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A Comparison of Microbial Protein Supply of Steers Grazing *Ad Libitum* Ryegrass or Fodder Beet

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A dissertation
submitted in partial fulfilment
of the requirement for the Degree of
Bachelor of Agricultural Science with Honours
at
Lincoln University 2014
by

Sophie Prendergast

Abstract of a dissertation submitted in partial fulfilment of the
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**A Comparison of Microbial Protein Supply
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Microbial protein synthesis of steers of mean starting liveweight 286kg was established on a control diet of *ad libitum* winter grass and a treatment diet of *ad libitum* fodder beet with 1kg DM of lucerne silage as a fibre source. The experimental design was a four steer by two diet treatment comparison (experiment 1 and 2) with five sub-trials in each experiment. These series of trials assessed *in vivo* digestibility of diet energy and protein, nitrogen balance, and diurnal variations of rumen parameters (pH, redox, and acid, ammonia and urea concentrations) and urine and faeces production and parameters (pH, nitrogen concentration) in housed individual metabolism crates for 11d. Microbial protein production was estimated from purine derivatives determined from total urine collection for 9d of this period. Outdoors, under industry standard grazing conditions, rumen evacuations (10am, 6pm and 2am) and serial placement of *in sacco* nylon bag (0, 2, 4, 6, 12, 24, 48h) methods were used to assess rumen fluid passage rates, pool changes and feed dry matter and nitrogen degradability for both treatments.

Voluntary dry matter intake and microbial protein supply to the steers, and the mean diurnal rumen pH, was higher in the fodder beet treatment than in the winter grass treatment. Fodder beet supplied almost twice as much microbial protein as winter grass despite a lower crude protein content of the diet. Total rumen VFA and ammonia concentrations, and mean redox, were lower for the fodder beet than winter grass but the rumen urea concentration was higher in the fodder beet treatment. The winter grass treatment rumen nitrogen pool size was only greater than the fodder beet at the 6pm rumen evacuation. The initial 0h DM and nitrogen % disappearance of fodder beet bulb was extremely high as

assessed by serial nylon bag placement and removal. However, the rate of DM and N disappearance (%/h) of fodder beet leaf after 6h incubation was higher than that of the fodder beet bulb and the winter grass. The higher voluntary intake, DMD% and rate of disappearance of fodder beet bulb and leaf compared with winter grass did not result in lower rumen pH or higher redox as would be expected by the current understanding of rumen function. The lower crude protein content and very low concentrations of rumen ammonia were not observed to reduce microbial protein production as would be expected by the current understanding of nitrogen kinetics in the rumen. The observed high microbial protein supply with fodder beet diets may be the result of ruminal and extra-ruminal adaptations to greater nitrogen recycling in low dry matter diets of high energy density not well described at present.

Keywords: Microbial protein production, rumen function, nitrogen recycling, fodder beet, pasture fed, *ad libitum* feeding, purine derivatives.

Acknowledgements

Firstly I would like to sincerely thank my supervisor Dr. Jim Gibbs for his very valuable time, expertise and direction with my honours project this year. I thoroughly enjoyed the research work and you ensured that the working environment was always positive no matter what the situation was that faced us. This project has well and truly exceeded my expectations and I am extremely grateful to have had this opportunity to learn from one of the best scientists in ruminant nutrition.

To Dr. Bernardita Saldias, I cannot thank you enough for the endless hours you dedicated to ensuring that my trials ran smoothly. You would happily give assistance at any hour of the day or night and I thoroughly appreciate this. I am also very grateful for all the help you gave me with editing, learning to use new programmes, and helping to find papers I could not access. I know this has been a very busy time for you but you would always help me when I needed it and I am very thankful.

To Dr. Terry Hughes, your input into my dissertation has been invaluable. By working with you, not only did my writing improve, but I learned about a wide variety of topics which I had very little knowledge of, and especially in ruminant nutrition. I greatly appreciate that

you took your own time to spend with me to help me to deepen my understanding of ruminant nutrition, so thank you very much.

To Rob McDonald, thank you for all the weekends, late nights, early mornings and all-nighters that you endured for my trial. You were very efficient and practical to work with and this helped to make the trial run smoothly. You also took a lot of pressure off me by helping with the processing of samples for analysis and I am very grateful for this.

To Ali, thank you for your help with the second half of the experiment. An extra pair of hands at a very busy time was thoroughly appreciated.

To Rosy Tung and the staff at Riddolls, thank you for the very prompt analysis of my samples, I am very grateful.

To Matt Price, your expertise in processing some of my data was invaluable and I could not have presented some of this data without your help so thank you very much.

To my family and friends who have supported me through this year in many different ways, I cannot name everyone but I truly appreciate that I have so many close friends and family that I can call on if I need to.

To my parents, you have been pillars of support for me not only this year but the last four years at university, and school prior to that. You expected me to set my goals high, respected my decisions and supported me to achieve my goals. For this I am exceptionally thankful.

To Stu, you have unwaveringly endured my endeavours over the last two years and your support has been exceptional. I know it has not been easy, but I will be forever grateful to have achieved this goal with your support and I look forward to the opportunities that this creates for us.

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Chapter 1
Introduction

Fodder beet (FB) has been grown in New Zealand for over 100 years, however, the current system of *ad libitum* grazing of FB *in situ* and with minimal supplementation is a new concept developed in the last five years here at Lincoln University by Dr Jim Gibbs. The FB crop has unique morphological characteristics which historically made the implementation of an *in situ* grazing system challenging and risky. A bulb crop, it has relatively low levels of crude protein (CP), fibre and minerals. However, there is a very high supply of water soluble carbohydrates (WSC) and therefore a high supply of rapidly fermentable metabolisable energy (FME). As a result of this, a transition period is required to allow rumen microbes to adapt to the high FME supply to the rumen when FB is grazed (Gibbs, 2014). Further, due to the low CP, fibre and mineral content of the feed, it has previously been considered to be marginal in terms of its supply of nutrients to the animal to support high levels of production.

Historically, FB has been a crop which was harvested and stored before feeding as a supplemental feed, but not as the main dietary source (Anonymous, 2013). The establishment of FB has been a challenge due to the morphology of seeds and lack of herbicide and pesticide programme specificity in New Zealand. This resulted in fodder beet cropping requiring specialist equipment or being a very labour intensive crop, and only small areas (<100ha) of FB were grown in New Zealand until the mid 2000's (Gibbs, 2011).

The use of other winter crops such as kale or swedes has resulted in liveweight gains of approximately 200 g/day in high producing beef systems (Woods et al., 1995). The current system of FB feeding is to graze animals *in situ* on an *ad libitum* diet of FB while supplying a supplemental fibre source such as lucerne silage or straw at approximately 1-2 kg DM/day. Over recent years, commercial scale trials have been conducted to record the liveweight gains of yearling steers fed in this manner. In 2014, liveweight gains of 1.1kg /day have been recorded for 290-530kg steers on this system of FB for 150d from March to September at stocking rates of 20+/ ha, a mean per hectare liveweight production of greater than 3000 kg in that period (Gibbs and Saldias, *unpublished data*). That these liveweight gains are very high is unexpected due to the low CP content (<14%) of the feed.

Due to the low use of FB grazing systems in New Zealand farming until recent years, there has been very little research into the physiological implications of using this feed. There has been a body of research conducted over recent years to investigate the rumen environmental parameters in FB fed animals, however, no specific research on protein metabolism for any FB fed stock is available (Gibbs, 2011). Specifically, given the apparent protein lack for the observed liveweight gains in young cattle fed on this system, there is a need for research to determine the microbial protein supply to the ruminant on an *ad libitum* FB diet with low levels of supplementation.

The objective of this research was to establish if there was a significant difference between the microbial protein production in steers fed a diet of *ad libitum* winter pasture compared with a diet of *ad libitum* FB with 1 kg DM supplementation. In addition, the influence of any differences in rumen function in each diet treatment on microbial protein supply would be determined, and the relative nitrogen use characteristics conferred by each diet treatment would be measured.

Chapter 2
Review of the Literature

2.1 Introduction

Fodder beet (*Beta vulgaris*) is a member of the Chenopodiaceae family and it has been grown in New Zealand for over 100 years. Historically, the crop required specialist ridge seeding, hand thinning and regular hand/hoe weeding for establishment. Recently, FB has gained popularity in both the dairy and the beef sectors due to the high dry matter yield and the high nutritive value of the crop. Fodder beet yields in 2014 have been recorded to be higher than 40t DM hectare $^{-1}$ (Gibbs, 2014). Given that crop establishment costs are constant per hectare, the cost of each kilogram of dry matter decreases as the yield increases. This can make FB a very cheap (5-10c kg^{-1} DM in 2014) way to feed stock on a small area. Fodder beet has a metabolisable energy (ME) content of approximately 12 MJME/kg DM, and has a very high sugar content (Clark et al., 1987). This provides a source of energy for the rumen microbes to utilise for microbial protein production. Sucrose has been shown to be a more efficient energy source to support ammonia assimilation by the microbes than starch (Chamberlain et al., 1993). Fodder beet has a very low CP content; the bulbs commonly contain less than 10% CP and the leaf commonly contains approximately 15% CP (Eriksson et al., 2004; Gibbs, 2014). Additionally, FB has a very low dry matter percentage; usually between 10-20% (Eriksson et al., 2004; Gibbs, 2014). A specific transition strategy has been developed to mitigate the risk of rumen acidosis due to the high content of WSC. The low DM content combined with the high proportion of readily soluble carbohydrates is likely to cause the FB to have a fast passage rate through the rumen. This will mean that rumen microbes will increase their energetic efficiency as they will be washed through the digestive tract more quickly and the maintenance energy requirements will be reduced. Bach et al. (2005) reported that when there is a high passage rate of digesta, microbes with fast growth rates are selected for, which decreases the maintenance energy required.

One of the benefits of FB in New Zealand is the flexibility to either graze it *in situ* or harvest the bulbs and store them for up to 4 months. Dairy cattle can be wintered on FB, and it can also be harvested and used to supplement lactation in the autumn and spring. Dry stock

farmers are able to finish cattle on FB or produce the feed and sell it without having stock on the property which reduces management requirements. Fodder beet is harvested using a flail mower which severs the leaf from the top of the bulb. The bulb is stored while the leaf is incorporated back into the soil. This causes a recycling of nutrients so reduces losses. Storage of the bulb requires little management as bulbs can be stacked in windrows and do not need to be covered. The quality of the bulb will not decline over storage for up to 4 months.

Over recent years the agronomy of FB has improved vastly. Fodder beet seeds have a rough exterior which makes the practice of precision drilling problematic as seeds are prone to getting stuck in the drill. To overcome this problem, seeds are now coated to make them into a uniform shape which is suitable for precision planting. Fodder beet has weak seedling vigor which means that weed and pest control is very important, especially in the early stages of development. If weeds are not controlled, the FB seedlings are likely to be outcompeted for light and nutrients, and will die out.

Furthermore, FB seedlings tend to be very sensitive to a number of compounds which are associated with many common herbicides and pesticides. Therefore, a number of sprays have been manufactured which are sensitive to the delicate requirements of FB, but are effective in controlling weed and pest problems. In the early stages of development, FB requires intensive management and the timing of sprays and fertiliser applications are vital to the success of the crop. However, once canopy closure is reached, the crop requires very little further management. Fodder beet has a relatively low requirement for N, however, it has a very high requirement for K and NaCl (Niazi, 2007). Due to the high requirement for K and NaCl, combined with the high herbicide and pesticide requirement, the crop has a high establishment cost per hectare – in 2014, $1700-2100 (Gibbs, 2014). However, if the early crop management is thorough, the yield is likely to be very high which will cause a dilution of the cost per unit of feed produced.

The use of FB as an unrestricted finishing feed in beef systems is now very common in New Zealand, the only country in which it has been developed. Given the high reported liveweight gains from FB and the low CP content of the feed, detailed information on the rumen microbial protein supply is required and of great interest, however, there are no available studies of this.

2.2 Microbial Protein Synthesis

In the rumen there are many microbes- bacteria, protozoa and fungi. The microbes digest feed as it stays in the rumen. They use this energy transfer from the diet for their own growth and reproduce. Bacteria are then passed through into the small intestine where they are absorbed as microbial true protein. A large proportion of protozoa are selectively retained in the rumen and may complete multiple life cycles without being passed into the small intestine. The microbial true protein makes up between 50 and 80 per cent of the protein supply to the ruminant (Bach et al., 2005); however, this can change depending on the system and the diet.

The three main factors influencing microbial protein production are nutrient supply, microbial population and rumen environmental conditions (Thomas, 1973). Nitrogen and energy are the most limiting nutrients for microbial protein synthesis. When either of these resources are in short supply, microbial growth rates will not be maximised and microbial protein synthesis will be inefficient. Ruminants possess a unique ability to survive solely on non-protein nitrogen (Dewhurst et al., 2000). However, microbial protein synthesis will not be maximised unless urea, amino acids and peptides are also present especially in low protein diets (Dewhurst et al., 2000; Firkins, 1996). Forming peptide bonds and amino acids is energetically inefficient; therefore microbes use preformed peptides and amino acids to increase efficiency of growth. Different species of bacteria will thrive on different proportions of ammonia: urea: amino acid: peptide chains (Maeng et al., 1976).

Ammonia is the central intermediate in the degradation and assimilation of dietary N in the rumen (Maeng et al., 1976). Russell and Strobel (1987) reported that microbial protein synthesis was limited if rumen ammonia levels were less than 50 mg/L rumen fluid in stock fed total mixed rations. Ammonia is a lipophilic compound which allows it to diffuse passively across membranes, therefore it can be lost through the rumen wall if it is not incorporated into microbial protein (Hume, 1970; Russell & Strobel, 1987). This ammonia will be transported to the liver where it will be converted to urea. A small proportion of the urea in the blood will be lost through urine, but the majority of the urea will be recycled to the rumen through the salivary glands (Firkins, 1996) and direct rumen infusion. Protein N can also be released in the catabolism of bodily tissues, this N will be transported in the blood and can also be re-cycled into the rumen. Microbes do not discriminate between N from dietary source, endogenous source or re-cycled urea. This means that almost all

protein N from dietary or endogenous origin will be available for use by the microbes. While microbial protein synthesis will be limited if rumen fluid ammonia levels are less than 50 mg /L, microbial ammonia concentrations levels will be much higher than 50 mg/L. Despite the fact that ammonia passively diffuses through cell wall, much of the free N in the rumen is in the lipophobic ammonium form. Therefore, it will not diffuse passively. Microbes are thought to possess active transport systems to accumulate N across the concentration gradient (Russell & Strobel, 1987). At low levels of extracellular ammonia (50mg /L fluid), intracellular levels were 160mg /L. While rumen ammonia levels are generally considered to be high in pasture fed animals, the ammonia levels in ruminants fed a diet of unrestricted FB are unknown.

The second major limiting nutrient to microbes is energy which is supplied to microbes in the form of carbohydrates. Carbohydrates can be classified as structural carbohydrates and storage carbohydrates. The structural carbohydrate component is made up of cellulose, hemicellulose and lignin, while the storage carbohydrates are predominantly mono, di and oligosaccharides. Storage carbohydrates are easily degraded and fermented by rumen microbes, while structural carbohydrates tend to take a longer amount of time to be degraded and are often less digestible than storage carbohydrates (Kolver, 2003). Microbes degrade carbohydrates and hydrolyse ATP in fermentation. Volatile fatty acids (VFA) are the end products of microbial fermentation, and these are absorbed through the rumen wall as energy for the ruminant. If the supply of energy to the microbes is insufficient, microbial protein synthesis will be restricted.

Different species of microbes digest different feed types. For example, cellulolytic bacteria digest cellulose but will not digest starch. The different bacterial and protozoan species work at different rates depending on the species present and the substrate being fermented. Starch based feeds are usually degraded more quickly than fibre based feeds. Such feeds provide the potential for the rapid production of VFA and the rapid release of hydrogen (H^+) ions into the rumen. The pH will then decrease which creates the potential to selectively inhibit or kill some species of microbes and this may reduce microbial efficiency (Dewhurst et al., 2000). Therefore, it is important that the carbohydrate ratio of rapidly fermented and slower fermenting components of a ruminant diet is closely monitored. A proportion of metabolisable energy (ME) can be classified as fermentable metabolisable energy FME. While ME is energy which can be used by the animal to satisfy daily energy requirement,

FME is energy which is used by microbes in the rumen to satisfy their specific energy requirements. Microbes transfer energy from the diet via fermentation which gives them energy to form peptide bonds from non-protein N sources. Microbes are typically unable to ferment feed sources such as fats or oils, or pre-fermented products such as lactic acid in silages. Although carbohydrates are the main source of energy for the microbes, proteins can also be deaminated and used for fermentation as well.

The majority of particles will not leave the sheep rumen unless they are 1mm or less, and cattle rumen unless <5mm (Poppi et al., 1985). Storage carbohydrates require little degradation to be able to leave the rumen. However, structural carbohydrates are more tightly bound together and often require physical degradation as well as extensive chemical degradation. Structural carbohydrates are regurgitated as a bolus to be re chewed and then swallowed again. This process may occur multiple times before the feed particles are small enough to leave the rumen. Parts of the structural carbohydrate will be indigestible, while some digestible components will be released with the physical mastication.

Rumen environmental conditions have a large impact on the efficiency of microbial protein synthesis. The confinement fed rumen is maintained at approximately 39°C and at a pH between 5.5-6.5 (Russell & Hespell, 1981), and the pasture based rumen at 37-41°C and 5.0-7.0 (Gibbs & Laporte, 2008). A low pH will cause some bacteria to spill energy across their membranes as heat which decreases the efficiency of growth of the microbes (Dewhurst et al., 2000; Firkins, 1996). This will cause a number of cellulolytic bacteria to be inefficient, while other bacterial species will die off completely. For example, *Fibrobacter succinogenes*, a major cellulolytic bacterial species, is unable to maintain a membrane potential at low pH and will be unable to absorb and transport glucose for their metabolism (Chow & Russell, 1992). They also found in confinement fed cattle that bacterial growth declined steadily from pH 6.5 – 5.5 but ceased completely at pH 5.4. Glucose uptake also ceased at pH 5.5. This will result in degradable fibre potentially being lost in the faeces which represents a further energy loss from low pH (Piwonka et al., 1994).

Mastication has been found to stimulate the production of saliva (Plaizier et al., 2009). Therefore the more fibrous a feed, the more mastication that will occur and the more saliva that will be produced. Saliva acts as a pH buffer and also brings nutrients to the rumen. The inorganic compounds such as sodium bicarbonate found in saliva help to neutralise the

organic volatile fatty acids (Baumont et al., 2006; Plaizier et al., 2009). It has been found that chewing enhances the production of saliva (Plaizier et al., 2009). Maekawa et al. (2002) reported that this is due to the need to reduce particle size of highly fibrous feeds in order to form a bolus. This means that as particle size of feed decreases and more readily soluble carbohydrates are available, there will be less chewing, therefore there will also be less saliva production while VFA production in the rumen will be rapid. This is likely to lead to a decrease in the pH as there are less buffering agents present and a high number of VFAs. However, Maekawa et al. (2002) found that while chewing will increase saliva production at the time of eating and ruminating, the total daily saliva production is similar in spite of the fibre content of the feeding ration. The diurnal pH fluctuations were also similar for diets differing in fibre content. For pasture based diets, the effect of ensalivation is less clear at present.

Owens et al. (1998) reported that there is a 6-10 hour lag time between the meal time and the minimum pH in the rumen in cattle fed TMR. This was also demonstrated in South Island cows on pasture diets (Gibbs & Laporte, 2008). This gives a significant amount of time for salivary secretions into the rumen which will help to buffer the decline in pH. In addition, nitrogen of endogenous origin which has been catabolised, or nitrogen which has left the rumen as urea will then be recycled through saliva for use by microbes. Methanogens also act as a mild and partial pH buffer and help to keep the pH constant (Kolver, 2003). Methanogens utilise hydrogen in the rumen to make methane which is then released from the rumen by eructation. At low pH the end products of fermentation will then change to favour propionate, lactate and butyrate. This is because acetate produces eight hydrogens atoms per glucose molecule compared with four hydrogen atoms produced to form propionate and butyrate. This is also a method of increasing energy efficiency as the production and eructation of methane is an energy cost to the animal (Kolver, 2003).

Rumen osmolality also facilitates the function of rumen microbes. Water levels in the rumen fluctuate to maintain a constant osmolality. When there is a fall in pH, osmolality increases rapidly. Water is then pulled in from outside the rumen to counteract the increase. If the fall in pH is severe, this influx of water can happen very rapidly at the cost of the rumen wall. Parts of the epithelium tissue are ripped off leaving lesions which can allow bacteria to enter the blood. Following this acidotic period, the rumen wall will mend to a higher strength than it originally was- a process call hyperkeratosis or parakeratosis. Papillae formed are thicker

and absorption of VFA from the rumen will take longer in the future (Owens et al., 1998). Rumen osmolality values range from 240-265 mOsm/L for roughage diets and 280-300 mOsm/L for concentrate diets. However, high intake cattle on high quality pastures in the South Island have been observed to generate similarly high mOsm/L to that of TMR fed feedlot cattle (Trotter, 2012). If rumen osmolality values reach or exceed approximately 350 mOsm/L the function of starch and fibre digesting microbes will be impaired (Owens et al., 1998).

After mastication, rumen microbes hydrolyse polymers and ferment sugars, starch and fibre to produce volatile fatty acids (Brock et al., 1982). Different microbial species have different functions in the digestion and fermentation of carbohydrates. Kolver (2003) stated that the microbial population can be categorised as either primary fermenters or secondary fermenters. Primary fermenters hydrolyse bonds in plant material to break complex carbohydrates down to simple carbohydrates so that they are available for digestion by secondary fermenters. Secondary fermenters produce substrates which are then absorbed through the rumen wall and can be used by the ruminant. Different species of primary and secondary fermenters will digest different substrates (Stewart et al., 1997). Some microbial species digest a broad range of substrates such as *Butyrivibrio fibrisolvens* which digests starch, cellulose, xylan and pectins. Whereas other microbial species will be specific in the substrates they digest. *Ruminobacter amylophilus* will only digest starch and *Fibrobacter succinogenes* will only digest fibrous compounds. *Selenomonas ruminatium* is a microbial species which has not been found to hydrolyse polymers but will utilise the products of hydrolysis by hydrolytic species.

2.3 Dry Matter Content

It has long been thought that by decreasing the dry matter content of a feed below a critical threshold, the dry matter intake will decline. Dry matter intake is determined by the rate at which dry matter is consumed multiplied by the time spent grazing. Butris and Phillips (1987) saturated cut grass with water prior to feeding it to steers. This lowered the dry matter content from approximately 22% dry matter for the fresh forage to 14% dry matter for the soaked herbage. Herbage prehension time and mastication time declined as a result of the lowered dry matter content, though there was no effect on the biting rate of the steers.

While dry matter intake of the steers was reduced by an average of 22% with the low dry matter feed, this effect was not seen through a reduction in intake rate. The fresh matter intake rate increased from 127g /minute on the high dry matter to 196g /minute on the low dry matter feed. This caused the dry matter intake rates of the different dry matter feeds to be similar. However, the daily eating time was decreased by the lower dry matter content, which reduced the total daily dry matter intake. Offering silage or hay to the soaked feeds did not increase dry matter intake. Kenney et al. (1984) found a similar effect in a trial using sheep. As the dry matter content of the forage decreased from 94% to 15%, intake of fresh matter increased from 14g per minute to 60g per minute. However, once the DM content of the forage fell below 40%, the increased intake rate of fresh matter could not make up for the decrease in dry matter, and dry matter intake declined. Perhaps the difference in dry matter intake rate seen in this trial was due to the vast difference in dry matter content compared with the trial by Butris and Phillips (1987).

Results by Cabrera Estrada et al. (2003) conflicted with Kenney et al. (1984) finding that total daily dry matter intake and dry matter intake rate didn’t differ when forage dry matter content was reduced from 16.1% to 11.7%. Furthermore, these results conflicted with Butris and Phillips (1987) as daily eating time and ruminating time were also not affected by the reduced dry matter content. It was noted by Cabrera Estrada et al. (2003) that the addition of water to the rumen either by infusion directly into the rumen or by sprinkling of grass prior to ingestion, did not have an effect on the structure of the rumen contents. This was explained by the fact that saliva production in the rumen is approximately 18L /kg dry matter consumed, which amounts to between 250-350L per day (Cabrera Estrada et al., 2003). The total amount of fluid flowing through the rumen of a high intake cow is likely to be between 250-400L per day. Therefore, the addition of 30L of water perfused into the rumen or the 5L of water sprinkled onto the grass is miniscule compared to the total amount of water entering and leaving the rumen.

Moreover, it was observed that when dry matter content of the feed decreased, water intake by drinking also decreased which made the total water intake similar. John and Ulyatt (1987) found that in the first hour after ingestion, the vast majority of plant cells will be damaged due to mastication. The cell water will be released and free to flow through the gastrointestinal tract or be absorbed into the blood stream. Consequently, dry matter intake will not be limited by rumen distension from the addition of water to feed, or from a feed

which naturally has a high water content. No change in rumen fluid VFA content, pH or NH_3 levels were observed. Cabrera Estrada et al. (2004) confirmed that internal rumen mechanisms regulate the flow of water through the rumen as the fresh weight, dry matter content, and fractional outflow rates were not affected by dry matter content of the feed.

2.4 Purine Derivatives

Measuring purine derivatives (PD) in urine of cattle has been identified as a method of measuring the microbial protein supply to a ruminant animal (X. B. Chen et al., 1990). Absorbed purines are extensively degraded to four main end products –allantoin, uric acid, xanthine and hypoxanthine, which are excreted in urine. Most ruminant feeds have a low purine content. Given that virtually all these purines will be degraded in the rumen, nucleic acids leaving the rumen are almost exclusively of microbial origin (X. B. Chen & Gomes, 1992). Purine derivative excretion is directly related to the purine absorption. This means that microbial protein absorption can be estimated from the urinary purine derivative excretion if the purine N: total-N ratio in microbial biomass is known. This method is a simple and non-invasive method of measuring microbial protein supply.

Microbial nucleic acids leaving the rumen undergo extensive digestion in the small intestine. Purine nucleotides are hydrolysed to purine nucleoside and free bases which can be absorbed from the intestinal lumen or utilised by intestinal mucosa. It is assumed that they are 85% digestible - the same as non-microbial nucleic acids. In the intestinal mucosa of cattle, almost all purine derivatives are converted to uric acid by the xanthine oxidase enzyme. Uric acid is unavailable for incorporation into tissue nucleic acids and will be transported to the liver and excreted via the urine. These xanthine oxidase enzymes are present in a large number of tissues in cattle, including the blood. As a result of the high activity of xanthine oxidase, no exogenous purines can be absorbed in cattle. Therefore, the animal must synthesise purines from amino acids. This means that endogenous purine derivative excretion in cattle is much higher than that for other species. As purines are mobilised from bodily tissue, the xanthine oxidase promotes the derivatives to enter the degradation pathway as opposed to the salvage pathway as seen in sheep.

In sheep, the endogenous excretion of purine derivatives was an average of 168 micro-moles /kg LWT $^{0.75}$ compared with the average of 514 µmols /kg LWT $^{0.75}$ seen in cattle(X. B. Chen et al., 1990; Kanjanapruthipong & Leng, 1998). Due to the xanthine oxidase, uric acid is the only

purine derivative found in the urine of cattle, whereas all four purine derivatives are found in sheep urine. X. B. Chen et al. (1990) found that when xanthine was incubated in plasma from cattle, uric acid concentrations increased, however, uric acid concentrations were unchanged when xanthine was incubated in plasma from sheep and pigs. This is due to the lack of xanthine oxidase in the plasma from sheep and pigs. Endogenous losses were found to be variable between animals (12%) however, X. B. Chen et al. (1990) expected that part of the variation was due to nutritional differences. There was also a lack of the uricase enzyme found in the plasma of cattle. This is likely to be another contributing factor to the high concentrations of uric acid found in urine from cattle.

Urinary disposal of purine derivatives is the primary method of excretion, and blood PD levels are cleared at a rate of approximately 30% per hour (X. B. Chen & Gomes, 1992). There is very little recycling of PD in the kidneys which means that excretion rate is a function of the filtration rare through the kidneys and blood plasma PD concentrations.

To measure PD in urine of cattle, total urine excretions must be collected for a number of days. It is ideal to collect urine for at least five days to ensure that day to day variations in purine concentrations are accounted for.

2.5 Rumen Epithelium Turnover

The rumen epithelium is vital in the absorption of VFA and ammonia produced during microbial fermentation in the rumen, while it also plays a critical role as a physical barrier protecting the extra-ruminal cavity from ruminal contents (Trotter, 2012). The inside of the rumen epithelium is covered in thousands of finger-like projections called papillae. These papillae vastly increase the surface area of the rumen wall, and the capacity to absorb nutrients across the rumen wall (Graham & Simmons, 2005). The epithelium is made up of four layers: (from the lumen) stratum corneum, stratum granulosum, stratum spinosum and stratum basale (Graham & Simmons, 2005). The stratum corneum is the initial barrier layer. This layer is made up of cornified keratinocytes which come in direct contact with ruminal contents and shields the lower strata layers from this material (Penner et al., 2011). The stratum granulosum layer is made of granulosa cells and is the second physical barrier layer in the epithelium. This layer contains tight junctions which means that ions and molecules

must pass through the cells by either diffusion or active transport to cross this stratum layer (Graham & Simmons, 2005). This is the dominant layer for barrier function in the epithelium. The stratum spinosum and stratum basale are metabolically active strata layers containing mitochondria (Penner et al., 2011). The stratum basale has the highest number of mitochondria, followed by stratum spinosum then stratum granulosum. This allows for active transport of ions across the epithelium. In addition, when the animal is fed and nutrients are crossing the rumen wall, the stratum spinosum and the stratum basale produce more ketone bodies from the catabolism of volatile fatty acids than the liver. This ketogenesis provides a large amount of the daily energy requirements for the ruminant (Penner et al., 2011).

When a feed with a high concentration of WSC is digested, there is a rapid increase in the concentration of VFAs in the rumen. If VFAs are produced faster than they can be absorbed across the rumen wall, the pH will decrease. Traditionally, ruminal acidosis was defined as occurring when there is a rapid drop of ruminal pH to levels between 5.5-5.0 and <5.0 for sub-acute and acute acidosis respectively. If sub-acute or acute acidosis occurs and pH remains <5.5 for more than 3 hours per day, damage can occur to the rumen epithelium cells (Steele et al., 2011). Unlike abomasal epithelium cells, rumen epithelium cells are not protected by mucus secretion, this makes them highly susceptible to damage in acidic conditions. However, this is not the case with pasture fed dairy cows, and apparently not in FB fed cows and steers. Gibbs and Laporte (2008) and Gibbs (2011) have demonstrated that typical rumen pH fluctuations in these forage diets will routinely reduce to 5.0 and climb again within a diurnal cycle to above 6.5, without apparent rumen function or epithelium impact.

The ruminal wall has been shown to adapt to increasing WSC levels by increasing epithelium proliferation and morphogenesis (Steele et al., 2011). When the diet changes from a fibre rich diet to a starch rich diet, rumen papillae will increase in size to compensate for the high production of VFAs. This is a coping mechanism to reduce the effects of ruminal acidosis. Bannick et al. (2012) reported that a 5% increase in rumen papillae size would result in an 18% change in absorptive area. However, this change takes a number of days and ruminal acidosis will occur if animals are not transitioned onto high starch diets. Steele et al. (2011) reported butyrate as the most prominent stimulant for the cellular proliferation and

inhibitor of apoptosis. However, this report also stated that the rate of cellular aging decreases with high grain-fed cattle, and parakeratosis is common.

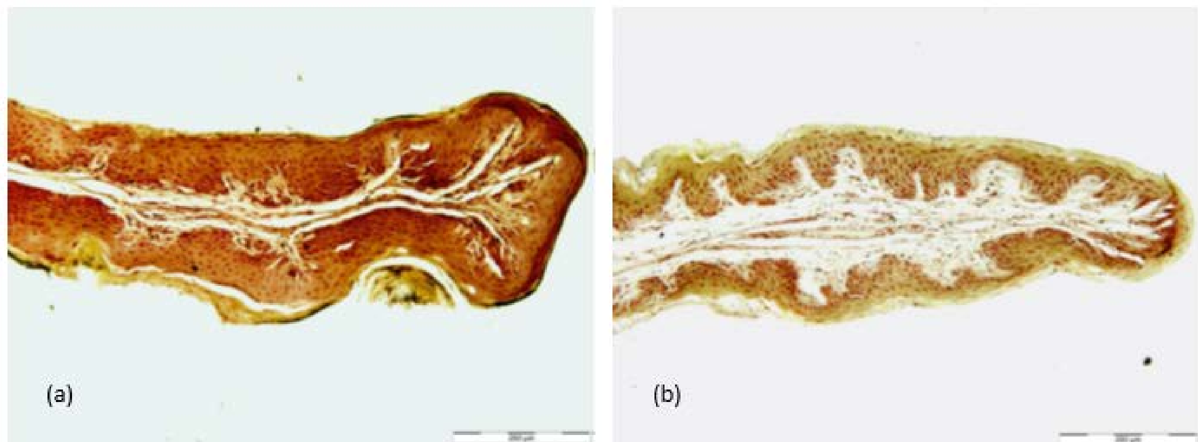

Figure 1. A and B show sections obtained by light microscopy of excised rumen papillae from a bull for winter and summer pasture respectively. Bar represents 200µm. Figure taken from (Trotter, 2012).

It is widely accepted that the rumen epithelium undergoes structural and morphological changes due to dietary changes (Bannick et al., 2012; Penner et al., 2011; Steele et al., 2011). In a trial conducted by Trotter (2012), the effect of seasonal variation in quality and quantity of pasture fed to bulls in a pasture based feeding system on rumen epithelium adaptations was investigated. In spring the mean total short chain volatile fatty acid (SCVFA) concentrations in the rumen were 43% higher than the SCVFA concentrations in winter. Figure 1 shows that rumen papillae have adapted to the higher SCVFA concentration by decreasing the epithelial thickness and increasing the vascular capacity of the papillae to absorb nutrients and transport them from the rumen. While width of papillae is reduced in spring, the depth is increased to increase surface area. This finding is supported by Bannick et al. (2012) who found that papillae became longer and thinner when animals were transitioned onto high concentrate diets. Bannick et al. (2012) proposed that epithelial growth was partitioned towards increasing surface area by increasing the size of papillae as opposed to maintaining the thickness of the rumen wall. Furthermore, Trotter (2012) surmised that the increase in SCVFA concentration decreased the mitotic activity of the s. corneum. Trotter (2012) also found that papillae excised in spring had deep furrows and corrugations compared with the smooth appearance of papillae excised in winter. This is another adaptive response to further increase surface area of the papillae to increase

absorptive capacity. While Trotter (2012) recorded very high SCVFA concentrations in the rumen (141 mMol/L in summer), there was no evidence of sub-acute ruminal acidosis.

The rumen epithelium can then be considered a major functional adaptation to high fermentable carbohydrate content in the diet, and to high water loads. Both of these impacts are mitigated by both anatomical and functional changes to the rumen epithelium.

2.6 Grazing Behaviour and Intake Regulation

When ruminants are on *ad libitum* feed allowance, they will have 3-4 major grazing bouts or 'meals' during the day with an additional 7-11 minor meals (Baumont et al., 2006; Gregorini et al., 2008). They will spend 5-9 hours of the day grazing and a further 5-9 hours of the day ruminating (Baumont et al., 2006). When fresh feed is offered, the first meal of the day will generally be the largest meal but the three main grazing bouts will be at dawn, midday and at dusk (Gill & Romney, 1994). It is thought that rumen fill is not maximised at the morning or afternoon grazing bouts, but at the dusk grazing bout (Gregorini et al., 2008). Different grazing bouts will provide the animal with different levels of energy intake and a different balance of nutrients. In the evening, plants have a low water content and a higher WSC to NDF ratio (Gregorini et al., 2008). This increases intake rate as there is less energy required for mastication to release the sugars from the plant cells. In the process of ingestive mastication the cell walls are broken allowing enzymes to access cell contents for digestion, while particle size is reduced to increase the surface area for digestion (Gregorini et al., 2008). During this process, approximately 65% of the intracellular water is released which allows particles to be packed efficiently into the rumen to maximise rumen capacity. Where intracellular water isn't released, rumen fill may increase and dry matter intake decline.

However, ingestive mastication is time consuming, and during the first grazing bout, mastication per gram of dry matter intake is lower than other times of the day to increase intake rate. This results in larger particles entering the rumen, and the ability of the rumen to pack the particles tightly decreases which then decreases the capacity of the rumen. The grazing bout is then likely to be cut short due to rumen fill. Gregorini et al. (2008) argue that grazing dynamics are strongly influenced by ruminal fill. As the animal becomes more

satisfied and full, it will search for the most profitable bites; bites which maximise energy gained for the energy expended in harvesting the bite.

However, as pasture availability may be decreasing further into the grazing bout, intake rate is likely to decline (Baumont et al., 2006). As the pasture availability declines and the selectivity of the ruminant increases through the grazing bout, ingestive mastication increases (Gregorini et al., 2008). This is due to an increase in time spent searching for the next bite. Gregorini et al. (2008) showed that ruminants would continue chewing as they searched for their next bite. This would result in smaller particles reaching the rumen later in the grazing bout which would then aid in the packing of the rumen. As pasture has a higher NDF content at the morning grazing bout, this will take longer to clear the rumen than pasture consumed during the afternoon or evening. This results in a longer lag time before the next grazing bout (Gregorini et al., 2008). Further, Baumont et al. (1990) found that as ruminal fill increases, ruminants will reduce intake via intake rate rather than the total time spent eating each day. However, the cattle did take smaller, more frequent meals. Grazing behaviour is known to change with different plant dynamics. If animals have to search for a profitable bite, intake rate will decline, whereas, if feed availability is high, animals are likely to have high intake rates as they do not need to spend a long time searching for efficient bites (Dougherty et al., 1989; Popp et al., 1997).

As the fibre content of a feed increases, the passage rate of the feed through the digestive tract is likely to decrease. Given that feeds will not leave the rumen until they are 5 mm or less, fibrous feeds will require a longer time to be broken down than more soluble feeds. This will result in fibrous feeds having a longer residence time in the rumen, and increasing the rumen fill. Therefore, as ruminal fill increases, the frequency and size of grazing bouts is likely to decrease.

Ruminants restrict their daily dry matter intake even when feed is offered *ad libitum* (Gill & Romney, 1994). Daily dry matter intake is influenced by the animal's feelings of satiety and malaise during and after a meal. Animals are satisfied when they consume adequate amounts of nutrients, but they will feel discomfort or malaise if they consume excesses of nutrients or toxins (Provenza, 1995). Over time, animals will acquire preferences for foods which give them feelings of satiety and avoid foods which give them feelings of malaise. Both physical and metabolic feedback contributes to satiety and malaise. Physical

constraints are the result of stomach distension factors and the rate at which feed leaves the rumen. Voluntary intake of feeds with a high content of soluble (storage) carbohydrates is more likely to be regulated by metabolic constraints as opposed to physical constraints. Metabolic feedback is very complex and is the result of a large number of signals in the ventromedial and lateral areas of the hypothalamus which are related to satiety and hunger (Baile & McLaughlin, 1987). The interpretation of these signals is likely to be different between animals and even in different time frames with the same animal depending on their mental and physiological state. These factors make metabolic constraints very difficult to predict. Physical and metabolic constraints are not mutually exclusive, and both constraints contribute to determining voluntary feed intake (Forbes, 2007). The digestibility of a feed is often positively correlated with voluntary intake (Forbes, 2007; Provenza, 1995). Given that particles must be less than 1mm to leave the rumen, a high cell wall content will increase the rumen residence time and decrease voluntary intake (Dulphy & Demarquilly, 1994).

During the morning grazing bout, as an average the number of chews per bite is highest (Gregorini et al., 2008). Furthermore, during a single grazing bout, the number of chews per bite will increase throughout the grazing bout as animals spend longer searching for the next bite. This means that energetic efficiency of pasture harvesting increased throughout the day but decreased throughout each grazing bout.

Physical factors are also found to influence grazing behaviour and dry matter intake of cattle. (Popp et al., 1997) found that as stocking rate increased and pasture availability decreased, the time spent grazing increased.

Dougherty et al. (1989) found that biting rate was stimulated by the allowance of fresh pasture, but this effect did not last long or result in an overall increase in DM intake. This may illustrate the concept of the profitable bite with animals increasing their intake rate when they can also increase their efficiency.

Furthermore, the faster the rate of breakdown in the gut, the higher the rate of positive post-prandial feedback will be to the brain which causes the ruminant to form preferences to particular feeds. Provenza (1995) reported that the level of metabolites in the portal and jugular blood increase within 15 minutes of the beginning of the meal. This enables the ruminant to innately sense the effects of nutrient intake and adjust dry matter intake accordingly. If the feed is nutrient poor, intake will be limited due to negative post prandial

feedback as the animal will experience feelings of malaise. Similarly, if the feed has an excessive nutrient content, the animal will again feel discomfort or malaise and it will decrease intake.

Illuis and Jessop (1996) propose a model where metabolic constraint is determined by the ability of the ruminant to dispose of surplus nutrients in order to achieve maximal production. If the diet is perfectly formulated to supply the ruminant with the exact nutrients required to achieve maximal production, bodily stores will remain unchanged and voluntary intake will not be restricted. If the diet is slightly limiting in nutrients, the ruminant may be able to mobilise bodily stores to compensate for this. Conversely, the ruminant may consume more of the diet to compensate for the limiting nutrient. For example, if protein is limiting to the animal and it increases its intake to compensate for this. This may result in the animal consuming excess energy which must be disposed of either by storing it as fat on the body, or by excreting it. Energy intake will reach its limit when the ruminant cannot increase the rate at which it stores energy as fat or the rate at which it disposes energy. Once this stage is reached, voluntary intake cannot increase, and production will not be maximal. It is assumed that metabolite will not be cleared from the blood fast enough causing a build-up. This will result in a negative feedback causing voluntary intake to decline. It is assumed that the animal will have a feeling of discomfort post-eating which will cause the reduced intake.

Tolkamp and Ketelaars (1992) have the approach that feed intake is related to the cost and benefit of grazing. The benefit of grazing for a non-reproducing animal is the energy consumed to use for maintenance and growth. The cost is the increased oxygen required, and the damage and ageing to the tissues. Given that the total amount of oxygen an organism can consume in its lifetime is thought to be fixed, it is likely the organism will attempt to maximise the efficiency of oxygen utilisation. This follows the theory of diminishing returns, where the cost of additional units of ME increases as intake increases and additional intake of NE reduces per intake of ME (Ketelaars & Tolkamp, 1992). The efficiency of feed intake is maximal when the intake of Net Energy is maximal per litre of oxygen consumed.

It appears that the signals for satiety change throughout the day but it is not yet known what the changes or the exact signals are (Gregorini et al., 2008). Further, Pittroff and Soca (2006) report that the greater the energy deficit, the greater the hunger signals. This is likely to

influence the size of each meal. If the animal hasn't grazed overnight, it is logical that the hunger signals in the morning will be the strongest, and therefore, the largest meal is taken at dawn.

There are no available studies on FB grazing behaviour. However, the body of literature above suggests that the relatively low DM content, the physical apprehension required of a bulb crop, and the expected low DM mass per bite of FB would all serve to limit voluntary DMI. While there are no grazing behaviour studies, there is a body of intake rate data from both cows and steers (Gibbs *unpublished data*) from several years of trials that demonstrates high DMI (>2-2.5% LWT/d) with FB grazing. This suggests that the physical characteristics of FB are not significantly limiting intake levels, but at present there is insufficient data as to why this is so.

Summary

Rumen microbial protein production is a primary driver of animal performance, and is principally influenced by both rumen N kinetics and FME supply, both of which are a function of the feed composition – energy density and DM content in particular - and degradation profile. There are further influences on the rumen environment – for example rumen pH, redox and metabolite concentration and intake regulation - on microbial protein production that are also largely a feature of the feed type. At present there are no available studies on either the microbial protein production of beef cattle grazing FB or the rumen function of that diet. However, there are several well documented features of FB – high DMD, high sugar content, low DM content – that may predispose to high microbial protein production, yet also some – low CP content for example – that may limit this. This study will determine the microbial protein supply of beef steers grazing FB under industry standard conditions, and compare this with that of a grass diet for which it is well described.

Chapter 3
Materials and Methods

This study aimed to establish the microbial protein supply of the low crude protein content fodder beet and the higher crude protein content winter grass diets to yearling Charolais steers. Four yearling Charolais steers of mean starting (March) liveweight 286 kg were surgically inserted with rumen cannula into the dorsal sac. Two experiments were conducted to determine the effects of feeding a winter perennial ryegrass (cv Bealey) (control) compared with feeding Fodder Beet (cv 'Brigadier', Seed Force, Christchurch) (treatment) on the microbial protein production of steers. In each experiment, the steers were placed in metabolism crates and a nine day digestibility trial with total faecal and urinary collection was conducted. Following the digestibility trial, the steers remained in the metabolism crates and ruminal pH was monitored *in situ* for 24h. On day 11, the steers remained in the metabolism crates and rumen, urine and faecal samples were collected every 2h over the diurnal period for analysis. On day 12, the steers were removed from the metabolism crates and returned to the paddock to graze. At 10am on day 13 and at 2am and 6pm on day 14, rumen evacuations were performed. At 10am on day 16, triplicate nylon bags were inserted into the rumen to be removed at 2, 4, 6, 12, 24, and 48h.

3.1.1 Experiment 1

3.1.1.1 Animals and Feeding

The steers were fed *ad libitum* on a ration of fresh winter grass. While steers were in metabolism crates from day one to day 12 of the experiment, the daily winter grass allowance was cut at 9am and carried to the steers. Steers were fed after 10am and 5pm daily. On day 12 of the experiment, steers were removed from the metabolism crates and were break fed on *ad libitum* allowance in the same Bealey ryegrass paddock as where the daily feed was collected while the steers were in the metabolism crates. The pasture was managed to remain between 1800-4000 kg DM/ha to mimic industry standard beef production models of use. The mean DM content of the pasture was 16% and the quality measurements of the pasture are shown in Table 4-1. The steers had constant access to water.

3.1.1.2 Digestibility Trial

Trial one of experiment one was a digestibility trial. From 10am day 1 to 10, the steers were placed in metabolism crates and remained on *ad libitum* feed rations of winter grass and had constant access to water. Steer intake was observed using the pre and post-grazing pasture mass and allocation in the crates was targeted at 10% refusal level. Daily dry matter intake (DMI) for the four steers averaged 4.6kg DM per day. Duplicate representative sub-samples of feed were collected each day to obtain dry matter content. Further sub-samples were obtained to determine feed quality and frozen at -20°C until freeze drying for analysis. Feed refusals were weighed for each individual animal, and oven dried at 68°C to a constant weight to obtain the DM content.

Total urine was collected and weighed daily and a 5% sub-sample was frozen for further analysis. Each day 500mL of 25% H_2SO_4 was placed into each urine tray to reduce the pH below 3 and prevent volatilisation of urine ammonia. Total faeces were collected and weighed daily. After thorough mixing duplicate representative samples for DM determination were obtained. A 10% sub-sample was obtained each day and bulked before freezing for further analysis.

3.1.1.3 Rumen pH, Rumen Digesta, Urine and Faecal Sampling

Trials two and three of experiment one was sequential 24h periods of firstly monitoring rumen pH *in situ,* and secondly collecting rumen digesta, urine and faecal samples at 2h intervals for the respective trials. From day 10-11, the rumen pH was monitored using an *in situ* rumen pH probe (Ionode IJ-44, Brisbane, Australia) placed on the floor of the ventral sac for 24h beginning at 10am on day 10. The pH probe was attached to a 1500 g steel paddle to ensure it stayed in place and pH was measured every 15 seconds over the diurnal period.

For 24h from 10am on day 11, rumen fluid and digesta samples were taken every two hours from the ventral sac to analyse for ammonia, urea, and volatile fatty acid (VFA) concentration. Rumen digesta was obtained by hand from the ventral sac region of the rumen. Rumen fluid samples were obtained by squeezing rumen digesta through two layers of cheese cloth. Urine and faeces were collected and weighed at each 2h sampling period. A 70mL subsample of urine was obtained and a 250mL subsample of faeces was obtained. For faeces, duplicate DM samples were obtained and oven dried at 68°C until a constant weight was reached. The pH of rumen fluid, rumen digesta, urine and faeces was measured at each

collection. Rumen fluid for ammonia analysis and urine samples were acidified with 1mL of 6M H_2SO_4 to inhibit ammonia volatilisation.

3.1.1.4 Rumen Evacuations

Trial four of experiment one involved three rumen evacuations which were performed 16h apart, beginning at 10am on day 13 and concluding at 6pm on day 14. Total rumen contents were evacuated from the rumen and weighed. Rumen contents were kept warm using a 50°C water bath. Rumen digesta was mixed thoroughly and rumen fluid and digesta samples were obtained from multiple sites within the container. Rumen fluid samples were obtained by squeezing rumen digesta through two layers of cheese cloth. These samples were used for analysis of ammonia and urea concentration, total N concentration, and VFA concentration, and the samples were placed in an ice slurry and then kept frozen to - 20°C until analysis. The pH of rumen fluid was measured at each collection. Rumen fluid samples were acidified with 1mL of 6M H_2SO_4 to inhibit ammonia volatilisation. Duplicate rumen digesta samples were also taken and weighed before placing in the oven to determine the DM content of rumen digesta.

An external marker polyethylene glycol (PEG) was used to determine passage rate of rumen fluid. Each steer was administered 64g in 500mls of water directly into the rumen via the cannula at 10am on day 13 and 14. At each evacuation, a 70ml sample of rumen fluid was obtained and kept frozen at -20°C until analysis.

3.1.1.5 Nylon Bags

Trial five of experiment one was an *in sacco* nylon bag trial was carried out to determine the disappearance rate of the dry matter and the nitrogen of the winter grass. A preliminary DM assessment of the pasture was done, then 5 g DM of winter grass was placed into each 10 x 15 cm nylon bag of 50 um pore size. Three nylon bags were placed into the rumen to be removed at each time interval. Time intervals were 0, 2, 4, 6, 12, 24, and 48h. A total of 18 nylon bags were placed into the rumen of each steer.

At removal, the bags were rinsed in cold water then frozen at -20 °C. In a single batch, all bags from experiment one and two were thawed and then washed for 30 minutes under gentle agitation in a standard commercial washing machine on cold cycle without detergent. The bags were then dried for 48h to a constant weight at 68°C. The contents of the bags were bulked within steer and within time interval. The bulked contents were ground in a

centrifugal grinder (1mm) and analysed for total N content using the elemental analyser Vario Max CN (based on the Dumas combustion method of Etheridge et al. (1998)).

To determine the indigestible component of the diet, in addition, another 12 bags of 10µm pore size were used with 5g DM each for each of winter grass weighted in the rumen of three non-lactating adult cows for 12d.

3.1.2 Experiment 2

3.1.2.1 Animals and Feeding

At the completion of experiment one, the steers were transitioned onto fodder beet. On the day one of transition, steers were fed 4kg DM each of lucerne silage and were let graze fodder beet for 2h with an allowance of 500 g DM per steer. On day two of transition, steers were given the same feed allowances as day one, but on day three the fodder beet allowance was increased by 500 g DM, taking them to a total fodder beet allowance of 1 kg DM per steer. Fodder beet allowance was increased by 500 g per steer every second day until steers were on *ad libitum* intake. Lucerne silage allowance was decreased over this period, and once the steers were on *ad libitum* intake of fodder beet, the lucerne silage allowance was 1 kg DM per steer per day. After transition, the steers were observed to be on *ad libitum* dry matter intake for two weeks prior to the commencement of experiment two.

At the beginning of experiment two, the steers were replaced into the metabolism crates. From day one of the experiment until day 12, while the steers were in the metabolism crates, steers were fed *ad libitum* on a ration of fresh fodder beet which was collected by hand and carried to the steers daily, along with 1kg DM lucerne silage at 10am daily. Fodder beet was cut to separate leaf and bulb, and the leaf to bulb ratio fed to the steers was 1:4. This ratio was calculated from five years of previous 'Brigadier' crop assessments by this research group. On day 12 of the experiment, steers were moved to graze fodder beet in the paddock for the remainder of the experiment.

The steers had constant access to water. The crop yield was assessed at 25 tonne DM/ha. The dry matter content of the fodder beet leaf was 14.3% and the bulb 10.2%, and the proximate analyses of all feed quality are shown in Table 4-1.

3.1.2.2 Digestibility Trial

Trial one of experiment two was a digestibility trial. The steers were placed in metabolism crates and remained on *ad libitum* feed rations of fodder beet and had constant access to water. Daily dry matter intake (DMI) for the four steers averaged 6kg DM per day. Duplicate representative sub-samples of all feeds were collected and dried each day to obtain DM content of the feed. Further sub-samples were obtained and kept frozen at -20°C for later feed quality assessment. Feed refusals were collected weighed, and oven dried to obtain the DM content. Urine and faecal collection and sub-sampling was carried out as described in experiment one 3.1.1.2.

3.1.2.3 Rumen pH, Rumen Digesta, Urine and Faecal Sampling

Trials two and three of experiment two involving the *in situ* rumen pH measurements and the rumen digesta, urine and faecal sampling were carried out as described in experiment one.

3.1.2.4 Rumen Evacuations

Trial four of experiment two involving rumen evacuations were carried out as described in experiment one.

3.1.2.5 Nylon Bags

Trial five of experiment two involved an *in sacco* nylon bag trial to determine the DM and nitrogen disappearance rate of fodder beet. The method used was that developed specifically for this application by this research group in previous fodder beet research (Gibbs *unpublished data*).

Preliminary DM assessment of the crop leaf and bulb was conducted a week prior to the trial to enable calculation of the required wet weights of leaf and bulb in a bag to provide 5g DM fodder beet which was divided into bulb and leaf. Three plants with bulbs of *c*.3kg fresh weight were obtained at 6am and cleaned of soil for use in the nylon bags. An additional 12 plants of similar size were obtained. Each plant had bulb and leaf material separated at the juncture of the lowest leaf, and the wet weight of the leaf material of the 12 plants was used with the wet weight of the stem component of the leaf material to establish the proportion of 'true leaf' and 'leaf stem'. From this proportion (50:50) the relative wet weight of each component included in the nylon bags to provide 5g DM in total was strictly conserved. Both components were cut into 2cm pieces.

The bulb was quartered and two quarters were used for DM determination and proximate analysis, dried in an oven at 68 °C to a constant weight or kept frozen until freeze drying and analysis, respectively. The remaining quarters were carefully sliced longitudinally from crown to tip in a fine wedge of 50g wet weight, in a manner to conserve for each slice the same proportion of epidermis and core. Each steer at each time point had one bulb slice, individually identified, so triplicate samples at each time interval were produced.

By this method approximately 50 g wet matter was weighed and placed into each 10 x 15 cm nylon bag of a 50µm pore size. Three nylon bags were used for both leaf and bulb at each interval, and at 0h all bags were attached to a 2kg steel chain and placed into the rumen to be sequentially removed at each time interval for both leaf and bulb. Time intervals were 0, 2, 4, 6, 12, 24, and 48h. A total of 36 nylon bags were placed into the rumen of each steer.

To determine the indigestible component of the diet, in addition, another 12 bags of 10µm pore size were used with 5g DM each for each of fodder beet leaf and fodder beet bulb, weighted in the rumen of three non-lactating adult cows for 12d.

3.1.3 Sample Processing and Analysis

3.1.3.1 Dry Matter Analysis

Each day of the trial duplicate winter grass, fodder beet, lucerne silage and faecal dry matter samples were weighed and dried at 68°C to a constant weight. Samples were re-weighed using the same scales to determine dry matter content.

3.1.3.2 Rumen Samples, Urine and Faecal preparation

Urine samples were thawed under refrigeration at a temperature of 4°C. Daily samples for each steer were then mixed well and subsampled proportionate to their contribution to the 9d total to give a combined total of 100mL. This subsample was used or analysis of purine derivatives, ammonia, urea and total N content were also measured. Analysis of purine derivative concentration of urine was carried out according to the method from X. B. Chen and Gomes (1992). Ammonia was analysed using an RX Daytona analyser (based on the method of Cray et al. (2013)), total N was measured using the elemental analyser Vario Max CN (based on the Dumas combustion method of Etheridge et al. (1998)).

Bulked faeces were stored at -20°C then thawed under refrigeration at 4°C. The 9d bulk of each steer was then mixed thoroughly and subsamples were taken and freeze dried, then analysed for total N as described above.

Rumen fluid samples from the 2 hourly sampling were mixed thoroughly and two 2mL representative samples were used to measure the ammonia and urea concentration and the VFA concentration. These 2mL samples were centrifuged at 13,000 revolutions per minute for 30 minutes at 4°C. The supernatant was separated from the solid fragment and frozen at -20°C until analysis. Analysis for ammonia was conducted as described above. VFA concentration was determined using gas chromatography (GC) (Shimadzu GC-2010) by the method of H. M. Chen and Lifschitz (1989).

3.1.3.1 Calculations

Dry matter digestibility of each steer across the 9d period of each experiment was determined using the relationship between total DM intake and faecal dry matter.

To calculate the diurnal pattern for pH, the pH measurements recorded every 15 seconds were averaged for successive 10 minute periods for each diet treatment, resulting in 160 pH records (4 per minute for 10 minutes for 4 steers). The recorded pH of all steers from each diet treatment were used for each 10 minute block to calculate the proportion of total records within six bands: <5.0, 5.0-5.5, 5.5-5.8, 5.8-6.0, 6.0-6.4 and >6.4. 'Bout counts' were calculated using the mean recorded diurnal pH measurements from each steer in the winter grass and the fodder beet diet treatments. If the recorded pH was less than each of the six thresholds (above) for two minutes, it was recorded as a single bout for that threshold and the duration (in minutes) of that bout was calculated. The means for the bout counts and bout times were then calculated for the steers in each diet treatment.

The N balance was calculated for each steer in each experiment by the difference between the daily N ingested in feed and the daily N excreted in the form of urine or faeces, and expressed as a mean of the four steers in each treatment group.

Microbial crude protein production (MCP) was estimated using the daily purine derivative (PD) mass in urine from each steer via the method of X. B. Chen and Gomes (1992). Mean daily urine volume for each steer was used and PD concentrations of the subsampled bulked

urines to calculate the daily mass of PD excreted in the urine of each steer. This was used in Equation 1 of X. B. Chen (1998) to calculate the amount of microbial purines absorbed daily:

Equation 1 $\boldsymbol{Y = 0.85X + (0.385\, W^{0.75})}$

Where Y = the daily PD excretion and X = the daily purine absorption.

Once X is determined, the daily microbial N yield as calculated by Equation 2 of X. B. Chen (1998):

Equation 2 Microbial N (g N/d) = $\boldsymbol{\frac{(X(mmol\,/d)x\,70)}{0.116\,x\,0.83\,x\,1000} = 0.727X}$

The mean dry matter disappearance from the nylon bags of the four steers at each time interval for each experiment was used to construct a curve. This curve was used to fit a first order kinetic model to the data using the indigestible component % of the diets (Equation 3) by the method of Orskov and McDonald (1970). In the equation, *a* is the rapidly soluble fraction of the feed, *b* is the insoluble, slowly degradable fraction of the feed, *c* is the indigestible fraction and *y(t)* is the percentage loss at each time interval. There is no lag time incorporated in the equation.

Equation 3 $\boldsymbol{y(t) = a + b \times (1 - e^{(-c \times t)})}$

3.1.3.2 Statistical Analysis

Statistical analysis was carried out using GenStat 16 (Hampshire, UK). Analysis of variance (ANOVA) was carried out using feed (winter grass vs fodder beet) as the treatment and animal as the block structure. Least significance difference (LSD) test (P=0.05) was carried out to compare means with significant differences from ANOVA.

A t-test was performed to test for significance in the diurnal pattern of rumen digesta pH between the winter grass and the fodder beet diet treatments. The diurnal pattern was divided into three time periods: 2am-10am, 10am-6pm, 6pm-2am. A second t-test was performed to test for significant differences between the two diet treatments for the three 8h periods for digesta pH. A t-test was also performed to test for significance in the microbial protein synthesis between the two diet treatments.

Chapter 4

Results

4.1 Digestibility Studies

4.1.1 Feed Composition

The proximate analyses of the feeds used in these trials are displayed in Table 4-1.

Table 4-1. Feed composition of winter grass, lucerne, fodder beet bulb and fodder beet leaf.

%	Winter Grass	Lucerne	Fodder Beet	
			Leaf	Bulb
DM	16.3	59.2	14.3	10.2
OM	88.6	88.3	80.0	91.4
CP	16.3	20.6	17.5	11.9
NDF	39.1	39.5	29.2	12.3

Values are expressed as percentage (%) of dry matter (DM), organic matter (OM), nitrogen (N), and neutral detergent fibre (NDF).

4.1.2 *In Vivo* Digestibility and Nitrogen Balance

The *in vivo* digestibility of dry matter, organic matter and digestible organic matter for the winter grass and the fodder beet diet treatments are displayed in Table 4-2, with the voluntary daily DMI. Fodder beet was significantly higher ($P<0.05$) in DMD% and DMI compared with winter grass diet.

Table 4-2. *In vivo* dry matter digestibility (DMD), organic matter digestibility (OMD), digestible organic matter digestibility (DOMD) of the winter grass and fodder beet treatments.

	DMD%	OMD%	DOMD%	DMI (kg/d)
Winter Grass	76.2	83.4	73.9	4.5
Fodder Beet	78.5	83.8	75.2	6.6
S.E.M	0.007	0.004	0.005	0.19
LSD	0.32	0.018	0.022	0.84
P Value	0.032	0.076	0.056	0.004

The N use efficiency calculated as the N mass retained / N excretion of the winter grass treatment was 0.45g, and that of the fodder beet was 0.44g. The fodder beet treatment was observed to have a higher N intake due to the increased DMI and a concomitantly increased N mass excretion, with a similar N efficiency compared with the winter grass treatment.

4.1.3 Microbial Protein Production

The estimated daily post-rumen microbial protein supply in g N and in g N/ kg DMI to the intestines in both diet treatment groups is displayed in Table 4-3. There was a highly significant difference (P<0.01) between diet treatments in mean g microbial N /d, with the fodder beet treatment observed to provide greater supply, and a less significant (P<0.05) difference between treatments in mean g microbial N/ kg DMI. Mean daily urine production (kg/d) was 14.2 in the winter grass treatment and 29.7 in the FB treatment.

Table 4-3. Mean microbial nitrogen production (g N) and microbial nitrogen production per kg DMI (g N/kg DMI) of the steers for the winter grass and fodder beet treatments.

Treatment	Microbial Nitrogen (g)	Microbial N efficiency (g N/ kg DMI)
Grass	56.4	12.5
Range	49.0 - 69.5	10.1 - 14.5
Fodder Beet	104.7	15.5
Range	98.6 - 113.3	14.8 - 16.0

4.2 Diurnal Pattern of Rumen Digesta pH and Redox

The diurnal pattern of mean recorded rumen digesta pH for winter grass and fodder beet diets is displayed in Figure 2. The pH recorded was higher for the fodder beet treatment at most intervals, with a significant difference (P<0.001) in digesta pH between the two diet treatments, and a similar trend to 6h post prandial reduction from 2h post prandially in both treatments, which persisted for approximately 16h. There were highly significant differences (P<0.001) between the treatments for each 8h time interval (10am-6pm, 6pm-2am, 2am-10am).

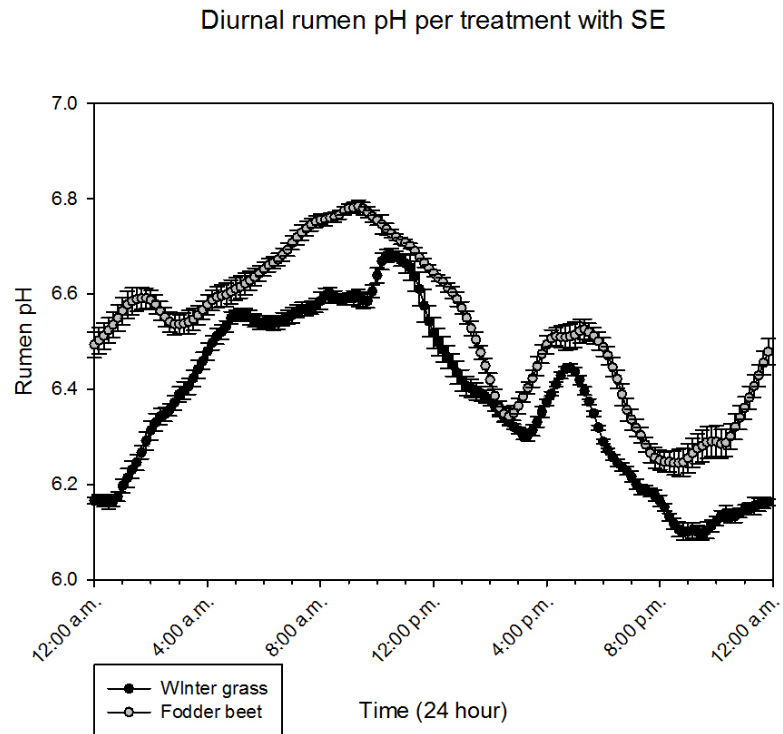

Figure 2. The diurnal pattern of mean recorded rumen digesta pH (±SEM) for winter grass and fodder beet diets.

The mean recorded rumen digesta redox diurnal pattern is displayed in Figure 3.

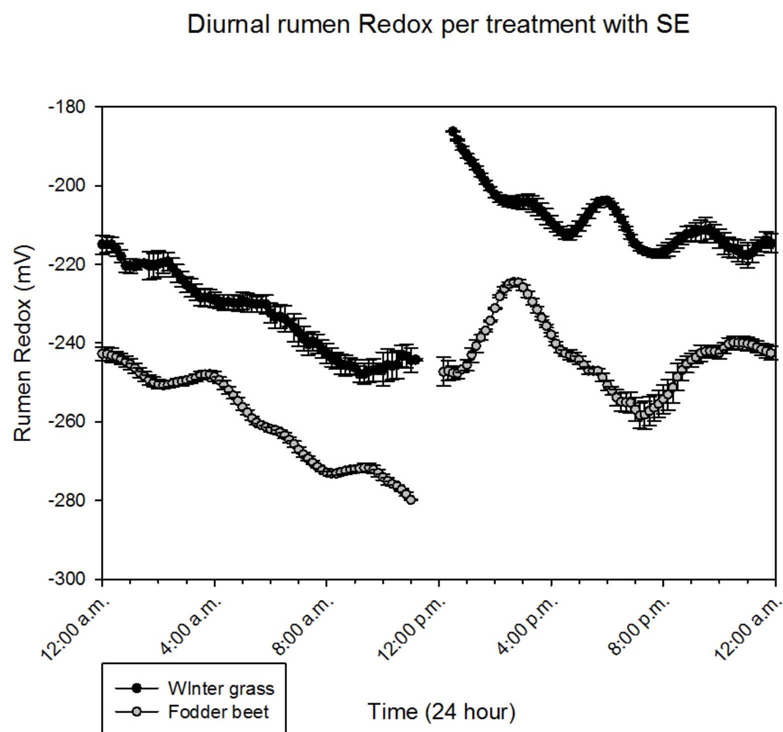

Figure 3. The diurnal pattern of the mean rumen digesta redox for the winter grass and the fodder beet diet treatments.

The diurnal distribution of the recorded rumen digesta pH values from the winter grass and the fodder beet diet treatments are displayed in Figure 4 and Figure 5. The winter grass treatment was observed to have a significantly (P<0.001) greater area under the curve of pH values below 6.0, and above 6.0. The FB treatment was observed to have very few recorded pH values below 5.8, largely maintaining pH in the range above 6.0. In both treatments increased area under the curve of lower threshold pH values occurred more commonly 10h post prandially.

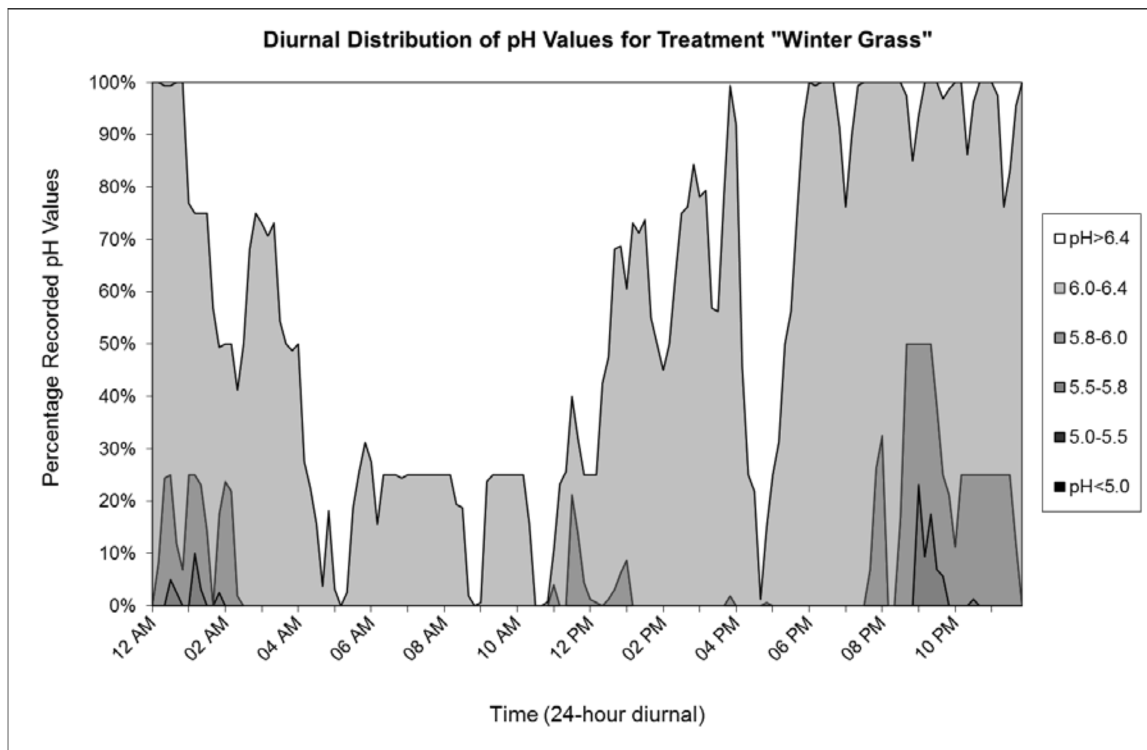

Figure 4. Diurnal pattern for the relative proportions of pH measured *in situ* for the winter grass diet treatment.

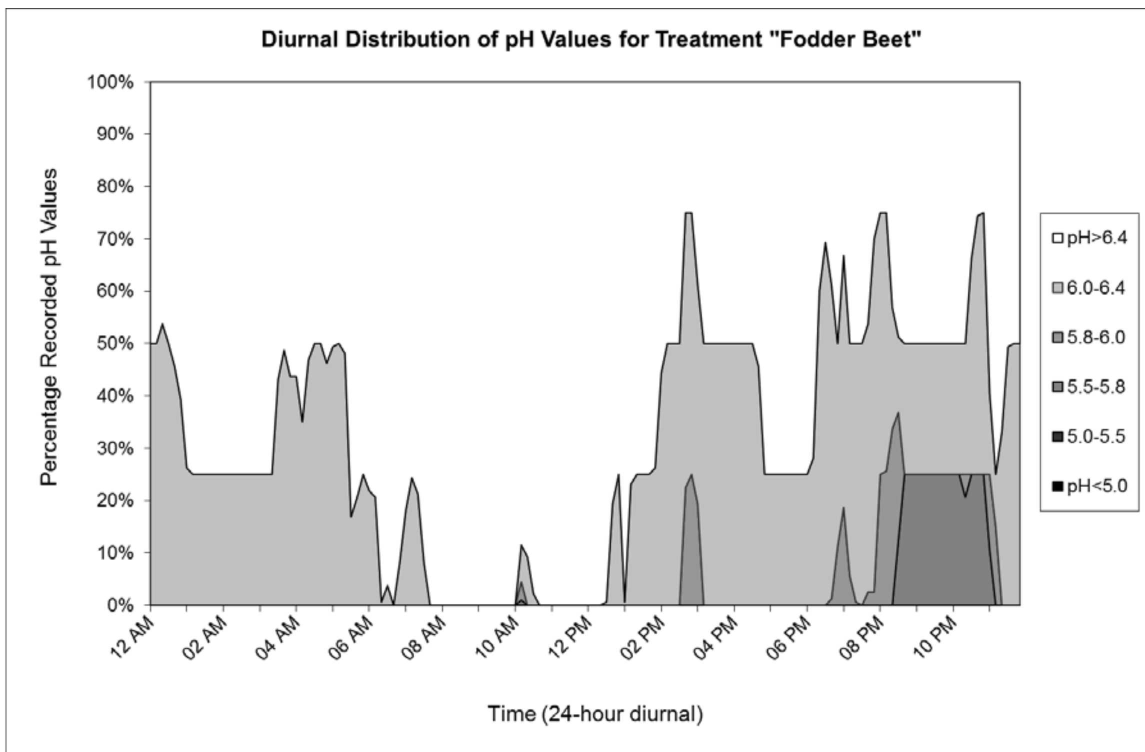

Figure 5. Diurnal pattern for the relative proportions of the pH measured *in situ* for the fodder beet diet treatment.

The mean number and duration of 'bouts' for the diurnal pattern of digesta pH for the winter grass and the fodder beet diet treatments are displayed in Table 4-4. The winter grass diet treatment was observed to have an increased number and duration of bouts under 6.0, and a fivefold increase in mean bout numbers under 5.8. However, in all thresholds the fodder beet treatment bout duration was longer, and particularly at lower thresholds.

Table 4-4. The mean number and duration of bouts of pH below three thresholds (<5.8, <6.0, <6.4) calculated from the diurnal pH measurements for the winter grass (grass) and the fodder beet (FB) diet treatments.

	Bouts <5.8:			Bouts <6.0:			Bouts <6.4:		
Feed:	Mean bouts per day:	Mean mins per bout:	Mins per bout SE:	Mean bouts per day:	Mean mins per bout:	Mins per bout SE:	Mean bouts per day:	Mean mins per bout:	Mins per bout SE:
FB	0.24	148.5	0	0.97	59	45.913	6.09	71.57	35.789
Grass	1.23	5.05	1.062	2.46	35.23	10.346	8.13	86.52	23.746

4.3 Rumen Fluid, Rumen Digesta, Urine and Faecal Sampling

4.3.1 Rumen Fluid pH, VFAs, Ammonia and Urea

The diurnal pattern of mean rumen fluid pH obtained by direct sampling is displayed in Figure 6. The pH ranged from 5.8 to 6.7 for winter grass and 5.5 to 6.9 for fodder beet, and declined after feeding for both diet treatments. Fodder beet resulted in a significantly higher (P = 0.01) rumen fluid pH than winter grass for the 4pm-6am sampling periods.

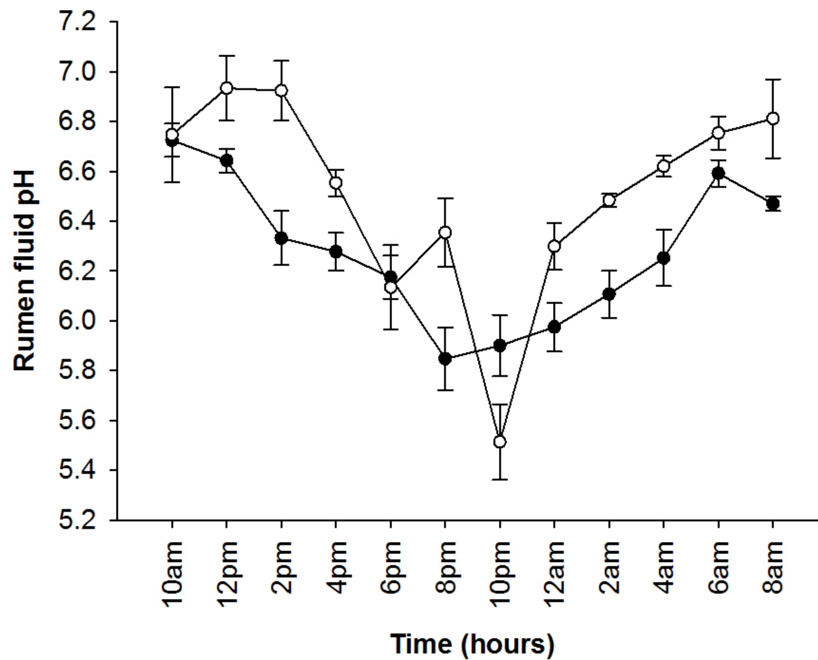

Figure 6. Diurnal pattern of rumen fluid pH of steers fed winter grass (●) and fodder beet (○). Values are means ± SEM.

The diurnal pattern for the total VFA concentration in rumen fluid obtained from the ventral sac for the winter grass and fodder beet diet treatments are displayed in Figure 7. There was a consistent diurnal pattern of VFA concentration for both diet treatments, with approximately 50% increase within 12h of feeding. The winter grass diet treatment resulted in a significantly higher ($P<0.001$) VFA concentration at the 8pm-10am sampling period.

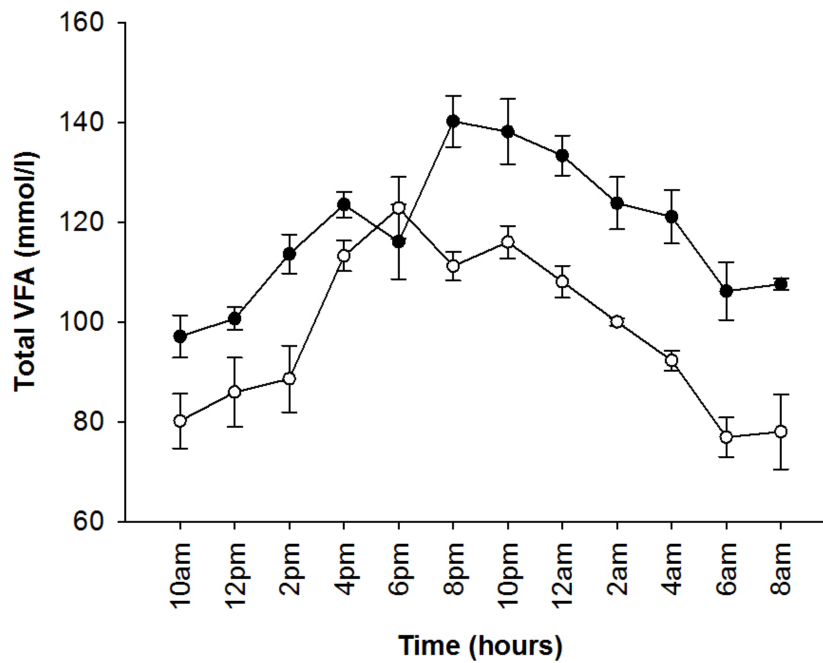

Figure 7. Diurnal pattern of total rumen volatile fatty acid (VFA) concentration (m mol/l) of steers fed winter grass (●) and fodder beet (○). Values are means ± SEM.

The statistical significance of the differences in rumen fluid pH and total rumen VFA concentration at each 2h sampling time over the diurnal period is displayed in Table 4-5. There were significant ($P<0.05$) differences in both pH and VFA concentration notably in the period beginning 10h post prandial to the morning feeding (8pm-4am), but also at a single period at 4pm prior to the afternoon feeding.

Table 4-5. P values for rumen fluid pH and total rumen VFA concentrations for the winter grass vs. fodder beet diet treatments for the diurnal period.

Time	Rumen Fluid pH	Total Rumen VFA
10am	0.922	0.007
12pm	0.089	0.110
2pm	0.062	0.087
4pm	0.024	0.028
6pm	0.871	0.306
8pm	0.016	0.001
10pm	0.045	0.035
12am	0.043	0.007
2am	0.039	0.023
4am	0.035	0.007
6am	0.091	0.021
8am	0.117	0.024

The diurnal pattern for the mean rumen fluid ammonia concentrations obtained from the ventral sac for the winter grass and the fodder beet diet treatment are displayed in Figure 8 and the statistical significance of the differences between treatments at three 8h periods is displayed in Table 4-6. There was a significant difference (P<0.001) between the treatments in ammonia concentration, with winter grass consistently greater, in each period. The range of values in the winter grass treatment was 33-188 mg/l. There was a clear diurnal pattern in the winter grass diet treatment with ammonia peaks between 2pm and 8pm approximately four fold higher than those observed between 2am and 8am. However, the range in values from the fodder beet treatment was 2.8-42 mg/l and peaks in the fodder beet treatment were not observed.

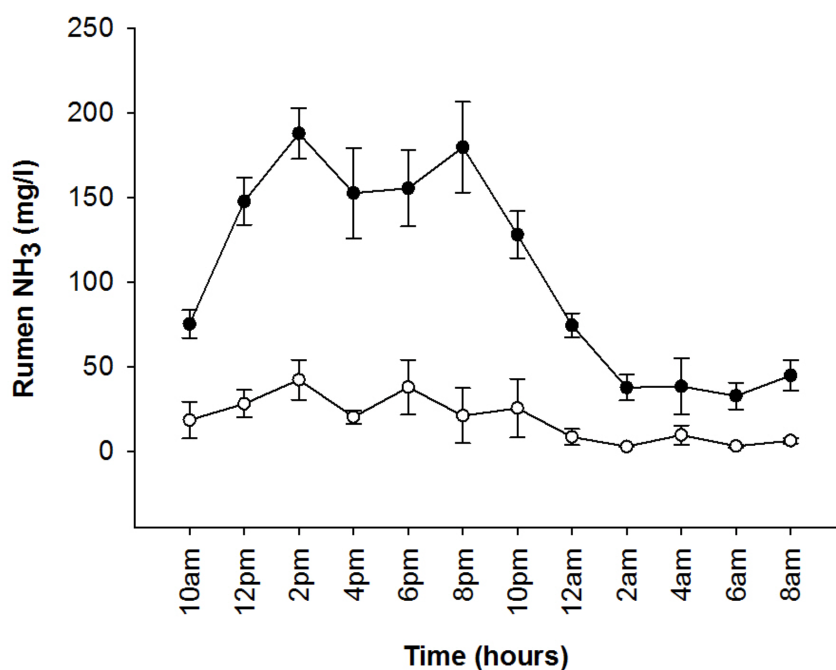

Figure 8. Diurnal pattern of rumen ammonia (NH_3) concentration (mg/l) for steers fed winter grass (●) and fodder beet (○). Values are means $\pm$ SEM.

The diurnal pattern for the mean rumen fluid urea concentration for the winter grass and the fodder beet diet treatments obtained from the ventral sac are displayed in Figure 9, and the statistical significance of the differences between treatments at three 8h periods is displayed in Table 4-6. There was a significant difference (P<0.001) between diet treatments at each 8h period, and the fodder beet treatment consistently higher at every sampling time. The range of values for the winter grass treatment was 1-5mmol/l, while the range of values

from the fodder beet treatment was 5-13mmol/l. There was a distinct difference between treatments in the diurnal pattern with the fodder beet treatment observed to have a three-fold increase within 12h of feeding, while that of the winter grass treatment only increased less than two-fold during the same time interval.

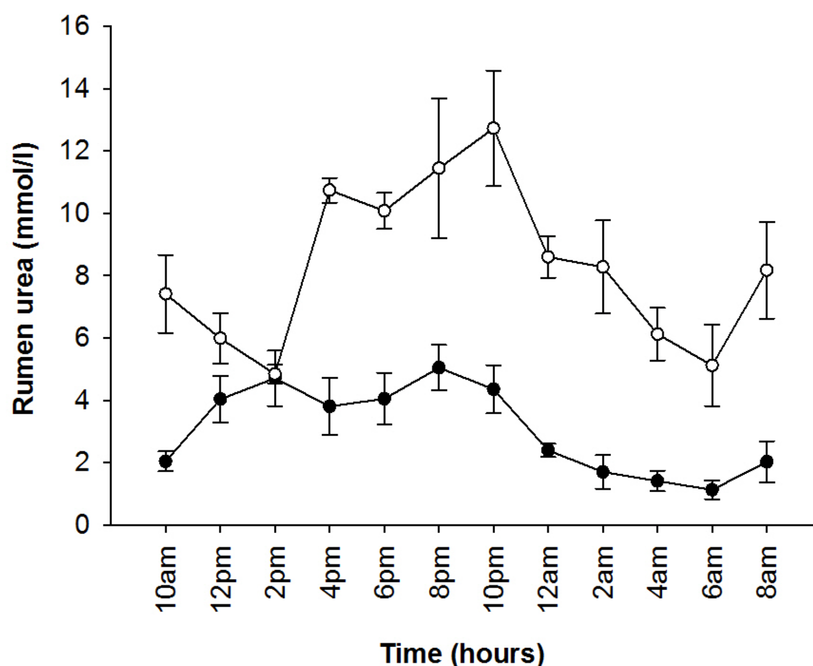

Figure 9. Diurnal pattern of rumen urea concentration (mmol/l) for steers fed winter grass (●) and fodder beet (○). Values are means ± SEM.

Table 4-6. Area under the curve for rumen ammonia and urea concentrations blocked into 8 hour periods for steers fed winter grass and fodder beet. Values are means.

		Winter Grass	Fodder Beet	S.E.M	LSD (5%)	P value
Ammonia (mg/l)	10am-6pm	152.6	31.5	11.13	50.08	0.005
	6pm-2am	119.7	18.8	8.18	36.82	0.003
	2am-10am	49.8	9.1	5.49	24.69	0.014
Urea (mmol/l)	10am-6pm	4.0	7.8	0.68	3.06	0.028
	6pm-2am	3.7	10.5	1.36	6.10	0.038
	2am-10am	1.76	7.02	1.08	4.85	0.041

4.4 Rumen Evacations

The mean total dry matter and nitrogen pools obtained from the three rumen evacuations at 10am, 2am and 6pm for the winter grass and the fodder beet treatments are displayed in Table 4-7. There was no significant difference in the dry matter pool between the two diet treatments from any of the 8h periods. There was a significant difference in the nitrogen pool between the winter grass treatment and the fodder beet treatment at the 6pm rumen evacuation, where the rumen DM pool was greatest for both treatments. There was a highly significant difference between treatments in mixed rumen pH at the 10am and 2am and a trend towards significance at the 6pm rumen evacuations. There was a significant difference between treatments for the rumen wet weight at 6pm only.

Table 4-7. Total dry matter (DM) and nitrogen pools from rumen evacuations at 10am, 2am and 6pm for winter grass and fodder beet trials in steers.

Time (hours)	Winter Grass	Fodder Beet	S.E.M	LSD (5%)	*P value*
		Rumen pH			
10am	5.60	6.86	0.044	0.20	<0.001
2am	5.67	6.67	0.08	0.35	0.003
6pm	5.63	6.12	0.111	0.499	0.052
		Total Rumen Wet Weight (kg)			
10am	24.5	23.1	2.13	9.59	0.675
2am	26.1	29.5	2.30	10.37	0.377
6pm	30.72	36.61	1.27	5.72	0.047
		% Rumen DM			
10am	0.104	0.075	0.001	0.051	0.172
2am	0.101	0.073	0.005	0.023	0.030
6pm	0.106	0.085	0.003	0.001	0.010
		Dry matter Pool (kg)			
10am	2.6	1.8	0.44	1.97	0.288
2am	2.6	2.2	0.29	1.30	0.385
6pm	3.3	3.2	0.20	0.89	0.718
		Nitrogen Pool (kg)			
10am	0.106	0.056	0.017	0.076	0.128
2am	0.102	0.067	0.008	0.036	0.055
6pm	0.128	0.098	0.003	0.014	0.006

The mean rumen fluid outflow rate (%/ h) for each diet treatment calculated from the PEG concentrations in rumen fluid for each interval, between rumen evacuations is presented in Table 4-8. There was no significant (P>0.05) rumen fluid outflow rate observed between the diet treatments.

Table 4-8. The mean rumen outflow rate (% /h) for winter grass and fodder beet diet treatment for 48h from a PEG marker, with standard error of the mean (SEM).

	Mean Rumen Outflow Rate
Winter Grass	21.5
Fodder Beet	20.5
S.E.M	0.55
LSD (5%)	2.5
P Value	0.286

4.5 Nylon Bags

The mean dry matter (DM) disappearance from nylon bags, and the statistical significance of the differences between the two diet treatments at each incubation period for 48h is displayed in Table 4-9 and Figure 10. The mean DM disappearance calculated by first order kinetics is displayed in Table 4-10. Fodder beet bulb had the greatest initial DM disappearance but the rate of disappearance of the winter grass, fodder beet leaf and bulb was similar during the first 6h of incubation after that.

However, there was a significant difference between the rate of disappearance of winter grass and fodder beet leaf between 6-48h and the rate of disappearance of fodder beet bulb was significantly different ($P<0.05$) from the winter grass and fodder beet leaf during the same incubation period. Fodder beet bulb DM disappearance was 86.5% after 12h, while DM disappearance for winter grass and fodder beet leaf was 69.6% and 64.8% respectively. The rates of DM disappearance for 0-6h were 9%, 7.3% and 12.9% /h for winter grass, fodder beet leaf and fodder beet bulb respectively. The rates of DM disappearance for 6-48h were 0.84%, 1.16% and 0.46% /h for winter grass, fodder beet leaf and fodder beet bulb respectively.

The rate of disappearance of dry matter from nylon bags calculated by the first order kinetic equation for winter grass and fodder beet is displayed in Table 4-10. There were highly significant differences at each time interval. Fodder beet bulb was observed to have a greater disappearance at each time interval. Though, the rate of disappearance from the time zero (washed) interval for fodder beet bulb was lower than either winter grass or fodder beet leaf.

Table 4-9. Mean dry matter disappearance (%) from nylon bags at each incubation time for winter grass, fodder beet leaf and fodder beet bulb.

Time (hours)	Winter Grass	Fodder Beet Leaf	Fodder Beet Bulb	S.E.M	LSD (5%)	*P* value
0	40.5	32.2	70.4	0.82	3.21	<0.001
2	42.6	33.4	69.3	0.97	3.39	<0.001
4	52.2	39.1	73.7	0.66	2.28	<0.001
6	54.0	43.9	77.2	0.96	3.31	<0.001
12	69.6	64.8	86.5	0.87	3.02	<0.001
24	82.9	87.0	94.8	0.62	2.15	<0.001
48	89.3	92.5	96.4	0.57	1.97	<0.001

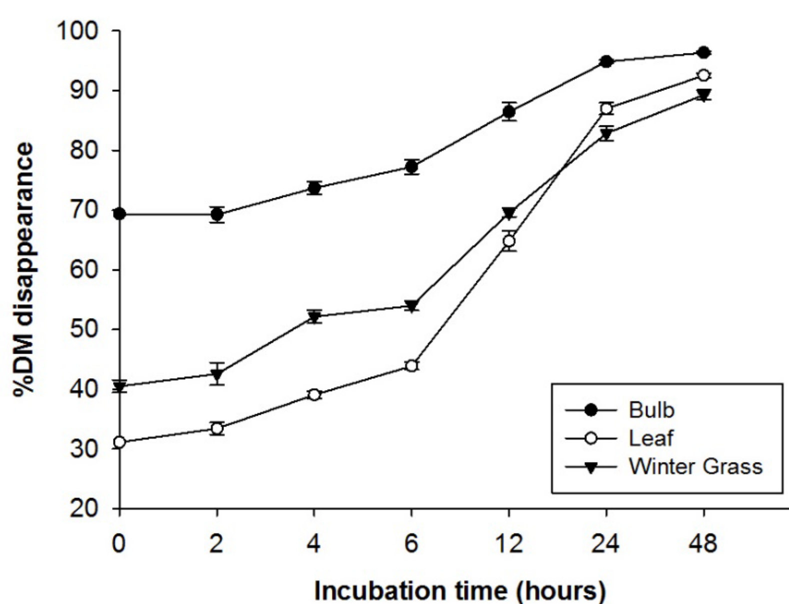

Figure 10. Dry matter disappearance percentage (%DM) of winter grass, fodder beet leaf and fodder beet bulb at each incubation period for 48 hours.

Table 4-10. Estimated mean dry matter disappearance calculated by first order kinetic equation.

Time	Winter Grass y(t) (%)	Fodder Beet Leaf y(t) (%)	Fodder Beet Bulb y(t) (%)	S.E.M	LSD	P Value
0	39.3	31.4	68.8	0.000	0.003	<0.001
2	49.0	39.1	71.4	0.007	0.020	<0.01
4	56.1	45.9	73.6	0.010	0.039	<0.001
6	62.2	51.8	75.7	0.014	0.049	<0.01
12	74.7	65.1	80.7	0.016	0.056	0.001
24	85.4	79.8	87.1	0.009	0.034	0.004
48	89.7	89.8	92.6	0.002	0.007	<0.001

LSD= Least significant difference (α<0.05). Significant difference P value = (P<0.05)

The mean nitrogen (N) disappearance from nylon bags at each incubation period for 48h is displayed in Table 4-11 and Figure 11. There was a marked loss of N immediately from the bulb. There was a ten-fold increase in N disappearance from the fodder beet bulb compared with the fodder beet leaf and a five-fold increase in N disappearance from the fodder beet bulb compared to the winter grass. There was a significant difference (P<0.001) between the N losses of the bulb and the other two treatments, which were similar. From 6-48h there was no significant difference in disappearance between the winter grass and the fodder beet leaf. The rates of N disappearance for 0-6h were 7%, 5.6% and 14.4% /h for winter grass, fodder beet leaf and fodder beet bulb respectively. The rates of N disappearance for 6-48h were 1.17%, 1.36% and 0.24% for winter grass, fodder beet leaf and fodder beet bulb respectively.

Table 4-11. Mean nitrogen disappearance (%) from nylon bags for each incubation period for winter grass, fodder beet leaf and fodder beet bulb.

		Fodder Beet				
Time (hours)	**Winter Grass**	**Leaf**	**Bulb**	**S.E.M**	**LSD (5%)**	***P* value**
0	17.5	8.8	85.8	1.45	6.54	<0.001
2	20.9	11.8	83.8	1.56	5.39	<0.001
4	37.9	22.3	84.3	2.08	7.18	<0.001
6	42.1	33.5	86.3	2.04	7.04	<0.001
12	70.1	65.1	90.5	1.40	4.84	<0.001
24	87.1	85.8	94.8	0.59	2.04	<0.001
48	91.4	90.5	96.2	0.50	1.71	<0.001

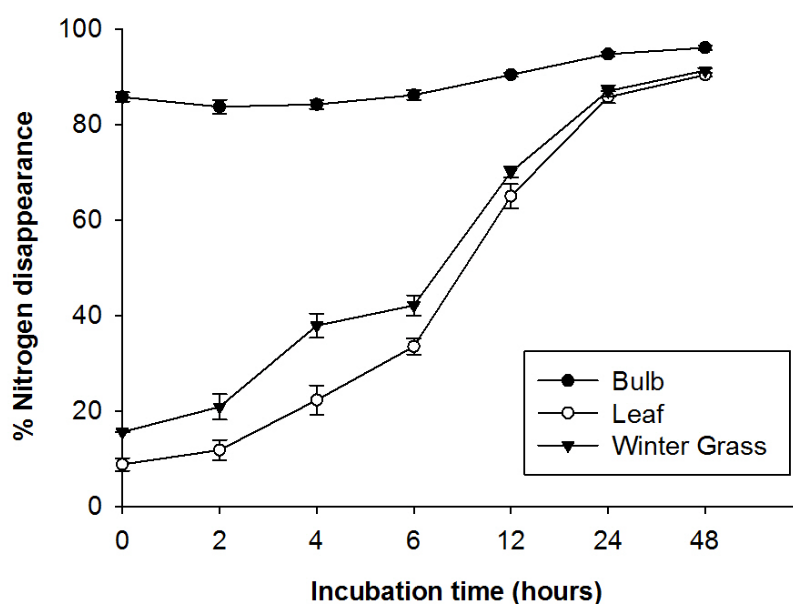

Figure 11. Nitrogen disappearance percentage (%) of winter grass, fodder beet leaf and fodder beet bulb at each incubation period for 48 hours.

Chapter 5

Discussion

5.1 Introduction

The objective of this study was to establish if there was a difference in the microbial protein production between diets of *ad libitum* winter grass and fodder beet given that the current research for both diets suggests certain features that promote high microbial protein supply, but that fodder beet is a significantly reduced crude protein content feed. Both high quality ryegrass and fodder beet diets are known to have high rumen outflows. Further, high quality ryegrass is usually associated with a high crude protein content of very rapidly degradable protein sources. Recent 2014 animal performance trials using steers fed *ad libitum* fodder beet diets with limited supplements and overall diet protein contents of 10-15% have observed liveweight gains of 1.1 kg /day for 290-530kg steers (Gibbs, *unpublished data*).

The experimental design required long term adaptation to this system of fodder beet feeding to allow rumen microbes to adapt to the high supply of rapidly fermentable WSC and to alleviate the risk of ruminal acidosis due to this. Winter grass diet was used as the control treatment as it required no adaptation period, has high feed quality and has been demonstrated to provide high rumen nitrogen in cattle (Gibbs, 2009). A conventional experimental approach would normally use a cross over design to mitigate the period effect however, in an experiment where a long adaptation period is required with substantial rumen and extra rumen physiological effects, and the feed supply is seasonal, this is not possible. Consequently, this experimental design used the four steers in blocked serial treatment comparison. It is acknowledged that there are possible unknown effects of increasing maturity, liveweight and previous diet exposure, but the parameters measured in addition to those required directly for microbial protein supply estimation were intended to mitigate this uncertainty.

The most effective methodology of establishing microbial protein supply in intact cattle is the use of the X. B. Chen and Gomes (1992) method of purine derivative excretions. Trial one in experiments one and two used this methodology of total urine collections and *in vivo* digestibility for this purpose.

Microbial protein production is known to be heavily influenced by both the components of the diet and the rumen environment, however, adaptations which can occur to relatively low dietary CP include physiological mechanisms of rumen N recycling. Therefore, the N balance of these respective diets was established in trial one of experiments one and two, and the rumen environment of the respective diets was defined closely by trials two, three and four including the effect of diurnal variation. Finally, trial five of experiments one and two defined closely the inherent disappearance values for DM and N for both diet treatments as these effect both the FME supply and the rapidly available N supply in the rumen, although they are not completely independent of the rumen environment.

This series of experiments with sub-trials was used with both diet treatments (winter grass and fodder beet) to firstly establish the microbial protein supply and secondly to establish as closely as possible the factors most likely to influence the microbial protein supply in a forage based diet.

In trial one of experiment one and two, the fodder beet diet resulted in a significantly higher microbial protein production than the winter grass diet despite the lower CP content of the fodder beet diet treatment. In trials two, three and four, significant differences in the rumen environment and function between treatments were observed. Relative to winter grass, fodder beet displayed higher rumen pH and urea concentrations (Figure 6 and Figure 9), and lower redox, VFA and ammonia concentrations (Figure 3, Figure 7 and Figure 8). Trial four in experiment one and two demonstrated little difference between the treatments in either DM or N pool size, with only a significant difference only at the 6pm rumen evacuation in the N pool size. Disappearance of DM and N, measured in trial five of experiment one and two, was greater in both the leaf and bulb of fodder beet than in the winter grass with bulb greater than leaf within the fodder beet treatment (Table 4-9 and Table 4-11).

5.2 Microbial Protein Production and Efficiency

This is the first study investigating the differences between microbial crude protein production on pasture compared to *ad libitum* fodder beet and it is the first report of a significantly increased microbial protein production on fodder beet compared with pasture. Microbial protein production (g N/ d) and efficiency (g N/ kg DMI) was observed to be

significantly higher on the fodder beet diet than on the winter grass diet. The twofold increase in urine from the steers on the fodder beet diet, with rumen outflow rates determined by the PEG marker Table 4-8, demonstrate the increased water loading of the rumen in the FB treatment, due to the low DM content of the feed and the high voluntary DMI.

As previous literature suggests, an increase in passage rate will increase the efficiency of microbial protein production due to lower cell lysis and microbial N recycling in the rumen (Dewhurst et al., 2000). However, as the rumen outflow rates were not significantly different between the treatments, this suggests that the increased water was leaving the rumen at an increased rate via the rumen epithelial route. This is the first reported observation of this in winter crop fed cattle that I am aware of, and suggests there must be substantial physiological adaptations to grazing FB beyond the rumen to maintain high DMI and subsequent production.

There is indirect evidence for this proposition in the relatively low total VFA concentrations and high rumen pH observed in the FB treatment (Figure 7; Table 4-5), despite the very high ME intake and very rapid early DM disappearance (Figure 10; Table 4-10) of the bulb that indicates high FME release. Both would suggest a low pH and a high total VFA concentration should result, but neither did. Rumen VFA removal is twofold, either via rumen outflow in fluid passage, or via the epithelial transport. It is striking in this study that with no significant difference between outflow rates, and in the face of greater energy supply, there is less VFA and this indicates that high epithelial transport of VFA is occurring. The mechanism for this is greater epithelial blood flow (Storm et al., 2012), and this will also increase water removal, and potentially, urea recycling, which is discussed in 5.3 below.

As discussed, a limitation of the experimental design employed in this research is the period effect. Therefore, over the course of the experimental work, the steers gained weight. The mean liveweight of the four steers in experiment one was 286kg, while the comparable mean liveweight of the steers in experiment two was 320kg, an approximate liveweight gain of 1.0kg/d, which is consistent with previous trial work results in this research group. Therefore, as the mean liveweight of steers increases, the mean DMI (kg) is also expected to increase. However, the increases in DMI (36.1%) observed were threefold greater (Table 4-2)

than the concomitant liveweight gain observed (11.9%), and this suggests that voluntary DMI (kg/ kg LWT) maybe inherently higher for FB diets compared with grass diets.

Due to the increase in mean liveweight of the steers, there would also be an expected increase in microbial protein production in kg/d, however, the magnitude of the increase in mean daily microbial protein production (84.5%: Table 4-3) was also greater than what was expected due to the increased mean steer liveweight. This also suggests that some characteristics of the fodder beet feed were factors in the increased microbial protein supply as opposed to simply the increased liveweight and increased DMI. Given it is not the high water content producing greater rumen outflow rates, it would appear to be associated with the greater rumen efficiency of N use (Table 4-3).

Therefore, when MCP supply (g) is expressed against DMI (12.5 vs. 15.5g N /kg DMI for winter grass and fodder beet respectively), this efficiency of MCP supply is significantly different between the two treatments. While the increase in mean daily microbial protein production between the treatments was unexpected given the available literature on drivers for DMI (Chapter 2) and the data at hand for fodder beet intakes, the increase in efficiency of microbial protein production was expected due to factors such as the high rumen fluid passage rates anticipated from the high water loading, and the high supply of FME to the microbes. However, this was not the case with no differences in passage rates observed, and the low NDF content (Table 4-1), high DMD and DOMD% (Table 4-2), DM disappearance rates (Table 4-9) and strong redox activity (Figure 3) of the winter grass and FB all suggest ample available FME was present in both treatments. The high supply of FME in both diets supports high microbial growth rates and suggests that microbes will not be limited by energy supply. Therefore, it would appear that there are other reasons for the increased rumen microbial N efficiency in the FB treatment, and perhaps the most likely explanation involves N recycling, and the relative abundances of rumen ammonia and urea.

5.3 Rumen Ammonia and Urea, and N Pool Changes

It would not automatically be expected from the available literature that total microbial protein production would be higher on the fodder beet diet than on the pasture diet due to the relatively low CP content of the fodder beet diet. Generally rumen N supply for microbes

is assessed in terms of ruminal ammonia concentration. It is commonly accepted that ruminal ammonia concentrations need to be between 50-100 mg/l for microbial survival and function (Satter & Slyter, 1974). In this study, rumen ammonia concentrations for the FB treatment were below 50mg/L (Figure 8), yet apparently high microbial production was observed.

However, what was also observed in this study in the FB treatment was an unusually high rumen urea concentration at all times during the diurnal period and most prominently when the ruminal ammonia concentrations were low. This is the first reported observation of the rumen environment of beef steers on an *ad libitum* diet of FB. The significant differences between treatments in the rumen ammonia and rumen urea concentrations in this experiment (Figures 8 & 9; Table 4-6) and the singularly high concentration of ruminal urea recorded in the FB treatment have not been reported previously in cattle studies. It is possible that this is it evidence of unusually high N recycling with this diet, perhaps due to a combination of low CP content of the FB diet, the very high FME and water loads. This would be supported by the fact that N is recycled in the form of urea rather than ammonia (Kennedy & Milligan, 1980). If N recycling was the driver for increased microbial efficiency, this recycling may be through saliva or it may be through direct secretion into the rumen.

The literature (Kennedy & Milligan, 1980) suggests that when urea enters the rumen, it will be degraded to the form of ammonia, as low rumen ammonia levels drive urea uptake back into the rumen. This is what makes the present finding unusual. One potential explanation for this finding is that almost all previous studies, as reviewed in Kennedy and Milligan (1980) for example, have been conducted on high DM feeds, with few forage studies available, and in general terms there are few low CP content forages of low DM with high FME supply. Therefore, I am suggesting that the high rumen urea concentrations may represent a novel adaptation process in the presence of such diets. The high water load of the FB treatment and concomitant high rumen epithelial blood flow to facilitate uptake would rapidly reabsorb any ammonia produced from recycled urea. However, urea itself would be less likely to be transported than ammonia, and if transported, cost less energy in recycling than the ureagenesis of ammonia in the liver. Hence there are several sound physiological reasons for suggesting this explanation.

Another supporting evidence from within this study is the relatively small differences observed between N pools between the treatments. High intake NZ dairy cows grazing very high quality pasture, have been observed to have significant differences between rumen N pool sizes at similar intervals around the diurnal period as this experiment investigated (Saldias, 2014). At times, the differences in the size of the N pools in the dairy cows were two-fold. Trial four of experiment one and two showed that there was relatively little variation in the rumen N pools within the winter grass treatment and between treatments. The only time when a significant difference was recorded in the size of the N pools was at 6pm. This suggests that in general terms, microbes in both rumen environments have a similar amount of N to utilise for microbial growth. However, contrary to the winter grass treatment, in the FB treatment the rumen ammonia concentrations were low and the rumen urea concentrations were high. This means that while the microbes in the fodder beet treatment had a different N substrate to utilise, the total N pools have been similar at most sampled intervals. This suggests that the urea concentration was also used successfully for microbial production in the FB treatment, and supports the idea that this is an energy effective adaptation to some rumen or extra-ruminal environment produced with the FB diet.

5.4 Nylon bag DM and N Disappearance

Trial five in experiment two employed a new methodology for the *in sacco* nylon bag trial. Due to the variability in plant morphology of FB, the feed needed to be highly standardised in order to draw accurate conclusions for DM and N disappearance of fodder beet components. There are very few *in sacco* studies of FB disappearance, but those available are severely limited in conclusions on disappearance able to be drawn by ineffective methods of representative sampling and DM inclusion in nylon bag (Jenkins & Bryant, 2014), which effectively invalidates comment on either DM or N release in the rumen for the FB components of the diet.

In this study, in the preparation of the feed put into the nylon bags, a truly representative proportion of bulb cortex and epidermis was allocated to each individual nylon bag (bulb). Further, the proportion of 'true leaf' to 'stem leaf' was determined by weighing each respective component, and allocating these proportions to each nylon bag (leaf). This

method resulted in the proportion of the different plant components which accurately represented what would be consumed by animals in a grazing situation being highly standardised.

From the results of the trial, it was observed that the bulb component of the feed had a very high immediate disappearance rate after placement in the rumen. After 2h, 69% of the bulb DM had disappeared and there was an ultimate digestibility of 96% (Table 4-9; Figure 10). This results in a very high supply of FME to the ruminal microbes during the post prandial period. While the FB leaf had a lower initial DM disappearance, the subsequent rate of DM disappearance was higher than that of FB bulb. After 6h, the leaf DM disappearance was 43.9% which further results in a high FME supply to microbes in the post prandial period. Winter grass had a higher initial DM disappearance than did the FB leaf, but the subsequent rates of disappearance were similar to the FB leaf component, and consistent with previous NZ grass *in sacco* studies (Chaves et al., 2006; Sun et al., 2010).

As the initial DM disappearance for bulb was greatly higher than the DM disappearance for the leaf, it is likely that the large quantity of WSC contained in the bulb were degraded during this period and that the yet undegraded component was the fibrous constituent of the feed. Given that the ultimate digestibility of the bulb and leaf components were similar, and the rate of leaf degradation over the subsequent period was higher than that of the bulb, this suggests that the fibrous content of the bulb was less digestible than that of the leaf, which has not been previously reported. Further, the more gradual and even degradation of the leaf will result in a more constant supply of energy and protein to the microbes, while the major energy source in the bulb will have already been degraded.

The N disappearance from the nylon bags showed a similar trend to the DM disappearance. There was a very rapid initial N disappearance from bulb (86% after 6h), but the rate of disappearance after the first 6h was very slow. Whereas, the leaf showed a small initial N disappearance, however very high N disappearance rates were observed after the initial 6h. The winter grass treatment showed significantly higher initial N disappearance than fodder beet leaf, however, after the first 6h, the N disappearance rate was faster for leaf than it was for the winter grass and after 48h, there was no significant difference in the total N disappearance. This was also broadly in line with previous New Zealand studies in this field (Sun et al., 2010).

5.5 Rumen pH and Environmental Conditions

As described briefly in 5.1, due to the very high WSC content of the fodder beet diet, pH values were expected to be lower for the fodder beet diet than the grass diet (Gibbs, 2014). However, this did not occur and the ruminal pH (digesta and fluid) was observed to be higher on the fodder beet diet than on the winter grass diet (Figure 2, 4, 5, 6; Table 4-4). Given the low supplement inputs which minimise rumination, it is unlikely that rumen buffering is a significant reason for a high pH on the fodder beet diet, and as the rumen outflow rates were similar between diets, that does not explain the difference in rumen pH either.

Rumen pH is primarily modulated by the removal of acid from the rumen, and this is by either by absorption through the rumen wall or by way of passage through the gastrointestinal tract (Dijkstra, 1994). While in this study the exact mechanisms for this removal of acid were not examined, what was observed was that for the high intake of rapidly FME, there was a low rumen VFA concentration and a high rumen pH in the FB treatment compared with the winter grass treatment. The winter grass had a lower FME value than FB, but gave a lower rumen pH which is highly unusual. Therefore, this is the first reported observation of a relatively high rumen pH on an *ad libitum* FB diet. Previous work by this research group (Gibbs, *unpublished data*) observed a rapid decrease in post prandial pH of steers fed a restricted diet of FB. Rumen pH values were recorded as low as 5.0-5.2 (Figure 12), a very different pattern to that seen in the present study. This suggests that the diurnal pH pattern and the rumen environment is influenced by restricted feeding level and grazing behaviour as a result of feeding level.

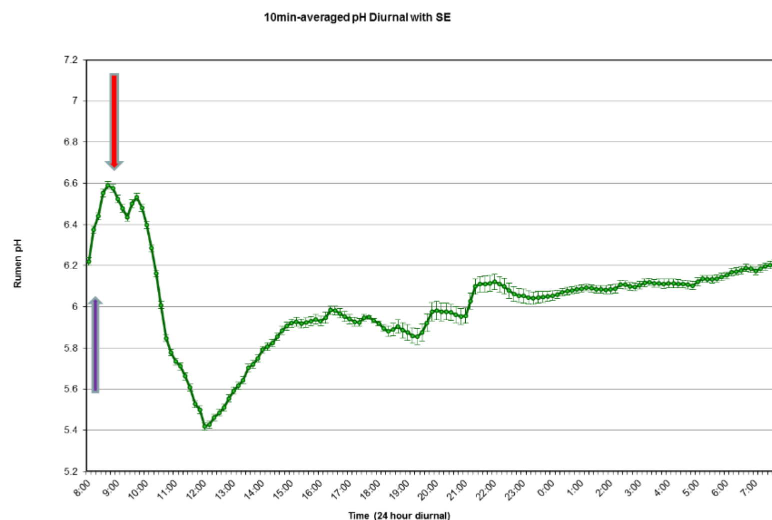

Figure 12. Diurnal pattern of rumen pH of steers fed a tightly restricted diet of fodder beet (Gibbs, *unpublished data*). The blue arrow is time of supplement feeding, the red arrow time of fodder beet feeding

When ruminants are on a restricted intake, the passage rate out of the rumen is likely to be slower than on an *ad libitum* diet, but the digestibility of feed is likely to be higher (Leaver et al., 1969). Furthermore, when animals are on a restricted diet, their grazing behaviour is likely to be different to if they are on an *ad libitum* diet. When ruminants are on an *ad libitum* diet, they are likely have 3-4 major grazing bouts per day, and 10-11 minor grazing bouts during the day (Baumont et al., 2006).

However, if the diet is restricted, the ruminant is likely to eat very quickly in the initial grazing bout and is likely to have fewer but larger grazing bouts (Gregorini et al., 2009). If feeding level is significantly restricted, there may only be one grazing bout. In these instances, there may be a relatively large intake of rapidly fermentable WSC which will not be passed to the small intestine as quickly as they would be in the *ad libitum* diet. If the diet contains a high quantity of readily fermentable WSC, this loading of FME will result in a rapid pH decline as a result, and that pattern is seen in the restricted FB diets.

Given the multifaceted control of grazing intakes, this difference between restricted and *ad libitum* FB diets suggests that at least some adaptations to the latter for cattle may involve extra-ruminal mechanisms – grazing behaviour pattern shifts, for example. Despite the extra-ruminal mechanisms that may be operating in *ad libitum* FB diets, one effect of the flattening of intake across the diurnal cycle would be to encourage a milder rumen environment of higher pH and less diurnal variation that is conducive to microbial protein production. This may be a factor in the higher efficiency of microbial protein production observed in this study.

5.6 Summary and Conclusions

The research of this dissertation has demonstrated that *ad libitum* diets of winter grass or FB had significant differences in the supply of microbial protein to beef steers. Voluntary DMI observed on the FB diet was higher than that of winter grass, and resulted in an increase in N intake, but neither explained fully the increased efficiency observed. Further, the

efficiency of microbial protein production increase was apparently independent of rumen outflow rates, and with similar FME available. The diurnal pattern of rumen pH was unexpectedly higher on the fodder beet diet, the VFA concentrations were lower, and there was a marked shift to higher concentrations of rumen urea, and lower rumen ammonia, low enough to be considered an impediment to normal rumen microbial function. However, there was no evidence of this reduced function, which may indicate a shift to increased urea use by microbes in this system. Dry matter and N disappearance from nylon bags was extremely fast which provided a large and rapid supply of WSC and N to rumen microbes to support high microbial growth rates.

These findings quantify the animal production results that have been observed in the form of high daily liveweight gains on a commercial scale. These results show that on an *ad libitum* diet, despite the low CP of the feed, microbial protein supply is adequate to the steers for high growth rates (>1 kg/ d) and due to the high ME content of the feed, energy supply is also adequate. It is likely that the only limiting factor to further improvements in growth rates is the DMI of animals. This system provides potential for growth in the beef industry and an opportunity to maximise liveweight gains all year round as winter is typically a period of the shortest feed supply.

5.7 Future Research

This is the only research into microbial protein production and the attendant rumen environmental conditions of beef cattle on *ad libitum* diets of fodder beet currently available. There are four novel findings in this work that are currently without a certain explanation in nutritional physiology. First, the key drivers of the increase in DMI on FB diets relative to grass diets are unknown, and contrary to what may have been expected from the extant literature on the basis of DM limits, ME intake and physical mastication limits. The unusual rumen urea: ammonia concentrations are previously unreported, and the suggestion of this being a function of accelerated recycling of N is support for this area to be given more study. The actual mechanism for this adaptation is unclear but this also provides an opportunity for further research as this appears to increase microbial protein production on a low CP diet, which is of interest for N reduction policies regionally. This work would be furthered by also analysing rumen microbial populations to investigate the recycling of N in

the animal and establish whether these adaptations are modulated by the rumen or if entire animal regulating mechanisms are involved. Finally, the suggestion in these results that a mechanism of increased rumen epithelial transport of water, and possibly VFA, in the absence of increased rumen outflow rates, is intriguing and deserves future attention on the grounds it offers an insight into rumen pH control mechanisms that are currently poorly understood.

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