Substitution of maize with high fibre by-products in concentrates supplemented to dairy cows grazing kikuyu/ryegrass pasture during spring

by

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DECLARATION

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ABSTRACT

Title: Substitution of maize with high fibre by-products in concentrates supplemented to dairy cows grazing kikuyu/ryegrass pasture during spring

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Kikuyu over-sown with ryegrass forms the basis of pasture based systems in the Southern Cape. During early spring, energy is the first limiting nutrient in kikuyu/ryegrass pasture, supplementation is thus essential. Supplementation consists mainly of high starch concentrates (high maize inclusion), which is expensive and could negatively affect rumen parameters. The objective of this study was to determine if milk production could be improved or maintained, and if the rumen environment would be improved, by replacing high starch concentrates with low starch (high by-product) concentrates for dairy cows on kikuyu/ryegrass pasture.

Forty-five multiparous, high producing, lactating Jersey cows [body weight, 340 ± 34.7 kg; milk yield (MY), 19.6 ± 2.23kg/d; days in milk (DIM), 153 ± 33.5; lactation number, 3.6 ± 1.85; (mean ± SD)] were used in the production study. A randomised block design was used. The forty-five cows were allocated to fifteen groups of three each (blocking) on the basis of MY, DIM, and lactation number. Cows from each group were randomly allocated to one of three treatment groups (high starch, medium starch and low starch concentrate supplementation). Cows were fed 6kg (3kg during each milking) concentrate per day and were allocated fresh pasture *ad lib* after each milking. There were no significant differences ($P > 0.05$) found in milk yield and fat corrected milk yield between treatment groups. Milk fat percentage was significantly higher ($P < 0.05$) in the low starch treatment than in the high starch treatment. Milk fat yield was significantly higher ($P < 0.05$) in both the low starch and the medium starch treatment when compared to the high starch treatment. Milk protein and lactose percentages, as well as milk urea nitrogen and somatic cell count, did not differ significantly ($P > 0.05$) between treatments. Live weight change, as well as body condition score change, was unaffected ($P > 0.05$) by treatments indicating body reserves were not used to maintain milk production in the low starch treatment.

A rumen metabolism study was also done with ten lactating, cannulated Jersey cows [body weight, 332 ± 56.3 kg; MY, 17.3 ± 1.73kg/d (mean ± SD)] were used. The cows were divided into two groups of five each, on the basis of lactation number, DIM, and MY. The five cows from each group were randomly allocated to one of two treatment groups (high starch and low starch concentrate supplementation) and used in a cross-over design. Cows were fed 6kg concentrate per day and were allocated fresh pasture *ad lib* after each milking. The volatile fatty acid (VFA) concentration was significantly higher ($P < 0.05$) in the high starch treatment when compared to the low starch treatment. The individual VFA's, acetic-, propionic- and butyric acid concentrations were also significantly higher ($P < 0.05$) in the high starch treatments when compared to the low starch treatment. The acetic to propionic acid ratio was unaffected ($P > 0.05$) by treatment. Rumen ammonia-nitrogen concentration was significantly higher ($P < 0.05$) in the high starch treatment. Rumen pH was unaffected ($P > 0.05$) by supplementation type. The *in sacco* dry matter and neutral detergent fibre digestibilities of the kikuyu/ryegrass pasture were unaffected ($P > 0.05$) by treatment type.

Results indicated that milk production could be maintained with low starch concentrates which also improved milk composition. Results further suggested that the rumen environment was relatively unaffected by low starch concentrate supplementation.

UITREKSEL

Titel: Vervanging van mielies met hoëvesel-neweprodukte in kragvoer vir aanvulling van melkkoeie op kikoejoe/raaigras-weiding gedurende die lente

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Kikoejoe, oorgesaai met raaigras, vorm die basis van weidingstelsels in the Suid-Kaap. Tydens die lentemaande is energie die eerste beperkende voedingstof op kikoejoe/raaigras weidings, wat kragvoeraanvulling noodsaaklik maak. Aanvulling bestaan grootliks uit hoëstysel-kragvoer (hoë mielie-inhoud) wat nie net duur is nie, maar dit kan ook die rumenomgewing benadeel. Die doel van die studie was om vas te stel of melkproduksievlakke onderhou kan word en of rumenomgewing verbeter kan word deur die vervanging van hoëstysel-kragvoer met laestysel-kragvoer (hoë neweprodukinhoud) vir melkkoeie op kikoejoe/raaigras weidingstelsels.

Vyf-en-veertig meervoudige pariteit-, hoë produserende, lakterende Jerseykoeie [liggaamsmassa, 340 ± 34.7 kg; melkproduksie, 19.6 ± 2.23kg/d; dae in melk, 153 ± 33.5; laktasienommer, 3.6 ± 1.85; (gem ± standaardafwyking)] is gebruik vir die produksiestudie van die proef. Daar is gebruik gemaak van ‘n ewekansige blokontwerp. Die vyf-en-veertig koeie is opgedeel in vyftien groepe van drie elk, gebaseer op melkproduksie, dae in melk en laktasienommer. Koeie in elke groep is ewekansig aan een van drie behandelings (hoëstysel-, mediumstysel- of laestysel-kragvoeraanvulling) geallokeer. Koeie is daagliks 6kg (3kg tydens twee milkings) kragvoer gevoer en vars weiding was *ad lib* beskikbaar na elke melking. Daar was geen beduidende verskil ($P > 0.05$) in melkopbrengs of vet-gekorrigeerde melkopbrengs tussen die drie behandelings nie. Bottervetpersentasie was beduidend hoër ($P < 0.05$) in die laestyselbehandeling in vergelyking met die hoëstyselbehandeling. Bottervetopbrengs was beduidend hoër ($P < 0.05$) in beide die laestysel- en mediumstyselbehandelings in vergelyking met die hoëstyselbehandeling. Melkproteïen- en melklaktosepersentasies, asook melkureumstikstof en somatise seltelling, was onveranderd ($P > 0.05$) tussen behandelinge. Liggaamsmassa en liggaamskondisietelling het geen verskille ($P > 0.05$) getoon tussen behandelings nie, wat daarop dui dat liggaamsreserwes nie gebruik is om melkproduksie in die laestyselbehandeling te onderhou nie.

‘n Rumenmetabolismestudie is ook uitgevoer met tien lakterende, gekannuleerde Jerseykoeie [liggaamsmassa, 332 ± 56.3 kg; melkproduksie, 17.3 ± 1.73kg/d (gem ± standard afwyking)]. Die koeie is in twee groepe van vyf elk verdeel, gebasseer op laktasienommer, dae in melk en melkproduksie. Die vyf koeie van elke groep is in ‘n omslagontwerp gebruik en is ewekansig aan een van twee behandelings (hoëstysel- of laestysel-kragvoeraanvulling) geallokeer. Koeie het daagliks 6kg kragvoer ontvang en vars weiding was *ad lib* beskikbaar na elke melking. Die vlugtige vetsuurkonsentrasie was beduidend hoër ($P < 0.05$) in die hoëstyselbehandeling as in die laestyselbehandeling. Die individuele vlugtige vetsure naamlik asynsuur, propionsuur en bottersuur, was ook beduidend hoër ($P < 0.05$) in die hoëstyselbehandeling as in die laestyselbehandeling. Die asynsuur- tot propionsuurverhoudings het geen verskille ($P > 0.05$) tussen behandelings getoon nie. Rumen-ammoniakstikstof was beduidend hoër ($P < 0.05$) in die hoëstyselbehandeling as in die laestyselbehandeling. Rumen-pH het geen verskille ($P > 0.05$) getoon tussen behandelings nie. Die *in sacco* droëmateriaal- en neutraalbestande veselverteerbaarhede van kikoejoe/raaigras weiding het geen verskille ($P > 0.05$) tussen behandelings getoon nie.

Die resultate het aangedui dat melkproduksie onderhou kan word en dat melksamestelling verbeter kan word met laestysel-kragvoeraanvulling. Resultate het ook daarop gedui dat die rumenomgewing nie noodwendig verbeter word deur die aanvulling van laestysel-kragvoer vir koeie op kikoejoe/raaigras weidings nie.

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CHAPTER 1
Introduction

Dairy producers, whose most important product is milk, are of great importance to human society. Recently, emphasis has shifted from solely quantity of milk produced to also include the quality of the milk being produced, with the fat and protein content of the milk important determinants of the price paid to the producer. The ever increasing demand for milk and milk products exerts great pressure on dairy producers to increase productivity and efficiency. Pasture based systems were responsible for approximately 65% of the total milk production and contributed 75% to the total number of dairy cows present in South Africa during 2009 (2010, Dr K. Coetzee, personal communication, Milk Producers' Organization, koos.coetzee@mpo.co.za). Increasing production costs due to rising labour costs, increased machinery and housing costs and a decrease in product prices and subsidies, has necessitated a transformation in grassland management practices in recent years (Dillon, 2006; McEvoy *et al*., 2009). Increasing the proportion of grazed forages in the diet should be a key objective for producers, seeing that grazed forages are currently the cheapest nutrient source for dairy cows and could therefore be the key to obtaining a lower cost feeding system (Clark & Kanneganti, 1998; McEvoy *et al*., 2009).

Traditionally, the success of pasture-based systems was based on high herbage utilization accompanied by high stocking rates. Such systems often compromised performance of individual animals. The future success of pasture based systems will thus have to be based on obtaining higher productivity per animal and the efficient exploitation of grazed pasture via grazing systems designed to maximise daily herbage intake per cow and the maintenance of a sufficient quantity of high quality pasture throughout the whole growing season (Dillon, 2006). Pasture systems for dairy cows are commonly based on temperate grass species (Bargo *et al*., 2003), with perennial ryegrass (*Lolium perenne*) under irrigation providing a useful basis for winter pastures (Van Oudtshoorn, 2004). Temperate pasture species, such as ryegrass (*Lolium* spp.), can play an important role in the animal production sector, especially in the dairy industry, by providing the least expensive forage source to fill the winter-spring feed-gap characteristic of the tropical species (Fulkerson *et al*., 1993; Dickinson *et al*., 2004). Botha *et al*. (2008) developed a system whereby kikuyu was over-sown with annual ryegrass during autumn to improve the seasonal dry matter (DM) production of kikuyu pastures during spring. Ryegrass pastures during early spring are high in protein, have low neutral detergent fibre (NDF) content and have a high digestibility (Wales *et al*., 2001). The metabolisable energy (ME) content of ryegrass is above 11.9 MJ/kg DM from winter until the end of September, after which it falls markedly to concentrations of less than 10 MJ/kg. A shortage of energy is, thus, the first limiting factor for milk production of high producing dairy cows grazing high quality ryegrass

pasture during early spring (Fulkerson *et al*., 1998). For this reason, there exists a need to supplement grazing animals with energy, and/or protein (Schwarz *et al*., 1995; Penno *et al*., 2001).

There are several benefits to supplementing dairy cows on ryegrass pastures with concentrates. These benefits include increased milk production per cow, increased stocking density and milk production per unit land, improvement in pasture utilisation, maintenance of the animal's body condition score at acceptable levels which can improve reproduction, increased lactation periods during times of pasture shortages and an increase in the dairy farm's profitability (Bargo *et al*., 2003). Delagarde *et al*. (1999) stated that carbohydrate supplementation, whatever the source, increases milk production. Concentrates are, however, expensive and can make up two thirds of the cost of a dairy cow's ration (Meeske *et al*., 2006). Currently, the main ingredients in the majority of supplement concentrates of grazing dairy cows are highly digestible carbohydrates, such as the starch found in maize. Although supplementation with these high starchy concentrates improve milk production, they are expensive, animals are more prone to develop health related issues and pasture utilisation is often negatively affected. In addition, the recent increase in the cost of high starch sources, such as maize and soybean oilcake, has drastically increased the input costs associated with supplementation with concentrates consisting of predominately these sources (Meeske *et al*., 2009). As result, altering concentrates of dairy cows on pastures, via altering the composition of concentrates, could markedly decrease input costs, while still maintaining milk production and potentially improving milk composition. Muller *et al*., (2001) suggested that starch in the concentrate could be replaced with non forage fibre sources such as cottonseed hulls, soy hulls, beet pulp, distiller's grains, citrus pulp, wheat middlings, whole cottonseed and some other by-products, in order to provide more fermentable fibre. These alterations would reduce concentrate costs and could have massive implications in the dairy industry, resulting in large savings for dairy producers as well as increased production efficiency and improved cow health.

The management of pasture systems is often more complicated when compared to that of total mixed ration systems, due to factors such as pasture substitution rate and because the rumen environment is subjected to two different diets (pasture and concentrates), causing a less consistent rumen environment. The feeding of highly fermentable carbohydrates separate from the main roughage source (pasture) results in extreme rumen environments that could lead to reduced pasture utilisation rates, decreased milk production and eventually health problems. Rumen pH is one of the most variable factors that can influence rumen microbial populations and volatile fatty acid production (Ishler *et al*., 1996). If rumen pH decreases below 6.2 it may cause a reduction in fibre digestion due to the environment being suboptimal for microbial growth (Pitt *et al*., 1996). The optimal pH for ruminal digestion of pasture was found to be 6.35, according to De Veth & Kolver (2001), while the highest milk yield occurred at a rumen pH of between 5.8 and 6.2. These findings are important, because even when ryegrass is supplemented with low levels of non structural carbohydrates, it causes a reduction in rumen pH (Bargo *et al*., 2003). Higher amounts of highly fermentable carbohydrates do improve production but may, however, result in acidosis,

with the pH decreasing in the rumen as a result of large increases in lactic acid production (Tajima *et al*., 2000). The most severe changes in the rumen microbial population occur with dietary changes, such as a change between roughage and a high grain concentrate, or pasture to concentrate feeding. This reduction in rumen pH is most probably brought about by a change in the molar ratios of volatile fatty acids (VFA) in the rumen, with the molar proportions of acetic acid decreasing and the molar proportions of propionate increasing (Sayers *et al.*, 2003). This may negatively impact on fibre and pasture digestion. Fibre digesters, such as cellulolytic and methanogenic bacteria, are most effective at a pH of between 6.2 and 6.8, with effectiveness decreasing as the rumen pH decreases below 6.0 (Ishler *et al*., 1996). In order to accommodate the requirements of all the different rumen micro organisms, it is important that normal feeding practices maintain a rumen pH of between 5.8 and 6.4 (Ishler *et al*., 1996). The extreme changes in rumen environment and pH often apparent when grazing dairy cows are fed a high starch concentrate could be eliminated by feeding fibre-based concentrates. Such fibre based concentrates have been shown to result in a decrease in milk production, but an increase in the milk fat percentage (Bargo *et al*., 2003). The addition of non forage fibre to the concentrate of dairy cows may also improve the stability of rumen environment in dairy cows grazing pasture, leading to increased pasture intake.

Including low starch concentrates, usually derived from by-products and thus replacing high starch (maize) products for grazing dairy cows could be a viable option. Not only could it lead to the same milk production as indicated by Kibbon & Holmes (1987), Spörndly (1991), Fisher *et al*. (1996) and Sayers *et al*. (2003) or even improved milk production as indicated by Meijs (1986), Khalili & Sairanen (2000) and Meeske *et al*. (2009), but it would decrease the total capital input cost, due to ingredients in the concentrate costing less. Even if milk production was to stay the same, the gross capital margin could still increase due to the lower input costs associated with cheaper feed. Meijs (1986) and Meeske at al. (2009) indicated that this dietary change could have a positive effect on milk fat percentage and could lead to increased fat corrected milk yields. Dairy farm profitability can be maximised by maintaining nutrient levels while managing feed costs as well as improving overall health (Ishler *et al*., 1996). A study to determine the effect of feeding low starch concentrates to cows grazing ryegrass/kikuyu pasture under South African conditions is therefore most important. Positive results could lead to substantial capital savings to the dairy industry.

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CHAPTER 2

Literature review

2.1 Introduction

Dairy production systems are of great importance to human society. They are managed commercially under mostly intensive conditions and provide several products of value to humans. The most important of these products is milk. The ever increasing demand for milk and milk products exert great pressure on dairy producers to increase their productivity and efficiency.

Another factor adding to the increased pressure put on dairy farmers, other than that of the quantity of milk produced, is the quality thereof. Now, more than ever, emphasis is placed on the quality of milk being produced and the fat and protein content in the milk largely influence the price paid to the farmer. To ensure sustainability and profitability of dairy farms, farmers must produce products complying with market demands.

Towards the end of 2009, South Africa had an estimated 540 000 dairy cows, with an average herd size of 280 cows. Of these, approximately 66% were Holsteins and 26% were Jersey cows. Although pasture based systems were responsible for approximately 65% of the total milk production in South Africa, more than 75% of the total number of cows were in these systems. This discrepancy is as a result of the fact that a lower milk production is obtained from animals fed in this way (2010, Dr K. Coetzee, personal communication, Milk Producers' Organization, koos.coetzee@mpo.co.za).

Transformations in grassland management practices in recent years have been necessitated by increasing production costs, such as decreasing of subsidies, rising labour costs, increased machinery and housing costs and a decrease in product prices (Dillon, 2006; McEvoy *et al*., 2009). Environmental and animal welfare concerns associated with intensive systems also influence this shift (Dillon, 2006).

If these costs continue to rise in future, then increasing the proportion of grazed forage in the diet should be a key objective to farmers (McEvoy *et al*., 2009). Grazed forage is the cheapest nutrient source, therefore feeding pastures to dairy cows results in a lower cost feeding system (Clark & Kanneganti, 1998). However, additional supplementation for high producing dairy cows on pasture based systems is still needed to eliminate any shortfall in nutrient provided by pasture. Only when the additional value of the milk produced as well as the added gain from improved body condition scores of cows from feeding supplements, exceeds the total cost of the supplement, the cost of storage and cost of feeding, should supplementation of dairy cows on pasture be considered (Penno *et al*., 2001).

Millions of years of evolution have allowed ruminants to digest and metabolize predominantly forage diets (Van Soest, 1994). Concentrate diets, although high in nutrients, have limited amounts of effective fibre

and often result in metabolic disorders, reduced dry matter intake (DMI) and fibre digestion, and milk fat depression (Meijs, 1986).

According to Meeske *et al*. (2006), concentrates can make up two thirds of the cost of the dairy cow ration. Cost can therefore be reduced by increasing pasture intake and reducing concentrate intake. Dairy farm profitability can be maximised by maintaining nutrient levels while managing feed costs. When optimal nutrition is obtained, cows will perform better and give a higher quantity and quality of milk. Overall health will also improve as a result, thereby further saving costs (Ishler *et al*., 1996).

Nutrition is a vital component in the performance, health and welfare of cows, as well as the composition of the milk produced (Poppi *et al*., 2000; Ishler *et al*., 1996). As mentioned above, concentrate supplementation is responsible for large capital input costs in dairy production systems from pasture. Possible alterations to the diet (e.g. concentrate supplementation) of dairy cows on pasture based systems could have a marked decrease in input costs while upholding dairy production and even improving milk composition. These dietary alterations could have massive implications in the dairy industry and could lead to large savings for dairy producers as well as increased efficiency and cow health.

2.2 Pasture based systems

2.2.1 Introduction

In the past the performance of pasture-based systems was based on high herbage utilization accompanied by high stocking rates, and the performance of individual animals was often compromised. However, because of factors such as the increased emphasis on product quality and environmental issues associated with nitrogen leaching, soil compaction, greenhouse-gas emissions and animal welfare and with the increased selection of modern higher producing dairy cows, pasture based systems of the future will require higher productivity per animal. For that reason the efficient exploitation of grazed pasture will require the development of a grazing system which is designed to maximise daily herbage intake per cow and at the same time maintaining higher-quality herbage at greater quantity over a whole grazing season (Dillon, 2006).

Pasture systems for dairy cows are commonly based on temperate grass species. These can be described as young leafy pastures or high quality pastures and usually contains 18 to 24% dry matter (DM), 18 to 25% crude protein (CP), 40 to 50% neutral detergent fibre (NDF), and 6.40 to 6.98 MJ/kg DM of net energy for lactation) NE_L (Bargo *et al*., 2003).

Of these grazed forage, early spring ryegrass pastures are the most digestible, but have a low NDF content and are high in protein (Wales *et al*., 2001). The first limiting factor for milk production of high producing dairy cows grazing high quality ryegrass pasture is usually a shortage of energy (Fulkerson *et al*., 1998).

2.2.2 Type of pasture used

Perennial ryegrass (*Lolium perenne*) is an evergreen, tufted, grass. It has narrower leaves than that of the annual ryegrass. The upper surface of a mature leaf is a dull dark green colour and is ribbed while the lower surface is shiny and lighter green (Dickinson *et al*., 2004).

Ryegrass is best adapted to the cool, moist, high altitudes of the country where summers are mild. The plants grow best under irrigation. They play an important role in the animal production sector, especially in the dairy industry, bridging the gap with fodder flow problems (Dickinson *et al*., 2004). Perennial ryegrass under irrigation is particularly useful as a winter pasture (Van Oudtshoorn, 2004). Although adapted to a wide variety of soils, it thrives on deep, fertile soils with good moisture retaining properties (Dickinson *et al*., 2004).

Ryegrass is called a 3 leaf plant because each tiller has 3 leaves (Figure 2.1) and as the fourth leaf begins to emerge, the oldest one starts to senesce (Fulkerson & Donaghy, 2001). The interval of appearance for each new leaf is mainly affected by temperature and by soil moisture (Fulkerson & Donaghy, 2001). The interval between defoliations during winter and spring has a major effect on the ryegrass' ability to survive during summer (Donaghy *et al*., 1997).

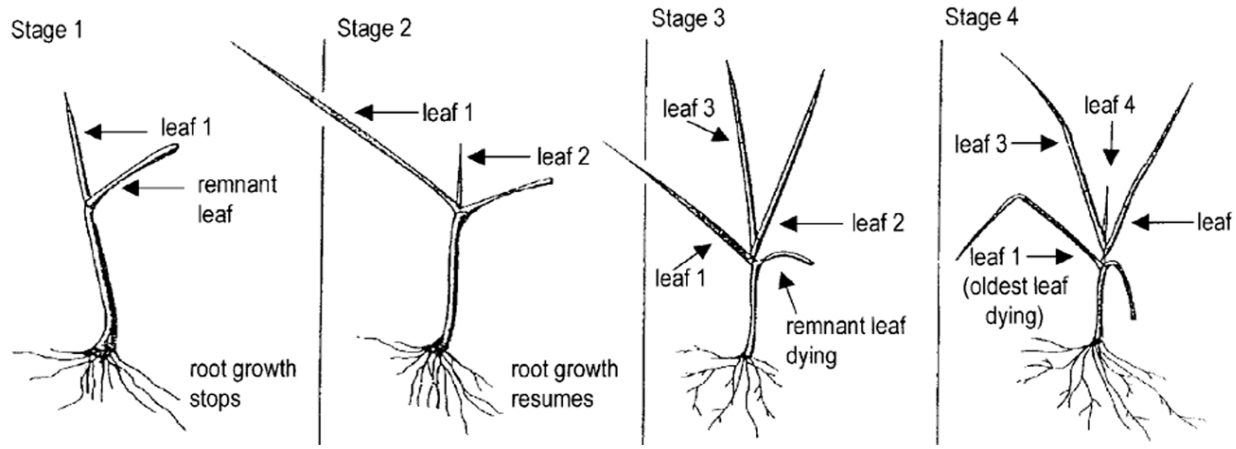

Figure 2.1 Leaf stages of the Ryegrass species (Donaghy, 1998)

Kikuyu (*Pennisetum cladestinum*) is a C_4 pasture species and is well adapted to areas of the Western Cape province of South Africa where it is used for milk production (Van der Colf *et al*., 2009). Growth is however restricted in some instances by a lack of tolerance to cold and dry conditions (Marais, 2001). During summer and autumn months, kikuyu is highly productive but the DM production is low during winter and spring (Van der Colf *et al*., 2009).

Energy is usually the major limiting factor in the productivity of high producing dairy cows on kikuyu pastures. This is because of the low digestibility of structural components in the diet and the lack of highly

digestible non-structural carbohydrates (Marais, 2001). Studies were done by Botha *et al*., (2008a) & Botha *et al*., (2008b) to determine the potential of kikuyu over-sown with other pasture types, in an attempt to improve pasture quality during these months.

2.2.3 Over-sowing of kikuyu

Botha *et al*. (2008a) found increases in DM production of kikuyu pastures during spring time if annual ryegrass, perennial clover or perennial ryegrass-clover were incorporated into kikuyu pasture. They found no negative effect on the DM production of kikuyu during summer and autumn months. They also indicated that kikuyu-ryegrass pastures which were fertilized with nitrogen had a higher DM production than kikuyu-clover pastures.

During summer time, the ryegrass would be dominant in the kikuyu-ryegrass pasture. It would then transform from ryegrass-dominant, to kikuyu-dominant pasture during summer and only kikuyu in autumn. Therefore, incorporating C_3 grasses such as Italian ryegrass and Westerwolds ryegrass into kikuyu increased the seasonal production of pastures (Botha *et al*., 2008a).

Van der Colf *et al*. (2009) did a study to determine which of the species (annual or perennial) and varieties (Italian or Westerwolds) had the highest production in a dairy system. They showed that the Westerwolds ryegrass had lower growth rates during November causing an increased component of kikuyu during summer. However with Italian and perennial ryegrass the opposite occurred. These two had higher growth rates in November which led to a reduced kikuyu component during summer. The perennial ryegrass also had a better growth rate in winter and spring during the second year because of the carry-over effect, and showed a significantly higher ($P<0.05$) DM production than both the Italian and Westerwolds ryegrass during this year, although they had similar DM production during the first year.

Van der Colf *et al*. (2009) showed that although the perennial ryegrass did not have a significantly higher ($P>0.05$) milk production per cow than that of Italian and Westerwolds ryegrass, there was a significantly higher ($P<0.05$) milk production per hectare obtained from perennial ryegrass pastures as opposed to Italian and Westerwolds ryegrass. This was due to the higher grazing capacity of the perennial ryegrass (Van der Colf *et al*., 2009). According to Clark & Kanneganti (1998), high milk output per unit land was the key to an efficient pasture based system, as opposed to high milk output per unit cow in confinement systems.

Annual ryegrass like Italian and Westerwolds ryegrass is however used more often for several reasons (Botha, 2003). These include the fact that annual ryegrass have a higher production during winter. The seasonal availability of water affect perennial ryegrass to a greater extent than annual ryegrass, and droughts can lead to bigger losses of pasture with perennial ryegrass. Perennial ryegrass is more expensive to plant because a planter is needed where this is not the case with annual ryegrass. Annual

ryegrass compete better with kikuyu in the pasture and can be planted earlier in the season thus providing more pasture available for grazing during late winter and early spring months (Botha, 2003).

2.3 Pasture Utilization

2.3.1 Pasture allowance

Many factors affect DMI in pasture. These include the pregrazing pasture mass, expressed as the amount of pasture per unit area (kg DM/ha) as well as the pasture allowance. Pasture allowance (PA) is the amount of pasture allocated to a cow per day, expressed as kg DM/cow per day (Bargo *et al*., 2003). Pasture allowance is usually estimated at ground level or to 4 to 5 cm above ground level because it is accepted that the material below that height is not available for grazing (Dillon, 2006).

Bargo *et al*. (2003) summarized seven studies to describe the relationship between DMI and PA. It was found that the optimum pasture allowance to obtain the maximum DMI of 21.9kg/day was a 110kg DM/cow/day. Pasture DMI increased 0.26kg/kg of increase in pasture allowance up to 110kg/cow/day. These studies demonstrated that the maximum DMI was obtained when 3 to 5 times the DMI of cows was allocated to grazing cows.

To maximize pasture DMI, high producing dairy cows have to be given pasture of unrestricted quantity and quality. This will however lead to low pasture utilization and will in turn lead to a decrease in pasture quality later in the season. As the pasture allowed to cows increased, so also did the amount of refused pasture and this would inevitably lead to a decrease in pasture quality in subsequent grazing rotations (McEvoy *et al*., 2009).

High producing dairy cows on a total mixed ration or on pasture supplemented with concentrates still had a higher DMI than high producing cows on unrestricted pastures (Bargo *et al*., 2003). To ensure that pasture utilization did not deteriorate Bargo *et al*. (2003) recommended a pasture allowance of only twice the DMI when cows were fed supplementation as well.

2.3.2 Herbage Mass

Pasture allowance is not the only important factor affecting the DMI and milk production of dairy cows. In pastures with a high herbage mass (more than 2200kg DM/ha) the swards have a lower digestibility than low herbage mass swards (McEvoy *et al*., 2009). Studies have shown that milk yield was depressed in the situation of high pasture herbage mass, compared to low herbage mass at a standard pasture allowance (Holmes *et al*., 1992).

The number of grazing days per hectare increases as the herbage mass increases. This should result in a higher performance per hectare but the increased amounts of dead material as well as stem material will reduce the overall quality of the pasture. This excess build-up of herbage of lower quality can be

prevented by shortening the rotational grazing intervals. This would lead to increased sward utilization as well as higher quality pasture (McEvoy *et al*., 2009).

With increased grazing pressure, total pasture DM yield was reduced, whereas the leaf DM proportion tended to be increased. During early summer, the organic matter (OM) content of the high herbage mass pastures declined at a higher rate than that of the low herbage pastures mass. This also coincided with the reproduction phase of the ryegrass, when new tillers were formed (McEvoy *et al*., 2009). Severe grazing during this time would decrease tiller formation causing lower herbage mass and better quality pastures (Hurley, 2007).

McEvoy *et al*. (2009) found no difference in the milk production of cows grazing medium or high herbage mass pastures, showing that high herbage mass at high pasture allowance would not improve milk production. They however also found that in the second half of the season the effect of the medium herbage mass pasture became apparent when milk production improved for the medium herbage mass pasture when given a pasture allowance of 20kg DM/cow/day.

McEvoy *et al*. (2009) concluded that giving cows a medium (1700kg/ha) herbage mass over the main grazing season supported higher stocking rates, as well as higher milk production. This was because of the better quality herbage available in the latter part of the grazing season thanks to the severe grazing in the earlier season. They suggested that in a rotational grazing system, cows be offered a medium herbage mass at 20kg DM/cow/day above a 4cm level.

2.3.3 Grazing method

Ryegrass is an important winter and early spring forage for dairy cows, but lacks persistence (Donaghy *et al*., 1997), since grazing management is usually focused on meeting the requirements of the animals rather than that of the plant (Fulkerson & Donaghy, 2001). Grazing management however should be based on the interaction between the plants and the grazing animals and should not be separated as often happens (Fulkerson & Donaghy, 2001)

The key to high quality forage is to know when the canopy is ready to be grazed, also how much residual to leave before moving to the next camp and how long it takes to get to that residual. It is important to stock pasture with cow densities which would reach the desired residual quickly and result in a more uniform distribution of excreta (Muller, 2003b).

There are three methods used to allocate pasture to animals. The first is daily rotation grazing which is the least precise method because it doesn't take climatic variation into account which could affect re-growth and the amount of pasture available. Secondly, pasture height is somewhat more precise but does not take into account pasture density, the species in the pasture, soil type and soil fertility or the availability of moisture. A third method using pasture mass is the most precise. It does not really examine whether pasture is ready to be grazed, but is instead used as an animal-related indicator. To maximize

production and persistence, grazing management should rather be based on plant–related indicators (Fulkerson & Donaghy, 2001). Pasture should be grazed so as to provide the best potential for persistence and re-growth, therefore any shortcomings in the animal's requirements should be supplemented (Fulkerson & Donaghy, 2001).

As mentioned before, ryegrass is called a 3 leaf plant because each tiller has 3 leaves (Figure 2.1) (Fulkerson & Donaghy, 2001). The interval between the appearances of each new leaf is affected by many factors. The ryegrass' ability to survive during summer is also dependent on the interval between defoliations during winter and spring (Donaghy *et al*., 1997). The best method of grazing ryegrass is to defoliate the grass to an average of about 5cm above ground level, and then to re-graze during the maximum growth rate period which is just before the ceiling yield is obtained (Fulkerson & Donaghy, 2001). Ceiling yield for ryegrass is obtained at 3.5 – 4 leaf stage (Fulkerson & Slack, 1994). The fourth leaf is the dying or dead leaf, and is replaced by a new leaf.

Measurement of standing crop is essential for investigating herbage production, determining stocking rates and evaluation of management strategies (Ganguli *et al*., 2000). It is also a key element in budgeting forage in a grazing system (Sanderson *et al*., 2001). Correct planning for the utilisation of forage requires regular measurement and assessment of pastures (Sanderson *et al*., 2001). The most dependable method of determining herbage yields is by mechanical cutting of pasture and subsequent weighing thereof. Many problems are associated with this method, such as its destructive nature, indicating the need for a better system to be developed (Symons & Jones, 1971).

The amount of standing crop for pastures has traditionally been estimated using hand- or mechanically clipped quadrates. Unfortunately, because these methods are time- and labour intensive, farmers are unwilling to use them (Ganguli *et al*., 2000; Sanderson *et al*., 2001).

Visual observations are probably the most commonly used method but limited by a lack of objectiveness and correlation between observers, as well as a lack of quantitative values (Tucker, 1980). Rapid measurements of canopy height (using measuring sticks, plastic disks and plates) can be difficult due to subjectivity associated with measurements (Ganguli *et al*., 2000).

There are also several double sampling techniques which can reduce labour and expenses (Sanderson *et al*., 2001). These methods use the development of a regression relationship which includes plant height, cover, vegetation density, leaf area and age. They are laborious and destructive until the regression has been developed. These techniques include the canopy analyzer, visual obstruction techniques and the rapid measuring of canopy height (Ganguli *et al*., 2000).

One of these double sampling methods includes the use of a rising plate meter (RPM). The RPM consists of a shaft with a circular disc of about 0.5m in diameter at the bottom. A measurement is taken by placing the shaft on the soil, sliding through the disc which is resting on the grass. The pasture height is

determined by the distance between the disc and the surface of the soil (Gabriels & Berg, 1993). The RPM measurement integrates the volume of the above ground forage when compressed and forage density. The measurement also describes vegetation height, density and compressibility (Ganguli *et al*., 2000; Sanderson *et al*., 2001).

Indirect measurement methods however are not always accurate (Sanderson *et al*., 2001). The RPM and visual obstruction methods appeared to be the most accurate. They were also fast and inexpensive (Ganguli *et al*., 2000). A study done by Rayburn & Rayburn (1998) found an error of only 10% for pasture yields when using RPMs. However, Sanderson *et al*. (2001) found a 26% error using the RPM. This would indicate that at least a region specific regression needed to be developed (Sanderson *et al*., 2001).

The method of choice is dependent on its practical application and the environment (Tucker, 1980). The RPM provides a reasonably simple and accurate measure of pasture height and pasture mass, it can be easily carried around in the field, practically performed and is relatively inexpensive (Rayburn & Rayburn, 1998).

2.3.4 Post grazing residual

High post grazing residual heights of pasture has long been associated with well fed cows. This is because high milk production as well as high DMI was reported to increase with the increase of pasture allowance (Bargo *et al*., 2003). As mentioned earlier the problem with leaving too high post grazing residual was that it negatively affected pasture quality later in the season.

Pasture allowance is affected by both the herbage mass as well as the area of pasture allocated to the cows (Lee *et al*., 2008). In a study done by Lee *et al*., (2008) it was found that pasture could be grazed to a lower (4.1 to 5.1cm) post grazing residual height without affecting the milk production adversely. This contradicted findings by Gibb *et al*., (1997) who found a positive correlation between pasture allowance and milk production.

Fulkerson & Donaghy (2001) stated that the best method of grazing ryegrass was to defoliate the grass to an average of about 5cm above ground level, giving a high milk yield while not causing deterioration of pasture quality in the later season. This agreed with the findings of Lee *et al*., (2008).

2.3.5 Dry Matter Intake

The nutritive value of pasture, specifically with relation to ruminant animals, is regarded as the product of the interaction between intake of pasture and its digestibility. Of these, intake appears to be the more important of the two (Mott & Moore, 1969). There are three factors which affect the DMI of dairy cows on grazing pasture. These are the nutrient requirement of the animal (also called the feeding drive), distension of the alimentary tract and associated factors (also called physical satiety) and thirdly pasture and animal factors limiting pasture DMI (also known as behavioural constraints) (Hodgson & Brookes, 1999).

A decreased DMI of pasture can directly lead to a decrease in milk production on a grazing system (Leaver, 1985). Total DMI was found to be lower for cows on a pasture-only diet when compared to pasture plus concentrate diet (Beever & Thorp, 1997). Factors responsible for the lower DMI on pasture only diets could be the physical constraints of the diet, the consumption of water associated with pasture intake and the rate of forage removal from the rumen (Bargo *et al*., 2003). The lower DMI is more likely the cause of the energy shortage and lower milk production, than the actual energy content of the pasture (Kolver *et al*., 1998).

Ribeiro Filho *et al*. (2005) found that increasing the pasture allowance of ryegrass did increase the DMI of cows by 0.17kg OM/cow/kg DM offered. They also found that 65% of total pasture mass was harvested by cows with low pasture allowance, and 45% with high pasture allowance, indicating that low pasture allowance lead to better utilization of pasture. On average milk production was also increased 1kg/kg pasture DM intake increase (Ribeiro Filho *et al*., 2005). There was however a decrease in milk fat content of 0.21g/kg for each kg DM increase above 5cm of pasture height as well as an increase in milk protein content of 0.19g/kg for each kg DM above 5cm (Ribeiro Filho *et al*., 2005).

Dry matter content of ryegrass in early spring is low, due to the high moisture content which poses a physical constraint which limits the intake of pasture (Chilibroste *et al*., 2000). Intake can be described as:

Pasture intake = grazing time x biting rate x bite mass (Muller, 2003a).

Bite mass, grazing time and biting rate are the three main grazing behaviour variables (Forbes, 1988). Grazing time and biting rate are mostly determined genetically or from animal related trademarks, but bite mass however influences DMI the most (Forbes, 1988). Pasture related characteristics such as pasture height and density usually determine bite mass (Bargo *et al*., 2003). Pasture height has a higher influence on bite mass in both supplemented and unsupplemented dairy cows (Gibb *et al*., 1997). Supplementation decreases the grazing time with no effect on the biting rate (Rook *et al*., 1994). Grazing time was reduced by 8 to 12min per kg of energy concentrate fed to dairy cows (Bargo *et al*., 2003). The same results were found in a study done on ryegrass where once again the grazing time was decreased when concentrate was given to dairy cows (Gibb *et al*., 1997).

Grazing time usually increases in response to changes in dairy cows' physiological status and Laca *et al*. (1994) found that just by moving cows more often to a new pasture, for example from one to six reallocations, milk production could be improved.

In a study done by Phillips *et al*. (2000) it was found that when cows were given hay instead of silage as a supplementary feed, the biting rate was reduced. This could be due to the need for more mastication of the hay, or the fact that the cows were more selective when feeding on the hay (Phillips *et al*., 2000).

In a study done by Chilibroste *et al*. (2000) on the re-growth of pasture it was found that cows grazed the longest when re-growth of pasture was only 9 days to compensate for a reduced bite mass and intake rate. Bite mass was the smallest for day 9 and the largest on day 30. Grazing time reduced as the time of re-growth (d16, d22 and d30) increased, as did the volatile fatty acids (VFA) in the rumen. When grazing 6 days after re-growth, the grazing time did not increase because of a physical limit in grass prehension that was reached (Chilibroste *et al*., 2000). This coincides with the findings of Meijs & Hoekstra (1984) that cows did not graze to a stubble height of lower than 4cm, irrespective of the pasture allowance or concentrate allowance.

Improving DMI of cows on pasture systems by improving and changing rotation time of cows, the amount and type of concentrate fed as well as moving cows to new pasture more frequently could have a marked effect on production and efficiency of dairy cows.

2.4 The Rumen

2.4.1 Introduction

To properly understand the effect and influence of concentrates on ruminant animals, their rumen environment and their production, it is necessary to understand certain concepts about the rumen anatomy and physiology as well as the interaction thereof with the rumen micro-organisms.

2.4.2 Rumen anatomy and physiology

A ruminant animal has four stomach compartments, the reticulum, the rumen, the omasum, and the abomasum. Of these, the rumen is the largest and can contain up to 150 to 230 litres of material (Ishler *et al*., 1996). The reticulo-rumen (reticulum and rumen are often considered together) constitutes up to 85 percent of the total capacity of the stomach (Mcdonald *et al*., 2002). The rumen acts as a fermentation vat and is the site of microbial activity. There are an estimated 150 billion microorganisms present in every teaspoon of rumen fluids (Ishler *et al*., 1996).

In ruminant animals microbial digestion takes place prior to enzymatic digestion and it is this characteristic of the ruminant's digestive physiology which enables them to utilize forages and fibrous roughages as a food source which monogastric animals are not able to do (Van Soest, 1994).

Another characteristic of ruminant animals is that of rumination. Rumination is the ability of an animal to complete eating at a rapid rate and finish chewing at a later stage. It involves the regurgitation of feed in the form of a bolus or cud, the re-mastication thereof, re-salivation and finally the re-swallowing of the rumen digesta. A major secretion into the digestive tract is that of saliva. The volume of saliva produced is directly related to the time spent eating and ruminating by dairy cows (Ishler *et al*., 1996).

Balch (1958) found that for every 5kg of hay fed to dairy cows they produced only 21-28kg of saliva while for concentrate this was only 6-8kg of saliva. Beauchemin *et al*. (2008) however indicated that forage source did not affect the rate of salivation (213 g/min). The eating rate did however differ between forage sources (g of DM/min). In an earlier study done by Beauchemin and Buchanan (1989) they indicated that increasing NDF content of the feed quadratically increased rumination and chewing time. Increases in chewing time in turn lead to differences in ensalivation of forages, in other words gram of saliva produced per gram of DM and gram of saliva produced per gram of NDF (Beauchemin *et al*., 2008). Saliva produced was greatest for straw (7.23g saliva/g DM) and was similar for barley silage, alfalfa silage, and alfalfa hay at 4.15, 3.40, and 4.34 g saliva/g of DM, respectively.

Saliva is important because it contains mineral ions such as sodium, phosphate and bicarbonate which serve as buffering agents in the rumen. Volatile fatty acids produced during fermentation are neutralized by saliva, helping to maintain an ideal rumen environment for microbial growth (Ishler *et al*., 1996).

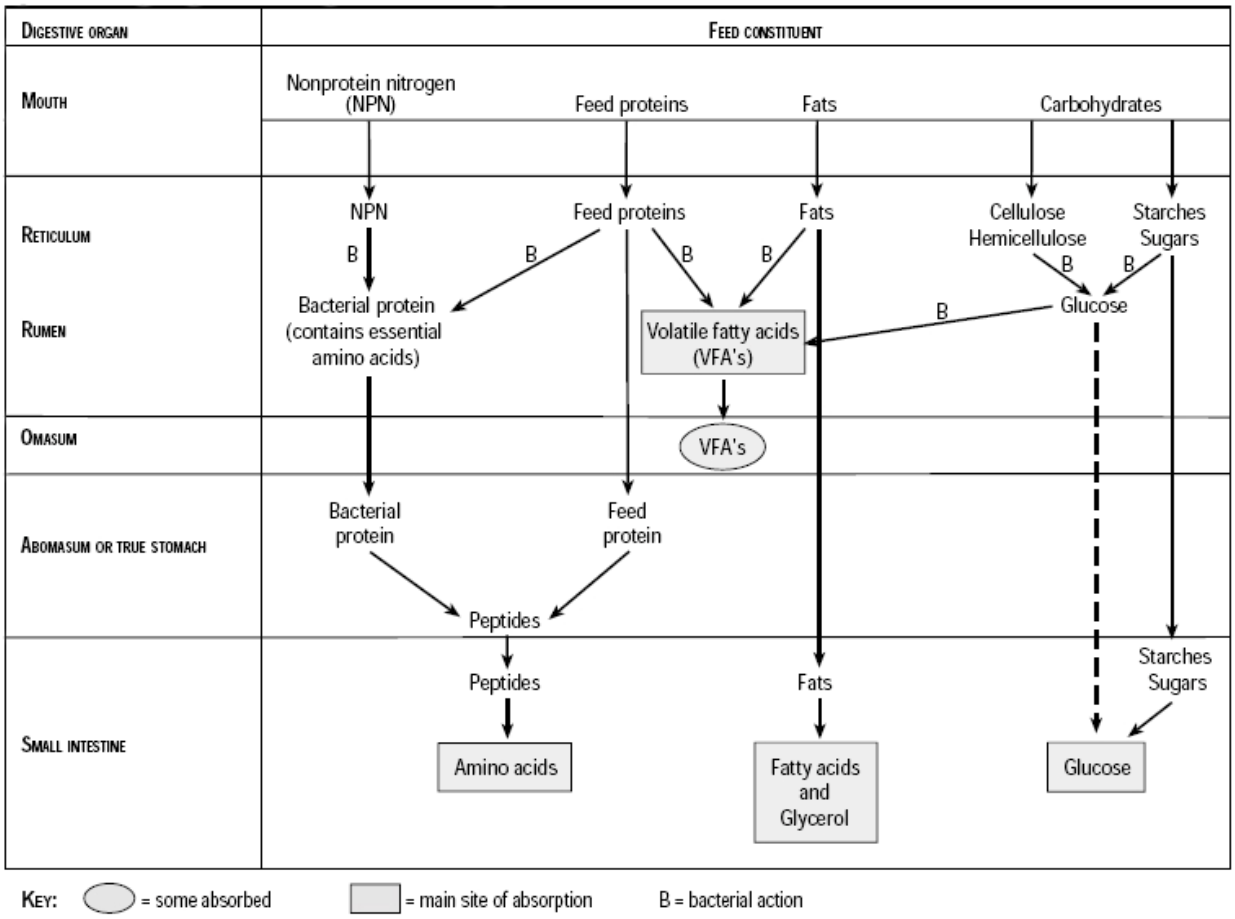

Figure 2.2 Summary of digestion and absorption in the ruminant (Ishler *et al*., 1996)

2.4.3 Rumen Microbiology

To obtain a rumen environment that maximizes microbial growth and production, nutritionally balanced diets are needed and both the needs of the rumen microbes as well as that of the cow need to be considered. Therefore, in the search for optimization of the diet, compromises to the cow and/or microbes may occur.

The rumen microbial population consists of bacteria, protozoa and fungi of which the bacteria are the majority with numbers ranging between 10^{10} to 10^{11}cells/gram rumen contents. Bacteria can be categorized into distinct groups according to the type of substrate they utilize. These include cellulose, hemicellulose, starch, sugars, intermediate acids, protein, and lipids. However, most species are capable of utilizing more than one type of substrate (Ishler *et al*., 1996).

Most of the bacteria groups are specialized in polysaccharide hydrolysis and the fermentation of sugars resulting from the hydrolysis, and for this reason animals fed the same diets would have microbe populations that are very similar (Firkens & Yu, 2006). However, as shown by Weimer *et al*. (1999) microbial populations can differ between animals fed the same diet making the integration between rumen microbiology and dairy cow nutrition a very difficult matter.

2.4.4 Rumen pH

One of the most variable factors that can influence rumen microbial populations and volatile fatty acid production is rumen pH (Ishler *et al*., 1996).The most severe changes in rumen microbe population structure occur with dietary changes, such as a change between roughage and a high grain concentrate, or pasture to concentrate feeding. The change to higher amounts of highly fermentable carbohydrates is done to improve production. This may however result in acidosis, with the pH decreasing in the rumen as a result of large increases in lactic acid production (Tajima *et al*., 2000).

There are two groups of bacteria which function at different pH levels in the rumen. The starch digesters are better suited to more acidic environments at a pH of 5.2 to 6 (Ishler *et al*., 1996). The major cause of lactic acidosis is *Streptococcus bovis*, which easily ferments starch and produces lactate (Tajima *et al*., 2000).

Fibre digesters in contrast thrive at a pH of 6.2 to 6.8, and cellulolytic and methanogenic bacteria decrease as the pH decreases below 6.0 (Ishler *et al*., 1996).Only three commonly found bacterial species in the rumen are considered cellulolytic although many others have been reported. These are *Fibrobacter succinogenes*, *Ruminococcus flavefaciens* and *Ruminococcus albus* and strains of these bacteria cause the solubilisation of cellulose and hemicellulose (Flint, 1994).

Low initial pH in batch cultures decreased fibre degradation (Mourino *et al*., 2001). But Calsamiglia *et al*., (2002) showed that in a continuous culture with continuous feeding, decreases in fibre degradation were small or insignificant at lower pH values. Extreme daily variations in ruminal pH can be more harmful to rumen microbes than a constant low pH because of the constant metabolic readjustments needed by rumen micro organisms (Mertens, 1979). Therefore, feeding supplements to dairy cows on a pasture system could be detrimental to rumen microorganisms because of the fluctuations they caused in the rumen pH.

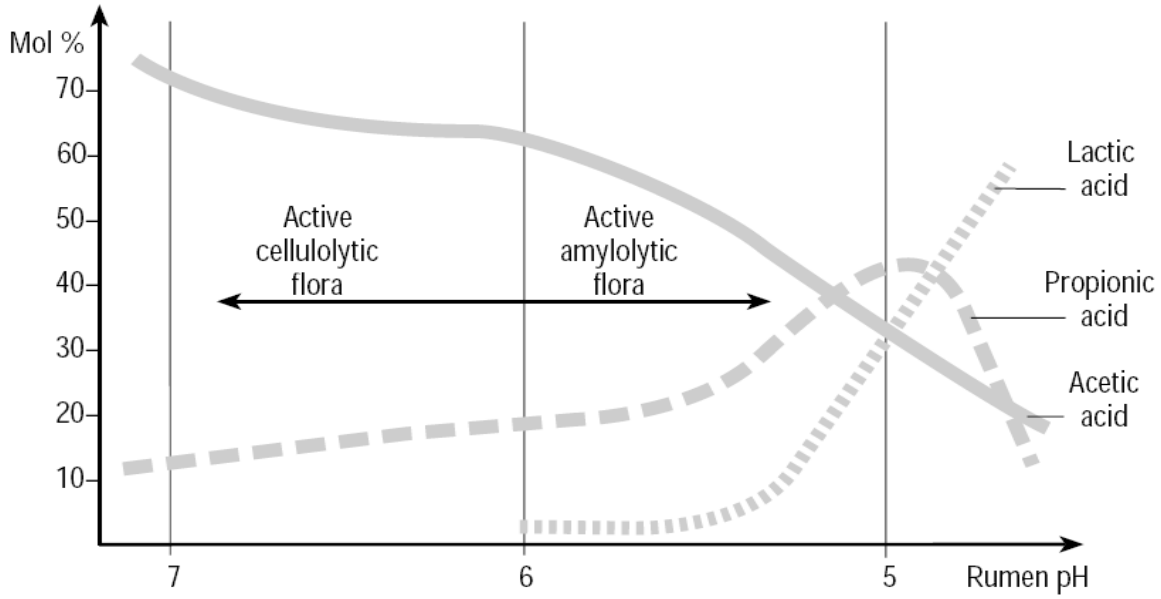

Figure 2.3 Ruminal fermentation as a consequence of adaption due to pH regulation (Ishler *et al*., 1996).

In a study done with high starch and low starch concentrates, increasing the proportion of the supplement in the diet depressed rumen pH ($P< 0.05$). This happened particularly with high starch supplementation and could be the cause of the lower intakes of pasture (Sayers *et al.*, 2003). The high starch concentrate resulted in a rumen pH of 5.8 while in the case of the low starch concentrate; the pH was higher at 5.96. They also indicated that during the study, 7 animals developed lameness with 5 of these animals being on the high starch concentrate. The reason for this was that the increased lactic acid in the rumen entered the blood stream and caused increased blood pressure, leading to damage of blood vessels in the feet (Sayers *et al.*, 2003).

As mentioned earlier, saliva neutralizes VFA produced in the rumen causing a better rumen environment for microbial growth. By controlling a ruminants diet the production of saliva can be encouraged. The feeding management as well as the concentrate composition, such as ingredient and particle size, are factors which affect rumination and saliva production. Rumination would for instance be significantly reduced by feeding high amounts of concentrates and finely chopped forages (Ishler *et al*., 1996).

In order to accommodate the requirements of all the different rumen micro organisms, it is important that normal feeding practices maintain a rumen pH of 5.8 to 6.4 (Ishler *et al*., 1996).

2.4.5 Rumen Protein

A function of rumen microbes is to synthesize microbial protein. Rumen ammonia nitrogen is utilised for protein production. Ammonia nitrogen is derived from three sources, namely dietary protein and non protein nitrogen, hydrolysis of recycled urea and degradation of microbial protein. Rumen ammonia is removed by way of protein synthesis by microbes, absorption through the rumen wall and flushing to the omasum. Figure 2.4 is a schematic summary of nitrogen utilization by ruminants. Microbial protein is of better quality than that of plant protein and often rivals that of animal protein. Microbes can however not produce certain essential amino acids and therefore supplementation is needed to achieve high levels of milk production (Ishler *et al*., 1996).

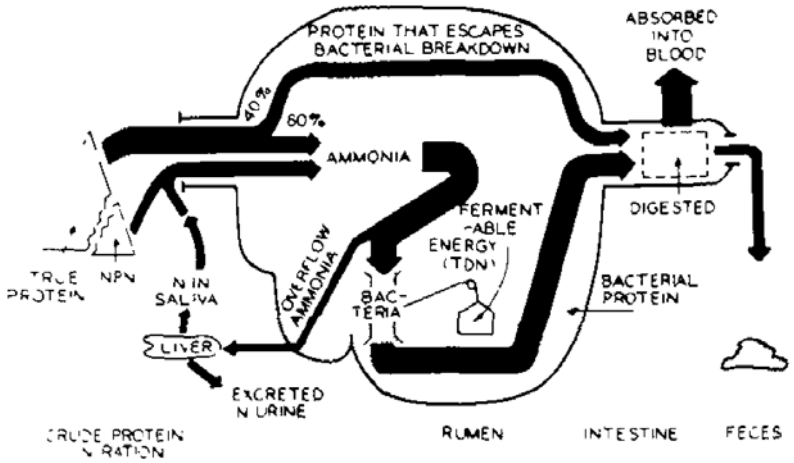

Figure 2.4 A schematic representation of the rumen of dairy cows and the nitrogen utilization by ruminants (Satter & Roffler, 1975)

Rumen ammonia utilization is affected by two factors. One is the amount of bacteria in the rumen, and the second is how rapidly the microbial population grows. Microbial mass and growth and therefore utilisation, will depend on the amount of energy available to bacteria. Therefore feeds high in total digestible nutrients are more fermentable and thus provide more energy to bacteria (Satter & Roffler, 1974). Rumen degradable protein is not utilized when ruminal ammonia nitrogen is in excess of 5mg/dL (Satter & Roffler, 1974). Therefore, when rumen ammonia exceeds 5mg/dL nothing more is gained from further supplementation of degradable protein (Satter & Slyter, 1973).

Under typical ryegrass and tropical high protein pastures, concentrates containing more than 12-13% CP is not efficiently utilized, however lactating dairy cows in the first third of lactation can benefit from dietary protein levels as high as 16 -17 % (Satter & Roffler, 1974). Under nitrogen limiting conditions microbial

protein production is severely reduced, VFA however showed only a slight decrease. Volatile fatty acids were also not affected by non protein nitrogen supplementation (Satter & Slyter, 1973).

2.5 Supplementation on pasture

2.5.1 Introduction

High producing dairy cows are cows which produce more than 25kg milk per day during early lactation and about 20kg per day during late lactation (Bargo *et al*., 2003). For Jersey cows these figures could be lower. High producing dairy cows need an estimated 11.9 MJ ME/kg DM if their intake is 17.5 kg DM/cow/day. The metabolisable energy (ME) values of ryegrass are above 11.9 MJ ME/kg DM from winter until the end of September, it then however falls markedly to less than 10 MJ ME/kg, but is subject to region and environmental conditions. There is therefore a need to supplement energy or energy and protein to grazing animals on ryegrass pastures (Schwarz *et al*., 1995). From October, energy supplementation or intake would need to increase to maintain productivity of high producing dairy cows (Fulkerson *et al*., 1998).

There are many benefits to feeding concentrates to dairy cows on these pastures. These include:

1) Increased milk production per cow
2) Increased stocking density and milk production per unit land
3) Improvement in pasture use
4) Maintaining the animal's body condition score and improved reproduction
5) Increase in lactation period during times of pasture shortages
6) Increasing the dairy farm's profit (Bargo *et al*., 2003).

As stipulated previously, feeding supplements to dairy cows on pasture should only be considered when the additional value of the milk produced as well as the added gain in improved body condition scores of cows from feeding supplements exceeds the total cost of the supplement, the cost of storage and cost of feeding (Penno *et al*., 2001).

In order to correctly supplement high producing dairy cows on pastures systems, it is necessary to better understand the effect of different types of supplement on DMI, performance, and nutrients which better compliment those nutrients in the pasture itself (Bargo *et al*., 2003). Supplementary foods formulated to complement pasture are of little use if the supplements are used to overcome a total food deficit (Penno *et al*., 2006)

High quality pasture may have nutritional imbalances and deficiencies and fail to meet the nutrient requirements for high producing dairy cows and therefore need to be supplemented. It has high CP

content while the Rumen Undegradable Protein (RUP) is low. The Rumen Degradable Protein (RDP) may be sufficient but there may be a lack of highly fermentable carbohydrates (Muller *et al*., 2001). The fibre content in high quality pastures may be low, which could lead to low milk yields from dairy cows on ryegrass pasture given concentrate supplements. This may be attributed to insufficient NDF (Mertens, 1997). Cows require additional NDF for effective rumination (Wales *et al*., 2001). The low NDF could also lead to severe milk fat reduction as well as acidosis (Mertens, 1997; Wales *et al*., 2001; Muller *et al*., 2001). Minerals such as Ca, P, Mg, S, Zn and salt are usually inadequate while the concentration of vitamins A and E are high (Muller *et al*., 2001).

Energy supplementation was found to be more related to the quantity fed as opposed to the type of supplement fed. Milk response in cows is however dependent on more factors, for example the pasture allowance, the nutritional value of both the pasture and the supplement, the cow's genetic capability and the amount of concentrate fed (Bargo *et al*., 2003).

2.5.2 Types of supplementation

a. Protein

Proteins that reach the small intestine are derived from three sources. The first is dietary protein that was not broken down by rumen microbes, the second is protein in micro organism cells that leave the rumen and the third is from endogenous proteins that come from sloughed cells and secretions into the abomasums and intestine (Ishler *et al*., 1996).

Fresh herbage supplies enough protein for dairy cows producing up to 35kg of milk per day (Journet & Demarquilly, 1979). The response obtained from protein supplementation is however dependent on the amount of pasture taken in and the relative protein content thereof, and of the cow's need (Dillon, 2006).When cows grazed swards with a CP content less than 140 g per kg DM herbage intake was increased with additional supplementation of concentrates with low levels of degradable protein, however there was no response to supplementation of protein when CP of the herbage was greater than 160 g per kg DM (Delagarde *et al*., 1997).

When giving grain supplementation to dairy cows on high quality pasture the CP normally does not have to be more than 120g/kg DM of supplement. Pasture CP is usually highly degradable and may result in a deficiency in proteins and amino acids in the small intestine. Supplementing dairy cows grazing only ryegrass pasture with a CP source such as soybean meal also reduced rumen pH, which resulted in an increase in VFA concentration in the rumen (Delagarde *et al*., 1997). This was confirmed by Russell *et al*., (1992) who stated that the yield of non structural carbohydrate fermenting bacteria was improved by up to 18.7% when proteins or peptides were available.

Most studies have shown RUP to be beneficial to high producing dairy cows. This is confirmed by the findings of Bargo *et al*. (2003) who showed a significant positive correlation between RUP in the

concentrate and milk yield. Milk urea nitrogen was also found to be higher on pasture only diets than when concentrate was given (Muller *et al*., 2001). Studies demonstrated that supplementing pasture with RUP rather than RDP did not have an effect on DMI (Bargo *et al*., 2003); however in a study on ryegrass it was found that higher RUP increased DMI of cows (Schor & Gagliostro, 2001).

b. Non-fibre carbohydrates / Non-structural carbohydrates

The primary source of energy for ruminants is carbohydrates and easily consists of 700 to 750 g/kg on a DM basis (Eastridge, 2007). Carbohydrates can be divided into two main groups namely structural and non structural carbohydrates. Non-structural carbohydrates consist of cell contents such as sugars, starches, pectins, and β-glucans (Ishler *et al*., 1996).

Table 2.1 Estimated production of volatile fatty acid for different substrate forms in the rumen of dairy cows (Murphy *et al*., 1982)

		Proportion of fermented substrate converted to			
Substrate	**Group**	**Acetate**	**Propionate**	**Butyrate**	**Valerate**
Soluble carbohydrate	R [a]	.6894 ± .0640	.2050 ± .0140	.1056 ± .0545	.0000 ± .0000
	C [b]	.4476 ± .0330	.2077 ± .0368	.3026 ± .0394	.0421 ± .0016
Starch	R	.5948 ± .0430	.1415 ± .0247	.2050 ± .0505	.0586 ± .0004
	C	.3987 ± .0124	.3020 ± .0133	.1955 ± .0051	.1038 ± .0058
Hemicellulose	R	.5670 ± .0557	.1804 ± ,0329	.2065 ± .0488	.0461 ± .0016
	C	.5578 ± .0106	.2574 ± .0149	.1090 ± .0064	.0738 ± .0003
Cellulose	R	.6579 ± .0962	.0866 ± .0031	.2280 ± .0913	.0276 ± .0034
	C	.7880 ± .0015	.0575 ± .0004	.0650 ± .0002	.0894 ± .0010
Protein	R	.4507 ± .0669	.3023 ± .0429	.1773 ± .0295	.0697 ±.0018
	C	.3553 ± .0309	.3722 ± .0066	.1995 ± .0256	.0729 ± .0006

[a]R = roughage group, mean ± SE; n = 10
[b]C = concentrate group, mean± SE; n = 8

Although not usually the case on pasture based systems, the most important factor determining dairy farms' profitability is milk production per cow. The main energy source for high producing dairy cows worldwide is non-fibre carbohydrates (NFC). By including NFC the energy density of dairy cow supplementation can be increased and therefore the overall milk production will increase (Carver, 2007). Non-fibre carbohydrates are very palatable and easily digestible compared to NDF, and fulfil the high energy demands of a lactating dairy cow. This unfortunately is at the expense of NDF, which could lead to rumen problems (NRC, 2001; Ishler *et al*., 1996).

The NFC fraction contained in cereal grains used in concentrate supplement contains up to 80% starch (Ishler *et al*., 1996).Cereal grains such as maize and barley form the basis of concentrate supplementation in diary production systems. Shifting focus to not limiting DM intake by maintaining

rumen pH thus maximizing digestibility of the diet and optimizing microbial protein synthesis, would optimize efficiency of milk production and improve animal health (Eastridge, 2007). It is very important to manage a good balance between carbohydrate fractions in order to maintain a healthy rumen environment for optimal metabolism (Ishler *et al*., 1996).Processing of cereal grains also affects the rate of fermentation, usually by speeding up fermentation. Processing includes grinding, steaming and ensiling and affects the ruminal availability of starch (Ishler & Varga, 2001).

A problem with concentrates high in NFC is that it decreases the rumen pH (Ishler & Varga, 2001). The cause of this is the excess fermentation of starch to VFA in the rumen, which may overwhelm the absorption and buffering capacity of the dairy cow. This could lead to decreased DMI in high producing dairy cows (Knowlton *et al*., 1998).

In Table 2.1 it can be seen that concentrates (high grain) impair the acetate to propionate ratios obtained from hemicellulose digestion when compared to that of a forage diet (Murphy *et al*., 1982). Even with cellulose the acetate to propionate ratios was 13.1 for forage diets and 7.3 for high grain diets, showing the effect high grain diets have on fermentation in the rumen (Ishler *et al*., 1996).

c. High fibre supplementation / structural carbohydrates

The primary source of energy for ruminants is carbohydrates which can be divided into two main groups' namely structural and non structural carbohydrates. Fibre is composed of several potentially digestible fractions, and an indigestible fraction that occupies space in the gastrointestinal tract of ruminants (Mertens, 1997).

Structural carbohydrate consists of the plant cell wall material and is normally defined as NDF. Neutral detergent fibre mainly consists of cellulose, hemicellulose and lignin and includes some pectin. Acid detergent fibre is the fraction of the structural carbohydrates consisting solely of cellulose and lignin (Ishler *et al*., 1996). Feed is digested in the rumen through fermentation by rumen microbes and physically broken down through rumination (Ishler & Varga, 2001). Hemicellulose can be found in various agricultural residues such as sugarcane bagasse, rice straw, wheat straw, maize stover, maize fibre, wheat bran and hominy chop, and these usually contain 20 to 40 percent hemicellulose. Hemicelluloses are heterogeneous polymers of pentoses, hexoses and sugar acids and unlike cellulose are not homogeneous (Saha, 2003).

The NDF level of forage is not only important functionally in promoting digestive processes, but can also limit energy intake if levels are two high, and could have a negative effect on performance. The rumen's physical capacity has an upper limit and as the rate of fibre digestion decreases, slowly digestible OM in the rumen increases. Initial fibre particle size, rate of particle size reduction (chewing, rumination), particle density, and rate of digestion all influence the retention time of fibre in the rumen and the time the fibre is exposed to fibrolytic enzymes (Zinn & Ware, 2007).

These more complex carbohydrates often degrade at a slower tempo and more often than not are incompletely digested. Ruminants are also unable to digest lignin, therefore the digestibility of the diet decreases as the lignin content increases (Ishler et al. 1996). The type of concentrate fed to dairy cow's changes and influences bacterial populations in the rumen which is necessary in order to successfully digest the food.

d. Forage Supplementation

Feedstuffs that contain more than 35% NDF are classified as forage (Zinn & Ware, 2007).When there is sufficient pasture available for dairy cows and ryegrass silage was given it was found that there was a reduction in milk yield as well as protein yield and the effect on milk fat yield was variable (Dillon, 2006). Supplementation with grass silage caused a large reduction in herbage intake, and this reduction is mainly due to the reduction in grazing time (Phillips, 1988).

When enough pasture is available, supplementation of forage would lead to a reduction in quality of pasture due to poor utilization of the herbage. However, in times of an herbage shortfall, supplementation with forage will lead to an increased DMI by dairy cows (Dillon, 2006). Forage supplementation as opposed to concentrate supplementation has a higher substitution rate due to the fact that the forage has a bulkier form, leading to a higher forage fill value (Dillon, 2006).

e. Silage

Maize silage as a supplement showed positive effects on milk yield when low amounts of pasture were offered but when pasture allowance was adequate maize silage seemed to have little effect on milk production but caused a decrease in DMI of pasture (Dillon, 2006). Maize silage is an excellent supplement for cows on high quality pastures because it provides the needed energy while diluting the high protein levels in pasture (Muller *et al.*, 2001).

Feeding maize silage to dairy cows which had a restricted pasture allowance was beneficial, but when pasture was not restricted, the maize silage decreased DMI of pasture (Bargo *et al*., 2003). Milk production could also increase if pasture was restricted and maize silage was given as a supplement (Bargo *et al*., 2003). In a study done by Phillips *et al*. (2000) ryegrass was supplemented with either ryegrass silage or ryegrass and white clover mixture silage. The ryegrass silage contained 995g ryegrass/kg DM and 5g clover/kg DM. The mixture contained 874g ryegrass/kg DM and 126g clover/kg DM. The rest of the chemical composition was similar. It was found that if ryegrass was supplemented with the ryegrass silage, the dairy cows' grazing time increased. The cows spent a longer time eating the mixed silage than the ryegrass silage and this could explain the increased grazing time for the ryegrass silage (Phillips *et al.*, 2000). Phillips *et al*. (2000) found that neither the milk yield nor the milk composition was significantly affected by either one of the pasture and silage supplementation combinations. Their results could be due to the fact that cows with low average yields were used for the study.

2.6 Concentrate supplementation

Grazing dairy cows are offered concentrate supplementation when there is a shortfall in grass supply, or energy is the first limiting factor. It can also lead to an increased overall dry matter intake of the cows. The efficiency of the supplement is expressed by the kg of milk gained per kg of supplement given (Dillon, 2006).The effects of supplementation such as DMI and energy intake increase are superior to that which is obtained from pasture-only diets (Bargo *et al*., 2003).

When feeding concentrates to dairy cattle on spring ryegrass pastures, it is expected that the increased energy provided would cause an increase in milk production. However a study in southern Australia found only small increases in milk production under such circumstances (Stockdale, 1999a). This coincides with a study done by Fulkerson *et al*. (2006) in which dairy cows on ryegrass were fed increasing amounts of cereal based concentrates. Fulkerson *et al*. (2006) found that increasing amounts of cereal concentrates had no effect on performance of dairy cows if increased from 4.75kg to 7.5kg (a 12% increase). He did however find a significant effect on substitution rate, where substitution rates were increased as concentrate intake increased.

Table 2.2 Marginal milk response (kg milk/kg concentrate) obtained by different authors at different concentrate levels

	LC (1-3kg)	MC (4-6kg)	HC (6-8kg)	HHC (>8kg)
Author	Increase kg MY/kg C	Increase kg MY/kg C	Increase kg MY/kg C	Increase kg MY/kg C
Wales et al., 2001	-	1.00	-	-
Meeske et al., 2006	1.25	0.78	0.54	-
Sayers et al., 2003	-	-	-	0.55
Stockdale et al., 1987	1.2	0.98	-	0.54

LC - Low concentrate levels
MC - Medium concentrate levels
MHC - Medium high concentrate levels
HC - High concentrate level

Sayers *et al.* (2003) showed that feeding the higher level of supplementation increased milk yield ($P < 0.05$). They only had a marginal milk response to the additional supplement of 0.55kg milk per kg supplement DM given. But it should be noted that they only took the difference in kg milk/kg supplement between 5kg of supplement and 10kg, a level at which the marginal milk response is already lower.

Wales *et al*. (2001) in contrast found that cows grazing pastures high in perennial ryegrass during springtime responded with an acceptable marginal milk response of 1 kg milk/kg DM when cereal grain

was supplemented. They also showed that on ryegrass pastures under irrigation, cows grazing only pasture could select pasture that had sufficient energy for 20.1kg FCM/day (Fat corrected milk/ day). When 4.5kg cereals were added, the milk yield increased to 24.5kg FCM/ day (Wales *et al*., 2001). This supports the statement of Delagarde *et al*. (1999) that carbohydrate supplementation, whatever the source, increased milk production.

In an earlier study done by Stockdale *et al*. (1987) in which cows were fed ryegrass and clover pasture, the amount of high energy pellets was increased. It was found that the milk production of the dairy cows was linearly related to the pellet intake, with 0.7kg to 1.8kg of increased milk for every kg of increased pellet intake. It was also found that the increases in milk yields from feeding pellets decreased as the lactation period increased (Stockdale *et al*., 1987).

This was also proved by Meeske *et al*. (2006) who did a study with varying levels of concentrate supplementation, and showed that as the level of concentrate increased, the level of milk production increased as well. On the high concentrate diet, which consisted of 7.2kg of concentrate, the fat corrected milk production increased by 0.54kg/kg of concentrate. For the low level of concentrate, the gain in FCM was 1.25kg/kg concentrate. Meeske *et al*. (2006) stated that as the concentrate level increased, the overall effect on FCM fat corrected milk production decreased, as can be seen in Table 2.2.

This coincides with the findings of Kellaway & Porta (1993) who stated that as the amount of concentrate increased the marginal milk per kg of concentrate decreased. Further support for this was given by Bargo *et al*. (2003) who evaluated 3 different studies, and who found a significant quadratic regression between milk yields and concentrate DMI. This showed a reduction in marginal milk response as concentrate levels increased (Bargo *et al*., 2003).

According to Stockdale *et al*. (1987) the reduction in marginal milk response was due to the reduced digestibility of concentrates with increased intake. There are increasing amounts of starch escaping digestion with increasing amounts of supplement given, leading to lower milk responses. Another possible reason for this was given by Penno *et al*. (2001) who stated that as concentrate levels, and therefore energy levels, increased, more of the energy was partitioned toward body reserve increases rather than milk production increases. According to Kellaway & Porta (1993), cows with higher genetic merit would respond better to increased supplementation because they partitioned more of the nutrients to milk production and not to live weight gain and therefore they lost more body condition during early lactation.

Pasture allowance also has an effect on the response to concentrate supplementation. In the study done by Bargo *et al*. (2002) dairy cows had a higher milk yield to concentrate ratio on low pasture allowance than cows on high pasture allowance. Low pasture allowance produced 1.36kg milk/kg concentrate given and the high pasture allowance only 0.96kg of milk/kg of concentrate. When concentrate was given to cows on pasture, the DMI of the pasture decreased but the total DMI of the cow increased (Bargo *et al*., 2003).

According to Meijs (1986) the rumen pH was reduced excessively when a high starch concentrate was given as a supplementation to dairy cattle on highly degradable pasture. The low pH inhibited fibre digesting bacteria and prevented them from degrading fibre at an optimum rate. This decreased the DMI of pasture for the animals because the retention time of the pasture was increased.

Studies also showed a reduction in milk fat percentage when high levels of concentrates were fed to dairy cows (Bargo *et al*., 2002; Walker *et al*., 2001), however fat yield increased because of the higher milk yields (Walker *et al*., 2001). Unlike milk fat percentage, the milk protein percentage increased with an increase in concentrate (Sayers *et al.*, 2003). Penno *et al*. (2001) found that feeding supplements to animals on spring pasture resulted in smaller increases in milk solids while gaining more live weight. Penno *et al*. (2001) also showed that with an increase in supplementation, the immediate effect on milk solids was usually a negative one. Sayers *et al.* (2003) found that irrespective of the type of supplementation, milk butterfat content was reduced ($p < 0.001$) and milk protein yield increased ($p < 0.001$) when animals were offered the higher level of supplementation. Concentrate level had no significant effect on live weight gain of dairy cows but the body condition score of dairy cow tended to rise with an increase in concentrate amount (Meeske *et al*., 2006).

According to Wales *et al*. (2001) there are two options available for increased milk production:

(1) Feeding concentrate supplementation or

(2) Increasing pasture allowance

When pasture growth rates are slow and stocking rates are too high, feeding grain appears to be the preferred option. However when there is enough pasture and the pasture re-growth is sufficient, then providing more pasture by good pasture management, could be the preferred option.

2.7 Substitution rate

2.7.1 Substitution rate and milk response to substitution

Substitution rate can be defined as the decrease (kg) found in the DMI of pasture per unit (kg) of concentrate, when cows are given supplementation on a grazing system (Kellaway & Porta, 1993; Dillon, 2006). This substitution rate is the cause for variations in milk response when supplementation is given (Kellaway & Porta, 1993).

Pasture allowance is the first and one of the major factors affection the level of substitution of pasture by supplement (Leaver, 1986). Substitution rate increase with a higher pasture allowance and in turn the milk response is negatively influenced (Meijs & Hoekstra, 1984). According to Grainger & Matthews (1989) an increase in pasture allowance decreased the milk response of a cow per kg concentrate. This was confirmed by Meeske *et al*. (2006) who stated that an increased pasture allowance resulted in lower pasture utilization with a resulting lowering of stocking rates and reduced profit per hectare. Substitution

rate varied from 0 for cows on pasture under high grazing pressure, to 0.6 to 0.8 for cows grazing at low grazing pressure (Dillon, 2006). The strong relationship between substitution rate and milk production is shown in Figure 2.5.

In a study done by Meijs & Hoekstra (1984) in which the substitution rate was measured for different pasture allowances, it was found that with an increase in concentrate supplementation (0.8, 3.2 and 5.6 kg per cow) with low pasture allowance the substitution rate was only 0.1 kg herbage OM/kg concentrate OM. However, with the same supplementation rate on high pasture allowance the substitution rate increased to 0.5 kg herbage OM/kg concentrate OM (Meijs & Hoekstra, 1984).

A second factor which influences substitution rate is the concentrate composition. This was demonstrated in a study done by Kolver *et al*. (1998) in which four diets which were based on high quality pasture, were given to dairy cows with increasing amounts of starch. The digestibility of OM did not differ between diets, but the digestibility of the NDF and CP decreased linearly with an increase in starch content. Concentrations of rumen NH_3 nitrogen were also decreased with the increase in starch levels (Kolver *et al*., 1998; Bargo *et al*., 2003). Higher inclusion levels of highly fermentable carbohydrates lead to increased substitution rates and could be prevented by the reduction of total non-structural carbohydrates in the diet (Kolver *et al*., 1998).

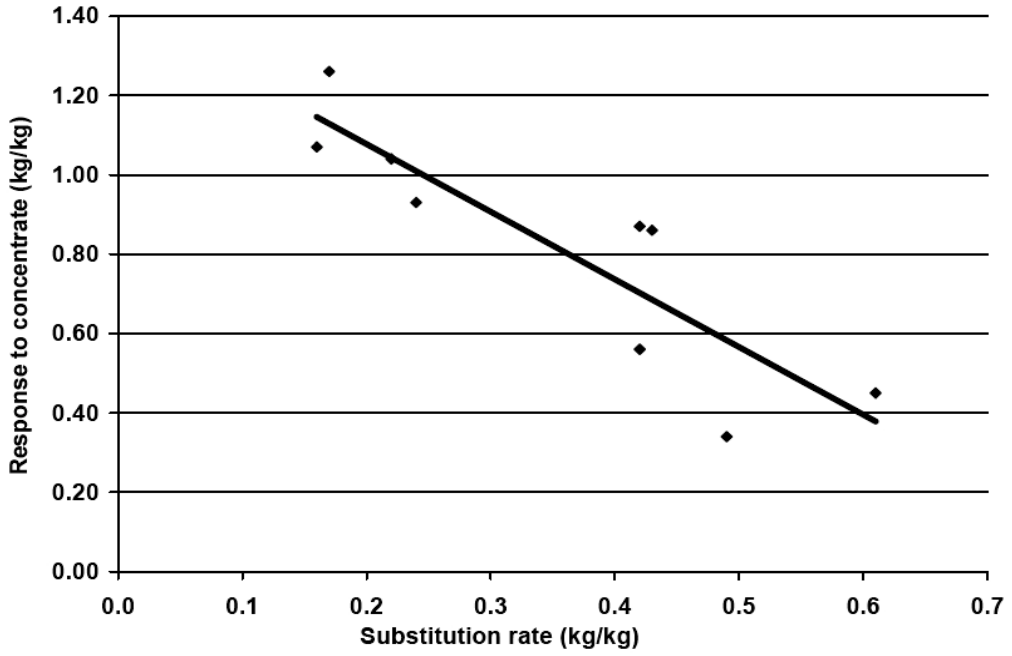

Figure 2.5 The relationship between milk production response to concentrate supplementation and substitution rate of pasture for concentrate (Horan *et al*., in press)

In a feeding study done by Meijs (1986) it was found that cows grazing on perennial ryegrass had a higher pasture intake and milk production when given low starch concentrates than cows on high starch

concentrates. It was found that when the high-fibre concentrate was fed, the mean substitution rate of herbage by concentrates had decreased from 0.45 kg herbage OM/(kg concentrate OM) on the high starch concentrate, to 0.21kg herbage OM/(kg concentrate OM) on the low starch concentrates. For these cows the daily intake of herbage was approximately 9.9 kg OM for the high-starch supplement and 10.5 kg OM for the low starch supplement (Meijs, 1986).

A third factor which is evident in influencing substitution rate is the amount of concentrate given to dairy cows. In a study done by Fulkerson *et al*. (2006) in which dairy cows on ryegrass pasture were fed increasing amounts (4.75kg to 7.5kg) of cereal based concentrates, no effect was seen on milk production, but the substitution rate was increased to 0.58 at 5.33kg of concentrate and increased even further to 1.18 at 7.5kg of concentrate fed. For every 9% increase in concentrate intake the pasture digestibility decreased by 8% (Fulkerson *et al*., 2006). According to Fulkerson *et al*. (2006) this showed that there was an upper limit to feeding concentrates to dairy cows on a pasture based system.

Bargo *et al*. (2003) stated that it was important not to give more than 10kg DM/day/cow of concentrate in order to prevent metabolic diseases such as acidosis. They found that it was possible to feed higher amounts of concentrate with a NDF of more than 50%. They also suggested that high producing dairy cows would react more favourably to supplementation because they partitioned more nutrients to milk production, resulting in a decrease in body weight and condition, but with higher milk yields.

Dillon (2006) showed that since 1990, higher efficiencies and lower substitution rates have been observed by researchers than before 1990. He showed from nine published studies, that the substitution rate was 0.4, with an efficiency of 0.92kg of milk produced for every kg of concentrate given. This higher efficiency of high genetic merit cows as opposed to low genetic merit cows could be attributed to more nutrients being partitioned to milk production (Dillon *et al*., in press)

2.7.2 Cause of substitution

The reduced herbage intake observed when supplements were offered could be a result of a reduction in cellulose digestion in the rumen and a reduced rate of digesta passage (Campling, 1966). According to Dixon & Stockdale (1999) the cause for the pasture substitution could be that the concentrate diets provided a lot of energy to the lactic acid-producing bacteria in the rumen. This in turn caused a reduction in rumen pH and a decrease in the activity of the fibrolytic and cellulolytic bacteria. Cellulolysis is very sensitive to pH and a pH of not less than 6.0 is suggested as optimum (Sutton *et al*., 1986). The reduction in DMI of the cow on pasture as a result of the decrease in fibre digestion resulted in a slower passage rate of feed.

This was confirmed by Steg *et al*. (1985) who also found that the decreased rumen pH caused by concentrate supplementation could explain the differences in herbage intake. Sayers *et al.* (2003) stated

that increasing the proportion of the supplement in the diet, depressed rumen pH (P< 0.05), particularly with high starch supplementation, which could lead to the lower intakes of pasture. High concentrations of easily fermentable substances such as certain proteins, starch and soluble sugars caused an increase in the concentration of VFA and lactate. The resulting lowering of the pH negatively affected microbial fibrolytic activity in the rumen and therefore reduced the rate of breakdown of fibrous particles as well as the rate of breakdown in the reticulorumen. The resulting increase in the amount of non-fermented residue increased the degree of rumen-fill and possibly restricted intake of new feed. The increased concentration of VFA, where the proportion of propionic acid to acetic acid was found to be higher, resulted in a reduction in milk fat percentage and an additional lowering of rumen pH (Steg *et al.*, 1985).

The time spent consuming a supplement instead of the pasture contributes to a reduction in DMI of pasture (McGilloway & Mayne, 1996). Concentrates are usually only fed twice a day while cows can graze several times during the day, thus causing a less stable rumen environment than with a TMR (total mixed ration). This clearly reveals that supplementation of high producing dairy cows on pasture is complex and that the priority in a pasture-based system should be the pasture management (Muller *et al*., 2001).

2.8 Possibility of replacement of starch with high fibre by-products in concentrate

Muller *et al*., (2001) suggested the replacement of starch in the concentrate with non forage fibre such as cottonseed hulls, soy hulls, beet pulp, distiller's grains, citrus pulp, wheat middlings, whole cottonseed and some other by-products, to provide more fermentable fibre. The increase in the cost of maize and soybean oilcake in recent times has increased input costs dramatically and Meeske *et al.* (2009) suggested the replacement of maize and soybean oilcake with low starch containing by-products like hominy chop, maize gluten and bran.

When ryegrass was supplemented even with low levels of non structural carbohydrates, it caused a reduction in rumen pH (Bargo *et al*., 2003). This reduction in rumen pH was caused by a change in the molar ratios of VFA in the rumen, with the molar proportions of acetic acid decreasing and the molar proportions of propionate increasing (Sayers *et al.*, 2003). When given a fibre-based concentrate, pasture DMI intake of cows increased. The fibre based concentrates resulted in a decrease of milk production but caused an increase in milk fat percentage (Bargo *et al*., 2003).

A reduction in rumen pH below 6.2 caused a decrease in fibre digestion, mainly because a pH below 6.2 is suboptimal for microbial growth (Pitt *et al*., 1996). Further reductions in pH to between 5 and 5.5 caused severe reductions in OM digestibility (Wales *et al*., 2004). It was found that at a pH of 5.6 the OM digestibility was lower than when compared to a pH of 6.1. This was due to the direct result of a decrease in fibre digestion (Wales *et al*., 2004). According to Meijs (1986) the rapid formation of VFA and lactic acid

in the rumen could be reduced by decreasing the amount of easily fermentable substrates such as starch from the concentrate, (Meijs, 1986).

Ruminal digestion of pasture was optimal at a pH of 6.35 according to De Veth & Kolver (2001), although the highest milk yield occurred at a rumen pH of 5.8 to 6.2, it was still somewhat low for optimal TMR (total mixed ration) utilization (Wales *et al*., 2004). High milk yields could be produced from well managed pasture with only moderate supplementation levels (4 - 6kg) for high genetic merit cows. The effect of the type of energy source on milk production only really becomes apparent at higher levels of supplementation in high producing dairy cows (Sayers *et al.*, 2003).

Muller *et al*., (2001) suggested that half of the supplementation mix during springtime be in the form of non forage fibre.

2.9 Low starch supplementation research

A limited amount of research has been done on the replacement of high starch supplements with low starch supplements and all these have had variable results. Nine studies are summarized in Table 2.3 for the purposes of this review. Seven of the nine studies were conducted on perennial ryegrass pasture. Of the seven studies, only three were conducted during spring (Meijs, 1986; Kibbon & Holmes, 1987; Meeske *et al*., 2009). Except for the study of Meeske *et al*. (2009) who conducted their study on Jersey cows, all the other studies on ryegrass made use of Friesian cows

Meijs (1986) conducted a study on spring calving Dutch Friesian dairy cows grazing on perennial ryegrass pastures. In another study six ruminally cannulated Dutch-Friesian cows grazing on perennial ryegrass were given either a high starch supplement at 1kg or 7kg, or 7kg of a low starch supplement (Van Vuuren *et al*., 1986). Kibbon & Holmes (1987) did a study on 30 spring calving British Friesian dairy cows grazing two different heights of perennial ryegrass pasture. They were offered 3kg of either a cereal based concentrate or a sugar beet pulp based concentrate as a daily supplement. In a study done by Fisher *et al*. (1996), fifty-two Holstein-Friesian cows grazing on perennial ryegrass were given either a barley based supplement as a high starch supplement, or a molassed sugar beet pulp based concentrate as a low starch supplement. Sayers *et al*. (2003) did a study on high producing Holstein-Friesian dairy cows grazing on perennial ryegrass. They investigated the effect of low starch and high starch supplements at two different levels on milk production and rumen parameters. Meeske et al. (2009) did a study using 60 high producing Jersey cows grazing on perennial ryegrass. Cows were given three different supplements, each with decreasing amounts of starch (maize) and increasing amounts of fibre (hominy chop based).

Three other studies were also included. A study was done by Spörndly (1991) in which 20 Swedish red and white cows were given fresh cut herbage ad lib, 2kg of hay and two different levels (4kg or 7kg) of

either a high starch or low starch containing supplement. Schwarz *et al*. (1995) did a study on Simmental X Red Holstein Friesian dairy cows receiving freshly cut herbage *ad lib*. The effect of a low starch supplement based molassed sugar beet pulp on milk production was compared to that of maize based supplement and to that of an oats, wheat and soybean meal concentrate. Khalili and Sairanen (2000) did a study in which a high starch concentrate was compared to a fibre based concentrate. Four kilogram of supplement was given to high producing Holstein-Friesian cows which were grazing on perennial timothy and meadow fescue pasture (Khalili & Sairanen, 2000).

2.9.1 Production Studies

a. Milk Production data

Milk production and milk components demonstrate rather variable responses to low starch supplementation when compared to that of high starch supplementation.

Milk yield was significantly increased in studies done by Khalili & Sairanen (2000). In studies done by Meijs (1986) and Meeske *et al*. (2009) there were no significant effects on milk yield, but fat corrected milk was significantly higher for the low starch supplement. Several authors found no significant effect on milk production at all when low starch supplements were compared to high starch supplements (Kibbon & Holmes, 1987; Spörndly, 1991; Fisher *et al*., 1996; Sayers *et al*., 2003). This contradicts the findings of Schwarz *et al*. (1995) who found a significantly higher milk yield for both high starch (maize and cereal mixture) supplementations when compared to low starch supplementations. In the study of Schwarz *et al*. (1995) ryegrass constituted only 5% of the composition of the pasture provided to animals and could be the cause of the different result.

Milk fat content was significantly increased by low starch supplementation in a study done by Meeske *et al*. (2009). This contradicts the findings of Spörndly (1991) who found that high starch supplements resulted in significantly higher milk fat percentages. Fisher *et al*. (1996) found that milk fat content tended to be higher with low starch supplementation but this result was not significant. Meijs (1986) found no significant effect between low starch and high starch treatments on milk fat percentage, but did find a significant increase in milk fat production for low starch supplementation. Several authors found no effect between low starch and high starch supplementation on milk fat percentage (Kibbon & Holmes, 1987; Schwarz *et al*., 1995; Khalili & Sairanen, 2000; Sayers *et al*., 2003). Only one study (Spörndly, 1991) found a higher milk fat content on low starch supplement. This unexpected result could be ascribed to the fact that the cows received an extra 2kg of hay with their concentrates.

Table 2.3 A comparison of nine studies on the effect of type of energy supplement on milk production parameters of grazing dairy cows.

Author	Concentrate	Supplement Intake kg DM/d	Herbage intake kg DM/d	Production				BW change kg/d
				Milk yield kg/d	Fat g/kg	Protein g/kg	Lactose g/kg	
Meijs, 1986	Starch: Maize based	5.4 [1]	11.5 [1]	25.6	39.6	34.0	-	0.28
	Fibre: Molassed sugar beet pulp based	5.3 [1]	12.6 [1]	26.9	41.0	33.7	-	0.11
Van Vuuren *et al*., 1986	Starch: Maize/Tapioca based	0.8	13.4	19.3	41.0	33.0	-	-
	Starch: Maize/Tapioca based	5.4	11.3	20.0	38.0	35.0	-	-
	Fibre: Sugar beet pulp based	5.2	12.8	18.9	41.0	33.0	-	-
Kibon & Holmes, 1987	Starch: Cereal based	3.8 [1]	13.6 [1]	28.5	38.5	-	-	-0.20
	Fibre: Sugar beet pulp based	3.8 [1]	14.0 [1]	28.3	38.5	-	-	-0.09
Spröndly, 1991	Starch: Barley/Soybean meal	3.3	14.7 [2]	20.5	46.8	33.9	44.8	0.6
	Starch: Barley/Soybean meal	5.8	13.0 [2]	22.0	44.4	35.0	45.3	0.6
	Fibre: Beet pulp/Wheat bran/Soybean meal	3.3	14.4 [2]	19.9	45.0	34.4	44.9	0.6
	Fibre: Beet pulp/Wheat bran/Soybean meal	5.6	12.5 [2]	22.0	43.2	34.1	45.6	0.6
Schwarz *et al*., 1995	Starch: Maize based	4.8	12.6	22.4	38.1	33.9	47.7	-
	Starch: Oats/Wheat/Soybean meal	5.4	11.8	22.9	39.5	32.9	47.6	-
	Fibre: Molassed sugar beet pulp based	5.5	12.1	20.8	39.7	33.1	46.7	-
Fisher *et al*., 1996	Starch: Barley based	5.0	12.6	23.7	39.4	30.3	46.5	-0.14
	Fibre: Molassed sugar beet pulp based	5.0	13.5	24.7	40.7	30.3	45.3	0.04
Khalili & Sairanen, 2000	Starch: Barley based	4.0	-	19.7	38.5	34.2	47.6	-
	Fibre: Molassed sugar beet pulp based	4.0	-	21.0	37.6	34.9	47.5	-
Sayers *et al*, 2003	Starch : Barley based	5.0	11.7	33.3	37.5	33.5	48.5	-1.29
	Starch: Barley based	10.0	9.7	37.3	30.8	34.4	49.5	-0.68
	Fibre: Sugar beet pulp/Citrus pulp based	5.0	12.4	34.0	38.1	31.9	49.0	-0.99
	Fibre: Sugar beet pulp/Citrus pulp based	10.0	9.6	36.0	35.8	32.5	49.5	-0.83
Meeske *et al*., 2009	Starch: Maize based	6.0	-	21.0	36.6	34.5	-	0.48
	Medium Starch: Maize/Hominy chop based	6.0	-	20.8	40.3	35.5	-	0.56
	Fibre: Hominy chop/Maize gluten/Bran	6.0	-	20.1	44.1	34.2	-	0.54

[1] kg OM/d
[2] 2kg of herbage taken in is supplemented hay
DM = Dry matter, BW = Body weight

The reason for the decreased milk fat when high starch concentrates were fed was suggested by Meijs (1986) to be that increased starch in the diet of dairy cows increased the concentration of propionate in the rumen. This in turn increased blood glucose and insulin levels. The higher blood insulin levels caused an increased nutrient uptake by tissues by lipogenesis and a reduction in lipolysis, which can cause a reduced amount of triglycerides in the blood plasma available to the mammary gland, thus causing a reduced milk fat composition.

Milk fat concentration declines by an average of 5g/kg for every unit reduction in acetate plus butyrate: propionate ratio (Sutton, 1984). Sayers *et al.* (2003) found similar rates of 5.3g/kg decline per unit fall in acetate to propionate ratio observed. The fact that milk fat increased when animals were supplemented with this non forage fibre, illustrates the lack of effective fibre in high quality pastures (Muller *et al*., 2001).

Milk protein yield was increased by low starch supplementation for dairy cows (Khalili & Sairanen, 2000). Sayers *et al*. (2003) found milk protein to be higher with a high starch supplementation. Several authors found no significant differences for milk protein content between low starch and high starch supplementations for grazing dairy cows (Meijs, 1986; Kibbon and Holmes, 1987; Schwarz *et al*., 1995; Fisher *et al*., 1996; Meeske *et al*., 2009).

Apart from the work of Khalili & Sairanen (2000) who found milk lactose yield to be higher for high starch supplemented grazing dairy cows, and Schwarz *et al*. (1995) who found high starch supplements to have higher milk lactose content than low starch supplements, most authors found no effect on lactose content of the milk between low starch and high starch supplement (Kibbon & Holmes, 1987; Spörndly, 1991; Fisher *et al*., 1996).

The results of the nine studies are variable when high starch supplements are replaced with low starch concentrates. Often no significant difference was obtained between treatments, as was the case for milk yield and milk solids. Low starch supplementation mostly consisted of cheaper by-products which resulted in lower feed cost and an overall increase in profit margins in cases in which milk production parameters remained constant.

b. Live weight

Seven studies recorded live weight change of dairy cows. Kibbon and Holmes (1987), Spörndly (1991), Khalili & Sairanen (2000) and Sayers *et al*. (2003) all found no significant difference in live weight change of animals between low starch and high starch supplementation. Fisher *et al*. (1996) and Meeske *et al*. (2009) also found no significant effect on live weight change as well as body condition score. Meijs (1986) found a 0.17kg difference in mean daily live weight between low starch and high starch supplementation, with the latter being the higher one. The large decrease in live weight when the low starch treatment of Meijs was compared to the high starch treatment could be due to the decreased energy available from this concentrate and thus cows relied more on body reserves to keep milk production constant. Except for

the study of Meijs (1986) it seems evident that supplementation type has little effect on live weight change or body condition score of lactating dairy cows.

c. Herbage intake

Sayers *et al*. (2003) found that supplementation type, in this case low starch compared to high starch, had no significant effect on herbage intake but the amount of supplementation fed did affect herbage intake. This agreed with Spörndly (1991) who also found no significant effects between supplementation types on herbage intake. Fisher *et al*., (1996) found that supplementation type did not significantly affect herbage intake, although the low starch concentrate did increase herbage intake by 0.9kg DM/day. Kibbon and Holmes, (1987) found that on low pasture length (grazed to a height of 5cm) there was no difference in herbage OM intake, but on high pasture length (grazed to a length of 6.5cm) the high starch concentrate (a cereal based concentrate) did suppress herbage OM intake. Meijs (1986) found a significant effect on herbage intake between two treatments with the low starch supplement having a significantly higher herbage intake than the high starch concentrate. He also indicated a significantly lower substitution rate for dairy cows fed the low starch supplementation, with the low starch supplementation having a substitution rate of 0.21kg herbage OM compared to the 0.45kg of the high starch concentrate.

Results indicate mostly no significant difference for herbage intake between low starch and high starch supplements, although Meijs (1986) found a potentially positive effect on herbage intake when low starch concentrates were fed.

2.9.2 Rumen studies

a. Rumen ammonia-nitrogen

Three of the studies mentioned above included rumen studies and recorded rumen ammonia levels (Van Vuuren *et al.*, 1986; Khalili & Sairanen, 2000; Sayers *et al*., 2003). These are summarized in Table 2.4. There were differences for rumen ammonia nitrogen, with the high starch concentrate resulting in significantly higher ruminal ammonia nitrogen (NH_3-N) levels than the low starch concentrate (Khalili & Sairanen, 2000).

This contradicts the findings of Van Vuuren *et al*. (1986) who found that ruminal NH_3-N content did not differ significantly between supplementation types when cows were given 7kg (5.2-5.4kg actual intake) supplement, however the 1kg supplementation level had a significantly higher NH_3-N value. Van Vuuren *et al*. (1986) ascribed this to the fact that more pasture was taken in at a lower level of supplementation than at the higher levels of supplementation, and that the pasture had a higher rumen degradable protein content than the supplement had and therefore increased the rumen ammonia concentration.

Table 2.4 A comparison of three studies on the effect of type of energy supplement on rumen fermentation parameters of grazing dairy cows.

Author	Concentrate	Volatile Fatty acids						NH_3 mg/dL	pH
		Total mM/L	Acetate mM/L	Propionate mM/L	Butyrate mM/L	A : P [1]	Sutton's Ratio (A + B):P [2]		
Van Vuuren *et al*., 1986	Starch: Maize/Tapioca based – 0.8kg	127.0	-	-	-	3.20	-	32.4	6.00
	Starch: Maize/Tapioca based – 5.4kg	127.0	-	-	-	2.80	-	22.1	5.90
	Fibre: Sugar beet pulp based – 5.2kg	130.0	-	-	-	3.20	-	20.4	5.90
Khalili & Sairanen, 2000	Starch: Barley based – 4.0kg	127.0	81.4	24.3	15.9	3.35	4.07	32.2	6.17
	Fibre: Molassed sugar beet pulp based – 4.0kg	132.0	84.5	27.1	15.3	3.12	3.72	21.8	6.01
Sayers *et al*, 2003	Starch : Barley based – 5-10kg	121.6	68.1	31.6	17.0	2.26	2.82	12.0	5.80
	Fibre: Sugar beet pulp/Citrus pulp based – 5-10kg	122.5	73.5	25.7	18.4	2.94	3.69	13.6	5.96

mM/L = mmol/L
[1] Acetate to propionate ratio
[2] Acetate plus butyrate to propionate ratio

Sayers *et al*. (2003) found no significant difference for NH_3-N when low starch and high starch concentrates were compared. The rumen NH_3-N level were however lower in the study of Sayers *et al*. (2003) than was the case in the study of Van Vuuren *et al*. (1986). This could possibly be because of the higher levels of concentrate given in the study of Sayers and the effect thereof on pasture intake and rumen degradable protein than was described by Van Vuuren *et al*. (1986).

Ammonia concentration peaked at 24h00, the same time VFA peaked (Van Vuuren *et al*., 1986). This agrees with Khalili & Sairanen (2000) who found that NH_3-N concentration was higher especially after 11h00. Khalili & Sairanen (2000) ascribed this to the fact that there was probably a considerable intake of pasture after evening milking. All three studies had rumen ammonia concentrations above the suggested minimum level of Satter and Roffler (1974) of 5mg/dL.

b. Volatile Fatty Acids

There was very little difference in diurnal variation of propionate and butyrate ratios (Khalili & Sairanen, 2000). There were no significant differences for molar proportions of acetic, propionic or butyric acid between two treatments (Khalili & Sairanen, 2000).

This agrees with Van Vuuren et al, (1986) who found no significant difference in volatile fatty acid concentration between treatments. They showed that the volatile fatty acid diurnal pattern was the inverse of the ruminal pH pattern. Van Vuuren et al (1986) stated that concentrate supplementation had a less pronounced effect on the rumen because of pasture, and thus the composition of the concentrate would have an even smaller effect.

Differences in molar proportions where however found by Sayers *et al*. (2003) who indicated that high starch concentrates had a significantly lower molar proportion of acetate and butyrate, when compared to low starch concentrates, and also had significantly higher propionate and valerate proportions. This led to significantly lower acetate to propionate ratio as well as Sutton's ratio (acetate plus butyrate to propionate ratio) for high starch concentrates (Sayers et al., 2003).

c. Rumen pH

All three authors who recorded rumen pH found no significant difference in pH between high starch and low starch treatments (Van Vuuren *et al*., 1986; Khalili & Sairanen, 2000; Sayers *et al*., 2003). However, Van Vuuren *et al*. (1986) found a significant difference in the rumen pH at 08h00, where the rumen pH was inversely proportional to the amount of highly fermentable carbohydrates taken in.

The lowest pH values recorded by Van Vuuren *et al*. (1986) were at 24h00, which agreed with the findings of Khalili & Sairanen (2000) who stated that rumen pH lowered to under pH 6.0 during the evening. Khalili & Sairanen (2000) ascribed this to the fact that there was probably a considerable intake of pasture after evening milking.

d. Rumen *In sacco* digestibility

The equation $P = a + b\ (1 - e^{-ct})$ was used to explain the disappearance of DM from nylon bags in the rumen where *P* is the proportionate amount of DM that disappeared in time *t* (hours). The rapidly soluble fraction is represented by *a*, *b* represents the potential degradable fraction and *c* the rate of degradation for time *t* (Ørskov & McDonald, 1979; McDonald, 1981).

In the study done by Sayers *et al*. (2003) supplementation type had no effect on DM or NDF degradability of ryegrass in the rumen. The DM of the concentrate had a significantly higher *a* value, a significantly lower *b* value and a significantly higher *c* value for the high starch concentrate compared to the low starch concentrate. This led to a significant overall higher DM degradability for the high starch concentrate (Sayers *et al*., 2003). Khalili & Sairanen (2000) found that *a*, *b* and *c* for hay incubated in the rumen was not affected by treatments. Low starch concentrates showed a significantly higher rumen fluid out flow rate than the high starch concentrate at the same level of supplementation, and this was attributed to the higher pasture intake with the low starch concentrate (Van Vuuren *et al*., 1986).

2.10 Conclusion

During the last decade a combination of factors have led to a shift in dairy production to include more pasture based systems. It is cheaper to feed dairy cows using pasture systems but new challenges and problems are encountered with their use. The lack of energy provided by early spring ryegrass pastures appears to be an important problem which indicates the need for supplementation. Presently most of the supplementation given to high producing dairy cows on these pastures includes high amounts of highly digestible carbohydrate, such as starch found in maize, the main ingredient in most supplement concentrates. Although the supplementation of the diets of dairy cows with these concentrates improves milk production, it is expensive and animals more easily develop health related problems and tend to underutilize pasture.

Pasture systems are more complicated than normal TMR systems by virtue of factors such as substitution rate and the fact that the rumen environment is subjected to two different nutrient sources. By causing rumen environment extremes such as feeding highly fermentable carbohydrates to dairy cows, pasture utilization and milk production could decrease and health problems could appear as well.

It was shown by several authors that the maize, barley and wheat in the concentrates of dairy cows on ryegrass pasture, could be replaced by by-products such as citrus pulp, sugar-beet pulp, wheat middlings, cottonseed, hominy chop and wheat bran without having a major effect on milk production. It was found that overall herbage substitution rates were higher with high-starch supplements when compared to low starch (by-product) supplements. High starch supplementation resulted in an increase in milk protein content and reductions in milk fat content. This demonstrated that high-fibre supplementation with high hemicellulose content could result in a higher rumen pH and increased milk production in dairy cows and could be a viable option for supplementation of ryegrass pastures during spring

Replacing high starch (high maize) concentrate supplementation for dairy cows on pasture based systems with a low starch concentrate, usually derived from by-products, could be a viable option. It would decrease the total capital input cost, due to ingredients in the concentrate costing less. It could lead to the same milk production as indicated by Kibbon & Holmes (1987), Spörndly (1991), Fisher *et al*. (1996) and Sayers *et al*. (2003) or even improved milk production as indicated by Meijs (1986), Khalili & Sairanen (2000) and Meeske *et al*. (2009). Even if milk production were to stay the same, the gross capital margin would still increase due to the lower input costs associated with cheaper feed. The effect of the dietary change on milk fat percentage could also be positive as indicated by Meijs (1986) and Meeske at al. (2009). This could lead to increased fat corrected milk yields.

A study to determine the effect of feeding low starch concentrate to cows grazing ryegrass/kikuyu pasture under South African conditions is therefore most important. Positive results could lead to substantial capital savings to the dairy industry.

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CHAPTER 3

Materials and Methods

3.1 General information

3.1.1 Location and duration of the project

The study was conducted at the Outeniqua research farm near George in the Western Cape Province of South Africa (Longitude 22º25.222' E, Latitude 33º58.702'S, altitude 193 m). The mean annual rainfall for this region from 1991 to 2009 was 775 ± 170 mm per annum, although the mean annual rainfall during 2009 only amounted to 449.7 mm. The mean rainfall for the period from August until October from 1991 to 2009 was 205.1 ± 116.4 mm, but only 135.4 mm was recorded during 2009. The average maximum and minimum temperatures during the study were 19.8 and 8.9 ºC, respectively.

This research was conducted from 30 July 2009 to 22 October 2009. Cows were allocated to their treatment groups a week prior to the start of the trial, and were made accustomed to the conditions and rituals during the study by the time of trial commencement. Two studies were undertaken simultaneously during this period, consisting of a production study and rumen study respectively.

3.1.2 Pasture Management

Primary pasture utilised for grazing during the study consisted 8.6 hectares of kikuyu (*Pennisetum clandestinum*) over-sown with annual Italian ryegrass (*Lolium multiflorum* var. *italicum cv*. Jeanne). A further 4.05 hectares of similar pasture was also available for grazing during periods when pasture growth rates were to slow to accommodate all experimental animals. The study area was characterised by an Estcourt soil type (Soil Classification Working Group, 1991). During the study period, which occurred from late winter to early spring, the kikuyu component of the pasture was dormant, resulting in pasture consisting of predominantly ryegrass. Pasture was fertilized with 56 kg N (LAN, limestone ammonium nitrate)/ha after each grazing by dairy cows.

3.2 Production Study

3.2.1 Experimental design

Forty-five high producing, lactating Jersey cows [body weight, 340 ± 34.7 kg; milk yield, 19.6 ± 2.23kg/d; days in lactation, 153 ± 33.5; lactation number, 3.6 ± 1.85; (mean ± SD)] from the Outeniqua research farm trust herd were used during the production study component of the study.

Cows were allocated to treatments using a randomized block design in order to eliminate variation and allow for valid comparisons to be made. The forty-five cows were allocated to fifteen groups of three each (blocking) on the basis of lactation number, DIM, and milk yield (MY) and is shown in Appendix A in Table

A.1, Table A.2 and Table A.3. First lactation cows were excluded from the study due to the variability often experienced in the milk production of such animals. The three cows from each group were randomly allocated to one of three treatment groups.

Animals within a treatment group were consistently fed one of the three possible diets (which made up the treatments) throughout the experimental period. A 14 day adaption period was followed by a 70 day trial period during which data was collected. All treatments were allocated to the exact same strip of fresh pasture, at the same time, after each milking. Pasture was allocated in order to allow for *ad lib* pasture intake by all animals, while still ensuring sufficient quality pasture would be available at a later stage during the study period. Treatment groups only differed in the composition of the concentrate supplementation fed to cows over and above the pasture consumed. The diet of the three treatment groups differed in regard to the starch and fibre concentrations in the concentrates fed to the cows. Table 3.1 presents the ingredient composition and chemical composition of the experimental diets. The dietary composition of the treatment groups were as follows:

Treatment 1 (High Starch): Basal diet of kikuyu ryegrass pasture plus 6kg of concentrate. Concentrate included 80% maize and 11% Soybean oilcake, and 0% Hominy chop, 0% wheat bran and 0% gluten 20.

Treatment 2 (Medium Starch): Basal diet of kikuyu ryegrass pasture plus 6kg of concentrate. Concentrate included 40% maize and 4% Soybean oilcake, and 25% Hominy chop, 11% wheat bran and 11% gluten 20.

Treatment 3 (Low Starch): Basal diet of kikuyu ryegrass pasture plus 6kg of concentrate. Concentrate included 20% maize and 0% Soybean oilcake, and 35% Hominy chop, 18% wheat bran and 18% gluten 20.

Red, blue and white coloured tags were attached to the neck of each cow to identify them as being part of one of the three different treatment groups. This eased management and ensured that animals received the correct diet at all times.

The energy values of the three different treatment concentrates were 12.71 MJ ME/kg, 11.58 MJ ME/kg and 10.96 MJ ME/kg dry matter (DM), respectively. The difference in the ME values can be attributed to the reduction in the maize concentration from 80.37% in the high maize diet to 20.67% in the high fibre diet. The hominy chop component was increased from 0 to 35% from the high maize diet to the high fibre diet, while soybean oilcake meal was reduced from 11% to 0% as the fibre content of the diet increased. The crude protein (CP) value was, however, maintained at a constant value of 13% on a DM basis. There was a reduction in the non fibre carbohydrate (NFC) content from 64.13% in the high maize diet to 45.39% in the high fibre diet and an increase in hemicellulose percentage from 6.04% in the high maize diet to 17.98% in the high fibre diet. The standard, high maize diet given to dairy cows was, therefore, changed to include higher concentrations of high-fibre by-products in order to test the viability of high by-product substitution. Maize could not be completely omitted because the energy content of the diet would then be too low. Additional energy could not be obtained from an increase in fat content of the diet because the upper inclusion level of fat had already been reached.

Table 3.1 Ingredient and chemical composition of concentrates used for experimental treatments fed to Jersey cows grazing kikuyu/ryegrass pasture during October.

Ingredient (g/kg)[1]	Treatment 1 High Starch	Treatment 2 Medium Starch	Treatment 3 Low Starch
Maize	804	407	207
Hominy chop	0.00	250	350
Wheat bran	0.00	110	180
Gluten 20	0.00	110	180
Soybean oilcake	110	40.0	0.00
Molasses	40.0	40.0	40.0
Feed lime	20.0	22.0	22.0
MCP	5.00	0.00	0.00
Salt	10.0	10.0	10.0
Sodium bicarbonate	5.00	5.00	5.00
MgO	3.00	3.00	3.00
Vit and Min Premix	3.30	3.30	3.30
Nutrient (g/kg)			
DM	891	889	887
CP	130	130	130
RUP (of CP)	602	542	503
ME (MJ/kg)	127	116	110
NDF	111	219	278
ADF	50.8	81.6	98.4
Hemicellulose	60.4	138	180
NFC	641	520	454
Starch	571	437	364
Fat	45.3	59.5	65.0
Ca	9.80	9.40	9.40
P	4.30	5.00	6.00

[1] MCP –Monocalcium Phosphate; MgO – Magnesium Oxide; DM – Dry matter; CP – Crude Protein; RUP – Rumen Undegradable protein; ME – Metabolisable Energy; NDF – Neutral detergent fibre; ADF – Acid detergent fibre; NFC – Non fibre carbohydrates; Ca – Calcium; P – Phosphate.

3.2.3 Feeding and milking program

Kikuyu/ryegrass pasture was made available *ad lib* for cows 24 hours a day, except for the duration of milking. The forty-five cows were allocated a strip of 15m by 150m (depending on pasture DM yield of the specific strip) of fresh pasture daily. All cows grazed on the same pasture, with fresh pasture made available after each milking. Fresh water was available *ad lib* at all times.

Cows were milked twice daily at 06h00 and 14h00. Cows received a daily allowance of 6kg of supplemented concentrate in two different feedings of 3kg each during the two milking periods. Proper milking procedures, aimed at maintaining udder health, were followed during each milking session. Each treatment group was

assigned a colour coded and numbered neck tag according to the treatment group a specific cow belonged to. Prior to entering the milking parlour, the cows were split into the 3 different treatment groups and fed and milked accordingly. Each cow received 3kg of their specific treatment group feed, which was weighed by hand and manually placed into the feeding troughs to ensure that each cow received the correct amount and correct type of feed. Feed troughs were thoroughly cleaned before each allocation by sweeping up all the refusals left by cows from the main herd during the previous feeding period.

3.2.4 Data collection

a. Feed and Pasture samples

Over the twelve week study period (two weeks adaptation period and ten weeks experimental period), daily grab samples were taken from each bag of concentrate supplement and pooled into fortnightly collection samples for each treatment diet. This pooled sample was then thoroughly mixed in order to obtain a representative sample of each feed type. From this pooled sample a grab sample was taken and placed into plastic containers, sealed and frozen in a refrigerator for later analysis. A total of six samples were thus collected per treatment group over the twelve week study period.

Pasture samples were also taken during the period of the study. Weekly pasture samples were taken every Thursday, with three samples cut from the pasture that the cows where scheduled to graze after the next milking. An iron ring with a height of 30 mm above ground level was randomly placed at different sites in the pasture. All plant material within the ring area was then cut to a height of 30 mm above ground level and placed in a paper bag. The pasture sample was weighed and dried for 72hr at 60 ˚C and then weighed again to determine the pasture DM content. The pasture sample was then milled through a 1mm sieve and placed into plastic containers, sealed and frozen in a refrigerator for later analysis. A total of twelve pasture samples were collected during the study period.

Chemical analyses were done to determine DM, ash, Crude Protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), neutral detergent insoluble nitrogen (NDIN), acid detergent insoluble nitrogen (ADIN) and ether extract (EE). Prior to chemical analysis feed particle size was reduced by grounding sample to pass through a 1mm screen. The methods followed during analyses are discussed in detail in section 3.4 of this thesis.

b. Milk yield and milk samples

Daily milk yields for each individual cow were recorded electronically by the Dairy Master Computer software and a 20 point swing over milking machine (Total Pipeline Industries, 33 Van Riebeeck Street, Heidelberg, 6665) during each milking, twice daily for the total period of the study. Milk samples were collected at three different instances during the study period. Approximately 10ml/L milk per cow per milking was siphoned off into a separate container during milking to get a representative sample of the milk. The fat composition of

morning milk differs from that of afternoon milk. To take into account that the butterfat content of morning milk differs from that of afternoon milk, samples taken in the morning and afternoon were mixed to form a composite sample. The interval between the morning and afternoon milking was 8 hours and between the afternoon and morning was 16 hours. Therefore the milk was collected in the ratio of 8ml from afternoon milk sample and 16ml from the morning sample as shown in Figure 3.1. The two samples were then composited, which ensured a representative sample. Samples were preserved with Potassium dichromate ($K_2Cr_2O_3$) after collection by inverting 14ml container gently.

Figure 3.1 Measuring of correct amount of milk during milk sampling to insure a representative sample between morning and afternoon milk.

Samples were taken in duplicate and were sent to two different laboratories, namely Lactolab (Irene), Pretoria and NIQL Laboratories, (Johannesburg), for analysis. Milk samples were analyzed for milk fat (BF), protein, lactose, and milk urea nitrogen (MUN) content using infrared technology by means of the Milkoscan 6000 (Foss Integrated Milk Testing FT 6000, Foss Electric, Hillerod, Denmark). The somatic cell count (SCC) of the samples was also determined using flow cytometry by means of the Fossomatic 5000 (Foss Electric, Hillerod, Denmark).

c. Body weight and body condition scoring (BCS)

Cows were weighed on two consecutive days prior to the commencement of the study and twice at the end of the study. The weighing was done after milking to ensure that udders were not filled with milk and affecting the recorded weights. Two weights were recorded and averaged to reduce variation caused by the variations in pasture intake and urination and defaecation. Body condition scoring (BCS) was done at the same time for all experimental animals. Condition was scored on a five point scale (NRC, 2001) where 1 = severe under conditioning (emaciated) and 5 = severe over conditioning. Because body condition scoring is done subjectively, each scoring was done by the same person to minimize variation.

3.3 Rumen Study

3.3.1 Experimental design

Ten lactating cannulated Jersey cows [body weight, 332 ± 56.3 kg; milk yield, 17.3± 1.73kg/d (mean ± SD)] from the Outeniqua research farm trust herd were used during the rumen study component of the study.

The ten cannulated cows were divided into five groups of two each on the basis of lactation number, DIM, and milk yield (MY). The two cows from each group were randomly allocated to one of two treatment groups. This was done to eliminate variation and allow for valid comparisons to be made. Effectiveness of blocking was reduced due to the smaller population size.

The study was undertaken over a period of 42 days, divided into two 21 day periods. The first period consisted of a 14-day adaptation period and a 7-day data collection period for the first treatment diet. Following this, a second 14 day adaptation period was followed by a 7 day trial period for data collection for the second diet. Thus, using a cross-over design, results were duplicated, since each cow received both diets during the course of the rumen study.

After each milking the cows were returned to the same feeding strip. Pasture was allocated in such a way as to allow for *ad lib* pasture intake while still ensuring sufficient quality pasture to be available at a later stage during the study period.

Treatments diets only differed in the composition of the concentrate supplementation fed to cows over and above the pasture consumed. Each treatment group had an increasing level of fibre and a decreasing level of starch. Table 3.1 presents the ingredient composition and chemical composition of the experimental diets.

Treatment 1 (High starch): Basal diet of kikuyu ryegrass pasture plus 6kg of concentrate. Concentrate included 80% maize and 11% Soybean oilcake, and 0% Hominy chop, 0% wheat bran and 0% gluten 20.

Treatment 3 (Low Starch): Basal diet of kikuyu ryegrass pasture plus 6kg of concentrate. Concentrate included 20% maize and 0% Soybean oilcake, and 35% Hominy chop, 18% wheat bran and 18% gluten 20.

Treatment 2 (Medium Starch) was not included in the rumen study like in the case with the production study.

3.3.2 Feeding and milking program

Cannulated cows at all times grazed together with the cows from the production study, with management procedures related to these cows not differing in any manner. The cannulated cows had the same neck tags and were also milked in exactly the same way as described above, together with the cows from the production study.

Cows were milked twice daily at 06h00 and 14h00. Cows received a daily allowance of 6kg of supplemented concentrate in two different feedings of 3kg each during the two milking periods. Proper milking procedures, aimed at maintaining udder health, were followed during each milking session. Each treatment group was assigned a colour coded and numbered neck tag according to the treatment group a specific cow belonged to. Prior to entering the milking parlour, the cows were split into the 3 different treatment groups and fed and milked accordingly. Each cow received 3kg of their specific treatment group feed, which was weighed by hand and manually placed into the feeding troughs to ensure that each cow received the correct amount and correct type of feed. Feed troughs were thoroughly cleaned before each allocation by sweeping up all the refusals left by cows from the main herd during the previous feeding period.

3.3.3 Data collection

a. Feed and Pasture samples

The same feed and pasture samples collected in the production study as described above (Section 3.4.2 a) were used for the rumen study.

b. Rumen pH profiles

Within each data collection period, the rumen pH of each cow was measured continuously every ten minutes for four consecutive days by using TruTrack Data Loggers (Model pH-HR mark 4, Intech Instruments LTD, NZ). The pH data loggers were calibrated before insertion into the rumen of the cannulated cow using the Omnilog Data Management Program Version 1.64 with buffer solutions of pH 4, 7 and 9 as shown in Figure 3.2.

A mounting for the pH loggers was designed and built to house the pH data loggers and electrodes in a manner so as to protect the data loggers in the rumen, while still allowing the electrodes to protrude from the mounting and water-tight fittings as shown in Figure 3.3. This enabled maximum exposure to rumen liquor and measurement of the rumen pH, while eliminating logger malfunction. A hole was cut into a cannula plug, a flexible but sturdy rubber pipe was connected to the hole in the cannula plug and the hole sealed to be watertight. The logger was then inserted into the mount and sealed to be watertight at the bottom of the mounting, thus allowing only the electrode to come into contact with the rumen liquor. In addition, the logger was sealed at the top with a screw cap and given a very small profile mounting to allowing the cow to continue everyday activities without any discomfort or danger of damaging the logger.

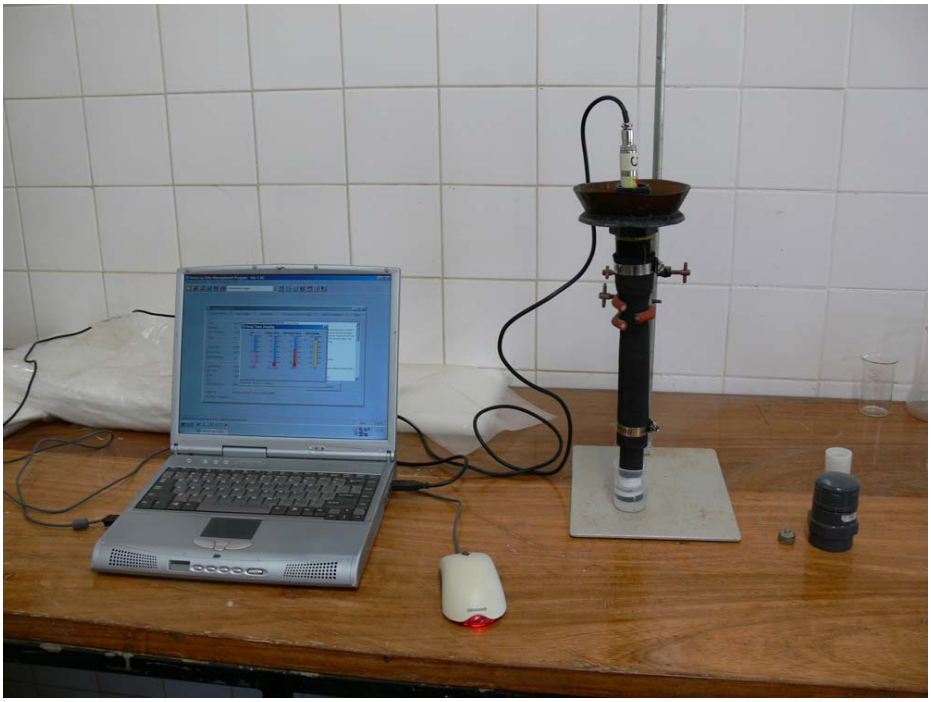

Figure 3.2 TruTrack Data Loggers (Model pH-HR mark 4, Intech Instruments LTD, NZ) connected to a laptop for logger calibration and data downloading with the Omnilog Data Management Program Version 1.64

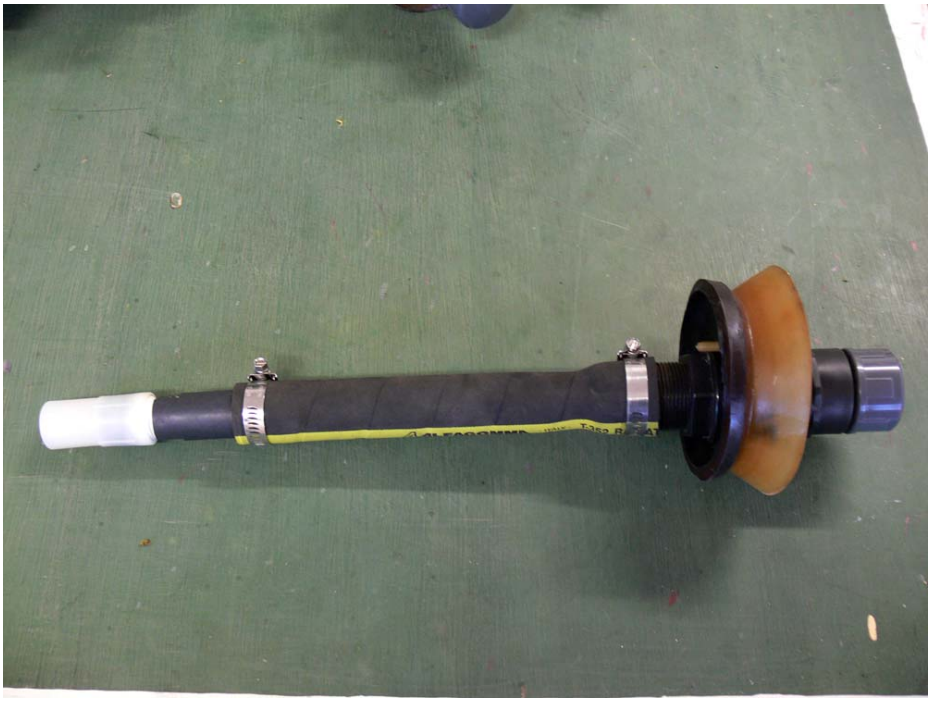

Figure 3.3 The TruTrack Data Logger (Model pH-HR mark 4, Intech Instruments LTD, NZ) used for rumen pH logging, inside cannula plug mounting ready for insertion into the rumen of cannulated cow

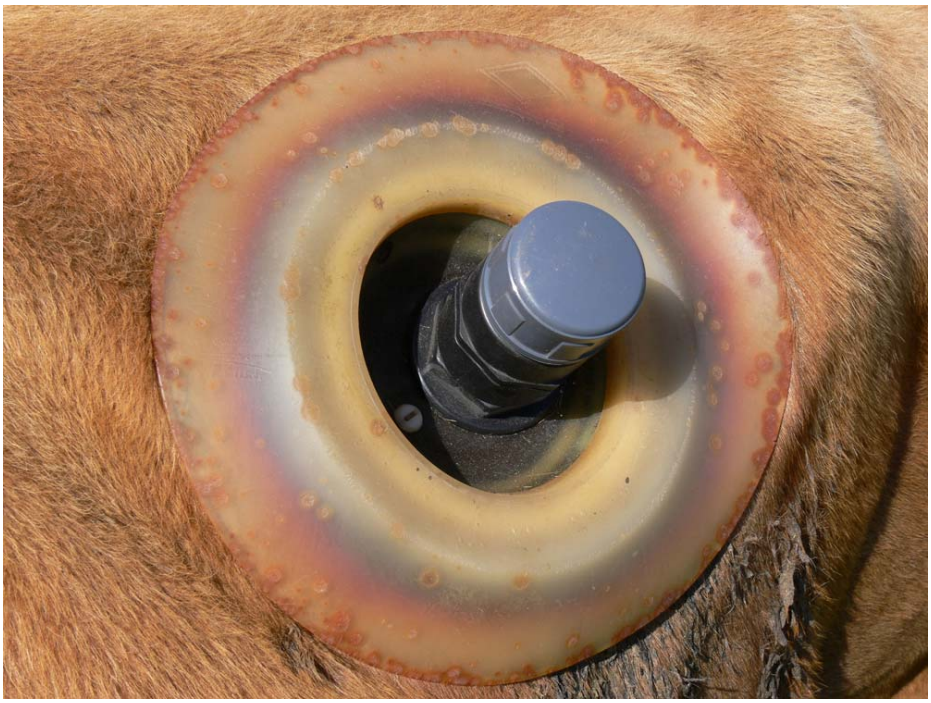

Figure 3.4 Rumen pH logger inserted and fitted in the rumen cannula for four days

The pH data loggers recorded pH for a period of four days, after insertion of the data logger mountings into the rumen of the cannulated cows as shown in Figure 3.4. Once the four day period had elapsed, the data logger mountings were removed and the recorded pH measurements downloaded from the data loggers onto a computer using the Omnilog Data Management Program, version 1.64 as indicated in Figure 3.2. All pH data was reduced to average half hourly values for statistical analysis and in order to construct graphs.

During the second part of the rumen study (after the “swing-over”), these same procedures were followed and care was taken to allocate the same pH data logger to the same cow in order to reduce variation.

c. Rumen fluid samples

Rumen sampling was carried out to further explain and understand the dynamics of the ruminal bacterial populations and their activity in the rumen. Bacteria produce three main volatile fatty acids (VFA) which include acetic, propionic and butyric acid. The nature of the diet determines which volatile fatty acid predominates in the rumen. High fibre diets promote acetic acid production, and high concentrate diets promote high propionic acid production from the rumen microbes. Rumen samples are taken at specific time’s periods during the cow’s eating patterns which consist of both pasture and concentrate intakes. This influences the resultant acid concentrations.

Figure 3.5 Rumen fluid collection from cannulated cows using a suction pump connected to a fluid container

Figure 3.6 Filtration of rumen fluid samples through cheesecloth to remove particulate matter

To obtain samples, the cows were first safely restrained in a crush that was either temporarily constructed at the pasture or the permanent crush at the milking parlour. A small hole of 5 mm was made in the cannula

plug of each cannulated cow and closed with a screw to prevent air contamination of rumen. During sampling, the screw was removed and a 50cm stainless steel tube inserted slowly through the hole into the rumen with care taken not to damage the rumen wall. The stainless steel tube was then connected to a plastic tube, leading to the container for the rumen fluid sample which was connected to a suction pump. One person would gently move the stainless steel tube inside the rumen while a second would ensure constant suction force on the pump as shown in Figure 3.5. Following sampling, the 5mm screw was replaced into the hole of the cannula plug.

During the collection of the sample the pH was also recorded using a hand held pH logger. The container was then closed and moved to a nearby laboratory. Each rumen sample was filtered through a double layer of clean cheese cloth to remove particulate matter from the rumen sample as shown in Figure 3.6.

Fifteen ml aliquots were transferred to airtight containers for each individual cow, labelled and frozen immediately pending analysis. No preservatives were added. Volatile fatty acid concentrations were determined by gas-liquid chromatography. Another 15ml aliquot was transferred to a second airtight container for each individual cow and was preserved with 2.5ml of 50% H_2SO_4 and frozen immediately pending ammonia nitrogen determination.

It was imperative to finish the procedure as quickly as possible, and to freeze samples immediately to terminate any further microbial action in the samples. Effective labelling was also of utmost importance. Samples were collected for both treatments during both replicates of the study at 06h00, 12h00 and 20h00.

d. In Sacco Dacron bag study

The In Sacco Method (“stocking procedure”) is the procedure carried out to determine the DM digestibility in the rumen of the available pasture as described by Cruywagen (2006). This method facilitates bag retrieval and prevents unnecessary exposure to air of bags intended for later removal. Air exposure effects microbial degradation so it is important to minimize the time when the cannula plugs are open during Dacron bag removal. The percentage DM disappearance of the test ingredient can then be accurately examined at specific time intervals, using this technique.

Kikuyu-ryegrass pasture was cut down to a 3cm level. Grass was then dried in brown paper bags for 72hrs at 60°C. The dried grass was removed and cut by hand with a pair of scissors into pieces approximately 7mm long. One hundred pieces were measured to ensure the grass was cut short enough.

Dacron bags were dried, and weighed empty. Thereafter five gram of the cut grass was placed into the Dacron bags. The bags were weighed again, and were then closed securely by a cable tie and then weighed for the last time as shown in Figure 3.7.

Figure 3.7 A dacron bag, labeled with a material marker, filled with five gram of kikuyu/ryegrass pasture and sealed with a cable tie, ready for insertion into stocking

Figure 3.8 Seventeen dacron bags inserted in four stockings and connected to the cannula plug with the “catch”, ready for insertion into the rumen of cannulated cows.

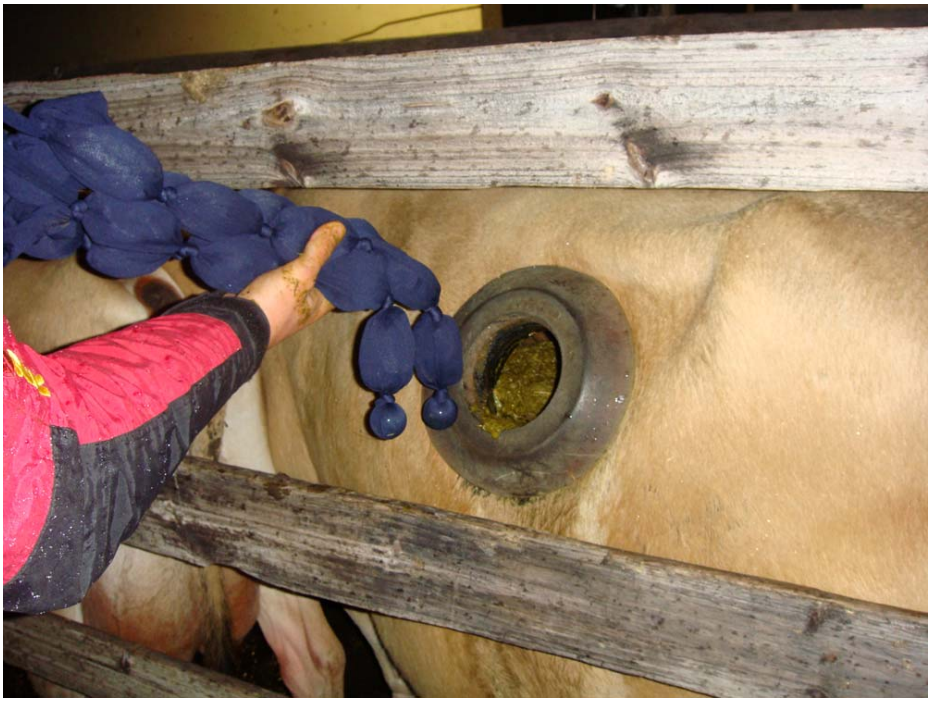

Figure 3.9 Dacron bags inserted into rumen through rumen cannula

Figure 3.10 Dacron bags removed from the rumen at specific times to determine pasture degradability between two treatments

Two bags were prepared per cow for hour 2, 4, 8 and 16, and three each for hours 30, 48 and 72. In total 17 bags were prepared for each cow. Bags were placed inside 40 deci-tex silk stocking. It was important to ensure that the stocking was not of the “anti-bacterial” type. Into the tip off each stocking, a weight was placed, and secured with a knot in the stocking. Each Dacron bag was kept separate from the other by a knot in the stocking. Three stockings with four bags each and one stocking containing five bags, were prepared for each cow. Stockings were attached to the inside of the cannula plug by a metal ring Figure 3.8.

Bags were inserted into cows and thereafter removed at the correct hourly interval as shown in Figure 3.9 and Figure 3.10. Bag numbers were recorded when removed and bags were immediately frozen. Once all the bags have been removed, they were thawed and washed gently in a washing machine for three six minute intervals until the water rinsed off was clear. All the bags were washed simultaneously to eliminate variability between samples.

Three 0 hour bags were also prepared in exactly the same manner, but were not inserted into the cannulated cows. Afterwards they were washed together with all the bags to determine the 0 hour disappearance of the sample.

After all the samples were washed, they were dried for 72hrs at 60°C and weighed. Sample residue in the bags was carefully removed and pooled for each hour for each individual cow, for determination of neutral detergent fibre (NDF) content. Unwashed grass was also stored for later analysis of NDF content.

The non-linear model, $p = a + b\ (1 - e^{-ct})$ was used to fit the disappearance data and to derive the parameters *a*, *b* and *c* (Ørskov & McDonald,1979), where *p* is the proportionate amount of DM that disappeared in time *t* (hours). The rapidly soluble fraction is represented by *a*, *b* represents the potential degradable fraction and *c* the rate of degradation for time *t* (Ørskov & McDonald, 1979; McDonald, 1981).

e. Body weight and body condition scoring (BCS)

The same procedure was followed as with the production study.

3.4 Analytical Methodologies

3.4.1 Feed sample and pasture sample analyses

a. Moisture

AOAC (2002) Official Method 934.041: Determination of moisture contents of feed.

Clean labeled porcelain crucibles were dried for two hours (or overnight) in an oven at 100°C. The crucibles were then placed in a desiccator and allowed to cool down for 30 minutes after which the weight of each crucible was recorded on a four decimal accurate scale. The scale onto which the crucible was placed was

then zeroed. Two grams of a feed test sample and the pasture test sample was weighed into the crucible, and recorded two the fourth decimal. The crucibles containing the samples were then placed in an oven at 100°C and dried for 24 hours. The crucibles were placed in a desiccator once more and allowed to cool down for 30 minutes which the weight of the dried samples were recorded.

% Moisture = [(dry crucible mass + sample mass – mass of dry sample in crucible) / (sample mass)] x 100 % DM = 100 - % moisture

b. Ash

AOAC (2002) Official Method 942.05: Ash

Two grams of moisture-free feed test sample and pasture sample was weighed into a moisture-free porcelain crucible. The crucible containing the sample was placed into a temperature-controlled furnace at 500°C for six hours. After six hours, the furnace was switched off and allowed to cool down for at least 2 hours (or overnight). The samples were then transferred directly into a desiccator, allowed to cool for 30 minutes and weighed (g) and recorded to the fourth decimal.

% Ash = [(mass of crucible and ash – empty dry crucible) / sample weight] x 100

% Organic matter = 100 - % Ash

c. Crude Protein

AOAC (2002) Method 990.03: Crude Protein in Animal Feed

Apparatus:	LECO FP-528, Protein/Nitrogen Determinator (Leco Corporation, St. Joseph, USA)
Accessories:	502-186 Tin Foil Cups
Sample Weight:	0.1000 g
Calibration Standard:	Alfalfa
Furnace Temperature:	850°C
Protein Factor:	6.25

Procedure:

1. The LECO FP-528 was at all times operated in accordance with manufacturer instructions (i.e. gas supply checked, necessary maintenance of crucibles, filters, reduction heater tubes, etc.).

2. Blanks (gas) were analyzed until a plateau was reached. Three to five additional blanks were analyzed and the blank standard was set using the data sets obtained.

3. For samples with a protein content lower than 20% Alfalfa is used as the calibration standard. Three alfalfa standards (using the Tin Foil Cups) at 0.10g were analyzed. The alfalfa standard should read within 3.96 to 4.04. If drift occurred calibration is needed. Drift was corrected when necessary.

4. An empty tin foil cup was placed on the scale and zeroed. A feed sample and pasture sample (0.1000 g) were weighed to the nearest fourth decimal into the empty tin foil cup. All weights were recorded on the FP 528 software. The foil cup was then closed and twisted, care should be taken to ensure that foil capsule is small enough to easily fall into LECO machine. Foil capsule was placed into the carousel sample tray, at the corresponding number for the recorded sample weight. The sample was combusted and the Nitrogen percentage was obtained.

5. % Crude Protein = % N x 6.25

d. Neutral detergent fibre (NDF)

Neutral detergent fibre was determined as described by Robertson & Van Soest (1981) with the aid of the Fibertech System M, 1020 Hot Extractor (SMM Instruments Pty Ltd., Cape Town, South Africa).

Neutral detergent solution (NDS) preparation:

- Firstly thirty grams of sodium-lauryl-sulphate were dissolved in 500 ml distilled water and was followed by the addition of 10ml 2-Ethoxyethanol.

- Secondly 18.61 g of EDTA ($Na_2EDTA.2H_20$) and 6.81 g of Sodium-borate decahydrate ($Na_2B_4O_7.10H_2O$) were weighed out into a 1 L Erlenmeyer-flask after which 200 ml distilled water was added. The solution was heated and stirred until everything was dissolved. The sodium-lauryl-sulphate solution was then added and stirred.

- Thirdly 4.56 g Di-sodium-hydrogen-phosphate (Na_2HPO_4) were added to 100 ml distilled water and dissolved where after it was added to the Erlenmeyer-flask containing the mixed solution.

- The Erlenmeyer-flask was then filled to volume (1 L) with distilled water.

Procedure:

1. An empty, clean cintered glass crucible was dried for 2 hours (or overnight) in an oven set at 100°C.

2. The crucible was removed from the oven and placed directly into a desiccator and allowed to cool down for 30 minutes. The crucible was then placed on a scale and the weight of the dried crucible was then recorded, to the fourth decimal.

3. The scale was zeroed and one gram of the feed sample and pasture samples were weighed into the glass crucible. The sample weight (WS) was then recorded to the fourth decimal.

4. The crucible (with sample) was placed in the heating unit of the Fibertech apparatus. Care must be taken to ensure that the crucibles are the exact same height and that upon insertion that there are no leaks between the crucibles and the apparatus.

5. The valves were closed and the water-tap opened for cooling purposes of the apparatus.

6. One hundred milliliters of cool NDS reagent was added into the crucible.

7. The heat was increased to 100°C (heat setting number six) until the solution reached boiling point.

8. Then 0.1 ml heat stable α-Amylase (Sigma number A3306) was added into the crucible.

9. The temperature was reduced to 65°C (heat setting number 4) and left to boil for one hour.

10. After one hour the heating unit was turned off and the liquid was filtered from the crucible with the aid of the apparatus' vacuum-system.

11. The sample was washed three times with warm distilled water and then rinsed one time with a little acetone. It is important to rinse off any particulate matter form the tubes of the apparatus into the glass crucible.

12. The crucible containing the feed sample and pasture sample was then removed from the apparatus and placed into a drying oven for 24 hours at 100°C.

13. After 24 hours the crucible was removed from the oven and immediately placed into a desiccator and allowed to cool down for 30 minutes. The weight of the crucible containing the dried feed sample and pasture samples were then recorded (W1).

14. The crucible was placed into a temperature-controlled furnace at 500°C for 6 hours. After 6 hours, the furnace was switched off and allowed to cool down for at least 2 hours (or overnight). It is imperative that the crucibles are allowed to cool down for at least two hours after incineration, to prevent the crucible from damage due to sudden changes in temperature.

15. The sample was then directly transferred into a desiccator, allowed to cool down for 30 minutes, after which the weight of the crucible with the ash (W2) was recorded.

%NDF was calculated as follow: $\%NDF = (W1 - W2) / WS \times 100$

e. Acid detergent fibre (ADF)

Acid detergent fibre was determined as described by Goering & Van Soest (1970) with the aid of the Fibertech System M, 1020 Hot extractor (SMM Instruments Pty Ltd., Cape Town, South Africa).

Acid detergent solution (ADS) preparation:

- Twenty grams of N-Cetyl-N,N,N-Trimethyl Ammonium Bromide (CTAB) was added to 1 L

Standardized H_2SO_4

- Standardized H_2SO_4 was prepared by measuring 28 ml of 98% $H_2SO_4$and filling to volume (1 L) with distilled water

Procedure:

1. An empty, clean cintered glass crucible was dried for 2 hours (or overnight) in an oven set at 100°C.

2. The crucible was removed from the oven and placed directly into a desiccator and allowed to cool down for 30 minutes. The crucible was then placed on a scale and the weight of the dried crucible was then recorded, to the fourth decimal.

3. The scale was zeroed and one gram of the feed sample and pasture samples were weighed into the glass crucible. The sample weight (WS) was then recorded to the fourth decimal.

4. The crucible (with sample) was placed in the heating unit of the Fibertech apparatus. Care must be taken to ensure that the crucibles are the exact same height and that upon insertion that there are no leaks between the crucibles and the apparatus.

5. The valves were closed and the water-tap opened for cooling purposes of the apparatus.

6. One hundred milliliters of cool ADS reagent was added into the crucible.

7. The heat was increased to 100°C (heat setting number six) until the solution reached boiling point. The temperature was then reduced to 65°C (heat setting number 4) and left to boil for one hour.

8. After one hour the heating unit was turned off and the liquid was filtered from the crucible with the aid of the apparatus' vacuum-system.

9. The sample was washed three times with warm distilled water and then rinsed one time with a little acetone. It is important to rinse off any particulate matter form the tubes of the apparatus into the glass crucible.

10. The crucible containing the feed sample and pasture sample was then removed from the apparatus and placed into a drying oven for 24 hours at 100°C.

11. After 24 hours the crucible was removed from the oven and immediately placed into a desiccator and allowed to cool down for 30 minutes. The weight of the crucible containing the dried feed sample and pasture samples were then recorded (W1).

12. The crucible was placed into a temperature-controlled furnace at 500°C for 6 hours. After 6 hours, the furnace was switched off and allowed to cool down for at least 2 hours (or overnight). It is imperative that the crucibles are allowed to cool down for at least two hours after incineration, to prevent the crucible from damage due to sudden changes in temperature.

14. The sample was then directly transferred into a desiccator, allowed to cool down for 30 minutes, after which the weight of the crucible with the ash (W2) was recorded.

%ADF was calculated as follow: %ADF = (W1 – W2) / WS x 100

f. Acid detergent lignin (ADL)

Reagents:

Sulfuric acid (72% by weight)

*S*tandardize reagent grade H_2SO_4 to a specific gravity of 1634 g/L at 20° C. Add 1200g 98% H_2SO_4 to 440 ml H_2O in 1 L MCA volumetric flask. Place the volumetric flask in an ice bath and add H_2SO_4 slowly (can take up to 1 day). Standardize this solution to 1634 g/L at 20° C specific gravity by removing solution and adding H_2O or H_2SO_4 as required.

ADS solution

As prepared in ADF determination (see 3.4.1. e)

Apparatus:

Filtration device: ANKOM Technology

F57 Filter Bags

Heat Sealer

Desiccator

2L & 3L Beaker

Procedure:

1. Label F57 filter bags with a material marker and place in a dry oven at 100°C

2. Bags were placed in a desiccator and allowed to cool for 30 minutes. Bags were then weight (W1) and recorded to the fourth decimal and the scale zeroed (tare).

3. 0.5 g moisture free feed and pasture samples were weighed (W2) and recorded to the fourth decimal and bags were sealed with a heat sealer. One blank bag were also prepared and included in digestion (C1). Sample was spread uniformly inside the filter bag by flicking the bag to eliminate clumping.

4. ADF determinations were performed using Fibre Analyzer.

5. After ADF determinations were performed, the dried bags containing samples were placed into 3L beakers. A sufficient quantity (approximately 250 ml) of 72% H_2SO_4 was added to the beakers, enough to cover all the bags. Bags should be completely dry and at ambient temperature before adding concentrate acid. Moisture may generate heat when reacting with H_2SO_4 and reaction could adversely affect the results.

6. A 2L beaker was placed inside the 3L beaker to keep bags submerged. Bags were rotated and mixed gently every 30 minute by pushing and lifting 2L beaker up and down approximately 30 times.

7. After 3 hours the H_2SO_4 was poured off and rinsed with tap water to remove all acid. This was repeated until pH was neutral, after which the bags was rinsed with approximately 250 ml of acetone for 3 minutes to remove and remaining water. Bags should not be placed in the oven until acetone is completely evaporated.

8. Samples were dried in an oven at 100°C for 2-4 hours. Upon removal bags were placed in a desiccator and allowed to cool for 30

9. Bags were then weighed (W3) and recorded to the fourth decimal.

Calculation:

ADL_{DM}(DM basis) = ((W3 - (W1 x C1)) x 100) / W2 x DM

W1 = Bag tare weight

W2 = Sample weight

W3 = Weight after extraction process

C1 = Blank bag correction (final oven-dried weight/original blank bag weight)

g. Neutral detergent insoluble crude protein (NDICP)

Apparatus: Fibertech System M, 1020 Hot Extractor

LECO FP-528, Protein/Nitrogen Determinator (Leco Corporation, St. Joseph, USA)

Accessories: 502-186 Tin Foil Cups

Sample Weight: 0.1000 g

Calibration Standard: Alfalfa

Furnace Temperature: 850°C

Reagent:

NDS solution

As prepared in NDF determination (see 3.4.1. d)

Procedure:

1. Feed and pasture samples were analyzed for NDF as described in 3.4.1. d.

2. Residue / remaining ADF fraction in crucibles were then analyzed for nitrogen percentage as described in 3.4.1 c

NOTE: Sample size may need to be increased in order to have a large enough NDF fraction for the LECO to work accurately and effectively.

h. Acid detergent insoluble crude protein (ADICP)

Apparatus: Fibertech System M, 1020 Hot extractor

LECO FP-528, Protein/Nitrogen Determinator (Leco Corporation, St. Joseph, USA)

Accessories: 502-186 Tin Foil Cups

Sample Weight: 0.1000 g

Calibration Standard: Alfalfa

Furnace Temperature: 850°C

Reagent:

ADS solution

As prepared in ADF determination (see 3.4.1. e)

Procedure:

1. Feed and pasture samples were analyzed for ADF as described in 3.4.1. e.

2. Residue or remaining ADF fraction in crucibles were then analyzed for nitrogen percentage as described in 3.4.1 c

NOTE: Sample size may need to be increased in order to have a large enough ADF fraction for the LECO to work accurately and effectively.

i. Ether extract (EE)

AOAC (2002) Official Method 920.39: Fat (Crude) or Ether Extract in animal feed.

Reagent:

Diethyl ether

Procedure:

1. Open the tap for the water before switching the heat on. Then switch on the oil bath and fan.

2. Aluminum fat beakers were placed in an oven to dry overnight at 100°C. Moisture free beakers were then placed in a desiccator and allowed to cool for 30 minutes.

3. Beakers were accurately weighed to the fourth decimal.

4. Extraction thimbles were placed on a scale and the scale was tarred.

5. Two grams of the feed and pasture sample was placed in the extraction thimbles, weighed and recorded to the fourth decimal. The thimbles were then plugged with a piece of cotton-wool to prevent the sample from washing out of the thimbles.

6. The aluminum beakers were filled with 50ml of diethyl ether

7. If the oil bath's "ready" light is flashing the thimbles can be placed in the extraction tubes in the correct position.

8. The corresponding aluminum beakers must be placed on the heating element underneath each thimble. The extraction tubes are then lowered to fit tightly on the aluminum beakers.

9. The thimbles were lowered into the aluminum beakers, with the leaver on "boiling" and the small taps on open. The thimbles were then boiled in the diethyl ether for 15min.

10. After 15 minutes the thimbles was raised by placing the leaver on "rinsing" and left for another 30 minutes.

11. The small taps were then closed to capture the diethyl ether and were left to boil for a further 15min.

12. The aluminum beakers were then removed and placed in a dry oven for 2 hours at 100° C.

13. The beakers were removed and placed in a desiccator and allowed to cool for 30 min.

14. Beakers were weighed and recorded to the fourth decimal.

%Fat was calculated as follow:

%Fat = (Mass of aluminum beaker after fat extraction – Mass of aluminum beakers before extraction)/Mass of sample x 100

j. In vitro dry matter digestibility (IVDMD)

Method used is that of Ankom® DAISY^{II} *in vitro* fermentation system (Ankom® Technology Corp., Fairport, NY, USA) as described by them. The buffer solution is modified from Goering & Van Soest (1970).

Apparatus:

Ankom® DAISY^{II} *in vitro* fermentation system (Ankom® Technology Corp., Fairport, NY, USA)

Heat sealer

2l *In vitro* bottles with lids

Dacron bags

pH meter

Cheese cloth

Thermal flasks

Reagents:

Medium

Distilled water (ml)	2250 ml
Macro mineral solution (ml)	1125 ml
Micro mineral solution (ml)	0.5625 ml
Buffer solution (ml)	1125 ml
Tryptose	11.25 g
Rezasurin	5.625 ml

Reducing solution

Cysteine HCl

Potassium OH pellets

Sodium sulphide nonahydrate

Rumen fluid

Reagent preparation:

Medium – To a 5l beaker add 2250 ml of distilled water. Thereafter, add 1125ml of the macro mineral solution (prepared below), 0.5625 ml micro mineral solution (prepared below), 1125 ml buffer (prepared below), 11.25 g tryptose and 5.625 ml rezasurin. A magnetic stir bar was used to slowly dissolve the mixture.

Macro mineral solution – A 2l volumetric flask was filled two thirds with distilled water. 11.4g of Na_2HPO_4, 12.4g KH_2PO_4, 1.18g $MgSO_4.7H_2O$ and 4.44g NaCl was added and dissolved. The Volumetric flask was made to volume.

Micro mineral solution – Into a 100ml volumetric flask 13.2 g $CaCl_2.2H_2O$, 10 g $MnCl_2.4H_2O$, 1 g $CoCl_2.6H_2O$ and 8 g $FeCl_3.6H_2O$ was added. The substances were dissolved in distilled water and the volumetric flask was made to volume.

Buffer solution - A 2l volumetric flask was filled two thirds with distilled water. 8g of NH_4HCO_3 and 70g $NaHCO_3$ was added and dissolved. The Volumetric flask was made to volume.

Rezasurin – Rezasurin is made to a concentration of 1.22g/l.

Reducing solution – The contents of beaker A and Beaker B, as prepared below, was mixed together.

Beaker A – Into a 200 ml volumetric flask, two thirds of distilled water was added. Thereafter 2.5g of Cysteine HCl was added as well as 40 KOH pellets. The chemicals were dissolved and the volumetric flask was made to volume.

Beaker B – Into a 200 ml volumetric flask, two thirds of distilled water was added. To this 3.75g sodium sulphide nonahydrate was added and dissolved. The volumetric flask was made to volume.

Rumen fluid – Rumen content of lactating cannulated dairy cows were removed and placed in two layers of pre-warmed cheese cloth. The cheese cloth was compressed and the rumen fluid was squeezed out into thermal flasks. Flasks are then sealed and transferred to the laboratory. In the laboratory the rumen fluid was filtered through two layers of pre-warmed cheese cloth to remove any further particulate matter. Rumen fluid of was then placed in a mixer and blended at low speed for 30 seconds. The pH of the rumen fluid was then measured. The rumen fluid was placed in a water bath at 39º C, the headspace gassed with CO_2, covered and left till use. It is of utmost importance to not let the rumen fluids cool down, or for them to be exposed to open air for extended periods of time. Preferably rumen fluids should not be collected more than an hour before use.

Procedure:

1. Dacron bags were numbered and placed into a drying oven at 100º C overnight. Bags were removed and placed in a desiccator and allowed to cool down for 30 minutes.

2. Dacron bags were weighed and recorded to the fourth decimal (BW). The scale was then tarred.

3. Into a dacron bag two grams of either concentrate or pasture sample were weighed and recorded (SW).

4. Each dacron bag was then sealed with a heat sealer. Each bag was sealed three times to ensure no sample were lost.

5. Bags were prepared in duplicate. Into a 2l *in vitro* bottle twelve pasture samples were placed (one from each sample) as well as nine concentrate samples (three from each diet). Duplicates were placed into a second bottle.

6. Into each bottle an 1130 ml of medium was added followed by 270 ml of reducing agent. Bottles were capped and placed into a pre-warmed Ankom® DAISY^{II} *in vitro* fermentation system incubator at 39º C to equilibrate and reduce the medium.

7. During this time rumen fluid was collected and prepared.

8. Once the medium had been reduced and the temperature had equilibrated 270 ml of rumen fluid was added to each bottle. Bottles headspace was gassed with CO_2 and for 1 minute, where after it bottles were capped again and placed back into the daisy.

9. The agitate switch was also switched on to insure that bottles turn to agitate samples.

10. Samples were left for 48 hours in the incubator at 39º C and the agitation on.

11. To terminate the incubation process bottles containing samples were emptied into a sink. Bags were flushed with clean cold water until the water became clear.

12. Bags with residue were placed in a drying oven for 24 hours at 100º C.

13. After 24 hours bags were removed from the oven and placed in a desiccator for 30 minutes and allowed to cool down.

14. Thereafter the bags and their residues were weighed and recorded to the fourth decimal (FW).

IVDMD calculation:

Sample weight DM = SW * (DM% / 100) (SWDM)

% IVDMD = (SWDM – (FW – BW) / SW) * 100

k. Metabolisable Energy

Apparatus: CP 500 Bomb Calorimeter

Gross Energy (GE) was determined using the CP 500 Bomb Calorimeter

IVDMD was determined as described above (section 3.4.1 j)

Calculations:

ME = GE x IVDMD x 0.84 for concentrates

ME = GE x IVDMD x 0.81 for pasture (ARC, 1984)

l. Starch analysis

A. Starch gelatinization and hydrolysis method

Modified from Holm *et al*. (1986)

Sample preparation:

Samples were moisture free and milled through a 1mm screen.

Reagents preparation:

0.1M sodium acetate buffer, (pH 4.5)

1. 13.61g of sodium acetate trihydrate was weighed into a 100ml glass beaker.

2. Approximately 30-40 ml of distilled water was added and a magnetic stirrer bar was inserted with no heat to aid in dissolving the sodium acetate.

3. A calibrated hand held pH meter was inserted into the solution

4. Concentrated HCl was added drop wise in order to adjust the pH of the solution to 4.5 while still constantly stirring. If solution becomes too acidic dilute sodium hydroxide could be added to raise the pH.

5. After the desired pH was reached the solution was transferred to a 1l volumetric flask, rinsing the 100ml beaker repeatedly to assure the transfer off all the chemicals.

6. The volumetric flask was then made up to volume.

7. The pH should be rechecked before used

Enzyme:

Heat-stable α-amylase, Sigma Aldrich no. A 3306

Amyloglucosidase, Sigma Aldrich no. A1602

Equipment:

Water baths: 93º C and 60ºC

Funnels

Erlenmeyer flasks

Volumetric flasks: 100ml and 50ml

Glass wool

Aluminum foil

Procedure:

1. 0.2 g of sample was weighed and recorded to the fourth decimal and placed in labeled Erlenmeyer flasks.

2. 20ml distilled water was added to each flask and was stirred with a magnetic stir bar.

3. 100µl heat stable α-amylase was then added to the sample and water and was gently stirred.

4. The Erlenmeyer flasks was covered with foil and placed in a 92-93º C water bath for 1 hour. Flasks was then removed and left to cool for 15 minutes.

5. Samples were filtered through funnels plugged with glass wool into 100ml volumetric flasks. The flasks should be thoroughly rinsed. The 100ml volumetric flasks should be made up to volume.

6. 2ml aliquots where transferred to 50ml volumetric flasks and 8ml of 0.1M sodium acetate buffer was added to each flask

7. 50µl of Amyloglucosidase was also added to each flask and was given a gentle swirl in order to mix.

8. The solution was placed in a 60º C water bath for 30 minutes, and was swirled every 10 minutes.

9. After incubation flasks were made to volume (50ml) and were ready for assay for glucose.

B. Glucose analysis method

Modified from Karkalas (1985)

Sample preparation:

Gelatinization and hydrolysis of starch in the sample as described above.

Reagents:

Glucose oxidase-peroxidase reagent

Glucose, ACS grade

Distilled water (dH_2O)

Equipment:

Vortex

Volumetric flasks: 100ml

Screw cap test tubes: 16 x 150mm

Water bath: 40º C

Spectrophotometer: λ = 505nm

Reagent preparation:

1. 300ml of distilled water was added to a 500ml volumetric flask and 4.55g of sodium phosphate, dibasic anhydrous (Na_2HPO_4, FW = 141.96) as well as 2.5g potassium phosphate, monobasic (KH_2PO_4, FW =136.09) was added to the water. Flask was swirled to dissolve chemicals.

2. Once chemicals were completely dissolved 0.50 g phenol (C_6H_5OH) and 0.075g 4-aminoantupyrine ($C_{11}H_{13}N_3O$, FW 203.2) was added to the solution and dissolved. The 4-aminoantupyrine is light sensitive.

3. To this solution 0,1411g glucose oxidase and 0.0479g peroxides was added and swirled gently.

4. The volumetric flasks was filled to volume (500ml), mixed and filtered through a Whatman GF/A microfibre glass filter paper into an amber bottle.

5. GOP should be stored at 4º C

Glucose standard solution preparation:

1. One gram of ACS grade glucose was weighed and recorded to the fourth decimal.

2. Glucose was added to a 100ml volumetric flask, dissolved and made to volume

3. The glucose concentration was calculated using the following formula:

Glucose stock µg/ml = [(Glucose g) x (DM% of Glucose) x (1 000 000 µg/g)]/100ml

4. Aliquots of the glucose standard solution was transferred to 100ml volumetric flasks

Dilutions for glucose standard solutions:

0 µg/ml = dH2O = standard blank

25 µg/ml = 0.250 ml stock/100ml dilution

50 µg/ml = 0.500 ml stock/100ml dilution

75 µg/ml = 0.750 ml stock/100ml dilution

100 µg/ml = 1.000 ml stock/100ml dilution

5. Volumetric flask was brought to volume (100ml).

Procedure:

1. One milliliter of each of the glucose standard solutions as well as the samples prepared in the gelatinization and hydrolysis of starch were transferred to each test tube.

2. Five milliliter of the prepared GOP reagent was added into each tube and vortexed.

3. Tubes were capped, placed in a tube rack and incubated in a water bath at 40º C for 45 minutes.

4. After 45 minutes the tubes were removed and left to cool to room temperature in a dark place.

5. Solutions were transferred to spectrophotometer vials and the absorbance was measured at λ = 505nm

6. The spectrophotometer was zeroed using the sample blanks for samples and standard blank for stock standards.

Calculations:

1. The concentration of glucose in the standard solution was calculated using the following formula:

Glucose µg/ml = [Glucose stock solution, µg/ml) x Va]/Vs

where,

Va = aliquot volume of stock solution (0, 0.25, 0.5, 0.75 or 1.00ml in above example)

Vs = the final dilution volume that the Va was diluted to

2. A graph was constructed with glucose concentration of the standard solutions (µg/ml) on the y axis, against absorbance values (λ = 505nm) of the standard solutions on the x axis. The regression was calculated on the basis of y = mx + c and took the following form:

Glucose µg/ml = value of slope x Absorbance + value of intercept.

3. The regression equation was then used to calculate the glucose (µg/ml) concentration of each sample.

4. The starch percentage of the sample was calculated as glucose (µg/ml) x 0.9

5. Dilutions were corrected for.

3.4.2 Rumen liquor samples

a. Volatile fatty acid concentrations

Rumen liquor samples (30 ml) were stored frozen at minus 10°C pending analysis.

Sample preparation:

Modified from Siegfried *et al*. (1984).

The calcium hydroxide solution (CHS) and the cupric sulfate solution (CSR) deproteinized the rumen liquor samples as well as removed the sugars. The resulting solution was a fairly clean one, consisting of fermentation products such as nonvolatile and VFA and short chain alcohols. Samples were analyzed for VFA via gas-liquid chromatography.

Reagents Preparation:

1. Calcium hydroxide solution (CHS) – 52.9 g of $Ca(OH)_2$was weighed into a 250 ml Erlenmeyer flask. A large stir bar and 200 ml of ultra-pure water were then added. The solution was suspended by inverting the stoppered flask several times. During the dispensing of the reagent a stir plate was used in order to maintain a homogenous slurry while pipetting.

2. Cupric sulphate solution (CSR) – 50.0 g of $CuSO_4.5H_20$ was weighed into a 500 ml volumetric flask and was dissolved in 400 ml of ultra-pure water. Two grams of crotonic acid (2-butenoic acid, Sigma Aldrich nr. C 4630) was then added to the solution. The solution was then mixed and the volumetric flask was made to volume (500 ml) with distilled water.

Procedure:

1. A 1.5 ml rumen fluid sample was transferred into a 1.7 ml centrifuge tube and was centrifuged for ten minutes at 12000 x g.

2. There after 600 µl of the supernatant was transferred to a duplicate empty 1.7 ml centrifuge tube. 600 µl of CHS and 300 µl of CSR were added to each tube. The tubes were then capped, vortexed and frozen.

3. The tubes were then thawed and centrifuged for 10 minutes.

4. Once more a 1000 µl of supernatant was transferred to a clean 1.7 ml centrifuge tube containing 28 µl of concentrated H_2SO_4. The tubes were then capped and frozen again.

5. The tubes were then thawed after which it was frozen a final time.

6. Tubes were thawed and centrifuged for 10 minutes for a final time.

7. The supernatant was transferred to HPLC vials, capped and stored in a refrigerator.

Notes:

1. 1.5ml centrifuge caps may pop as a result from freezing 1.5 ml samples in 1.5 ml tubes therefore 1.7 ml centrifuge tubes should be used to prevent loss of sample due to this.

2. During step 3 when samples are being centrifuged, sulphuric acid should be pipetted into the clean 1.7 ml tubes (step 4) to prevent prolonged exposure of the plastic to concentrated sulphuric acid. Prolonged exposure will damage the tubes.

3. The amount of supernatant transferred in steps 4 and 7 is not critical. More importantly care should be taken to obtain a clean supernatant, free of pelleted solids, rather than getting a quantitative transfer. Use of the internal standard crotonic acid (added with the CSR) allows adjustment of concentrations from samples of varying volumes transferred at steps 4 and 7.

HPLC method

HPLC solvent:

3.5l of ultra pure water was added to a 4l Erlenmeyer flask. 1.66ml of concentrated H_2SO_4 was also added as well as 0.40g of ethylenediaminetetraacetic acid (EDTA). This was heated on a plate for 2 min and was then cooled overnight while stirring in order to completely dissolve the EDTA. The Erlenmeyer flask was then made to volume with ultrapure water. The solution was vacuum filtered through a 0.2µm membrane. Filtrate is then transferred to HPLC reservoir bottle.

Apparatus:

Focus GC Model (Thermo Finnigan, Austin, Texas, USA)

Conditions:

Internal standard: Crotonic acid

Column: BPX21, 30m x 0.25mm ID, 0.25µm film

Initial temperature:	60º C, 5 min
Rate 1:	7ºC/min
Final temperature:	160º C
Detector:	FID, 260º C
Injector:	220º C
Split flow:	20:80
Split ratio:	80
Carrier gas:	Hydrogen, 1ml/min
Injection volume:	1µL
Run time:	35 min

Volatile fatty acid standard solution:

1. A 100ml volumetric flask was filled with 80 ml ultra-pure water after which the following was added:

Acetic acid	57.3 µl
Propionic acid	74.6 µl
Iso-butyric acid	92.8 µl
Butyric acid	91.8 µl
Iso-valeric acid	54.5 µl
Valeric acid	108.4 µl
Concentrated Sulphuric acid (H_2SO_4)	41.5 µl

2. The solution was then diluted to a total volume of 100 ml with ultra-pure water

Note:

Under these conditions, the appearance of peaks should be as follows:

Acetic acid	13.8 min
Propionic acid	16.5 min

Iso-butyric acid	18.8 min
Butyric acid	20.5 min
Iso-valeric acid	24.0 min
Valeric acid	29.5 min

b. Rumen ammonia nitrogen concentration determination

Modified from Broderick & Kang (1980).

Reagent preparation:

1. Phenol reagent – 0.05g of sodium nitroferricyanide ($Na_2Fe(CN)_5(NO)2H_20$) and 11ml of Phenol, 90% w/v (C_6H_5OH) was dissolved in 800ml of deionized water in a 1l volumetric flasks. The volumetric flasks was brought to volume and stored in an amber glass bottle.

2. Hypochlorite reagent – 5g sodium hydroxide (NaOH) and 20.07g of disodium phosphate (Na_2HPO_4) was dissolved in 800ml of deionized water in a 1l volumetric flask. 50ml Commercial bleach (5.25% sodium hypochlorite) was then added and the volumetric flask was brought to volume. The solution was stored in amber polyethylene bottle.

Standard solution preparation:

1. A 0.1N HCl solution was prepared.

2. The stock standard was prepared by weighing 0.6607g of ammonium sulfate ($(NH_4)_2SO_4$) and dissolved it in a 100ml volumetric flask filled two thirds with 0.1N HCl. Thereafter volumetric flask was made to volume with 0.1N HCl.

3. Aliquots of the stock standard solution was transferred to 100ml volumetric flasks

Dilutions for working standard solutions:

0 mM = 0.1 N HCl = standard blank

1 mM =1.000 ml stock/100ml dilution

2 mM = 2.000 ml stock/100ml dilution

4 mM = 4.000 ml stock/100ml dilution

5 mM = 5.000 ml stock/100ml dilution

8 mM = 8.000 ml stock/100ml dilution

10 mM = 10.000 ml stock/100ml dilution

15 mM = 15.000 ml stock/100ml dilution

20 mM = 20.000 ml stock/100ml dilution

4. Volumetric flasks were brought to volume (100ml) with 0.1N HCl.

Procedure:

1. 50µl of working standards and rumen fluid samples, control sample, and 0.1 N HCl (blank) were transferred into test tubes in duplicate.

2. 2.5 ml of the phenol reagent was added and mixed

3. Thereafter 2ml of hypochlorite reagent was added and mixed.

4. Tubes were placed in a rack and incubated in a water bath for 95º C for 5 minutes.

5. Tubes were removed and placed in an ice bath for 5 to 7 minutes.

6. Solutions were transferred to spectrophotometer vials and the absorbance was measured at λ = 630nm

7. The spectrophotometer was zeroed using the sample blanks for samples and standard blank for working standards.

Calculations:

A graph was constructed with the mM NH_3/l concentration of the working standard solutions on the x axis, against absorbance values (λ = 630nm) of the working standard solutions on the y axis. The regression was calculated on the basis of y = mx + c and took the following form:

The regression equation was then used to calculate the ammonia concentration of each sample in mM NH_3/l. The mM NH_3/l was then converted to mg NH_3/dl.

3.5 Statistical analysis

3.5.1 Production data

The experimental design was a randomized complete block design with 3 treatments (high starch, medium starch and low starch) repeated in 15 blocks (cows). An appropriate analysis of variance (ANOVA) was

done; the residuals (observed value-fitted value) of each variable were subjected to Shapiro Wilk's test for Non Normality (Shapiro & Wilk, 1965). A Student's t test was used to compare the treatment means at significant level of 5% (p=0.05) (SAS Institute Inc. 2008). Furthermore LSMEANS was used to calculate a pooled standard error of treat means (SAS Institute Inc. 2008).

3.5.2 Rumen data

The experiment was executed according to a cross-over design with two treatments (high starch and low starch) and the data were subjected to a main effects ANOVA. The residuals (observed value fitted value) of each variable were subjected to a Shapiro Wilk's test for Non Normality (Shapiro & Wilk, 1965). A Student's *t*-Test was used to compare treatment means and significance was declared at $P <= 0.05$ (SAS Institute Inc. 2008). Furthermore, LS Means was used to compare treatments and giving a pooled standard error (SAS Institute Inc. 2008).

3.5.3 In Sacco data

In sacco data were fitted to the non-linear model, $p = a + b\ (1 - e^{-ct})$ (Ørskov & McDonald, 1979) using SAS, to derive the *a, b* and *c* parameters. Data were analized according to a Main Effects ANOVA with main effects being animal, treatment and period. A Student's *t*-Test was used to compare treatment means and significance was declared at $P <= 0.05$.

3.6 References

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CHAPTER 4

Results

4.1 Pasture and concentrate intake

A rising plate meter (RPM) was used to estimate pasture dry matter (DM) yield for the pasture above 3cm height. A linear regression (Figure 4.1) was developed to estimate pasture dry matter intake (DMI) and to allocate sufficient herbage per cow per day. The regression is in the form of $y = mx + c$, where y is the DM yield and x is the height on the disk meter. The regression was calculated to be $y = 73.64x + 29.56$ and explains 57.9 percent of the variation obtained in sampling.

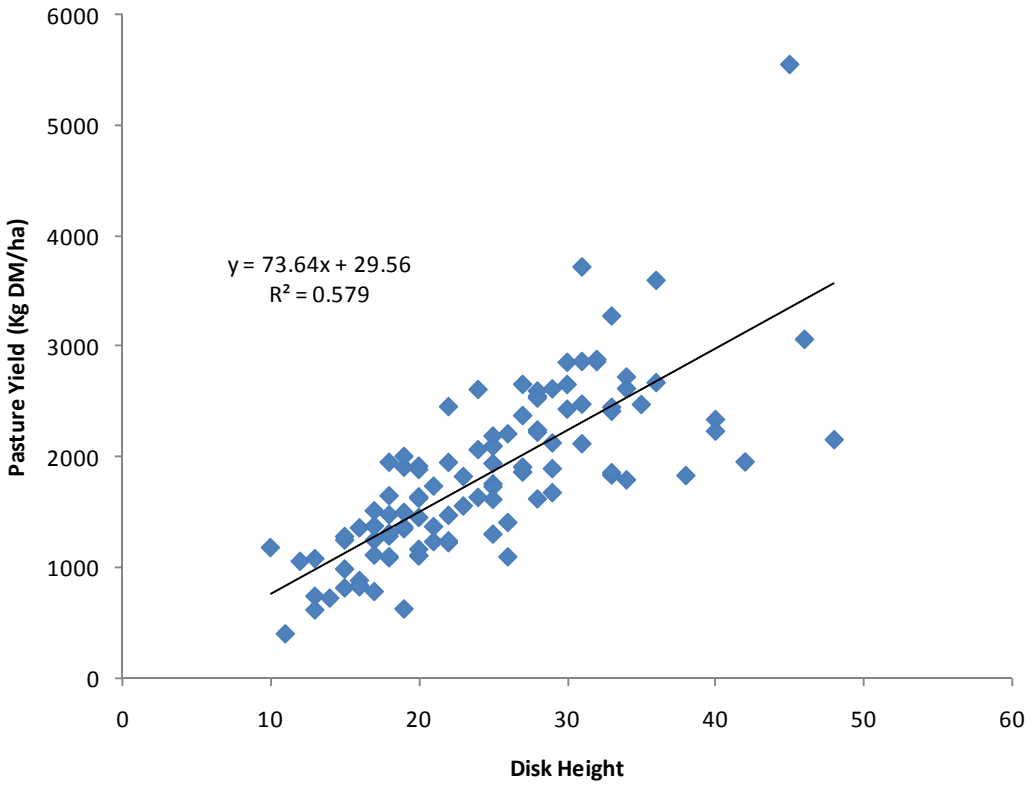

Figure 4.1 The relation between the rising plate meter height and dry matter yield of kikuyu/ryegrass pasture from pasture used for the duration of the study, and the regression developed for this relationship

The regression developed and illustrated in Figure 4.1 was used to allocate pasture to cows (Table 4.1). Cows were allocated pasture at 12.9kg DM/cow/day above 3cm level. Cows were allocated pasture when at an average height of 13.9cm, or 27.75 ± 6.56 (n = 105) on the RPM. This amounted to 2073 ± 483.3kg DM/ha (n = 105) for the pasture available above 3cm level. Post graze pasture height over the duration of the study was 11.40 ± 2.60 (n = 105) on the RPM which amounted to 5.7cm in height. Pasture intake was calculated to be 6.5kg DM/cow/day using the RPM. Cows were fed 6kg of concentrate per day on an as is basis which amounted to 5.3kg DM/cow/day.

Table 4.1 Mean daily pasture allocation, pasture intake prediction and mean estimated daily intake of pasture by dairy cows during the study as determined with the rising plate meter.

Parameter	kg DM/cow/day
Pasture allocated	12.9
Pasture Intake	6.5
Concentrate intake	5.3

DM – Dry matter

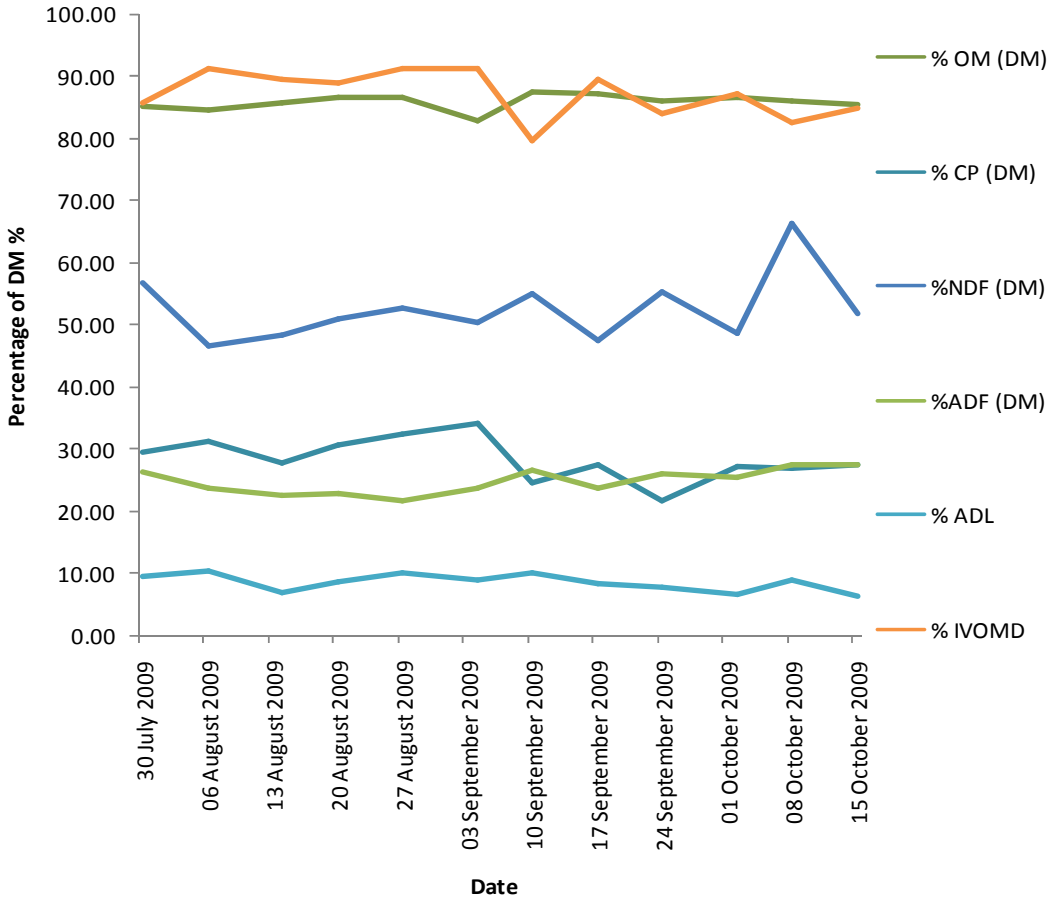

Figure 4.2 A graphic representation of the different pasture quality parameters over the duration of the study (30 July 2009 to 15 October 2009).

Table 4.2 Composition of concentrates and pasture fed to Jersey cows grazing kikuyu/ryegrass pasture during the study in spring (n = 6 for concentrate, n = 12 for pasture)

Nutrient [1]	Concentrate [2]			Pasture
	High starch	Medium Starch	Low starch	
DM (g/kg)	880 ± 4.7	874 ± 2.7	869 ± 3.9	147 ± 18.1
Ash (g/kg DM)	75.3 ± 0.7	85.1 ± 4.1	95.1 ± 1.9	135 ± 12.9
OM (g/kg DM)	925 ± 0.7	915 ± 4.1	905 ± 1.9	865 ± 12.9
CP (g/kg DM)	146 ± 4.8	140 ± 1.1	143 ± 1.5	259 ± 34.2
EE (g/kg DM)	37.6 ± 1.3	50.7 ± 3.2	53.5 ± 0.7	44.7 ± 10.1
NDF (g/kg DM)	186 ± 49.4	263 ± 31.6	322 ± 17.7	541 ± 54.3
NDIN (g/kg NDF)	17.3 ± 3.4	15.1 ± 0.7	13.5 ± 0.4	25.5 ± 5.6
ADF (g/kg DM)	59.8 ± 6.2	90.3 ± 15.4	100 ± 3.2	261 ± 19.8
ADIN (g/kg ADF)	32.3 ± 6.7	20.4 ± 1.7	13.1 ± 1.4	9.90 ± 1.5
Hemicellulose (g/kg DM)	126 ± 48.9	172 ± 40.3	222 ± 20.2	280 ± 44.9
ADL (g/kg DM)	13.6 ± 0.8	18.3 ± 1.3	28.7 ± 6.8	80.3 ± 13.7
IVOMD (g/kg DM)	938 ± 3.3	872 ± 0.5	836 ± 3.4	846 ± 38.1
Starch (g/kg DM)	517 ± 9.3	427 ± 16.7	371 ± 9.8	-
Starch:Hemicellulose	4.09	2.47	1.67	-
GE (MJ/kg)	15.3 ± 0.08	15.5 ± 0.01	15.6 ± 0.07	16.6 ± 0.16
ME MJ/kg DM	12.04 ± 0.05	11.36 ± 0.01	10.95 ± 0.05	11.37 ± 0.11

[1] DM – Dry matter; OM – Organic matter; CP – Crude protein, EE – Ether extract; NDF – Neutral detergent fibre; NDIN – Neutral detergent insoluble nitrogen; ADF – Acid detergent fibre; ADIN – Acid detergent insoluble nitrogen; ADL – Acid detergent lignin; IVOMD – *In vitro* organic matter digestibility; GE – Gross energy; ME – Metabolisable energy.
[2]High starch: Dairy concentrate containing 80% maize; Medium starch: Dairy concentrate containing 40% maize; Low starch: Dairy concentrate containing 20% maize.

Figure 4.2 illustrates the change in pasture quality over the course of the study. From the end of July to the end of October pasture neutral detergent fibre (NDF) content increased slightly while pasture crude protein (CP) content decreased. Composition of concentrates and pasture is shown in Table 4.2. Crude protein content was similar for all three diets at 143 g/kg DM and high in pasture at 259 g/kg DM. NDF increased from 186 g/kg DM in the high starch diet to 322 g/kg DM in the low starch diet. Starch content decreased from 517 g/kg DM in the high starch diet to 371 g/kg DM in the low starch diet. Similarly the metabolisable energy (ME) content of the high starch diet was the highest at 12.04 MJ ME/kg DM and the low starch diet the lowest at 10.95 MJ ME/kg DM. The hemicellulose content of the high starch diet was low and consistently increased to almost double in the low starch diet. This combined with the decrease in the starch content between the supplements led to a decrease in the starch to hemicellulose ratio from 4.09 in the high starch supplement to 1.67 in the low starch diet.

4.2 Rumen study

Rumen parameters are shown in Table 4.3. The total volatile fatty acid concentration between the two treatments differed significantly ($P < 0.05$) with the high starch diet (high starch) being the highest at 122 mM/L and the low starch diet found to be 113 mM/L. The acetic acid, propionic and butyric acid were the highest ($P < 0.05$) in the high starch diet. Iso-valeric and valeric acid both showed no difference to treatments although in the high starch diet group there was a tendency towards a higher concentration in both. The acetate to propionate ratio did not differ between the two treatments. Rumen ammonia nitrogen (NH_3-N) differed significantly between the two treatments, with the high starch diet having the higher concentration. No difference was found in the mean ruminal pH between the two treatments.

Table 4.3 Average daily ruminal volatile fatty acids, rumen NH_3-N and pH measurements of ten cannulated high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high or low starch concentrates during October (n = 10).

Parameter	Treatment [1]		SEM [2]	P
	High starch	Low starch		
Total VFA (mM/L)	122[a]	113[b]	1.92	0.01
Acetic acid (mM/L)	87.7[a]	82.6[b]	1.72	0.05
Propionic acid (mM/L)	19.0[a]	17.3[b]	0.368	0.01
Butyric acid (mM/L)	11.9[a]	10.4[b]	0.281	0.01
Iso-valeric acid (mM/L)	1.21[a]	0.99[b]	0.025	0.01
Valeric acid (mM/L)	1.29	1.08	0.071	0.07
Acetate : Propionate	4.90	4.99	0.102	0.56
NH_3-N (mg/dL)	21.2[a]	18.8[b]	0.687	0.04
pH	6.05	6.08	0.031	0.47

[1] High starch: Dairy concentrate containing 80% maize; Dairy concentrate containing 20% maize.
[2] Standard error of mean
[a, b] Means in the same row with different superscripts differ ($P < 0.05$)

Figure 4.3 illustrates the pattern between volatile fatty acids (VFA), rumen ammonia nitrogen and rumen pH. As illustrated in Figure 4.3 it is clear that rumen VFA and rumen pH are indirectly proportional. Rumen NH_3-N increased constantly with the high starch diet. With the low starch diet the rumen ammonia followed a similar trend although the concentration of rumen NH_3-N was lower at 12h00. Although the rumen NH_3-N was not exactly similar to the volatile fatty acid trend, it appears that the diurnal pattern is the inverse to that of rumen pH.

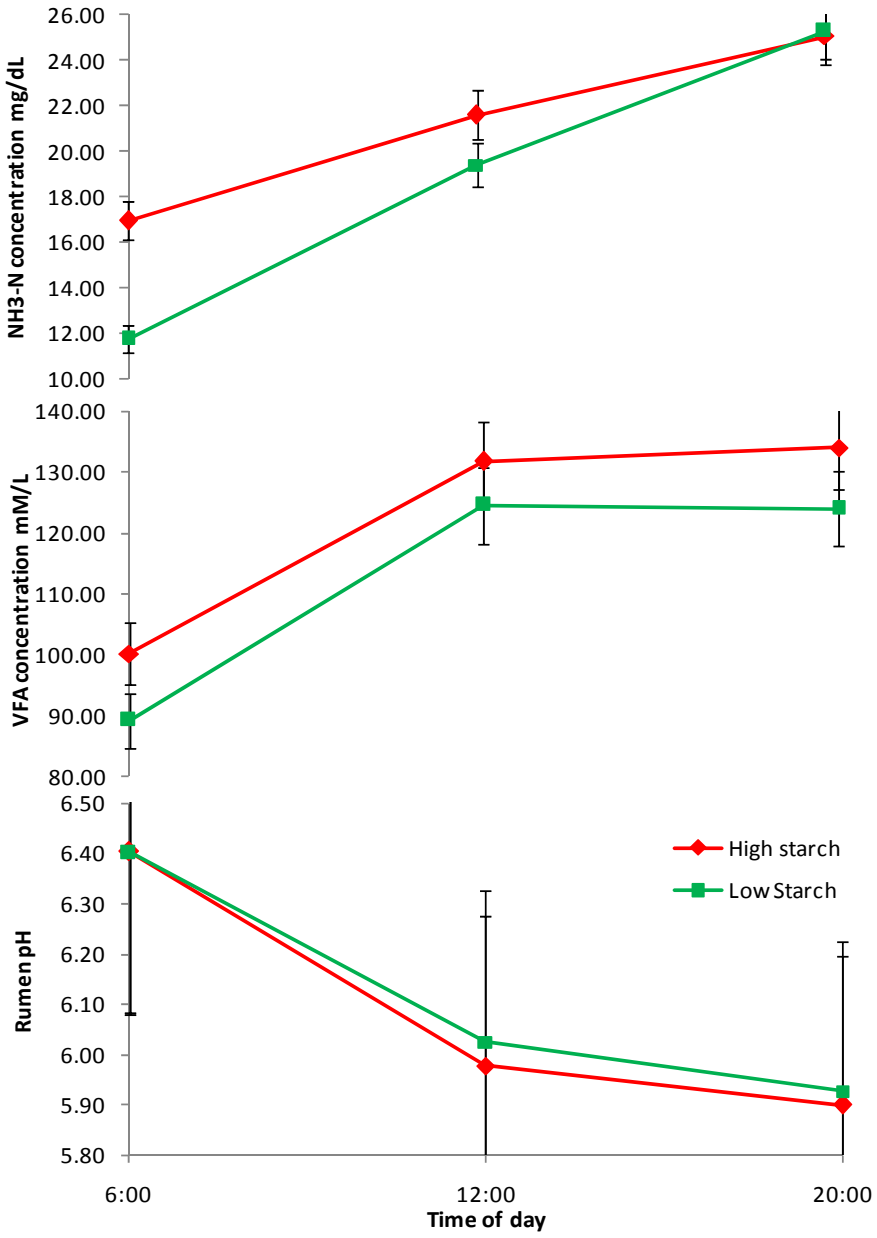

Figure 4.3 The ruminal pH, rumen volatile fatty acid concentration (mM/L) and rumen NH_3-N concentration (mg/dL) of ten cannulated high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high or low starch concentrates during October.

4.2.1 Volatile Fatty acids

Table **4.4** shows the rumen volatile fatty acid concentration at different times for the two different treatments and is illustrated in Figure 4.4. There were no significant differences for volatile fatty acid concentration at both 06h00 and 12h00. The high starch diet had a higher volatile fatty acid concentration at 20h00 than the low starch diet. The daily average rumen volatile fatty acid concentration was higher ($P < 0.05$) in cows fed the high starch concentrate compared to the low starch concentrate by 9.37mM/L.

Table 4.4 Average ruminal volatile fatty acid concentration (mM/L) measured at 06h00, 12h00 and 20h00 of ten cannulated high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high or low starch concentrates during October (n = 10).

Time	Treatment [1]		SEM [2]	P
	High starch	Low starch		
06h00	100	89	4.19	0.10
12h00	132	125	4.22	0.26
20h00	134[a]	124[b]	3.12	0.05
Daily average	122[a]	113[b]	1.92	0.01

[1]High starch: Dairy concentrate containing 80% maize; Dairy concentrate containing 20% maize.
[2] Standard error of mean
[a, b] Means in the same row with different superscripts differ ($P<0.05$).

The volatile fatty acid concentrations for the two separate periods are shown in Table 4.5. Results were similar in both periods for the high starch treatment. Results were more variable between the two periods for the low starch diet. Volatile fatty acid concentration was similar at 06h00 but for Period 2 the 12h00 was 20mM/L higher than that of Period 1. The 20h00 concentrations for the low starch treatment were 11mM/L higher in Period 1. The variation between the two periods for the high starch treatment was less, and never varied more than 6mM/L between the two periods for a specific time. This variation is illustrated in Figure 4.4. There was a significant difference between the two treatments in Period 1 at 12h00, with the high starch found to have a higher volatile fatty acid concentration. In Period 2 there was no significant difference at any time.

Table 4.5 Average ruminal volatile fatty acid concentration (mM/L) measured at 06h00, 12h00 and 20h00 of ten cannulated high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high or low starch concentrates during October for period 1 and period 2 (n = 5).

Time	Treatment [1]		SEM [2]	P
	High starch	Low starch		
Period 1				
06h00	102	88	5.86	0.13
12h00	129[a]	112[b]	5.03	0.05
20h00	136	130	5.23	0.36
Daily average	**123[a]**	**110[ab]**	4.08	0.06
Period 2				
06h00	98.1	90.3	7.16	0.46
12h00	134	137	6.47	0.79
20h00	131	119	6.57	0.20
Daily average	**121**	**115**	4.83	0.40

[1] High starch: Dairy concentrate containing 80% maize; Dairy concentrate containing 20% maize.
[2] Standard error of mean
[a, b] Means in the same row with different superscripts differ (P < 0.05).

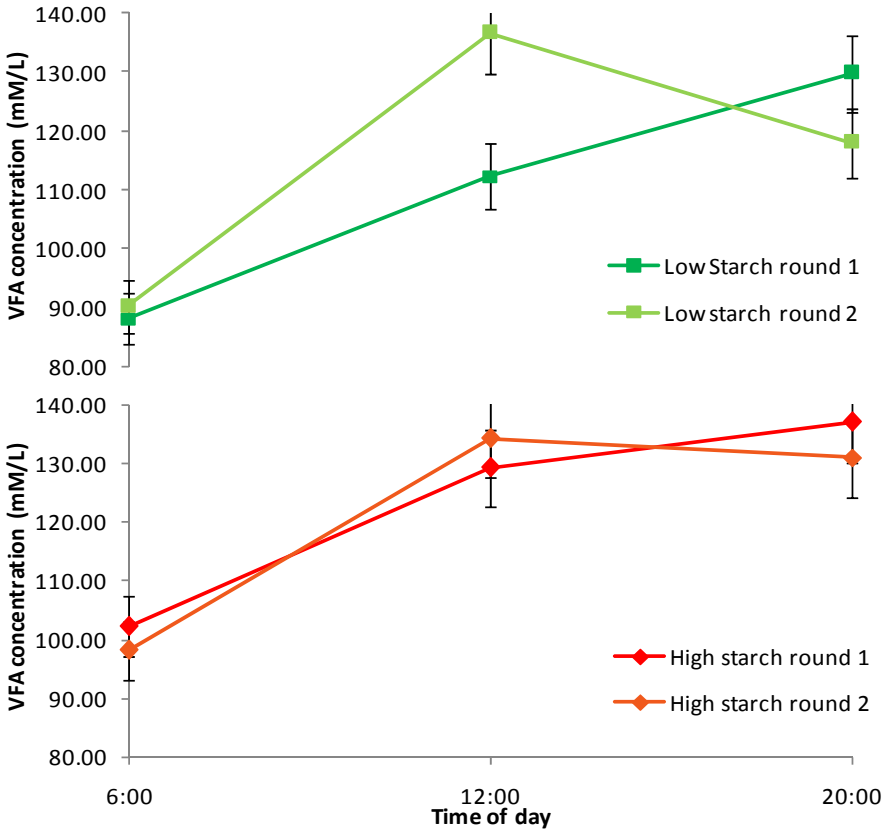

Figure 4.4 The diurnal variation in ruminal volatile fatty acid concentration between two treatments and between two periods of high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high or low starch concentrates during October

Table 4.6 shows the rumen acetic acid concentration at different times for the two treatments. The daily average of the high starch diet was 5.59 mM/L higher than that of the low starch diet and the difference was significant. There was no difference at 20h00, although the high starch diet did tend to have a higher concentration. There were no significant differences for the acetic acid concentrations at 06h00 and 12h00.

Table 4.6 Average ruminal acetic acid concentration (mM/L) measured at 06h00, 12h00 and 20h00 of ten cannulated high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high or low starch concentrates during October (n = 10).

Time	Treatment [1]		SEM [2]	P
	High starch	Low starch		
06h00	73.9	66.8	3.12	0.14
12h00	95.3	91.3	3.33	0.42
20h00	94.0	88.3	1.91	0.07
Daily average	87.7[a]	82.2[b]	1.72	0.05

[1]High starch: Dairy concentrate containing 80% maize; Dairy concentrate containing 20% maize.
[2] Standard error of mean
[a, b] Means in the same row with different superscripts differ (P < 0.05)

Table 4.7 shows the rumen propionic acid concentration at three different times for the two treatments. The daily average for the propionic acid concentration was significantly higher on the high starch treatment compared to the low starch concentrate treatment, with a difference of only 1.75 mM/L. There was no significant differences for the propionic acid concentration at either of the three sample times 06h00, 12h00 and 20h00.

Table 4.7 Average ruminal propionic acid concentration (mM/L) measured at 06h00, 12h00 and 20h00 of ten cannulated high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high or low starch concentrates during October (n = 10).

Time	Treatment [1]		SEM [2]	P
	High starch	Low starch		
06h00	13.6	12.0	0.717	0.16
12h00	20.2	18.9	0.616	0.17
20h00	23.3	20.9	0.955	0.12
Daily average	19.0[a]	17.3[b]	0.368	0.01

[1]High starch: Dairy concentrate containing 80% maize; Dairy concentrate containing 20% maize.
[2] Standard error of mean
[a, b] Means in the same row with different superscripts differ (P < 0.05)

Table 4.8 shows rumen butyric acid concentration at three different times for the two different treatments. The daily average butyric acid concentration for the high starch treatment was also significantly higher than that of the low starch treatment with a difference of 1.49 mM/L. There were no significant differences for butyric acid concentration at 06h00, but there was a tendency for the high starch treatment to have a higher concentration. The same tendency was found in the 20h00 sample. A significant difference was found

between the two treatments in the 12h00 sample with the high starch treatment having a higher butyric acid concentration.

Table 4.8 Average ruminal butyric acid concentration (mM/L) measured at 06h00, 12h00 and 20h00 of ten cannulated high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high or low starch concentrates during October (n = 10).

Time	Treatment [1]		SEM [2]	P
	High starch	Low starch		
06h00	9.61	8.21	0.502	0.08
12h00	13.0[a]	11.4[b]	0.364	0.02
20h00	13.0	11.5	0.503	0.07
Daily average	11.9[a]	10.4[b]	0.281	0.01

[1]High starch: Dairy concentrate containing 80% maize; Dairy concentrate containing 20% maize.
[2] Standard error of mean
[a, b] Means in the same row with different superscripts differ (P < 0.05)

As indicated in Table 4.9 the acetate to propionate ratio did not differ for any of the three times between the two treatments, neither did the average. The acetate plus butyrate to propionate ratio did also not differ between the two treatments. The acetate to propionate ratio was the highest at 06h00 and almost 1.5 units lower at 20h00.

Table 4.9 Molar proportions of ruminal acetate to propionate and acetate plus butyrate to propionate, of ten cannulated, high yielding Jersey cows grazing kikuyu/ryegrass pasture and fed 6kg (as is) of high or low starch concentrates during spring(n =10).

Ratio	Treatment [1]		SEM [2]	P
	High starch	Low starch		
Acetate : Propionate 06h00	5.73	5.71	0.157	0.91
Acetate : Propionate 12h00	4.80	4.92	0.094	0.41
Acetate : Propionate 20h00	4.16	4.33	0.105	0.28
Acetate : Propionate Avg	4.90	4.99	0.102	0.56
Acetate + Butyrate : Propionate	5.39	5.47	0.109	0.61

[1]High starch: Dairy concentrate containing 80% maize; Dairy concentrate containing 20% maize.
[2] Standard error of mean
[a, b] Means in the same row with different superscripts differ (P < 0.05).

Table 4.10 shows the percentage of each volatile fatty acid in the rumen as a percentage of the total VFA. There were no differences between treatments for any VFA except for valeric acid which had a significantly higher percentage in the high starch treatment. Iso-valeric acid concentration also tended to be higher in the high starch treatment.

Table 4.10 Ruminal percentages of acetic acid, propionic acid, butyric acid, valeric acid and iso-valeric acid of ten cannulated high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high or low starch concentrates during October (n =10).

Ratio	Treatment [1]		SEM [2]	P
	High starch	Low starch		
Total VFA (mM/L)	122[a]	113[b]	1.92	0.01
Acetic acid %	71.9	73.0	0.015	0.14
Propionic acid %	15.7	15.4	0.004	0.75
Butyric acid %	9.73	9.23	0.002	0.19
Valeric acid %	1.06[a]	0.95[b]	0.0003	0.02
Iso-valeric acid %	0.99	0.87	0.0007	0.06

[1]High starch: Dairy concentrate containing 80% maize; Dairy concentrate containing 20% maize.
[2] Standard error of mean
[a, b] Means in the same row with different superscripts differ (P < 0.05).

4.2.2 Rumen Ammonia-nitrogen

Table 4.11 shows the rumen NH_3-N concentrations at different times. There was a significant difference in the rumen ammonia content of animals at 06h00 with the high starch treatment having the highest concentration. There where however no differences at 12h00 and 20h00. There was a significant difference in the daily average for rumen NH_3-N concentration between the two treatments with the high starch treatment being the higher of the two.

Table 4.11 Average ruminal ammonia-N concentration (mg/dL) measured at 06h00, 12h00 and 20h00 of ten cannulated high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high or low starch concentrates during October (n = 10).

Time	Treatment [1]		SEM [2]	P
	High starch	Low starch		
06h00	17.0[a]	11.7[b]	1.316	0.02
12h00	21.6	19.4	1.288	0.26
20h00	25.1	25.3	0.669	0.80
Daily average	21.2[a]	18.8[b]	0.687	0.04

[1]High starch: Dairy concentrate containing 80% maize; Dairy concentrate containing 20% maize.
[2] Standard error of mean
[a, b] Means in the same row with different superscripts differ (P < 0.05)

Table 4.12 shows that there was a significant difference in rumen NH_3-N concentration at 06h00 during Period 1 of sampling, with the high starch treatment being higher. There were no further differences for Period 1. There was a tendency in the high starch treatment to have a higher average for rumen NH_3-N concentrations. In Period 2 there were no differences with almost identical values and high P values. Concentrations for Period 1 were lower than that of Period 2, especially for the low starch treatment.

Table 4.12 Average ruminal ammonia-N concentration (mg/dL) measured at 06h00, 12h00 and 20h00 of ten cannulated high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high or low starch concentrates during October of period 1 (n = 5).

Time	Treatment [1]		SEM [2]	P
	High starch	Low starch		
Period 1				
06h00	15.2[a]	7.38[b]	1.18	0.01
12h00	18.0	11.8	2.62	0.13
20h00	26.3	25.9	1.61	0.87
Daily average	**19.8**	**15.0**	**1.61**	**0.07**
Period 2				
06h00	18.7	16.1	3.04	0.57
12h00	25.2	27.0	1.66	0.47
20h00	23.8	24.7	2.23	0.79
Daily average	**22.6**	**22.6**	**1.80**	**0.99**

[1]High starch: Dairy concentrate containing 80% maize; Dairy concentrate containing 20% maize.
[2] Standard error of mean
[a, b] Means in the same row with different superscripts differ (P < 0.05)

4.2.3 Rumen pH

The average rumen pH as well as the pH at three different times during the day is illustrated in Table 4.13. No significant differences were found at any of the three times or for the averages. Figure 4.5 illustrates the diurnal pattern of the pH for the two treatments. The two patterns are very similar. The highest pH of 6.40 was measured at 06h00 for both treatments. Thereafter the pH declined drastically for three hours. From 09h00 the pH declined but at a slower rate. The pH then decreased again at 14h00. The lowest pH of 5.84 for the high starch treatment was found at 15h00 and the low starch treatment only reached a pH of 5.84 half an hour later at 15h30. From then on pH slowly rose through the evening to reach the highest point again at 06h00.

Table 4.13 Average ruminal pH measured at 06h00, 12h00 and 20h00 of ten cannulated high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high or low starch concentrates during October (n = 10).

Time	Treatment [1]		SEM [2]	P
	High starch	Low starch		
06h00	6.40	6.40	0.065	0.99
12h00	5.98	6.02	0.029	0.28
20h00	5.90	5.93	0.053	0.75
Daily average	6.05	6.08	0.031	0.47

[1]High starch: Dairy concentrate containing 80% maize; Dairy concentrate containing 20% maize.
[2] Standard error of mean
[a, b] Means in the same row with different superscripts differ (P < 0.05).

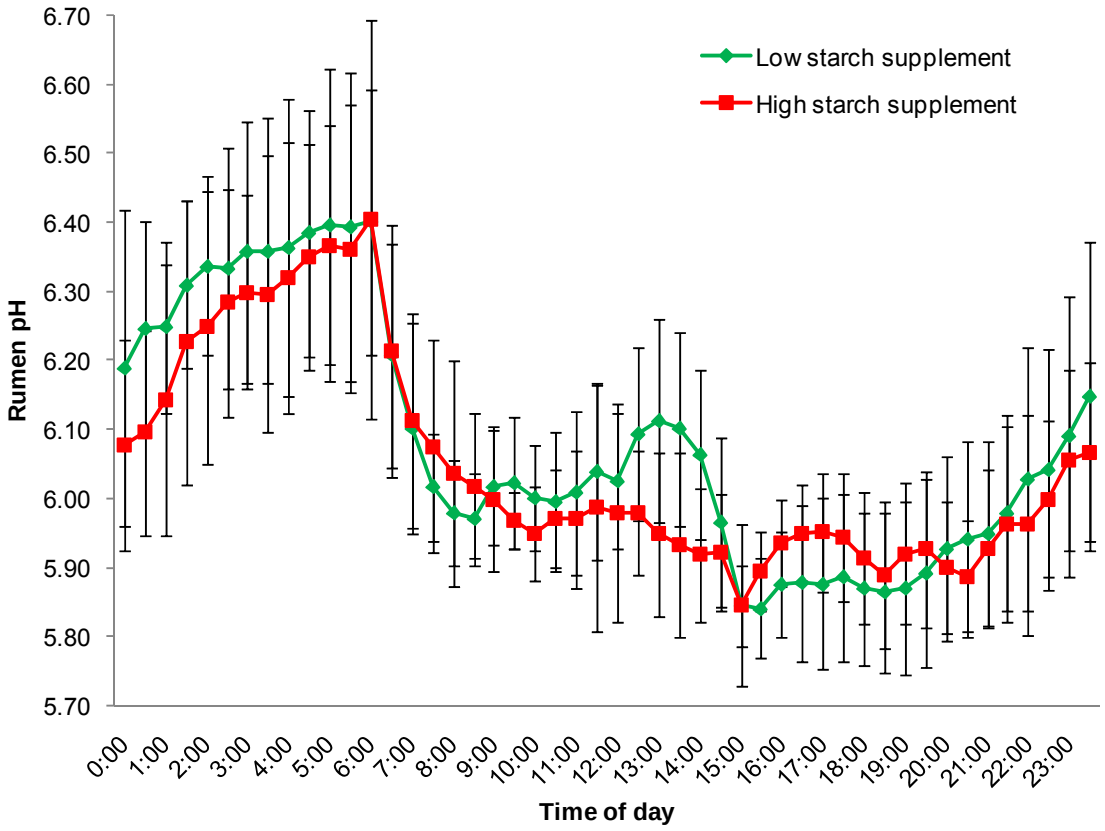

Figure 4.5 The diurnal pattern for rumen pH of high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high or low starch concentrates during October

Table 4.14 Average pH measured at 06h00, 12h00 and 20h00 of ten cannulated high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high and low starch concentrates during October of period 1 and period 2 (n = 5).

Time	Treatment [1]		SEM [2]	P
	High starch	Low starch		
Period 1				
06h00	6.23	6.44	0.092	0.13
12h00	5.94	6.07	0.120	0.46
20h00	5.86	5.96	0.122	0.59
Daily average	5.99	6.11	0.094	0.41
Period 2				
06h00	6.58	6.36	0.129	0.27
12h00	6.02	5.98	0.133	0.85
20h00	5.94	5.90	0.146	0.82
Daily average	6.10	6.05	0.116	0.77

[1] High starch: Dairy concentrate containing 80% maize; Dairy concentrate containing 20% maize.
[2] Standard error of mean
[a, b] Means in the same row with different superscripts differ (P < 0.05).

Table 4.14 shows the pH data for each individual period. There were no significant differences between treatments at any time for either period. The pH values were similar between periods. Table 4.15illustrates the time under certain pH levels. The pH dropped to under pH 5.8 for 4.5 to 5.15 hours per day for the low and the high starch treatment respectively, and showed no significant difference between the two treatments. The time during which the pH was lower than 6.0 was double the time during which it was lower than 5.8, with no significant difference found.

Table 4.15 Average pH measured under pH 5.8, 6.0 and 6.2 per day of ten cannulated high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high or low starch concentrates during October (n =10).

Time under (hours)	Treatment [1]		SEM [2]	P
	High starch	Low starch		
pH 5.8	5.15	4.50	0.635	0.49
pH 6.0	10.6	9.70	1.087	0.78
pH 6.2	16.7	15.	0.998	0.38

[1]High starch: Dairy concentrate containing 80% maize; Dairy concentrate containing 20% maize.
[2] Standard error of mean

4.2.5 *In sacco*

Table 4.16 shows the degradability coefficients for both the DM and NDF fractions between the two treatments. Constants were determined from the model $p = a + b\ (1 - e^{-tc})$ (Ørskov & McDonald, 1979). Neither *a*, *b* or *c* differed significantly between treatments for both DM and NDF. The 30 hour digestibility was determined using the model and did not differ significantly between the two treatments.

Table 4.16 Rumen degradability parameters of ryegrass pasture *in sacco* of ten cannulated high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high or low starch concentrates during October (n = 5).

Parameter	Treatment [1]		SEM [2]	P
	High starch	Low starch		
DM fraction				
a [3]	32.0	30.5	0.826	0.26
b [3]	56.3	56.0	1.275	0.22
c [3]	0.071	0.068	0.006	0.74
30 hour Digestibility	81.3	81.5	1.031	0.89
NDF fraction				
a [3]	7.41	5.22	1.119	0.24
b [3]	76.0	79.9	1.660	0.17
c [3]	0.080	0.074	0.007	0.55
30 hour Digestibility	76.1	75.8	1.421	0.92

[1]High starch: Dairy concentrate containing 80% maize; Dairy concentrate containing 20% maize.
[2] Standard error of mean
[3] Constants determined from $p = a + b\ (1 - e^{-tc})$; *a* = Rapidly soluble fraction; *b* = Potential degradable fraction *c* = Rate at which *b* is degraded in the rumen (Ørskov & McDonald, 1979)

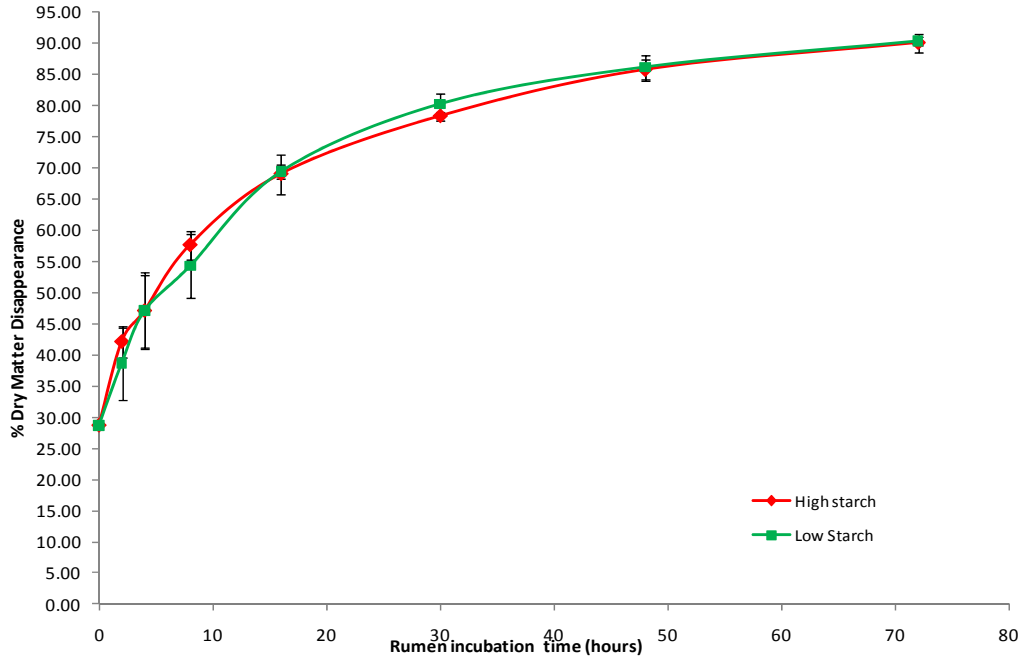

Figure 4.6 *In sacco* dry matter disappearance of ryegrass placed in the rumen of ten cannulated high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high or low starch concentrates during October.

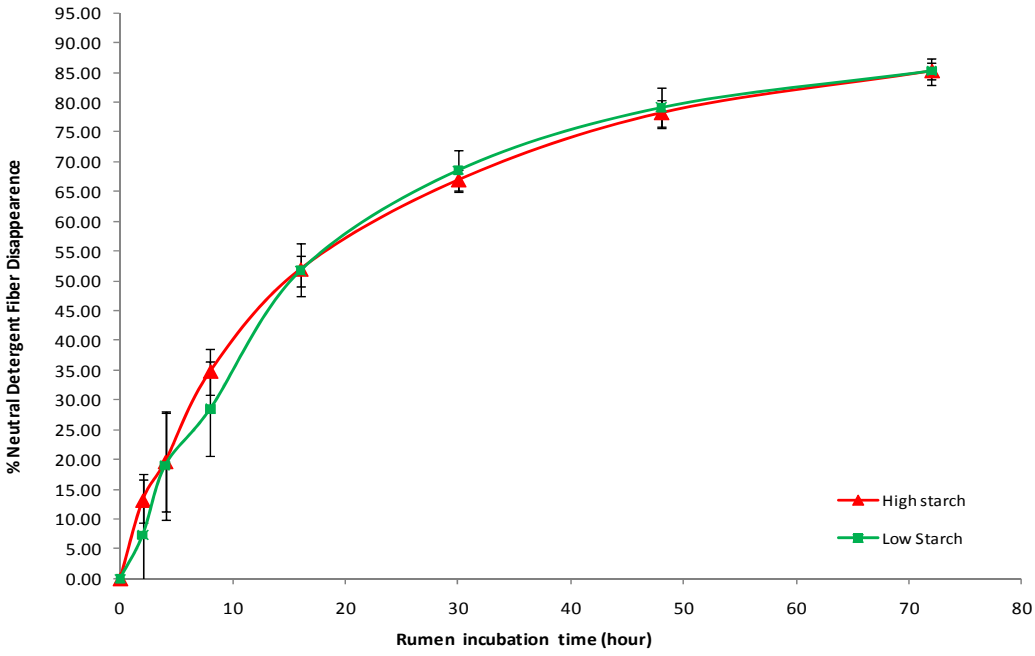

Figure 4.7 *In sacco* neutral detergent fibre disappearance of ryegrass placed in the rumen of ten cannulated high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high or low starch concentrates during October.

Figure 4.6 illustrates the actual DM disappearance of ryegrass placed *in sacco* into ten cannulated dairy cows. The zero hour disappearance of DM was 28.72%. There was no difference between the two treatments for DM disappearance at any of the removal times. Dry matter digestibility was almost identical at 72 hours between the two treatments. Figure 4.7 illustartes the actual NDF disappearance of ryegrass placed *in sacco* into ten cannulated dairy cows. There was no difference between the two treatments for NDF disappearance at any of the removal times. The NDF digestibility was, similarly to the DM, almost identical at 72 hours.

4.3 Production study

Milk production parameters are shown in Table 4.17. Milk yield and fat corrected milk yield did not differ between treatments (P > 0.05). Milk fat percentage was found to differ significantly (P = 0.01) between the high starch and the low starch treatment, with the low starch treatment being higher. There was no significant difference in the milk fat percentage between the high starch and medium starch treatment groups, although the medium starch treatment group had a tendency to be higher. The milk fat yield of cows fed the medium and low starch treatments was higher than that of the high starch treatments (P = 0.05). Milk protein and lactose percentages as well as milk urea nitrogen and somatic cell count did not differ significantly between treatments.

Table 4.17 Milk production and milk composition of high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high, medium or low starch concentrates during October (n = 15).

Parameter [1]	Treatment [2]			SEM[3]	P
	High starch	Medium Starch	Low starch		
Milk yield (kg/cow/d)	19.9	20.2	19.0	0.522	0.28
4% FCM (kg/cow/d)	20.0	21.6	21.1	0.579	0.17
Milk Fat (g/100g)	4.07[a]	4.49[ab]	4.75[b]	0.152	0.01
Milk fat yield (kg/d)	0.804[a]	0.901[b]	0.898[b]	0.031	0.05
Milk Protein (g/100g)	3.53	3.63	3.59	0.065	0.53
Lactose (g/100g)	4.59	4.71	4.69	0.041	0.11
MUN (mg/dL)	17.8	17.1	17.3	0.303	0.48
SCC	255	163	241	53.1	0.43

[1] FCM – Fat corrected milk; MUN – Milk urea nitrogen; SCC – Somatic cell count.
[2]High starch: Dairy concentrate containing 80% maize; Medium starch: Dairy concentrate containing 40% maize; Low starch: Dairy concentrate containing 20% maize.
[3] Standard error of mean
[a, b] Means in the same row with different superscripts differ (P < 0.05)

Table 4.18 shows the body weight and body condition score parameters for the duration of the study. There was no difference in bodyweight of animals between treatments. There was also no difference in bodyweight change for the duration of the study. There was no difference in body condition score between treatments at the start of the study, there was however a tendency for the medium starch treatment to be lower at the end

of the study. This led to a tendency for body condition score change to be lower for the medium starch treatment.

Table 4.18 Body weight and body condition score of high yielding Jersey cows grazing kikuyu/ryegrass pasture fed 6kg (as is) of high, medium or low starch concentrates during October (n = 15).

Parameter [1]	Treatment [2]			SEM[3]	P
	High starch	Medium Starch	Low starch		
BW start (kg)	333	337	349	7.46	0.29
BW end (kg)	357	366	373	7.37	0.31
BW change (kg)	+23.5	+29.3	+23.8	3.02	0.32
BCS start	2.10	2.08	2.18	0.054	0.39
BCS end	2.42	2.23	2.47	0.074	0.08
BCS change	+0.32	+0.15	+0.28	0.054	0.09

[1] BW – Body weight; BCS – Body condition score.
[2]High starch: Dairy concentrate containing 80% maize; Medium starch: Dairy concentrate containing 40% maize; Low starch: Dairy concentrate containing 20% maize.
[3] Standard error of mean
[a, b] Means in the same row with different superscripts differ (P < 0.05)

Table 4.19 shows the ME requirement for maintenance and lactation, as well as the body condition score increase of each treatment group as obtained from the NRC 2001. The total ME required was calculated from the three variables. The ME obtained from the concentrate of each treatment was then subtracted from each treatment group. The ME still required was assumed to be obtained from pasture. The pasture ME was 11.36 MJ/kg DM as shown in Table 4.2. The pasture intake of each treatment group was then calculated. The high starch treatment group had the lowest intake of 9.07kg DM/cow/day, while the low starch treatment had the highest intake of 10.07 kg/cow/day. This was a difference of 1.00kg/cow/day.

Table 4.19 The mean Metabolisable energy requirement for maintenance and lactation for each treatment group (high, medium or low starch concentrates) of high yielding Jersey cows grazing kikuyu/ryegrass pasture, as well as the mean pasture intake of each treatment group

Parameter[1]	Treatment[2]		
	High Starch	Medium Starch	Low Starch
ME required for maintenance (MJ) [3]	56.40	57.28	58.45
ME required for lactation (MJ) [3]	104.81	113.19	109.00
ME required for BCS gain (MJ)	5.68	2.66	4.97
Total ME requirement (MJ)	166.89	173.13	172.42
ME obtained from concentrate (MJ)	63.81	60.21	58.04
ME required from pasture (MJ)	103.07	112.93	114.38
Estimated Pasture intake (kg DM/cow/day)	9.07	9.94	10.07

[1]ME – Metabolisable Energy; BCS – Body condition score.
[2]High starch: Dairy concentrate containing 80% maize; Medium starch: Dairy concentrate containing 40% maize; Low starch: Dairy concentrate containing 20% maize.
[3] Obtained from NRC 2001

4.4 References

National Research Council (NRC), 2001. Nutrient requirements of dairy cattle. (7th Rev. Ed.). National Academy. Press, Washington, D.C., USA.

Ørskov, E.R. & McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. J. Agric. Sci. 92, 499-503.

CHAPTER 5
Discussion

5.1 Pasture and concentrate intake

As indicated in the results (Table 4.1) cows were allocated to pasture when at an average height of 27.8 on the rising plate meter (RPM), or 13.9cm. The total dry matter (DM) available above three centimeters amounted to 2073kg DM/ha. This was higher than the 1700kg DM/ha recommended by McEvoy *et al*. (2009) but still lower than the 2200kg DM/ha which was indicated to cause deterioration in pasture quality. In the study of McEvoy *et al*. (2009) pasture mass was determined above four centimeter level, but if the pasture mass was determined above a three centimetres level then the values used would have been closer to that in the current study.

At the above mentioned pasture height (27.8 on the RPM, or 13.9cm) cows were allocated pasture at 12.9kg DM/cow/day. This was lower than the recommended value by McEvoy *et al*. (2009) who suggested that 20kg DM/cow/day be allocated to grazing dairy cows. This recommendation was, however, made for Holstein Friesland cows. Bargo *et al*. (2003) stated that pasture allowance should be three to five times the cow's daily requirement in order to maximise dry matter intake (DMI), but this would lead to a reduction in pasture quality. To prevent this, a pasture allowance of only twice the cow's daily DMI was suggested (Bargo *et al*., 2003). This was similar to pasture allocations made in similar studies by Meijs (1986) who allocated 2.24 times daily DMI and Sayers *et al*. (2003) who allocated 2.1 times daily DMI. The pasture intake in this study was calculated at 6.5kg DM/cow/day using the RPM, resulting in a pasture allowance of 12.9kg DM/day, which was equivalent to 1.98 times that of the estimated pasture DMI. Although slightly lower than the two previously mentioned studies it was close to the recommendation of Bargo *et al*. (2003). Rayburn & Rayburn (1998) found an error of ten percent for pasture yields when using RPM, however, Malleson (2008) stated that the RPM was not sufficiently accurate to predict differences in intake between treatments. Most studies did not indicate any differences in intake between treatments of high starch and low starch supplements even with other methods of intake determination (Kibbon and Holmes, 1987; Spörndly 1991; Fisher *et al*., 1996; Sayers *et al*., 2003). In this study the RPM was sufficiently accurate to do adequate pasture allocations.

The post-graze pasture height over the duration of the study was 11.40 on the RPM which amounts to 5.7cm in height. This agrees with the findings of Fulkerson & Donaghy (2001) and Lee *et al*., (2008) who stated that the best method of grazing ryegrass was to defoliate the grass to an average of about 5cm above ground level. Dillon (2006) also indicated that a post-graze height of higher than 5-6cm would lead to a reduction in pasture quality during the latter part of the season.

Composition of concentrates and pasture is shown in Table 4.2. The crude protein (CP) content of pasture was 259 g/kg DM. This value was higher than expected if compared to that of Meijs (1986), Sayers *et al*. (2003) and Meeske *et al*. (2006) who found the CP to be 218.5g/kg, 238.8g/kg and 207g/kg DM, respectively for ryegrass pastures. The Metabolisable energy (ME) value of the pasture was higher than expected at

11.36 MJ/kg DM. Meeske *et al*. (2006) indicated a ME value for ryegrass pasture of 10.6MJ/kg DM. The NDF content of the pasture was within a normal range with a value of 541.2g/kg DM. This is similar to Fulkerson *et al*. (2007) who indicated a NDF value of 531.0 g/kg DM for annual ryegrass during spring, and Meeske at al. (2006), who under similar conditions, indicated a NDF value of 490.0 g/kg DM; with a range of between 552.3g/kg DM and 427.7 g/kg DM. All the above indicated that a pasture of very high quality was used during the study.

The CP content was similar for all three concentrates with a mean of 143 g/kg. The NDF content increased from 186 g/kg in the high starch supplement to 322.1 g/kg in the low starch supplement. The supplements did, however, contain very little, if any, effective fibre. Starch content decreased from 516.6 g/kg in the high starch supplement to 371.4 g/kg in the low starch supplement. This led to the decrease in metabolisable energy (ME) content from the high starch supplement which was the highest at 12.04 MJ ME/kg DM to the low starch supplement which was the lowest at 10.95 MJ ME/kg DM.

5.2 Rumen study

Figure 4.3 illustrates the relationship between volatile fatty acids (VFA), rumen ammonia nitrogen (NH_3-N) and rumen pH. Both the VFA and rumen NH_3-N were inversely related to the rumen pH measured at the same times. Both the rumen NH_3-N and the volatile fatty acid concentration were the highest at 20h00 while pH was the lowest at this time. In Figure 4.5 it can be seen that the actual lowest point of pH was between the 12h00 and 20h00 sampling times. The relationship between pH, rumen NH_3-N and VFA were similar to that found by Van Vuuren *et al*. (1986), although they found the lowest pH reached to be at midnight.

5.2.1 Volatile Fatty acids

Volatile fatty acid concentration results are shown in Table 4.4. The mean daily volatile fatty acid concentration differed between the two treatments ($P < 0.05$), with the high starch treatment being the highest at 121.94mM/L and the low starch treatment lowest at 112.57mM/L. The high starch treatment results for VFA were similar to that found in a study done by Sayers *et al*. (2003) and were similar to low starch studies done by Van Vuuren *et al*. (1986) and Khalili & Sairanen (2000). These results were almost similar to the average calculated by Bargo *et al*. (2003) of 120.9 mM/L from an analysis of six different studies. In the current study it was found that there was a significant difference in volatile fatty acid concentrations between treatments, which is different from the results found by many other authors (Van Vuuren *et al*., 1986; Khalili & Sairanen, 2000; Sayers *et al*., 2003; Bargo *et al*., 2003). The volatile fatty acid concentration in the low starch treatment was substantially lower than the average indicated by Bargo *et al*. (2003) and was most probably due to the lower ME value in the low starch supplement (Kolver *et al*., 1998).

The results from Table 4.4 are illustrated in Figure 4.3. There were no significant differences between treatments for the volatile fatty acid concentration at 06h00, 12h00, but at 20h00 the high starch diet had a significantly higher concentration ($P < 0.05$). The diurnal pattern of the volatile fatty acid concentration was similar for both treatments. Table 4.5 shows the volatile fatty acid concentration for the two separate periods. Data was similar in both periods for the high starch treatment indicating little variation. The results for the low starch treatment were more variable between the two periods. Volatile fatty acid concentrations were similar

at 06h00 for both periods. At 12h00 in Period 2, the low starch diet had a substantially (20mM/L) higher concentration than in Period 1 and at 20h00 the opposite was the case, with concentrations for the low starch treatment being 11mM/L higher than in Period 1. This variation is illustrated in Figure 4.4, and although the pattern is similar for both treatments, the variation between the two periods was less pronounced in the high starch treatment and never varied more than 6 mM/L between the two periods. It can be argued that the low starch treatment led to a more variable response in volatile fatty acid concentration. There was a significant difference between the two treatments in Period 1 at 12h00, with the high starch having a higher volatile fatty acid concentration. In Period 2 there were no significant differences at any time.

Table 4.10 shows the percentage of each volatile fatty acid in the rumen as a percentage of the total VFA. There are no differences between treatments for acetic, propionic and butyric acids as a percentage of total VFA, with percentages varying with less than one unit between treatments. The acetic acid comprised 71.92 and 72.95 percent for the high starch and the low starch treatments, respectively. This was approximately eight percent higher than what was found in the study done by Khalili & Sairanen (2000) and more than ten percent higher than the results of the study done by Sayers *et al*. (2003). The propionic acid percentage was lower than that found in previous studies (Khalili & Sairanen, 2000; Sayers *et al*., 2003; Bargo *et al*., 2003). The percentage of butyric acid was similar to those found in the studies done by Khalili & Sairanen (2000) and summarised by Bargo *et al*. (2003), but was lower than what was found in the study done by Sayers *et al*. (2003). The acetic acid percentage was higher, and the propionic and butyric acid percentages were lower than in the study done by Erasmus (2009) at the same location. The high acetic acid and low propionic acid percentages could be ascribed to the high NDF content of the pasture. Another reason could be fact that on fresh pasture the preferential degradation of starch instead of fibre by microbes as seen in high concentrate diets, is less pronounced (Kolver & De Veth, 2002).

The high acetic acid percentage and the low propionic acid percentage in this particular study would be expected to lead to higher butterfat percentages (Meijs, 1986). No difference would, however, be expected between treatments because the acetate to propionate ratio did not differ between the two the two treatments groups. Valeric acid had a significantly higher percentage in the high starch treatment and the iso-valeric acid concentration tended ($P < 0.1$) to be higher in the high starch treatment, but because of the small percentage of the total volatile fatty acid concentration the effect thereof on the overall system was negligible.

The acetic acid concentration did not differ significantly between the treatments although it tended to be higher in the high starch treatment. Both propionic and butyric acid concentrations showed significant differences in daily average concentrations between the two treatments, and in both cases the high starch treatment had the higher concentration. Butyric acid concentration was also higher at 12h00 for the high starch treatment. These differences were not due to a higher percentage of each volatile fatty acid, as indicated by Table 4.10, but to a higher overall volatile fatty acid concentration obtained in the high starch treatment even if the percentages were the same.

Table 4.6 shows rumen acetic acid concentration at different times for the two different treatments. There were no significant differences for acetic acid concentration between the treatments at both 06h00 and 12h00, but there was a tendency for the high starch diet to have a higher concentration at 20h00. The same data are shown in Table 4.7 for propionic acid concentration and similarly there was no significant difference at 06h00 and 12h00. The butyric acid, as presented in Table 4.8, showed more differences than acetic and propionic acid. A significant difference was found between the two treatments for the 12h00 sample with the high starch diet having a higher butyric acid concentration. There was a tendency for the high starch diet to have a higher concentration at 20h00. There was, however, no significant differences indicated at 06h00.

The data represented in Table 4.9 indicate that the molar acetate to propionate ratio did not differ for any of the three times between the two treatments, as well as for the daily average. The lower concentration of volatile fatty acid in the low starch treatment could be due to the dilution effect of the added pasture intake in this group. Thus indicating why there is no difference in the respective volatile fatty acid ratios. The acetate to propionate ratio was the highest at 06h00 and almost 1.5 units lower at 20h00. Although there are differences in volatile fatty acid concentrations between the treatments, this did not result in a difference in the acetate to propionate ratios between treatments. This was because there was no difference in the relative proportions of each of the VFA compared to the total volatile fatty acid concentration. For the same reason the acetate plus butyrate to propionate ratio (Sutton's ratio), also did not differ between the two treatments. Sutton (1984) indicated that for every unit fall in the acetate plus butyrate to propionate ratio the butterfat percentage decreased by 0.5g butterfat/100g milk produced. This was also confirmed by Sayers *et al*. (2003) who found a decrease of 0.53g butterfat /100g milk produced. This differs from the findings of this study, which found that there was no difference in the acetate to propionate ratio of Sutton's ratio, and yet there was a difference in butterfat percentage and in milk fat yield (discussed later).

5.2.2 Rumen Ammonia-nitrogen

Table 4.11 shows the rumen NH_3-N concentrations at different times. The daily average of 21.2 and 18.8 mg/dL for the high starch and low starch treatments, respectively, was similar to what was found in the study done by Van Vuuren *et al*. (1986), although there was no significant difference between the high starch and low starch treatment in that study. The results of Khalili & Sairanen (2000) were similar to the findings of this study in that the high starch diet had significantly higher rumen NH_3-N concentrations than the low starch diet. The rumen NH_3-N concentration of the low starch diet of Khalili & Sairanen (2000) was similar to that of this study and that of Van Vuuren *et al*. (1986), but in the high starch diet the rumen NH_3-N was higher in the study of Khalili & Sairanen (2000), than concentrations found in this study as well as that of Van Vuuren *et al*. (1986). Sayers *et al*. (2003) found no difference in rumen NH_3-N concentrations and also obtained lower concentrations than those found in this study. It can most probably be attributed to the higher levels of concentrate fed to the cows which in turn led to lower pasture intake.

Concentrations of rumen NH_3-N were lower in diets containing high levels of starch, than in diets containing pasture only, but the quantity of microbial and total protein available for absorption in the duodenum was unchanged (Kolver *et al*., 1998). The reason for the lack of change of available microbial protein in the small intestine was described by Satter &Roffler (1974) who indicated that a rumen NH_3-N level above 5mg/dL had

no effect on microbial protein production. McDonald *et al*. (2002) however stated that microbial protein synthesis would be slower at levels of 5mg/dL and indicated an optimum range of 8.5mg/dL to 30mg/dL. The above mentioned diets did, however, result in rumen NH_3-N levels well above the level stated by Satter & Roffler (1974) and fell within the limits proposed by McDonald *et al*. (2002). The level of rumen NH_3-N concentration in both treatments in this study was higher than the level indicated by Satter & Roffler (1974) and fell within the range of McDonald *et al*. (2002). It can therefore be expected that there would be little to no difference in the level of microbial protein reaching the duodenum as indicated in previous studies by Kolver *et al*. (1998).

As illustrated in Table 4.11, there was a significant difference in the rumen NH_3-N content of animals at 06h00 with the high starch treatment having the higher concentration of the two treatments. There was also a significant difference in the daily average of rumen NH_3-N concentration between the two treatments with the high starch treatment once again being the higher of the two. In theory, it would be expected that the low starch treatment animals would have higher pasture intakes due to the lower ME content the diet (Hodgson & Brookes, 1999), thus resulting in higher rumen NH_3-N levels (Van Vuuren *et al*., 1986). This was, however, not found to be the case with the high starch treatment having a significantly higher rumen NH_3-N content. However, if the pasture intake of both treatments were similar, it could possibly explain the lower rumen NH_3-N levels. The low starch diet had a lower ME content and lower level of highly fermentable carbohydrates, thus resulting in less energy available for proteolysis of rumen micro-organisms to degrade protein to rumen ammonia, resulting in the lower rumen NH_3-N levels observed in the low starch diet. The milk yield and milk protein yield were, however, unaffected by the lower levels of rumen NH_3-N, because both treatments were well above the lower limits described by Satter & Roffler (1974). Other explanations could be sampling time and possible sampling error.

Table 4.12 shows a significant difference in rumen NH_3-N concentration at 06h00 during Period 1 of sampling, with the high starch treatment being higher. There was also a tendency for the high starch treatment to have a higher daily average for rumen NH_3-N concentration and this tendency was probably caused by the low concentration of rumen NH_3-N levels at 06h00 for the low starch treatment. In Period 2, there were no differences with almost identical values and high P values. Concentrations for Period 1 were lower than those of Period 2, especially for the low starch treatment. As mentioned in the discussion on VFA, the low starch diet showed a more variable response for rumen NH_3-N to supplementation than did the high starch diet, possibly leading to unpredictable results.

5.2.3 Rumen pH

Figure 4.5 illustrates the diurnal pattern of rumen pH for the two different treatments. The two treatments had very similar patterns. The highest pH of 6.40 was measured at 06h00 for both treatments. This indicates that cows most probably grazed less during the evening, causing a decrease in fermentable substrate and less volatile fatty acid produced, therefore the higher rumen pH. It could show to the fact that this was the longest time without concentrate feeding which gave the rumen time to stabilize, thus leading to higher rumen pH. Cows most probably ruminated during the evening, while not necessarily grazing. The volume of saliva produced by dairy cows is directly related to the time spent eating and ruminating (Ishler *et al*., 1996). The

saliva secretion would in turn improve the buffering capacity of the rumen and increase the pH, leading to the peak at just before morning milking, as no new substrate is taken in. After 06h00 the pH declined drastically for three hours. This decrease was most probably caused by cows receiving 3kg of their supplement at milking in the morning. From 09h00 onwards the pH declined, but commenced at a slower rate. The pH then drastically dropped again at 14h00 which coincided with the second milking where they received another 3kg of supplement. The lowest pH of 5.84 for the high starch treatment was obtained at 15h00 and the low starch treatment only reached a pH of 5.84 half an hour later at 15h30. From then on the pH slowly rose through the evening to the highest point again.

No significant difference in rumen pH was found at any specific time, or in the daily average as indicated in Table 4.13. The daily average rumen pH was 6.05 and 6.08 for the high starch and low starch treatments respectively. This was similar to the results of Khalili & Sairanen (2000) but higher than what was found by Van Vuuren *et al*. (1986) and Sayers *et al*. (2003). Bargo *et al*. (2003) summarised eight studies in which an average pH of 6.03 were found which is similar to the results of this study. It would also seem fair to say that in pasture based systems a pH of 6 would be the norm. Van Vuuren *et al*. (1986), Khalili & Sairanen (2000) and Sayers *et al*. (2003) found no difference in rumen pH between high starch and low starch treatments, as was the case in the current study. The treatments probably have a smaller than expected influence on the rumen pH because of the low level of supplementation and because more than fifty percent of the diet consisted of pasture. This is supported by Sayers *et al*. (2003), who indicated that a higher level of supplementation decreased rumen pH and volatile fatty acid ratios. The reason for this was presented by Van Vuuren *et al*. (1986) who stated that concentrate supplementation had a less pronounced effect on the rumen because of pasture, and thus the composition of the concentrate would have had an even smaller effect. The lack of difference in rumen pH results in this study is, however, strange considering that the high starch treatments resulted in a significantly higher volatile fatty acid concentration. There were, however, no differences in the proportions of the VFA, which could have led to the decreased effect.

Table 4.14 shows the pH data for each individual period. There were no significant differences between treatments for any time and either period. The two periods seem to be the inverse of each other. In Period 1 the low starch diet has higher pH values at all three times as well as the daily average. In Period 2 the opposite happened with the high starch diet being higher at all three times and the daily average. This may indicate that the cow effect was larger than the effect of the diet itself. This was explained by Weimer *et al*. (1999) who stated that microbial populations can differ between animals fed the same diet making the integration between rumen microbiology and dairy cow nutrition a very complicated matter.

The pH dropped to under pH 5.8 for only short durations during the day. There was also no significant difference in pH between the two treatments. The mean daily pH of the cannulated cows for both treatments also never dropped below 5.8. The time below pH 6.0 was double that of the pH 5.8, with once again no significant difference between treatments. The time under pH 6.2 was between 16.65 and 15.35 hours for the high starch and low starch treatments respectively, a non-significant difference. There are two groups of bacteria which function at different pH levels in the rumen. The starch digesters are better suited to more acidic environments at a pH of 5.2 to 6 (Ishler *et al*., 1996) and the fibre digesters which, in contrast, thrive at

a pH of 6.2 to 6.8. A reduction in rumen pH below 6.2 causes a decrease in fibre digestion, mainly because a pH below 6.2 is suboptimal for microbial growth (Ishler *et al*., 1996; Pitt *et al*., 1996). This is due to the direct result of a decrease in fibre digestion (Wales *et al*., 2004). In the current study there was, however, no difference between treatments in time spent under pH 5.8 or 6.0 between the two treatments, therefore no difference would be expected in the digestibility of fibre and pasture for that matter. Ruminal digestion of pasture was optimal at a pH of 6.35 according to De Veth & Kolver (2001), although the highest milk yield occurred at a rumen pH of 5.8 to 6.2 (Wales *et al*., 2004). The rumen pH in this particular study fell within the optimum range set by Wales *et al*. (2004) and the high milk yield at this pH range was probably due to the fact that both fibre and starch digesters were able to perform normally.

A lower pH value would be expected from the higher volatile fatty acid concentration obtained in the high starch treatment (Mertens, 1997), although this was not the case.There are several possible reasons why the pH would not differ between the two treatment groups in this particular study. The first could be the lack of physical effective fibre in the low starch treatment, resulting in no added buffering effect from chewing or rumination. The second could be the lack of difference in the volatile fatty acid proportions in the rumen between the two treatments. The third could be that the effect of the cow could have been greater than that of the feed given, as indicated by Weimer *et al*. (1999). The last and probably the most important factor was the low level of supplementation, and therefore the fact that the pasture is the major component of the animals' daily diet. This was confirmed by Sayers *et al.* (2003) who stated that the effect of the type of energy source only really becomes apparent at higher levels of supplementation in high producing dairy cows.

5.2.4 *In sacco*

The degradability parameters for both DM and NDF are shown in Table 4.16. Constants were determined for the model $p = a + b\ (1 - e^{-tc})$ (Ørskov & McDonald, 1979). The proportion of DM that disappeared in time *t* (hours) is represented by *p*. The rapidly soluble fraction is represented by *a*, the potential degradable fraction is represented by *b* and *c* is the rate at which *b* was degraded (Ørskov & McDonald, 1979; McDonald, 1981). In this study neither *a*, *b* or *c* differed significantly between treatments for either DM or NDF of kikuyu/ryegrass pasture. The result for the DM and NDF fraction was similar to that found by Sayers *et al*. (2003) who demonstrated that supplementation type had no effect on DM or NDF degradability of ryegrass pasture. Khalili & Sairanen (2000) found similar results to thse of the current study in that *a*, *b* and *c* for hay incubated in the rumen, was not affected by treatment.

Figure 4.6 and Figure 4.7 illustrates the actual DM disappearance and the NDF disappearance of ryegrass placed *in sacco* into ten cannulated dairy cows. The zero hour DM disappearance was 28.7%, which indicates a high level of soluble sugars in the pasture. There was no difference between the two treatments for DM disappearance or NDF disappearance at any of the removal times. DM digestibility was almost identical at 72 hours.

The similarities in die DM disappearance and the NDF disappearance of pasture could be attributed to the similarities in the rumen pH between the two treatments. If rumen pH was similar, the fibre digesters would not have had an advantage in the low starch diet, therefore no difference would occur in the disappearance of the pasture. According to Meijs (1986), the rapid formation of VFA and lactic acid in the rumen could be reduced by decreasing the amount of easily fermentable substrates such as starch from the diet, thereby improving rumen pH, but the current study found the opposite. This result could be explained by Calsamiglia *et al*. (2002) who showed that in a continuous culture with continuous feeding, decreases in fibre degradation were small or insignificant at relatively low pH values. Another factor could be that extreme daily variations in ruminal pH can be more harmful to rumen microbes than a constant low pH because of the constant metabolic readjustments needed by rumen microorganisms (Mertens, 1979). In the current study, the high starch treatment did not result in significantly lower rumen pH levels than the low starch treatment and therefore fibre digestion of kikuyu/ryegrass pasture was not negatively affected. It would be expected that the lack of difference in the DM disappearance and the NDF disappearance of ryegrass between the two treatments, would not result in an improved intake of pasture by the low starch treatment group. However, it was observed that the rumens of the cannulated cows were rarely filled to capacity, which could render the argument of increased pasture intake due to increased DM and NDF digestion of no worth. In the case of the rumen not filled to capacity, pasture intake would then rather be determined by physiological stimuli, that of energy shortage, rather than physical stimuli namely rumen fill, as was suggested by Forbes (1996).

5.3 Production study

5.3.1 Milk yield and composition

As shown in Table 4.17, there was no significant difference in milk yield between treatments. This was also the case for 4 % fat corrected milk yield (FCM). It could be argued that the high starch treatment should result in higher milk yields because of the higher energy content of the supplement. This was, however, not the case. This is in accordance with several authors who found no significant effect on milk production when low starch supplements were compared to high starch supplements (Kibbon & Holmes, 1987; Spörndly, 1991; Fisher *et al*., 1996; Sayers *et al*., 2003). The milk yields obtained in the current study were similar to the results of a study done by Meijs (1986) and Meeske *et al*. (2009) in which there were no significant effects on milk yield, but these authors found the fat corrected milk yield to be significantly higher for the low starch supplement. Milk yield was also significantly increased in a study done by Khalili & Sairanen (2000). It can be speculated that, if the variation that occurred in the low starch treatment of the current study had been lower, the difference in 4% FCM might have been significant.

The butterfat contents were 4.07, 4.49 and 4.75 g/100g milk for the high starch, medium starch and low starch treatments, respectively. The butterfat percentages were higher than reported in similar studies (Meijs, 1986; Van Vuuren *et al*., 1986; Kibbon & Holmes, 1987; Schwarz *et al*., 1995; Fisher *et al*., 1996; Khalili & Sairanen, 2000; Sayers *et al*., 2003; Meeske *et al*., 2009), but most of these studies, except that of Meeske *et al*. (2009), made use of Holstein Friesland cows. Although the difference in butterfat percentage between high and low starch treatments in the current study were similar to that of Meeske *et al*. (2009) who

also used Jersey cows, the current study still had a higher average butterfat percentage. The reason for this could be due to a higher NDF in the pasture during the current study.

Butterfat percentage differed significantly between the high starch and the low starch treatments, with the low starch treatment being higher in butterfat. There was no significant difference between the high starch and medium starch treatment for butterfat percentage, although the butterfat content of the medium starch treatment had a tendency to be higher than that of the high starch treatment. This is in agreement with the observations of Meeske *et al*. (2009) who indicated a significant difference in butterfat percentage between high starch and low starch treatments. Most authors, however, found no effect on butterfat percentage between low starch and high starch supplementation on pasture based systems (Meijs, 1986; Kibbon & Holmes, 1987; Schwarz *et al*., 1995; Khalili & Sairanen, 2000; Sayers *et al*., 2003). Milk fat yield (kg/d) was also significantly higher for the medium starch and low starch treatments when compared to the high starch treatment. This was similar to the findings of Meijs (1986) who obtained a significant increase in milk fat yield for low starch supplementation on a ryegrass pasture system. It could be expected that there would be no difference in butterfat percentage and milk fat yield, if the rumen pH data as well as the rumen volatile fatty acid ratios were taken into consideration. The reasons for the higher butterfat percentages are, however, unclear.

The milk protein contents were 3.53, 3.63 and 3.59 g/100g milk for the high starch, medium starch and low starch treatments, respectively and showed no significant differences between treatments. This was similar to several authors who found no significant differences in milk protein content between high starch and low starch supplementations for grazing dairy cows (Meijs, 1986; Kibbon and Holmes, 1987; Schwarz *et al*., 1995; Fisher *et al*., 1996; Meeske *et al*., 2009). Milk protein yield was increased by low starch supplementation for dairy cows (Khalili & Sairanen, 2000), but milk protein percentages were fairly similar. The main factor contributing to milk protein content is the ME content of the total ration (Schwarz *et al*., 1995). The lower ME content of the low starch diet was probably made up for by the high ME content of the pasture, hence the small effect shown on milk protein content. McCarthy *et al*. (1989) stipulated that an increase in protein content of the milk would be an indication of increased DMI. If this holds true, one would expect the two diets to have a fairly similar DMI of pasture. No difference was expected in milk protein percentage if the CP content of the diet was taken into consideration and the fact that both treatments had ample rumen NH_3-N for microbial protein.

Lactose percentages of 4.59, 4.71 and 4.69 g/100g milk produced were found for the high starch, medium starch and low starch treatments, respectively and showed no significant differences between treatments. This result agreed with most similar studies (Kibbon & Holmes, 1987; Spörndly, 1991; Schwarz *et al*., 1995: Fisher *et al*., 1996; Khalili & Sairanen, 2000). Most of these authors found no effect on lactose content of the milk between low starch and high starch supplements (Kibbon & Holmes, 1987; Spörndly, 1991; Fisher *et al*., 1996; Khalili & Sairanen, 2000). This result was expected because lactose content of milk is the least amendable to change (Kennelly & Glimm 1998) and always stays in the region of 4.7 g/100g milk produced for Jersey cows (Gibson, 1989). Khalili & Sairanen (2000) found milk lactose yield to be higher for high

starch supplemented grazing dairy cows, but this was most probably caused by an increased milk yield, with lactose percentages staying fairly similar.

Milk urea nitrogen content showed no significant differences between treatments. The milk urea nitrogen content was 17.8, 17.1 and 17.3 mg/dL milk for the high starch, medium starch and low starch treatments, respectively, and fell within the acceptable range of 12 – 18 mg/dL indicated by De Villiers *et al*. (2000). The observations also agreed with those of a similar study done by Meeske *et al*. (2009). These values are at the upper limit of the acceptable range and may indicate a possible lack of highly fermentable carbohydrates for utilisation of rumen ammonia or a protein excess, with the latter being the most probable cause.

Somatic cell count showed no significant differences between treatments. The average somatic cell count for the three treatments were 219 000/ml of milk produced. This is well below the acceptable range of 500 000/ml as stipulated in South African law (Regulation 1555 of the Foodstuffs, Cosmetics and Disinfectants Act, No 54 of 1972), below the 400 000/ml as required by the European Union for export and below 300 000/ml which is considered abnormal and indicative of subclinical mastitis (De Villiers *et al*., 2000).

5.3.2 Live weight

Live weights and body condition scores are presented in Table 4.18. There was no difference in live weight of animals between treatments. There was also no difference in live weight change over the period. There was, however, a tendency for body condition scores of the medium starch treatment to be lower at the end of the study. This led to a tendency for body condition score change to be lower for the medium starch treatment. The results are similar to most previous research on this subject with most authors indicating that supplementation type has little effect on live weight change or body condition score of lactating dairy cows (Kibbon & Holmes, 1987; Spörndly, 1991; Fisher *et al*., 1996; Khalili & Sairanen, 2000; Sayers *et al*., 2003; Meeske *et al*., 2009). The fact that cows did not lose bodyweight or body condition score between treatments also means that cows did not use more body reserves to maintain milk production in the low starch treatment than in the high starch treatment. The implication of this is that the pasture and the supplements provided sufficient energy to maintain the milk production.

5.4 Intake

The ME requirement for maintenance (based on the average live weight of each treatment group) and lactation (based on the average milk yield of each treatment group), as well as the body condition score gain of each treatment group, as obtained from the NRC (2001) are shown in Table 4.19. Using these requirements for each individual treatment group, a reverse calculation was made to estimate the pasture intake needed to maintain the level of production of each treatment group. The results indicated that a daily pasture intake of 9.07 kg DM pasture/cow/day for the high starch treatment, 9.94 kg DM pasture/cow/day for the medium starch treatment and 10.07 kg DM pasture/cow/day for the low starch treatment was required.

Thus, cows supplemented with the low starch treatment would have ingested 1.00 kg DM/cow/day of pasture more than the high starch treatment. This makes sense when the lack of difference in live weight gain between the different treatment groups was taken into consideration.

Both Erasmus (2009) and Malleson (2008) reported an under-estimation of pasture intake using the RPM. If the reverse calculation is used, it would seem that it was the same for this study. As mentioned earlier, Rayburn & Rayburn (1998) found an error of only 10 % for pasture yields when using RPM. The difference in pasture intake between the high starch and low starch treatments in this study was approximately 10 %. This would make the RPM too inaccurate to determine pasture intake differences between two treatments. This was also stated by Malleson (2008) who said that the RPM is not sufficiently accurate to predict differences in intake between treatments. Most studies did not indicate any differences in intake between treatments of high starch and low starch supplements, even with other methods of intake estimation (Kibbon and Holmes, 1987; Spörndly 1991; Fisher *et al*., 1996; Sayers *et al*., 2003). However, for the purpose of pasture allocations, the RPM appears to be was accurate enough. Because of the variation obtained with the use of a RPM based on the findings of previous studies concerning intake and the RPM, no attempt was made to determine differences in pasture intake.

Cows were allocated concentrates at 5.3 kg DM/cow/day. The mean total DMI of cows was estimated at 14.99 kg DM/cow/day. The intake as a percentage of average live weight (352.6kg) was 4.25 %. This is a higher intake than what was found in the studies of Kibon & Holmes (1987) who indicated a total DMI of 2.95 % of live weight. The NDF ingested daily from concentrates was 1.36 kg DM/cow/day and from pasture it was estimated to be 5.24 kg DM/cow/day. This amounted to a total NDF intake of 6.60 kg DM/cow/day or 1.87 % of body weight. This was higher than that indicated by Bargo *et al*. (2002) and probably resulted from the lower level of effective fibre in the diet.

Taking into consideration the lower ME content of the low starch concentrate, as well as the unchanged milk production between treatment groups, and the fact that there was no difference in live weight change between treatment groups, it would seem that energy had to be made up somewhere. The one explanation could be that of the calculation shown in Table 21 and the fact that pasture intake must have been improved by the low starch treatment to sustain milk production; the other could be that of an improved utilization of the low starch concentrates.

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CHAPTER 6

Economic Evaluation

For the economic evaluation it was assumed that all factors were the same for all three concentrate treatments and that cows consumed the same amount of pasture daily. Calculations were done for a herd of 280 cows in milk (280 cows is the average heard size in South Africa). The only variables taken into account were that of feed price and milk price, based on milk composition, as all the rest was assumed to be the same for all treatments. The differences in daily income, daily input costs, and profits were expressed relative to the numbers of the high starch treatment, i.e. the high starch treatment values for these parameters were set as 0. The feed price was obtained from NOVA feeds at the start of the study and the milk price was obtained from Nestlé, in September 2010, and is presented in Table 6.1

Table 6.1 Milk composition and price for high starch, medium starch and low starch concentrate treatment as well as the feed price for each individual feed.

Parameter	Treatment		
	High starch	Medium starch	Low starch
Milk yield (kg/cow/day)	19.9	20.2	19.0
Milk yield (kg/280 cows/day)	5572	5656	5320
Milk Fat (g/100g)	4.07	4.49	4.75
Milk Protein (g/100g)	3.53	3.63	3.59
Lactose (g/100g)	4.59	4.71	4.69
MUN (mg/dL)	17.8	17.1	17.3
Milk price (R/L)	**R 3.07**	**R 3.21**	**R 3.23**
Milk income (R/280 cows/day)	R 17,106.04	R 18,155.76	R 17,183.60
Increase in daily income	R 0.00	R 1,049.72	R 77.56
Feed price (R/ton)	**R 2,810**	**R 2,450**	**R 2,280**
Feed price (R/cow/day)	R 16.86	R 14.70	R 13.68
Feed price (R/280 cows/day)	R 4,720.80	R 4,116.00	R 3,830.40
Decrease in daily input cost (R)	R 0.00	R 604.80	R 890.40
Pasture price (R/kg)	**R 1.11**	**R 1.11**	**R 1.11**
Pasture price (R/cow/day)	R 10.07	R 11.03	R 11.18
Pasture price (R/280 cows/day)	R 2,818.96	R 3,089.35	R 3,129.76
Increase in daily input cost (R)	R 0.00	R 270.40	R 310.80
Net daily profit	R 0.00	R 1,384.12	R 657.16
Net monthly profit	R 0.00	R 42,215.78	R 20,043.38

Using the average milk production of each treatment group the production per day for 280 cows in milk was estimated. The high starch treatment would produce 5572 kg milk/day amounting to R17 106.04 with the stated milk price. The medium starch amounts to 5656 kg milk/day and R18 155.76. The low starch had the

lowest yield with only 5320 kg milk/day, but also had the highest milk price and which amounted to a total of R17 183.60. The medium starch had the highest daily income and the high starch had the lowest. On a monthly basis the medium starch treatment resulted in an increased income of R32 016.46 for 280 cows in milk compared to the high starch treatment. The difference between the high starch and low starch treatment would be less at R2 365.58.

Cows were fed 6 kg of supplement daily on a as is basis. This amounted to R16.86, R 14.70 and R13.68 per cow per day for the high starch, medium starch and low starch treatments respectively. With 280 cows in milk this daily concentrate cost of each treatment would be R 4720.80, R4116.00 and R3830.40 for the high starch, medium starch and low starch treatments respectively. On a monthly basis the medium starch treatment resulted in a decrease in input cost of R18 446.40 for 280 cows in milk. The decrease in input cost when changing from the high starch to the low starch treatment would be even larger at R27 157.20. If only feed cost was taken into consideration, the low starch treatment would be the most economical option. This was, however, expected due to the lower cost of by-products.

As was calculated in Table 4.19, each treatment group had a different pasture intake. Pasture price was set at R1.11/kg DM (Meeske *et al*., 2009). For the medium starch treatment this resulted in a daily increase in costs of R 270.40 for 280 cows in milk over that of the high starch treatment, and for the low starch treatment the daily increase caused by increased pasture intake amounted to R 310.80 more when compared to the high starch treatment.

When the milk price calculation was combined with that of the feed cost and pasture cost, the medium starch treatment would lead to a monthly net gain of R42 215.78 over that of the high starch treatment. The low starch treatment would lead to a net gain of R20 043.38 over that of the high starch treatment. If both the gain in milk price, as well as the reduction in feed cost, were taken into consideration, the medium starch treatment would be the most economical option. The possibility of replacing maize with low starch (high fibre) by-products and the savings that coincides with the change is subject to maize price as well as by-product prices.

5.1 References

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CHAPTER 7
Conclusion

During the rumen study ten cannulated, high yielding Jersey cows were allocated the same strips of pasture and given two different additional supplements. The treatments consisted of a high starch and a low starch treatment with only the composition of the supplements differing. The ten cannulated cows were used in a crossover design. It was found that the high starch treatment increased volatile fatty acid concentrations significantly. This was also the case for the individual volatile fatty acids (VFA), such as acetic, propionic and butyric acid, with each of the above mentioned having a higher concentration in the high starch treatment. The ratios of each volatile fatty acid, when compared to the total volatile fatty acid concentration were, however, unchanged by the supplement composition. The ratios between the two diets were almost identical for acetic, propionic and butyric acid. This was also the case for the acetic acid to propionic acid ratios, as well as the acetic acid plus butyric acid to propionic acid ratio. The rumen ammonia-nitrogen levels were also higher in the high starch treatment. Both treatments were, however, above the minimum requirements for ammonia-nitrogen, as stipulated for the rumen and were within the normal range for the rumen, and thus would not lead to an improved effect on production. Another factor with the low starch treatments is that it showed increased variability in the response to supplementation, whereas with the high starch supplementation the response was more predictable.

The rumen pH was unaffected by the supplementation type. The time below pH 5.8, 6.0 and 6.2 was the same in both treatments. This was most probably due to the moderate level of supplementation in the particular study. The DM and NDF digestibilities of ryegrass was unaffected by the supplementation type. This was probably the result of the lack of difference in the rumen pH and the fact that the time spent below pH 5.8 was fairly short. It seems that, at the current rate of supplementation (6 kg per day “as is”, divided into two feedings of 3 kg per feeding), the rumen environment was relatively unaffected. Although the volatile fatty acid concentrations were lower in the low starch treatment this did not lead to higher rumen pH. The rumen environment was not improved with the low starch supplementation if rumen pH is used as an indicator of rumen environment, as was the case with the digestibility of the ryegrass pasture.

During the production study, forty-five high producing Jersey cows were grouped into three groups of fifteen and were allocated the same strips of pasture and given a high starch, medium starch or low starch supplement. There were no differences between treatments for daily milk yield (kg/cow/day) and there were also no differences between treatments for fat corrected milk. This means that no production was lost when cows were given a low starch diet containing less ME on a kikuyu/ryegrass pasture based system. Pasture intake must have been improved in the low starch treatment to sustain milk production, if it was taken into consideration that the low starch concentrate had a lower ME content than the high starch treatment, milk production between treatment groups was unchanged and that there was no difference in live weight change between treatment groups.

Butterfat content (g/100 g) and butterfat yield (kg/cow/day) was improved ($P < 0.05$) by the low starch treatment, as was the butterfat yield of the medium starch diet. This improvement in butterfat content of the milk could not be explained by the rumen study. The result is, however, of great importance as butterfat affects milk price. The milk protein content, milk urea nitrogen, and somatic cell count showed no difference between treatments.

The above mentioned implies that it is possible for dairy producers on kikuyu/ryegrass pasture based systems during spring to make use of by-products such as hominy chop, wheat bran and gluten as a main source of supplementation to dairy cows on pasture based systems without losing milk production. The inclusion of the by-products reduces the need for expensive protein sources like soybean oilcake. Because by-products are usually cheaper, this leads to lower input cost while maintaining milk production output. The improved butterfat content of the milk from the low starch treatment would also lead to a higher potential milk price. This research allows the producers to make an informed decision on which type of concentrate to use at different times. When the maize price is very low, then staying on a high starch concentrate supplementation would not have negative effects. However, during times of high maize prices, it would be feasible to opt for higher by-product inclusion rates. In times when milk buyers set quotas to producers, it would also be a good method to keep milk production constant while decreasing input cost. This research allows the dairy producer to make a decision based on several factors.

Future studies should look at the effect of a higher level (8 - 10 kg/cow/day) of supplementation for dairy cows grazing kikuyu/ryegrass pasture during late winter and early spring. A higher level of supplementation could lead to more pronounced effect on the rumen environment and larger differences in milk production.

CHAPTER 8
Critical Evaluation

Determining pasture intake between treatments: Pasture intake between treatments was not determined. The main reason for this is the lack of an effective, practical and accurate method to determine pasture intake. Previous studies have shown that using the rising plate meter (RPM) is not accurate enough to determine differences between treatments. Using markers is expensive and has been shown to overestimate intake and is not necessarily accurate enough to indicate differences between treatments. The need for an effective and accurate method is of great importance to the research society. Improving pasture intake and being able to statistically prove it would cause great improvement in this field.

Milk meter readings: During the study period several problems occurred with the milk meters. All problems were dealt with effectively, but it restricts the size of the study that can be done. With more accurate milk recording larger scale studies can be run to get a better perspective of results for a whole heard.

Milk samples: Milk samples are of utmost importance. During the current study milk samples were taken three times. For less variation even more samples could have been taken. Care must be taken that all the milking points are clean. Holes must be clean so that milk is siphoned off in correct ratios to ensure that butterfat percentages are representative for each cow.

Dacron bag study: During the current study the dacron bag study was only done for one period, and was not repeated after cross-over. I would have like to do a second period. With the current study, it would have not made a difference, most probably, but for future reference it should be done like this.

Rumen study sampling schedule: Rumen fluids were only collected at three times during each period. An extra one or two sampling times during a 24 hour period would produce a better picture of the rumen dynamics, and allow for better interpretation between parameters.

Adaption period for rumen study: The adaption period in the current study was 14 days. This is long enough for research purposes and allows for sufficient adaption. In this particular case, the cannulated animals were also needed for a later study, so no extra time could be wasted with them. It could, however, be advantages to allow for a 21 day adaption period, if time allows. It could potentially lead to less variation in sampling.

Level of supplementation: On a pasture based system, low levels of supplementation would not necessarily have different responses between treatments. Doing a study with higher levels of supplementation could be beneficial and could lead to more significant results.

APPENDIX A

Cows used during the production study

Table A.1 Cows grouped in the high starch treatment group, labeled with red neck tags and blocked according to milk yield, lactation number and days in milk with other treatment groups

Block number	Name & Number		ID Number	Calving date	Days in milk	Lactation Number	Milk Yield (kg/day)
1	Liz	8	548	2009/05/17	74	6	22.0
2	Paulet	2	537	2009/06/30	30	7	20.3
3	Arna	7	423	2009/04/09	112	3	23.9
4	Sally	4	611	2009/05/22	69	4	18.2
5	Berta	50	666	2009/05/04	87	2	19.7
6	Amsa	34	620	2009/05/02	89	3	19.8
7	Etna	2	613	2009/06/09	51	4	19.7
8	Susa	37	647	2009/05/11	80	2	19.6
9	Lin	15	672	2009/06/17	43	2	20.4
10	Susa	23	615	2009/06/23	37	4	22.4
11	Lass	4	564	2009/03/29	123	5	15.7
12	Tes		504	2009/03/14	138	7	19.4
13	Lua	21	528	2009/06/27	33	2	19.3
14	Wanda	15	701	2009/06/13	47	2	16.0
15	Liz	24	708	2009/07/14	16	2	16.0

Table A.2 Cows grouped in the medium starch treatment group, labeled with blue neck tags and blocked according to milk yield, lactation number and days in milk with other treatment groups

Block number	Name & Number		ID Number	Calving date	Days in milk	Lactation Number	Milk Yield (kg/day)
1	Sally	2	571	2009/06/11	49	4	23.0
2	Dora	99	495	2009/06/09	51	7	21.7
3	Berta	25	568	2009/04/30	91	4	23.4
4	Etna	6	687	2009/05/14	77	2	21.0
5	Santa	8	665	2009/05/14	77	2	17.7
6	Amsa	17	565	2009/05/02	89	5	19.1
7	Lass	7	696	2009/07/04	26	2	18.2
8	Arna	8	475	2009/05/10	81	3	17.4
9	Santa	11	704	2009/06/06	54	2	19.7
10	Berta	29	600	2009/06/24	36	4	22.6
11	Susa	28	442	2009/04/06	115	3	16.5
12	Tes	2	575	2009/03/18	134	5	20.9
13	Amsa	49	694	2009/06/30	30	2	19.6
14	Paulet	13	706	2009/05/07	84	2	19.1
15	Amsa	57	708	2009/07/19	11	2	16.0

Table A.3 Cows grouped in the low starch treatment group, labeled with white neck tags and blocked according to milk yield, lactation number and days in milk with other treatment groups.

Block number	Name & Number		ID Number	Calving date	Days in milk	Lactation Number	Milk Yield (kg/day)
1	Sally	1	511	2009/06/03	57	7	24.2
2	Berta	5	492	2009/06/12	48	8	21.9
3	Amsa	13	535	2009/04/28	93	5	21.5
4	Hes	2	401	2009/05/05	86	4	19.0
5	Paulet	12	703	2009/05/13	78	2	18.7
6	Esme	2	554	2009/05/07	84	6	19.2
7	Amsa	32	468	2009/06/15	45	3	19.4
8	Tes	6	688	2009/04/30	91	2	18.1
9	Berta	54	691	2009/06/04	56	2	19.4
10	Paulet	11	669	2009/06/13	47	2	21.3
11	Lua	22	717	2009/03/27	125	2	17.7
12	Susa		473	2009/03/17	135	7	19.0
13	Berta	24	567	2009/06/20	40	4	21.5
14	Max	25	681	2009/05/26	65	2	16.6
15	Lua	20	711	2009/07/10	20	2	16.0