detergent fibre and rumen ammonia nitrogen (RAN) concentration ($\hat{Y}=-0.35439+0.1032X$, $\forall\ X\leq 8.0048$; $\hat{Y}=0.4719$, $\forall\ X>8.0048$; $R^2=0.9124$) (n=50; + = least square means of treatments), (Detmann *et al.,* 2009)

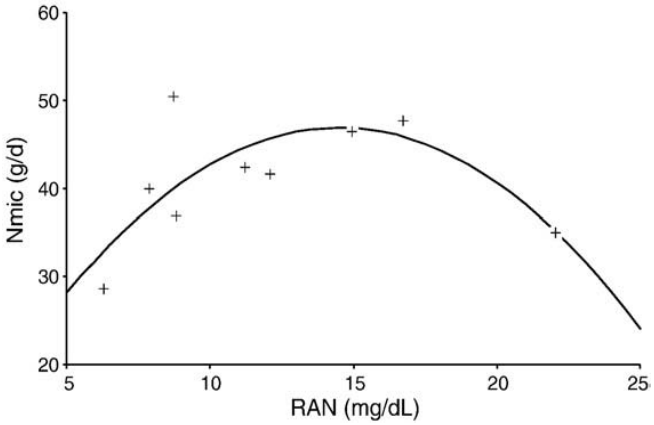

Figure 4.3.5 Relationship between intestinal flow of microbial nitrogen (Nmic) and rumen ammonia nitrogen (RAN) concentration ($\hat{Y} = 3.1825 + 6.0213X - 0.207380X^2$; $R^2 = 0.5485$) (n = 50; + = least square means of treatments), (Detmann *et al.,* 2009)

Detmann *et al*. (2009) implied that when the nitrogen availability in the rumen medium increased, the NDF followed first order kinetics. Thus nitrogen supplementation resulted in an increase of available microbial enzymes used for fibre digestion, while when the RAN concentration was below 8 mg/100 ml the degradation was limited by enzyme activity, characterized as a zero order reaction. According to these findings Detmann *et al*. (2009) concluded that the degradation of NDF of low-quality tropical forage in the rumen could be described as a second order kinetics process. When examining the pattern of LAG estimates, as illustrated by Figure 4.3.2, Detmann *et al*. (2009) implied that nitrogen deficiency at RAN concentrations below 8mg/ 100ml would cause a microbial deficiency in the synthesis of compounds needed for microbial adhesion on fibre and/or enzymes to start NDF and ADF degradation. The NDF intake (Tables 4.11 and 4.3.2) as a response to the RAN concentrations

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obtained in the current study indicated that the maximum response was not achieved. The NDF intake corresponded to a level of intake that was based on the RAN concentrations of 6.8, 5.3, 4.7 and 3.9 mg/100ml for daily, every 2nd day, every 3rd day and once weekly supplementation, respectively (Tables 4.1.1 and 4.3.2). When using these results in connection with Figure 4.3.3, it was noted that the NDF intake for all the treatments was between a range of 12 and 13 g/kg DM. This was in direct accordance to the actual NDF intake, as shown in Table 4.1.1. When focusing on the optimization of NDF degradation the RAN concentration of the current study (Table 4.3.2), it was observed that the average RAN concentrations for all the treatments fell beneath the required concentration of 8 mg/ 100ml.

The study of Detmann *et al.* (2009) was followed up by a study conducted by Detmann *et al.* (2014). The relationship between efficient nitrogen utilization and dietary CP and the concentration of RAN were described by using hyperbolic models. It was concluded that 108 g CP/kg DM and 6.30 mg NH_3 –N/100ml of rumen fluid were the estimated values that corresponded to the apparent equilibrium point. Drawing from the study of Detmann *et al.* (2014) it was observed that when using the results as a reference to the current study (Table 4.3.2) supplementing sheep every 2nd day, every 3rd day and once a week resulted in a negative efficiency of nitrogen utilization, while supplementing everyday had a positive efficiency of nitrogen utilization. In addition, according to the CP intake (Table 4.1.1) it was observed that supplementing once a week was the only treatment that had a negative efficiency of nitrogen utilization. In reference to the work of Detmann *et al.* (2009), depicted in Figure 4.3.6, it was observed that the RAN concentration of the current study (Table 4.3.2) did not resemble the amount of CP intake as shown in Table 4.1.1.

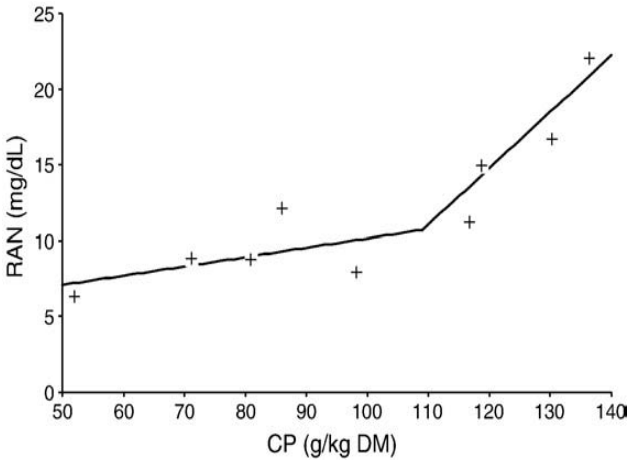

Figure 4.3.6 Relationship between rumen ammonia nitrogen (RAN) concentration and diet content of crude protein (CP) ($\hat{Y}$ = 4.012 + 0.06142X, X ≤ 109.038; $\hat{Y}$=–29,829+0.37178X, $ XN109.038; R^2=0.7520) (n=50; + = least square means of treatments). (Detmann *et al.*, 2009)

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Table 4.3.3 Purine derivatives and microbial protein production of wethers receiving *Eragrostis curvula* hay and supplemented intermittently with 250g of Voermol production lick, everyday (A), every 2nd day (B), every 3rd day (C) or once/week (D)

	Treatment				SEM*
	A	B	C	D	
Purine derivatives, mmol/day					
Allantoin	11.6	9.9	9.4	8.9	1.0
Uric acid	2.7^{a}	2.3^{b}	2.2^{b}	1.9^{c}	0.1
Hypoxantine	1.8$_{a}$	1.6$_{ab}$	1.6$_{ab}$	1.2$_{b}$	0.1
Xantine	0.2	0.2	0.2	0.2	0.0
Total purine derivatives, mmol/day	16.4	14.0	13.4	12.2	1.2
Microbial nitrogen, g/day	14.1$_{a}$	13.1$_{ab}$	11.5$_{ab}$	10.5$_{b}$	1.9
Microbial protein, g/day	88.1$_{a}$	81.8$_{ab}$	72.0$_{ab}$	65.5$_{b}$	5.3
NMICR#	0.4	0.5	0.5	0.5	0.1

a,b ,c Values bearing different superscripts in the same row differ significantly (P<0.05)
* Standard error of means
# NMICR = (g microbial N/ g ingested N), (Detmann *et al.,* 2014)

The allantion concentration had a tendency to decrease when the supplementation frequency decreased. Examining the uric acid concentration, it was observed that treatment A differed from treatments B, C and D, while treatments B and C did not differ from each other. The uric acid concentration was higher for treatment A and decreased as the supplementation frequency decreased from treatment A to D. The concentration of hypoxantine showed that there was no difference between treatments A, B and C as well as between treatments B, C and D. The concentration of hypoxantine is significantly higher for treatment A in comparison to treatment D. The amount of microbial nitrogen produced indicated that treatment A differed from treatment D, while treatment A and Treatment D did not differ from treatments B and C. The microbial protein produced was higher for treatment A and degreased as the supplementation frequency decreased from treatment A to treatment D. The NMICR did not differ between treatments, although treatment A had the lower value.

Discussion

Canesin *et al.* (2014) observed that supplementing steers (325 ± 65.7kg BW) with urea, citrus pulp and cottonseed meal, either once daily, once daily except Saturdays and Sundays or every 2nd day at 10, 14, and 20 g/kg BW per day respectively, showed no significant effect on nitrogen intake, duodenal bacterial nitrogen flow and bacterial nitrogen synthesis. These findings support the results of the current study (Table 4.3.3) in terms of the bacterial nitrogen synthesis. Wickersham *et al* (2008) fed a basal diet of prairie hay to steers (366 kg) while supplementing casein daily at 61 and 183 mg of N/kg BW and every 3rd day in amounts of 61, 183, and 549 mg of N/kg BW. It was observed that recycled urea provided 23% of the nitrogen in microbial protein for steers supplemented daily at 183

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mg of N/kg BW, but 42% for steers supplemented 549 mg of N/kg BW every 3rd day. Wickersham *et al.* (2008) found that the urinary N excretions and the urinary urea-N excretions were similar between supplementing 61 and 183 mg of N/kg BW. This indicated that there was no measurable inefficiency in the N metabolism when supplementing infrequently. This was not the case with the current study when using the results of Detmann *et al.* (2014) as a reference. It was indicative that supplementing every 2nd day, every 3rd day and once a week resulted in a negative efficiency of nitrogen utilization, while supplementing everyday had a positive efficiency of nitrogen utilization. The faecal N excretion can be linked to the aforementioned results, as it was found that the faecal excretion as a proportion of N intake (Table 4.3.1) increased when the supplementation frequency decreased. The reason for the difference between the results (Table 4.3.3) of the current study and the study done by Wickersham *et al.* (2008) was due to the amount of supplements provided per treatment. The amount of supplement provided in the current study remained at 250g per sheep for infrequent as well as daily supplementation, while Wickersham *et al.* (2008) increased the amount of supplements provided when supplementing infrequently.

Detmann *et al.* (2014) did a study using Holstein x Zebu heifers provided a basal diet of *Brachiaria decumbens* and supplemented with an increasing amount of nitrogenous compounds (casein, albumin, urea, soybean meal or a mixture of these sources). When applying the results of Detmann *et al.* (2014) to the current study, it was calculated that the NMICR (g microbial N / ingested N) when supplementing every 2nd day, every 3rd day and once weekly, al provided the same estimate of RNB (g ingested N – g N flowing out to the abomasum/ g ingested N). This finding was based on the quadratic and negative relationship between the relative production of microbial nitrogen and apparent relative nitrogen balance in the rumen, described by Detmann *et al.* (2014). When examining the results of the current study (Table 4.3.3) there was a tendency for the amount of N retained to be higher when the NMICR got lower. Supplementing daily, every 2nd day, every 3rd day and one a week had NMICR of 0.4, 0.5, 0.5, and 0.5 respectively, which was in direct connection and corresponded with the nitrogen retention results shown in Table 4.3.1, and the microbial protein production results shown in Table 4.3.3.

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CHAPTER 5

CONCLUSION

The DM, OM, NDF and ADF intake and total tract apparent digestibility showed no difference between the treatments, although there was a tendency for feed intake to be higher for daily supplementation, whereas the NDF and ADF digestibility tended to be higher when supplementing every 2nd day. It was observed that there was a tendency for the DM, OM, NDF, ADF and CP total tract apparent digestibility to decrease, as the supplementation frequency decreased. In relation to this, the propionic acid concentration decreased as the supplementation frequency decreased. It was noted that the non-glucogenic/glucogenic ratio as well as the acetic/propionic ratio was lower when supplementing everyday, every 2nd day and every 3rd day in comparison to supplementing only once a week. When considering microbial protein production, nitrogen retention, VFA concentrations and RAN, it was recognized that there was a tendency for daily and every 2nd day supplementation to be the preferable treatments. The microbial protein production tended to increase as the frequency of supplementation increased, although there were no differences between supplementing everyday, every 2nd day and every 3rd day. It was observed that the RAN concentrations of the sheep receiving supplementation every 2nd day, every 3rd day and once a week fell beneath the required RAN concentration (8mg/100ml) needed for efficient microbial fermentation (Table 4.3.2). Supplementing everyday fell beneath the required concentration, between 10:25am and 17:15pm as well as from 20:40pm onwards. The findings relating to the overall average RAN concentrations indicated that the sheep receiving supplementation everyday had the highest RAN concentration, although al the treatments came short of the required RAN concentration (8mg /100ml). This suggests that when supplementing infrequently it is advisable to increase the amount of supplements given when the frequency of supplementation decreases. The NGGR and acetic/propionic ratio surprisingly showed no difference between supplementing everyday, every 2nd day and every 3rd day. When supplementing only once a week resulted in an immensely low VFA concentration of 64.0 mmol/L rumen fluid, while supplementing everyday, every 2nd day and every 3rd day resulted in VFA concentrations of 154.1, 149.6 and 136.0 mmol/L rumen fluid, respectively. According to the DM, OM, NDF, ADF and CP digestibility, VFA concentrations, microbial nitrogen production and nitrogen retention, supplementing everyday, every 2nd day and every 3rd day could be implemented. In addition, when focusing on the efficiency of microbial fermentation in terms of the RAN concentrations, supplementing everyday turned out to be the only advisable treatment. It would be advised to increase the daily amount of supplement provided when supplementing less that every 2nd day when the aim is focused on efficient microbial fermentation, while it is definitely not advised to supplement only once a week at the same level of supplement.

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CHAPTER 6

CRITICAL EVALUATION

When reflecting on the main aim and the findings of this study, it's indicated that infrequent supplementation could hold promise as a new management strategy. The outcome of this study could have had a greater significant effect if the basal diet was of poor nutritive value or the supplement provided contained a higher concentration of protein when fed medium quality roughage. Future research should be focused on replicating the study under extensive poor rangeland conditions and in addition determining animal production. When linking the fermentative results with weight gain and reproduction a broader picture might be seen in terms of practicality and the cost saving efficiency.

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