Concentrate supplementation to Jersey cows grazing plantain and ryegrass

by

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DECLARATION

I, Zander Pretorius declare that the dissertation, which I hereby submit for the degree MSc (Agric): Animal Science (Animal Nutrition) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution. I declare that this dissertation for the degree in MSc (Agric): Animal Science (Animal Nutrition) at the University of Pretoria has not been submitted for a degree at any other university.

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SUMMARY

Title: Concentrate supplementation to Jersey cows grazing plantain and ryegrass

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When compared to TMR-based dairy production systems, pasture-based dairy production systems produce milk at a lower cost per litre. Nutrients provided by a pasture species need to fulfil a major portion of the dairy herd's nutritional requirements for the system to be successful. Kikuyu over-sown with ryegrass is the major herbage choice for pasture-based systems in South Africa's Southern Cape region. Climate and soil conditions are unpredictable, and the use of novel forage species is becoming ever more popular Plantain (Plantago lanceolate) is a perennial forage herb that can provide high amounts of high-quality forage. The forage herb is capable of adapting to drought and a wide range of soil conditions. The energy and mineral supply provided by plantain, decreases the need for high levels of concentrate supplementation. Plantain's low fiber content, however, may limit its application in pasture-based dairy production systems. The aim of this study was to see if milk production, milk solid production, body weight (BW), body condition score (BCS), and the rumen environment would stay the same or improve when Jersey cows grazing plantain during the day and ryegrass at night were given different levels of starch in dairy concentrates.

The study was carried out at the Outeniqua Research Farm in the Western Cape region of South Africa, near George. Perennial ryegrass and plantain pastures were divided into equal blocks to facilitate the measurement of pre-and post-grazing yields for estimation of pasture intake. Fifty-one multiparous lactating Jersey cows were used in a production study. They were blocked according to milk production, days in milk (DIM) and lactation number and randomly allocated to three treatments (high-starch, medium-starch and low-starch containing 80%, 50% and 20% maize respectively) in a randomised complete block design. Maize content was reduced from the high- to the low-starch group, by replacement with high-fibre by-products (hominy chop, wheat bran and soybean hulls). A 14-day adaptation period was followed by 34 days of data collection. Each cow received 6kg (3kg at each milking) of their respective concentrate treatments per day on an 'as-is' basis and strip-grazed plantain from 06h00-13h00 and ryegrass from 14h00-5h00. The BW and BCS of cows were determined at the beginning and end of the study over two consecutive days. Milk yield was recorded daily for individual cows and milk samples were taken every second week to determine milk solid production; sample collection commenced after the adaptation period. For the rumen study, six additional rumen-cannulated cows were randomly allocated to either the high- or low-starch treatments in a two-period cross-over design. Rumen pH, volatile fatty acids (VFA) and rumen ammonia nitrogen (NH_3-N) were determined and an *in situ* study was conducted to determine degradability of dry matter (DM_d), neutral detergent fibre (NDF_d) and the rate of NDF degradation (NDF_{kd}).

No differences (P>0.05) were found for milk yield and milk fat content between treatments and mean values were 20.9, 21.9 and 20.8 kg/cow/day and 4.88-, 4.91- and 4.90 % for the high-starch, medium-starch and low-starch treatment groups respectively. There was however a tendency for milk yield in the medium-starch group to be higher compared to the high-starch (P=0.10) and low-starch (P=0.07) treatments. Milk protein, milk lactose, somatic cell count (SCC), BW- and BCS change showed no difference between treatments (P>0.05). Milk urea nitrogen (MUN) was significantly higher in the medium-starch group compared to the low-starch group (P<0.05) and showed a tendency to be higher than the high-starch group (P=0.10).

Ruminal pH, and individual VFA concentration, rumen NH_3-N and the degradability parameters (DM_d, NDF_d and NDF_{kd}) did not differ between the high- and low-starch treatments. There was however a tendency for total VFA to be higher for the low-starch treatment.

It can be concluded that providing lactating Jersey cows with concentrate containing either 80-, 50- or 20% maize while grazing plantain and ryegrass caused no differences in production and ruminal parameters. It can be deduced that the medium-starch group performed best, because of the tendency for higher milk production. Lower cost associated with high fibre by-products compared to maize provides the opportunity for higher profit margins when feeding medium and low-starch levels as higher maize inclusion did not increase milk yield, milk fat or milk protein content.

LIST OF ABBREVIATIONS

°C	Degree Celsius
AA	Amino acids
ADF	Acid detergent fibre
AOAC	Association of Official Analytical Chemists
A:P	Acetate: propionate ratio
AR	Annual ryegrass
B	Barley
BCS	Body condition score
BP	Beet pulp
BW	Body weight
C	Cassava or control
Ca	Calcium
CIP	Citrus pulp
cm	Centimetre
Co	Cobalt
CP	Crude protein
Cu	Copper
D	Day
DIM	Days in milk
dl	Decilitre
DM	Dry matter
DMI	Dry matter intake

DM_d	Dry matter degradability
E	East
ECM	Energy corrected milk
EE	Ether extract
eNDF	Effective neutral detergent fibre
FB	Fibre-based
FCM	Fat corrected milk
Fe	Iron
g	Gram
G20	Gluten 20
GC	Ground corn
h	hour
ha	Hectare
HC	Hominy chop
HS	High-starch concentrate
IU	International units
IVOMD	In vitro organic matter digestibility
IVTD	In vitro true digestibility
K	Potassium
%	Percentage
K/AR	Kikuyu and annual ryegrass
K/PR	Kikuyu and perennial ryegrass
KCl	Potassium chloride

Kg	Kilogram
KK	Kikuyu
L	Litre
LAN	Limestone ammonium nitrate
LS	Low-starch concentrate
M	Molar
m	Metre
m^2	square metre
MCP	Mono-calcium phosphate
ME	Metabolizable energy
Mg	Magnesium
MgO	Magnesium oxide
MJ	Mega joule
ml	Millilitre
mm	Millimetre
Mn	Manganese
MS	Medium-starch concentrate
MUN	Milk urea nitrogen
N	Nitrogen
Na	Sodium
NDF	Neutral detergent fibre
NDF_d	Neutral detergent fibre degradability
NDF_{kd}	Neutral detergent fibre degradability rate

NEL	Net energy for lactation
NFC	Non-fibre carbohydrates
NH_3-N	Ammonia-nitrogen
NPN	Non protein nitrogen
No.	Number
NRC	National Research Council
NSC	Non-structural carbohydrates
O	Oats
OG	Orchard grass
P	Phosphorus
PKE	Palm Kernel expeller
PR	Perennial ryegrass
PTG/MFG	Perennial timothy grass and meadow fescue grass
PUFA	Poly-unsaturated fatty acids
RPM	Rising plate meter
RSA	Republic of South Africa
S	South
SB	Starch-based
SBH	Soybean hulls
SBP	Sugar beet pulp
SCC	Somatic cell count
Se	Selenium
SEM	Standard error of means

SF	Intermediate fibre- and starch-based
UDP	Rumen undegradable protein
UP	University of Pretoria
VFA	Volatile fatty acids
W	Wheat
WB	Wheat bran
WSC	Water soluble carbohydrates
Zn	Zinc

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CHAPTER 1

Introduction

The number of dairy farmers in South Africa has declined while dairy products are in higher demand (Scholtz & Grobler, 2009; Barkema *et al.,* 2015). The highest number of South-Africa's dairy producers are situated in the Western-Cape (Van Heerden, 2019). Refined management and genetic selection have created dairy cows with high production potential of quality milk. Milk quality determines the milk price, and the most important influencers are milk fat and protein content. Feeding the dairy cow to achieve its potential, while decreasing cost of feed, maintaining longevity and lowering the carbon footprint are the challenges faced by producers. The two primary systems are intensive total mixed rations (TMR's) and pasture based dairy production systems. Compared to TMRs, milk is produced at a cheaper cost per litre on pasture-based dairy production systems, and even though less milk is produced, larger profit margins are probable (Delahoy *et al.,* 2003). Additionally, pasture based dairy production systems decreases stress on cows, leading to a healthier herd with improved longevity. With dairy herds having the genetic potential for high milk yields, pasture only diets generally do not meet nutritional requirements (Charlton *et al*., 2011). Providing lactating dairy cows with concentrate supplementation increases milk production and ensures that cows maintain healthy body condition (Hills *et al.,* 2014). Over-supplementation decreases the intake of pasture and lowers the profit margin. The aim is to fulfil the nutritional needs of cows while still allowing for maximal pasture intake.

Concentrates fed to dairy cows are expensive and constitutes around 66% of total feed cost (Meeske *et al.,* 2006). To increase energy supply, highly fermentable carbohydrates such as starch are the main nutrients in a dairy concentrate. Maize having a high-starch content is normally included at 50- to 80% in the concentrate (Kolver, 2003). The availability of maize is dependent on weather conditions, causing the price to be volatile. Alternative energy sources, such as high-fiber by-products, have the potential to reduce feed costs (Lingnau, 2011; Van der Vyver *et al.,* 2019). Additionally, these alternative feed sources decrease the risk of rumen related metabolic disorders. These by-products contain higher levels of fibre necessary for a ruminant to maintain a healthy rumen pH (Banakar *et al.,* 2018). Previous studies (Van Wyngaard *et al.,* 2015; Cawood, 2016; Van der Vyver *et al*., 2019) found that concentrates containing high fibre by-products compared to high-starch feedstuff sustained milk production and, in some cases, increased milk fat production while sustaining milk production (Bargo *et al.,* 2003; Lingnau, 2011). Some studies showed increased milk production from high-fibre concentrates compared to high-starch concentrates (Meijs, 1986; Khalili & Sairanen, 2000; Meeske *et al.,* 2009).

The nutritional quality traits of pasture determine the extent of supplementation needed. Pasture species needs to be adaptable, have high yields and provide high amounts of nutrients to grazing animals. Forage herbs such as plantain (*Plantago lanceolate*) has been successful within pasture based dairy production systems (Sanderson *et al*., 2003; Minnee' *et al.,* 2017; Moorhead & Piggot, 2009). It serves as an alternative forage to increase feed security (Moorhead & Piggot, 2009). The perennial herb is becoming more popular with its potential to maintain high milk production and lower nitrate leaching (Edwards *et al.,* 2015; Mangwe *et al.,* 2020). It offers large amounts of high-quality herbage when ryegrass yields are low and of poorer quality (Glassey *et al.,* 2012). The forage herb has a high mineral content (Sanderson *et al.,* 2003). Additionally, plantain's tolerance to drought and wide acceptance to soil conditions makes it a viable candidate for further research (Stewart, 1996).

The low fibre content of plantain causes some concern. Moving from a starch-based to a fibre-based dairy concentrate is the potential solution that will be investigated in the following study. The aim was to find a balance of fibre and energy in the concentrate supplied to cows grazing plantain during the morning period and perennial ryegrass during the afternoon and night period. The effects on production parameters (milk yield, milk component yield and cow condition) were measured. A rumen study was also carried out to see how varied quantities of starch in a dairy concentrate affect the rumen fermentation dynamics of cows grazing perennial ryegrass and plantain. We hypothesised an increase in milk yield for cows receiving the high-starch concentrate with a decrease in milk fat g/kg compared to the low-strach group.

CHAPTER 2

Literature review

2.1 Introduction

With the dairy industry's dynamics shifting toward intensification, producers are under pressure to improve milk production per cow. While the potential for high milk production exists for TMR based dairy production, the high feed cost and short lifespan of cows within this production system decreases profit margins. Producing milk on a pasture-based system alleviates these pressures, but management of the pasture is the key to success.

Selection of the most suitable pasture species will determine if enough grazeable material of suitable nutritive value is available for a high production potential all year round (Lambert & Litherland, 2000). The selected pasture species should be able to thrive in its immediate environment and have a high concentration of nutrients to be considered successful.

Supplementation must be assessed considering the current circumstances on the farm. High-fibre concentrates are more likely to benefit pastures with high digestibility and quality than high-starch concentrates. This is especially true when the pasture’s energy supply is not a limiting factor. Supplementing cows with high-starch concentrates is better complimented when they receive lower quality pasture, as energy supply from pasture is the first limiting factor (Meeske *et al.,* 2006). Pastures with high quality and digestibility commonly have low NDF levels, thus the high-fibre concentrate is likely to result in a healthier rumen environment compared to high-starch concentrates (Sayers *et al*., 2003).

The following section focuses on the quality and management of plantain (*Plantago lanceolate*) and perennial ryegrass (*Lolium perenne),* which were the pasture species grazed by the experimental cows during the study.

2.2 Forage plantain

2.2.1 Morphology, production and origin

Plantain (*Plantago lanceolate)* is a rosette forming, perennial forage herb with an erect growth habit (Rumball *et al*., 1997). Vegetative shoots are dense and uniform (Stewart 1996; Rumball *et al*., 1997). Leaves are lanceolate to ovate lanceolate (Stewart 1996). The forage herb’s seed production is high with harvesting dates being 8 weeks after flowering (Rumball *et al.,* 1997).

The leaves contain parallel veins protected on both sides by sclerenchyma fibre. This along with a thick epidermis and collenchyma cell layer gives it a higher NDF value than chicory (another forage herb) (Sanderson *et al.,* 2003). It can grow in less fertile environments and is drought tolerant (Mook *et al.,* 1989; Stewart, 1996; Ayala *et al.,* 2011). Reproductive stems are unpalatable and has low feeding value. Cows avoid eating the reproductive stems, giving the plant a better chance of reseeding itself (Ayala *et al.,* 2011).

Trampling resistance of plantain is moderate (Blom, 1979). Stewart (1996) found that plantain yielded 84% of ryegrass grown in the same conditions, over an average of 4 years and under optimal conditions (environmental and management), plantain can yield up to 20 tonnes dry matter (DM)/ha/per year. Moorhead & Piggot (2009) found higher yields for plantain-based pastures than ryegrass-based pastures under homogenous management. They found this trend included all three years of sampling, confirming plantain's perenniality. The same study showed a decline in yield as the plants got older with seasonal yield ranging from 2000kg/ha in the winter to over 5000kg/ha in the summer. Fisher *et al.* (1996) found that plantain was not outcompeted by grasses. A study by Pembleton *et al.* (2016) found high infestation of weeds within a monoculture of plantain. Glassey *et al.* (2012) found that controlling weeds with herbicide before sowing increased plantain's establishment rate. Sanderson & Elwinger (2000) tested plantain's establishment rate and found that planting at 1cm compared to 3cm and 6cm depth yielded better results. If plantain reaches the 4-6 leave stage, it is fully established (Ries & Svejcar, 1991).

Plantain can grow in a wide range of soil acidity (4.2-7.8), textures, and organic matter levels (excluding severely saline and marshy soils) and it thrives in ryegrass and white clover-friendly soils (Stewart 1996). Less fertile soils increase plantain's competitiveness and higher pasture contribution is likely under these circumstances (Stewart 1996).

The first cultivar of forage plantain (*Plantago lanceolate*) was developed in New Zealand and given breeding rights in 1993 (Rumball *et al*., 1997). Selection aimed to create a plant with high biomass and seed production. Plantain cultivars that are both prolific and erect have been developed (Grassland Lancelot and Ceres tonic). The latter is taller and more active in the winter (Stewart, 1996).

2.2.2 Management

When nitrogen fertilizer is applied, it promotes leaf number, shoot growth, and overall biomass (Stewart 1996). Blacquire & Koetsier (1988) found that upon nitrogen depletion, leaf biomass and number decreased while root growth was largely unaffected. Due to its deep rooting system, nutrients can be utilised from lower soil levels (Foster, 1988). Herbicides can be used to remove unwanted species and increase plantain's pasture contribution. This is especially true in plantain's establishing phase. Ayala *et al.* (2011) concluded that plantain had a 64% botanical contribution after 3 years when herbicides were applied to a mixed pasture containing plantain, annual ryegrass (*Lolium multiflorum*) and lotus (*Lotus corniculatus*). Finding the correct herbicide that will kill or slow the growth of other species without damaging plantain is challenging, because of its broadleaf nature. Doing pre-planting control of species with glyphosate is an option suggested by Labreveux *et al.* (2006).

Studies show a consensus that plantain's performance is enhanced when 3-4-week grazing intervals are applied compared to 1-2 weeks (Labreveux *et al.,* 2006; Ayala *et al.,* 2011). Longer than 5-week grazing intervals causes qualitative and quantitative losses (Labreveux *et al.,* 2006; Ayala *et al.,* 2011). Regrowth after defoliation of plantain shows no differences between severely defoliated and lightly defoliated. The deep roots system of the plant allows it to regenerate after practically all the topsoil material has been removed (Labreveux *et al*., 2006).

2.2.3 Environmental impact

Urine nitrogen excretion has a direct influence on the environment through soil leaching and greenhouse gas emissions (Cheng *et al*., 2017). Lysimeter experiments done on plantain (*var.* ecotain) showed a 45% decrease in nitrogen (N) leaching when urine from animals grazing ryegrass pasture is applied to an ecotain sward. Eighty-nine percent reduction is observed when urine from animals grazing ecotain is applied to an ecotain sward (Carlton *et al.,* 2018). Soil N mineralisation is inhibited by secondary chemicals (aucubin and verbascoside) (Dietz *et al*., 2013). When animals consume ecotain instead of ryegrass pasture, urinary nitrogen levels and excretion are both lower (Cheng *et al*., 2017). Animals grazing plantain had lower nitrogen concentrations in their urine than those grazing perennial ryegrass-white clover pasture mix (Box *et al*., 2017).

2.2.4 Nutritive value

Plantain leaves have high production potential of good quality and palatable herbage (Fraser & Rowarth, 1996; Rumball *et al*., 1997, Sanderson *et al*., 2003). In mixed swards, cattle and sheep graze plantain in preference of grasses and legumes (Stewart, 1996).

Published studies for nutritive value of plantain are summarised in Tables 2.1a. Broadleaf herbs in general contain higher amounts of minerals than grasses (Sanderson *et al*., 2003). The levels meet or surpass the National Research Council's (NRC, 2001) recommendations for lactating dairy cows. Retention of calcium (Ca) is 4 times higher for cows grazing plantain, compared to ryegrass

(Stewart, 1996). High levels of potassium (K) may cause concern, as it inhibits the mobilisation of Ca from bone which can lead to milk fever (Judson & McFarlane, 1998). High levels of Mg decrease the risk of grass tetany (Sanderson *et al*., 2003). When compared to ryegrass, plantain has higher levels of sodium (Na), calcium (Ca), copper (Cu), and zinc (Zn), but lower levels of potassium (K) and manganese (Mn) (Rumball *et al.,* 1997). Variability of mineral concentrations within plants is also dependent on the underlying soil conditions, fertiliser application and irrigation (Waghorn & Clark, 2004). Table 2.2 summarises mineral concentrations of plantain and ryegrass respectively.

A range of biologically active compounds can be found at various quantities in plantain. Aucubin and catapol can constitute up to 3% and verbascoside 9% of DM in plantain. These compounds have antimicrobial properties, which may impair rumen fermentation (Stewart, 1996; Navarrete *et al*., 2016). In addition, these compounds along with plantain's mucilage also have a laxative effect (Stewart, 1996).

Plantain has low fibre concentrations which occasionally falls below 250-330g NDF/kg DM which is the minimum level recommended in the total DM of lactating dairy cow diets (NRC, 2001). Studies done by Box *et al.* (2017), Cheng *et al.* (2017), Fang *et al.* (2018), Mangwe *et al.* (2020) and Waghorn & Clark (2004) collectively show a NDF range of 212- to 299 g/kg DM. Metabolisable energy values for plantain falls in the range of 9.2- to 12.2 MJ/kg DM (Waghorn & Clark, 2004; Pembleton *et al.,* 2016; Box *et al.,* 2017; Mangwe *et al*., 2020). Crude protein levels of plantain are variable between studies, but the levels are comparable to that of ryegrass. Fang *et al*. (2018) and Sanderson *et al*. (2003) discovered lower CP levels of 136 g/kg DM and 134 g/kg DM, respectively, in their studies. Box *et al*. (2017) and Waghorn & Clark (2004), on the other hand, found CP levels of 250 g/kg DM and 223 g/kg DM, respectively. Because of its low NDF content, plantain has a high in vitro organic matter digestibility (IVOMD). These values ranged from 75 to 87.2 percent in studies by Cheng *et al*. (2017), Labreveux *et al*. (2006), and Mangwe *et al*. (2020). Sanderson *et al*. (2003) discovered a greater NDF (374 g/kg DM) with a high IVOMD value, raising concerns about the fibre's efficacy. Søegaard *et al.* (2008) found the opposite with a high NDF value (426 g/kgDM) and a low IVOMD value (63%) and Lee *et al.* (2015) reported a IVOMD value of 70.2% and NDF value of 30.4%.

2.3 Kikuyu/ryegrass pasture

2.3.1 Morphology, production and management

Kikuyu grass (Pennisetum clandestinum) is a tropical, C4 grass (Garca *et al*., 2014). Kikuyu has high summer production potential and low winter and spring production potential (Pearcy & Ehleringer, 1984; Gherbin *et al*., 2007; García *et al*., 2014). Milk production per cow is limited to 15-

16 L/day due to nutrient deficits (Reeves *et al*., 1996). For effective milk production, alternative grass species and/or concentrate feeding are required (Reeves *et al*., 1996; Garca *et al*., 2014). Botha *et al*. (2008) found that incorporating perennial ryegrass into a kikuyu pasture improved nutrient availability all year..

Perennial ryegrass (*Lolium perenne)* is a common temperate bunchgrass with dense tillering and an upright growth habit (Brock & Fletcher, 1993; Minnee', 2011; Yates *et al.,* 2019). Perennial ryegrass is highly productive and responds well to irrigation and nitrogen fertilisation (Pembleton *et al*., 2016). The three-leaf stage of growth is accepted as the rule of thumb for the initiation of grazing (Lee *et al.,* 2010). After the three-leaf stage, there is wastage through leaf senescence and a drop in forage quality is observed. If grazing commences before this stage, defoliation has a heavy toll on the plant's reserves and subsequent re-growth is retarded (Reeves *et al.*, 1996). It has high annual yields (15 tonnes per ha) which are generally consistent over three years (Moorhead and Piggot 2009).

As ryegrass ages, its nutrient content decreases due to a decrease in the leaf to stem ratio, resulting in lower pasture quality. Chen *et al.* (2019) found leaf to stem ratios of 4.6; 1.7; 0.6, and 0.4 after 2, 4, 6 and 8 weeks of growth respectively. Ryegrass also develops a reproductive stem, constituting 6% of DM, eight weeks after cutting (Chen *et al.,* 2019). Effective rotational grazing manages the quality by balancing defoliation intensity which limits the build-up of old material with sub-par quality. According to Waghorn & Clark, (2004) the quality of ryegrass declines the closer the sample is taken to the base.

2.3.2 Nutritive value

Table 2.1b shows the nutritive value comparisons determined by several published studies for ryegrass. These studies found NDF levels ranging between 395- to 521 g/kg (Reeves *et al.,* 1996; Clark & Kanneganti, 1998; Waghorn & Clark, 2004; Meeske *et al.,* 2006; Pembleton *et al.,* 2016; Cheng *et al.,* 2017). Waghorn & Clark, (2004) reported that ryegrass had 200 g/kg DM higher NDF that plantain with variable crude protein values which is comparable to values of plantain. Studies by various authors (Reeves *et al.,* 1996; Clark & Kanneganti, 1998, Waghorn & Clark, 2004; Meeske *et al.,* 2006; Pembleton *et al.,* 2016) found CP levels ranging between 158- to 252 g/kg DM. Metabolizable energy (ME) values for ryegrass-based pastures rarely fall below 10 MJ/kg DM and previous studies found a range between 10.4- to 11.5 MJ/Kg DM (Meeske *et al.,* 2006; Moller *et al.,* 2015; Van Wyngaard *et al.,* 2015; Cawood, 2016; Pembleton *et al.,* 2016). Higher IVOMD values, however, were published from several studies ranging from 80.2- to 84.2% (Reeves *et al.,* 1996; Moller *et al.*, 2015; Van Wyngaard *et al.,* 2015; Cheng *et al.,* 2017)

2.4 Pasture allocation determination

The direct method of pasture biomass determination is more accurate compared to other methods (Harmoney *et al*., 1997). The method entails removing pasture from an area with known size and determining the DM yield. Indirect methods are less labour intensive and saves time (Harmoney *et al*., 1997). The rising plate meter (RPM) was used to determine biomass in this study.

The RPM is equipped with a rod, metal plate and height meter. The rod passes through the middle of the metal plate, while allowing the metal plate to move freely up and down the rod. On the rod, gears are spaced 5mm from each other. Measurements are taken by placing the metal plate on top of the canopy and gently pushing the rod downwards. The metal plate then moves along the gears of the rod, summing the 5mm intervals. A reading is then displayed on the height meter. The RPM height is represented by the difference between the start and end readings (1 unit = 5mm).

The RPM is calibrated by regressing RPM height with its corresponding yield. This enables the user to estimate average yield when pasture is at a certain average height (Sanderson *et al*., 2001). Determining pasture biomass makes farming decisions (paddock selection, pasture allocation and predicting pasture shortages and wastage) more informative and aids in the monitoring of the quantitative factors of pasture.

RPM has a standard error of prediction of 26- to 33% (Sanderson *et al*., 2001). The study by Harmoney *et al.* (1997) found an R^2 value of 0.59 for the regression equation to determine yield. External factors such as slope, wind, wet pasture and pasture trampling contributes to the standard error (Harmoney *et al.,* 1997; Sanderson *et al*., 2001). The RPM's ability to estimate herbage mass is limited when the mass increases above 4000kg DM/ha (Lile *et al*., 2001).

Different calibrations are used for different herbage types (Lile *et al.,* 2001). Rising plate meter calibration equations for ryegrass is more refined compared to plantain. Plantain in its reproductive stage poses a problem for RPM estimation. It develops a rigid reproductive stem which grows higher than the rest of the plant. These stems are sturdy enough to keep the RPM's start reading at a height above the actual starting point of the leaves. An over-estimation of height and under- estimation of density leads to an over-estimation of available herbage. Frequent defoliation decreases the development of stems and this can improve the accuracy of the RPM (Waghorn & Clark, 2004).

Table 2.1a A comparison of the nutrient composition of plantain from several published studies

Reference	Nutrient composition[1] (g/kg DM or as stated)					
	DM	CP	IVOMD	ME[2]	NDF	ADF
Labreveux *et al.* (2006)	-	210	87.2	-	390	-
Waghorn & Clark (2004)	130	250	-	10.8	280	-
Cheng *et al.* (2017)	148	-	75.0	-	255	-
Sanderson *et al.* (2003)	-	134	78.3	-	374	-
Box *et al.* (2017)	98	223	-	11.4	299	224
Mangwe *et al.* (2020)	116	194	76.5	12.2	265	181
Pembleton *et al.* (2016) *	-	169	63.5	9.2	426	336
Fang *et al.* (2018)	-	136	-	-	212	170
Søegaard *et al.* (2008)	-	-	63	-	426	336
Lee *et al.* (2015)	-	-	70.2	-	-	-

[1]DM – dry matter; CP – crude protein; IVOMD – in vitro organic matter digestibility; ME – metabolizable energy; NDF – neutral detergent fibre; ADF – acid detergent fibre
[2]MJ/kg DM
*IVTD - *In vitro* true digestibility

Table 2.1b A comparison of the nutrient composition of ryegrass from several published studies

Reference	Nutrient composition[1] (g/kg DM or as stated)					
	DM	CP	IVOMD	ME[2]	NDF	ADF
Waghorn & Clark (2004)	190	160	-	-	480	-
Cheng *et al.* (2017)	215	-	83.0	-	514	-
Reeves *et al.* (1996)	-	252	84.2	-	395	177
Clark & Kanneganti (1998)	180-240	180-250	-	-	400-500	-
Meeske *et al.* (2006)	145	207	-	10.6	437	285
Pembleton *et al. (2016)*	-	158	71.9	10.6	521	319
Van Wyngaard *et al.* (2015)	128	153	80.2	11.5	493.9	301.5
Cawood (2016)	151	201	-	10.4	571	269
Moller *et al.* (2015)	135	246	82.2	11.2	494	255
Van der Vyver *et al.* (2019)	164	194	-	13.9	437	276

[1] DM – dry matter; CP – crude protein; IVOMD – in vitro organic matter digestibility; ME – metabolizable energy; NDF – neutral detergent fibre; ADF – acid detergent fibre
[2]MJ/kg DM

Table 2.2 A comparison of the mineral composition of plantain and ryegrass respectively

Reference	Macro minerals[1] (g/kg DM)					Micro minerals[2] (mg/kg DM)			
	Ca	**P**	**Mg**	**K**	**Na**	**Mn**	**Cu**	**Fe**	**Zn**
Plantain									
Sanderson *et al* (2003)	19	3.9	3.5	29	-	89	22	-	31
Cheng *et al (*2017)	-	-	-	23.5	9.9	-	-	-	-
Box *et al* (2017)	21.3	3.83	1.83	31.3	9.8	-	-	-	-
Ryegrass	-	-	-	-	-	-	-	-	-
Cheng *et al (*2017	-	-	-	13.4	0.9	-	-	-	-
Reeves *et al* (1996)	5.9	3.3	2.4	34.4	3.7	-	-	-	-
Meeske *et al* (2006)	6.7	3.6	3.6	33.9	-	60.5	6.9	194	42.9

[1]N – nitrogen; Ca – calcium; P – phosphorus; Mg – magnesium; K – potassium; Na – sodium
[2]Mn – manganese; Cu – copper; Fe – iron; Zn - zinc

2.5 Concentrate supplementation for grazing dairy cows

Jersey cows can produce 13kg of milk per day over a lactation from pasture only (Meeske *et al*., 2006). For cows to achieve their production potential, concentrate supplementation must be included in the diet. Cows exclusively grazing pasture lacks appropriate dry matter and energy intake (Valk *et al*., 1990). Protein supplied by pasture is highly degradable and an imbalance of protein and energy occurs, because of forages' low energy value (Carruthers *et al.,* 1997).

Supplementation decreases over-use of pasture and increases feed security (Meeske *et al*., 2006). Over-supplementation of grain-based concentrates leads to lower DMI of pasture due to substitution. The high cost of dairy meal should be taken in consideration and supplementing cows should yield an economic return. Historically, dairy concentrates were grain based. The use of by-products, however, is becoming ever more popular where starch is replaced with fibre (Sutton *et al.,* 1987). Increasing the level of concentrate feeding increases milk and milk component yield (Sayers *et al.,* 2003). Bargo *et al.* (2003) suggested that above 4-5kg DM/cow, milk yield response declines when pasture allocation and quality is relatively high. Maximising pasture intake to a point where nutrients are balanced with the inclusion of concentrate increases profit margins. The concentrate feed is usually fed during the milking process in the milking parlour. This enables personalized feeding based on a cow's potential for production. A flat rate feeding strategy of 4- to 8kg DM concentrate per day, split across two feedings, is used on many farms to facilitate management.

2.6 Production performance

Table 2.3a and Table 2.3b compares the effects of starch- and fibre-based concentrates on milk yield and milk component yield between published studies. This section also discusses the impact of plantain on production efficiency.

2.6.1 Milk production

According to Moorhead & Piggot (2009) plantain has a positive effect on milk production relative to ryegrass. Milk yield potential is often higher in forage species with higher ME values (Botha *et al*., 2008). Box *et al*. (2017) compared milk production from plantain (late lactation Jersey x Holstein) to a perennial ryegrass-white clover pasture mixture and found that the plantain treatment group had a higher milk yield (P=0.05). Mangwe *et al*. (2020) found that cows grazing plantain tended to produce more milk than when they grazed chicory or perennial ryegrass- white clover pature. Cows on ryegrass-white clover pasture spent more time ruminating but had lower DMI than those grazing plantain pasture (Mangwe *et al*., 2020). Minneé *et al*. (2017) discovered that using herbs like plantain in a sward mixture at 40% increased DMI, milk yield, and milk component yield. Plantain-containing swards had the same DMI as swards without it (Minnee' *et al*., 2017). Cows grazing swards of perennial ryegrass and white clover with 40% plantain produced 19% more milk and 17% more milk solids than cows grazing swards of perennial ryegrass and white clover without plantain (Minnee' *et al*., 2017).

There are disparities in the results of research comparing the milk production of grazing dairy cows fed high-starch vs. high-fibre concentrates. Cows fed high- and low-starch concentrate feeds produced the same amount of milk, according to Sayers *et al*. (2003). When the amount of concentrate fed to the cows was doubled in the same study, milk yield increased. Pressure placed on cows getting high levels of concentrate causes metabolic abnormalities and contributes to an increase in the replacement rate of animals, making the system less effective. Delahoy *et al.* (2003) reported no difference in milk yield when cows grazing orchard grass were supplemented with 8.2 kg of either high-starch or high-fibre concentrates. Cawood (2016), Lingnau (2011), Meeske *et al*. (2009), Van der Vyver *et al*. (2019), and Van Wyngaard *et al*. (2015) discovered no change in milk production between high- and low-starch concentrate treatments fed at 6kg/cow/day to Jersey cows grazing kikuyu-ryegrass-based pastures. When Holstein cows grazing perennial timothy and meadow fescue pasture were given 4kg of each of the two treatments, Khalili & Sairanen (2000) reported a significant increase in milk production for the low-starch concentrate (high fibre by-products) compared to the high-starch concentrate. Meijs (1986) also found higher milk yield for the low-starch compared to the high-starch treatment when Dutch-Friesian cows grazing perennial ryegrass received approximately 5.5kg of each treatment.

2.6.2 Milk components

The concentration of milk components, apart from milk volume, is a major factor determining milk price. Milk with higher concentrations of milk fat and protein commonly fetch a higher price per litre. Jersey cows produce higher quality milk, containing high levels of milk solids when compared to other breeds. Many factors (genetics, breed, disease, age and stage of lactation) play a role in milk component yield. Milk fat depression can be corrected within three weeks when cows are fed a properly formulated diet, while milk protein is more difficult to correct (Heinrichs & Jones, 2016). Proper rumen function is necessary for milk solid production. This means that enough protein and energy need to be supplied for maximal microbial growth. Fibre is essential for milk fat production. If energy levels of a diet increase, the inverse occurs with fibre levels and this might cause milk fat depression (Zebeli *et al*., 2008). If fibre levels are too high, energy is in short supply which causes lower milk and milk protein yield (Heinrichs & Jones, 2016). Even though plantain samples had lower NDF concentrations than ryegrass-white clover samples, Mangwe *et al*. (2020) reported higher milk solid yields for cows on plantain pasture compared to ryegrass-white clover pasture mix. This increase could have been due to higher energy supply to the rumen when cows grazed plantain rather than perennial ryegrass-white clover pasture mix (Mangwe *et al*., 2020).

2.6.2.1 Milk fat

The major precursor of milk fat is the volatile fatty acid (VFA) acetate, which is produced through fibre digestion in the rumen (Bauman & Griinari, 2003; Heinrichs & Jones, 2016; Banakar *et al*., 2018). High quantities of non-fibre carbohydrates (NFC) promote the production of propionate, which lowers the acetate to propionate ratio (Sairanen *et al*., 2006). Plantain is low in fiber and likely to cause milk fat depression if additional fibre is not supplied (Nkomboni *et al*., 2021)

Box *et al.* (2017) found that milk fat decreased with 0.36% ($P<0.05$) when cows grazed plantain instead of to perennial ryegrass-white clover pasture mix. In contrast, Mangwe *et al.* (2020) found an increase in milk fat when cows grazed plantain vs ryegrass- white clover pasture. Given the low NDF, this increase was thought to be produced by a larger energy supply to rumen microbes due to plantain's higher ME content. When compared to cows grazing ryegrass-white clover, cows grazing plantain had 68% more polyunsaturated fatty acids (PUFAs) in their milk (Mangwe *et al*., 2020). Differences for milk fat production between high- and low-starch treatments exists between studies. In theory, cows receiving higher amounts of effective fibre should produce more milk fat, but this is not always the case. Lingnau (2011) compared diets with high-starch levels to diets high in high-fibre levels and found that cows on the high-fibre by-product diet had higher milk fat yield ($P<0.05$). Meeske *et al*. (2009) compared three different concentrate treatments (high-starch, medium-starch, and low-starch) and discovered that the medium-starch group produced more milk fat than the high-starch group, with the same outcome for the medium- and low-starch groups. Khalili & Sairanen (2000) compared a pasture only treatment (perennial timothy and meadow fescue) with high- and low-starch supplementation given to cows grazing perennial timothy and meadow fescue pastures. The results of their study reported that the pasture only diet resulted in a more milk fat production than the high- and low- starch treatment groups with no difference between the supplemented cows. This can be attributed to higher effective fibre of pasture compared to both concentrates. Various authors (Meijs, 1986; Delahoy *et al.,* 2003; Van Wyngaard *et al.,* 2015; Cawood, 2016; Van der Vyver *et al.,* 2019) found no difference between high- and low-starch treatment groups.

2.6.2.2 Milk protein

Genetics, stage of lactation and environmental factors plays a role along with nutrition to determine milk protein yield. Hwang *et al.* (2000) stated that milk protein content has a stronger correlation to genetics than milk fat content. Microbial protein provides the mammary gland with amino acids (AA), which are needed for milk protein synthesis. Energy is needed for microbes to produce microbial protein. Milk protein concentration is regulated by energy intake and dietary density; thus, milk protein might indicate energy supply to lactating dairy cows (Coulan & Remond, 1991). Energy supply mainly comes from fermentation of carbohydrates to produce propionate as a precursor of glucose (Heinrichs & Jones, 2016). With an increased supply of glucose to the mammary gland, there is a higher potential for increased milk protein production (Mackle *et al*., 2000). Decreasing dietary concentrate by increasing roughage, lowers blood glucose levels, consequently decreasing the supply to the mammary gland (Evans *et al*., 1975). Emery (1978) compared 13 studies where substitution of concentrates with roughages occurred to determine the effect that energy supply had on milk protein synthesis. Average daily intake of energy among the studies ranged from 37.7- to 167 MJ ME/cow/day where milk protein ranged from 2.8- to 4.0%. Milk protein increased by 0.015 percent for every additional 4.184 MJ consumed, according to regression analysis (Emery, 1978). Because both parameters are linked to energy supply, milk protein content and yield have a positive relationship (Emery, 1978). Dietary crude protein content is more closely connected to milk yield than milk protein yield (Kirchgessner *et al*., 1967).

Studies by Cawood *(*2016) and Delahoy *et al.* (2003) found that milk protein content decreased when the dairy concentrate had higher inclusion of high fibre by-products and subsequently lower energy values. Van der Vyver *et al.* (2019) reported higher milk protein when maize was replaced with soybean hulls (P<0.05). According to studies conducted by Khalili & Sairanen (2000), Lingnau (2011), Meeske *et al*. (2009), Meijs (1986), and Van Wyngaard *et al*. (2015), there is no change in milk protein concentration between high- and low-starch concentrates. Box *et al*. (2017) reported no differences in milk protein yield between animals grazing plantain or ryegrass-white clover pasture, where Mangwe *et al.* (2020) reported higher protein yield for cows grazing plantain compared to those grazing perennial ryegrass-white clover pasture.

2.6.2.3 Milk lactose

Propionate produced in the rumen is converted to glucose in the liver (Aiello *et al.,* 1989). The mammary gland utilises glucose to produce lactose (McDonald *et al.,* 2001). Compared to other milk solids, lactose content is more difficult to manipulate through dietary means (Sutton, 1987). Jenkins & McGuire (2006) stated that milk lactose variation is caused by severe feeding conditions. High somatic cell count (SCC) and poor udder health can also alter milk lactose content (Kitchen, 1981). According to Welper & Freeman (1992), milk lactose content ranged from 4.61- to 5.04% across 6 different dairy breeds. Average milk lactose of approximately 4.85% was reported by the NRC (2001).

Khalili & Sairanen (2000) reported that although there was no difference in milk lactose between cows fed high- or low-starch concentrates (P<0.05), both yielded more milk lactose than cows only grazing pasture (P>0.05). No difference was found between the high- and low-starch concentrate treatments (P<0.05). Studies by Cawood (2016), Schwartz *et al.* (1995) and Van Wyngaard *et al.* (2015) found high-starch treatments resulted in higher milk lactose content. Van der Van der Vyver *et al.* (2019) reported that when maize was partially substituted with soybean hulls, the opposite was true, and Lingnau (2011) found no difference between the high- and low-starch treatments (P<0.05). When cows were given plantain pasture instead of ryegrass-white clover pasture, Box *et al*. (2017) showed a 0.1 % DM increase in lactose (P<0.05), however Mangwe *et al*. (2020) found no variation in milk lactose content for cows grazing perennial ryegrass-white clover. The additional energy available for microbial protein production when cows graze plantain was attributable to this increase.

2.6.2.4 Milk urea nitrogen

Milk urea nitrogen (MUN) content of milk reflects protein intake, degradation of dietary protein within the rumen and post ruminal supply of protein (Roseler *et al*., 1993). It acts as a guide to monitor nutritional status and detects imbalances (Kohn, 2007). Baker *et al.* (1995) found increasing levels of MUN when more highly degradable protein was given to cows. Because there is a positive association between rumen ammonia-N concentration, MUN, and blood urea nitrogen (BUN), MUN and BUN are indicators of rumen ammonia capture (DePeters & Ferguson, 1992). Milk urea nitrogen concentrations of cows receiving TMR's are usually lower when compared to cows grazing pasture (Bargo *et al.,* 2003). This agrees with Khalili & Sairanen's (2000) findings that reported that cows grazing pasture only had higher MUN levels than cows on high- or low-starch concentrate treatments (P<0.05). Standard reference levels indicate MUN levels should be between 11-17 mg/dl (Hwang *et al.,* 2000). Kohn (2007) recommends concentrations of 8-12 mg/dl which is more a reflection of cattle on a TMR diet. Beyond these ranges a shortage or surplus of protein is likely to have occurred. Shortages may cause a range of problems for the cow. The quality and quantity of milk can decrease, leading to lower profit margins. The reproductive efficiency is also compromised (Ferguson *et al.,* 1993). Surplus protein is not well used and is expelled in the urine, which has a negative environmental impact and is an energy-intensive procedure for the cow (Box *et al.,* 2017). The CP:ME ratio needs to be low enough for microbes to utilise nitrogen efficiently (Roseler *et al.,* 1993; Broderick *et al.,* 1997; Jonker *et al.,* 1999).

The recommended MUN levels have decreased over time. Many studies have been conducted in order to optimize the protein balance fed to cows and, as a result, to reduce the amount of protein lost in urine (Van Wyngaard *et al.,* 2015). Cows grazing plantain show lower MUN than cows on a ryegrass-white clover pasture mix (Mangwe *et al.,* 2020). Delahoy *et al.* (2003) reported that cows fed a low-starch diet had higher MUN values than cows on a high-starch diet (P<0.05). Van der Vyver *et al.* (2019) found a significantly higher MUN concentration for a low-starch treatment compared to both medium- and high-starch concentrate treatments. Cawood (2016), Lingnau (2011) and Van Wyngaard *et al.* (2015) found that there was no difference between the high- and low-starch concentrate treatment groups. (P>0.05).

2.6.2.5 Somatic cell count

Somatic cell count (SSC) reflects udder health. It provides a way of monitoring hygienic quality of milk (Skrzypek *et al.*, 2004). The SCC is affected by parity and environmental factors such as unhygienic conditions (Erdem *et al.,* 2007). Somatic cells primarily consist of leucocytes which is used to protect the udder. Presence of inflammation increases the amount, thus making it possible to detect mastitis based on SCC values. Somatic cell count in the range of 50 x 10^3 cells/ml of milk is a sign of a healthy udder (Skrzypek *et al.*, 2004). An SSC of 200 x 10^3 cells/ml is suggested to be the threshold between health and disease and milk with SCC above 500 x 10^3 cannot be used for human consumption (Skrzypek *et al.*, 2004). Comparing different groups of cows' SCCs based on treatment rarely yields any differences as it is usually more a case of individual cow health and general management (Van der Vyver *et al*., 2019).

2.6.3 Body weight and body condition score

A dairy herd's body weight (BW), body condition score (BCS), and fluctuations over time are essential management tools. At different ages and stages of lactation, differences will exist in body reserve mobilisation (De Villiers *et al*., 2000). A cow's body condition determines if enough body reserves are available to support high milk production (Heinrichs & Jones, 2016). Cows with low body condition have limited body reserves and this will influence their production and reproductive performance. To indicate if cows are meeting their nutritional requirements, BCS is a better measure than BW as BCS is more sensitive to changes (Bargo *et al*., 2002). According to Steyn (2012), a change in BW over a short period of time is negligible. The Wildman *et al*. (1982) body condition rating system, which ranges from emaciated to fat and is specified by a scale of 1-5 (thin-fat), is frequently used. The technician gives a score based on appearance and palpation of the animals back and hindquarters. Body condition score is subjective and structural differences exists between animals (Moran, 2005). The accuracy as well as comparability between studies depends on the competence of the scorers.

The high DMI, low internal parasite load and high ME value associated with plantain explains the weight gain experienced by ruminants grazing plantain (Carr, 2015). Pure plantain swards show weight gain of cows equal to that of endophyte-free perennial ryegrass (Stewart, 1996). Bargo *et al*. (2003), Cawood (2016), Meeske *et al*. (2009), Meijs (1986), Lingnau (2011), Sayers *et al*. (2003), and Van Wyngaard *et al*. (2015) found no difference between high- and low-starch concentrate treatments in terms of BCS or BW change. Van der Vyver *et al*. (2019) reported a substantial drop in BCS when soybean hulls were raised from 15% to 30% of the diet.

Table 2.3a Summary of the effect of starch- and fibre-based concentrates on production parameters of grazing dairy cows

Reference	Cow[1]		Pasture[2]		Concentrate[3]		Milk yield (kg/cow/day)	Milk components		
	Breed	DIM	Type	Intake*	Type	Intake		Milk fat (%)	Milk protein (%)	MUN[4] (mg/dl)
Delahoy *et al.* (2003)	Holstein Friesian	182	OG	12.1	S (Maize)	8.2	27.6	3.53	3.23[a]	14.9[a]
				12.0	F (BP, SBH)	8.2	27.4	3.63	3.19[b]	15.4[b]
Lingnau (2011)[5]	Jersey	153	K/AR	12.9	S (Maize)	6.0	19.9	4.07[a]	3.53	17.8
					SF (HC, WB, G20)	6.0	20.0	4.49[ab]	3.63	17.1
					F (HC, WB, G20)	6.0	19.0	4.75[b]	3.59	17.3
Cawood (2016)[5]	Jersey	96	K	10	S (Maize)	6.0	18.8	4.18	3.66[a]	10.2
					SF (HC, WB, G20)	6.0	18.9	4.14	3.53[ab]	10.3
					F (HC, WB, G20)	6.0	18.3	4.27	3.45[b]	9.26
Van Wyngaard *et al.* (2015)[5]	Jersey	82 - 89	K/PR	10	S (Maize)	6.0	21.3	4.63	3.54	17.7
					SF (PKE)	6.0	21.3	4.65	3.46	18.6
					F (PKE)	6.0	20.7	4.66	3.50	19.1
Van der Vyver *et al.* (2019)[5]	Jersey	127	K/AR	10.5	S (Maize)	6.0	19.3	5.12[c]	3.67[a]	8.30[a]
					SF (SBH)	6.0	19.4	5.48[d]	3.81[b]	8.54[a]
					F (SBH)	6.0	19.2	5.33	3.82[b]	9.36[b]

[1]DIM – pre- experimental days in milk
[2] OG – orchard grass (*Dactylis glomerata* L.); K/AR – kikuyu (*Pennisetum* cladestinum) and annual ryegrass (*Lolium* multiflorum); K – kikuyu; K/PR – kikuyu and perennial ryegrass (*Lolium perenne*)
[3]S – starch-based; F – fibre-based; SF – intermediate fibre- and starch-based; BP – Beet pulp; SBH – soybean hulls; HC – hominy chop; WB – wheat bran; G20 – gluten 20; PKE – palm kernel expeller
[4]MUN – milk urea nitrogen
[5]Concentrate intake on 'as is' basis
[a,b]Means in the same column with different subscripts differ (P<0.05); [c,d] P-value=0.06

Table 2.3b Summary of effect of starch- and fibre-based concentrates on production parameters of grazing dairy cows *cont.*

Reference	Cow[1]		Pasture[2]		Concentrate[3]		Milk yield (kg/cow/day)	Milk components		
	Breed	DIM	Type	Intake	Type	Intake		Milk fat (%)	Milk protein (%)	MUN[4] (mg/dl)
Khalili & Sairanen (2000)	Holstein Friesian	171	PTG/MFG	40*	C (Pasture only)	0	18.4[a]	4.12[a]	3.42	40.0[a]
					SF (B)	4.0	19.7[b]	3.85[b]	3.42	36.3[b]
					F (O, BP)	4.0	21.0[c]	3.76[b]	3.49	37.6[b]
Sayers *et al.* (2003)[5]	-	40	PR	23*	S (B, W, Corn)	5.0	33.3	3.75	3.35	-
					F (BP, CIP)	5.0	34.0	3.81	3.19	-
					S (B, W, Corn)	10.0	37.3	3.08	3.44	-
					F (BP, CIP)	10.0	36.0	3.58	3.25	-
Meijs (1986)	Dutch Friesian	60	PR	28*	S (Cassava, Corn)	5.5	25.6[a]	3.96	3.40	-
					F (BP, SBH, PKE)	5.4	26.9[b]	4.10	3.37	-
Meeske *et al.* (2009)[5]	Jersey	-	AR	-	S (Maize)	6.0	21.0	3.66[a]	3.45	17.8
					SF (HC, WB)	6.0	20.8	4.03[ab]	3.55	17.8
					F (HC, WB)	6.0	20.1	4.41[b]	3.42	18.1
Sutton *et al.* (1987)	Friesian	21 - 98	None	-	60S (B, W, C)	10.8	26.2	4.19	2.78	-
					60F (WF, SBP, CIP)	10.8	26.6	4.35	2.79	-
					80S (B, W, C)	14.0	29.8	2.26	2.76	-
					80F (WF, SBP, CIP)	14.0	26.5	3.62	2.91	-

[1]DIM – pre- experimental days in milk
[2]PTG/MFG – Perennial timothy grass (*Pleum pratense*) and meadow fescue grass (*Festuca pratensis*); PR - perennial ryegrass (*Lolium perenne*); AR – annual ryegrass (*Lolium multiflorum*)
[3]C – control; S – starch-based; F – fibre-based; SF – intermediate fibre- and starch-based; B – barley; O oats; BP – beet pulp; W – wheat; CIP – citrus pulp; SBH – soybean hulls; PKE – palm kernel expeller; HC hominy chop; WB - Wheat bran
[4]MUN – milk urea nitrogen
[5]Concentrate intake on 'as is' basis
[a, b] Means in the same column with different superscripts differ (P<0.05)
*Pasture allowance kg/cow/day 'as is'

2.7 Effects on rumen parameters

The effects of starch- and fibre-based concentrates on rumen pH, VFA production, and rumen NH3-N production are compared in Table 2.4. This section also discusses the impact of plantain on rumen parameters.

2.7.1 Rumen pH

Within the rumen, feed is fermented to produce VFA. These compounds reduce the ruminal pH (Erdman, 1988; Allen, 1997). Two to five hours (h) post feeding of concentrates, the rumen pH should be at its lowest (Cajarville *et al.,* 2006). The animal counteracts the reduction in pH mainly by supplying the rumen with saliva rich in buffers (primarily phosphates and bicarbonates). These compounds neutralise the pH by removing excess hydrogen ions from the rumen liquor (Allen, 1997). Saliva production is stimulated by chewing, which occurs primarily during rumination. Rumination is the process where coarser feed particles are returned to the mouth from the reticulum in the form of a bolus. The time the cow spends ruminating is directly related to the amount of saliva that will be produced and supplied to the rumen (Cassida & Stokes, 1984). Fibre content of a feed is highly correlated to rumination time and saliva production (Maekawa *et al.,* 2002). Dairy concentrates generally have high amounts of starch-based substances such as maize to ensure energy supply is sufficient. Fibre and starch content of a concentrate feed are inversely proportionate. It is necessary to provide the cows with additional fibre with high physical effectiveness. Allen (1997) discovered that ruminal pH and forage NDF have a positive correlation.

Rumen pH has a direct impact on the rumen microbial community, which influences nutrient availability and VFA concentration as fermentation end products. Rumen microbes are generally selective in terms of the nutrients they ferment, and they are sensitive to pH changes (Dijkstra, 1994). Shriver *et al.* (1986) and Varga *et al.* (1984) said that fibre fermenting microbes are more active within a pH range of 6.2 to 6.5. In comparison, microbes acting upon starch are more active at a lower pH range (5.2 to 6.0) (Cawood, 2016). Hoover (1986) stated that fibre digestion decreases drastically below a pH of 6.0. In the case where starch is over-abundant, fibre digesting bacteria are inhibited, decreasing the rumen’s ability to digest and ferment fibre (Cawood, 2016). A rumen pH of 5.8 to 6.4 is required for appropriate rumen activity and nutrient availability to the animal (Banakar *et al*, 2018). According to Owens *et al.* (1998) minimum pH values for subacute and acute acidosis are 5.6 and 5.2 respectively. The time ruminal fluid spends below a pH of 6 determines the extend of reduction in fibre digestion. If large diurnal variations in rumen pH exists, it requires constant adjustment in metabolic pathways. This constant variation of pH may have a detrimental impact on the rumen microbes (Mertens, 1997). Multiple feeding times of high-starch concentrates per day increases the risk of large diurnal variation of rumen pH (Sayers *et al.,* 2003).

Highest pH is usually found just before concentrate feeding (Cajarville *et al.,* 2006). Volatile fatty acids are absorbed via the rumen wall only in associated form (bound to hydrogen ions). If the pH is high, little of the VFA are in the associated form which decreases their absorption (Allen, 1997). Methods of determining rumen pH are variable among studies. Additionally, different parts of the rumen will have different pH values. This can create bias between comparisons with other studies.

Plantain has low levels of physical effective fibre (Table 2.1a). Minnee' *et al*. (2017) discovered that cows grazing a pasture containing plantain (20- or 40%), perennial ryegrass and white clover rumen pH falls faster than in perennial ryegrass-white clover pasture alone (P<0.001). In the same study, rumen pH had a tendency to be lower between 20h00 and 23h00. The bioactive allelochemicals especially, aucubin and verbascoside can inhibit rumen fermentation dynamics (Stewart, 1996; Dietz *et al.,* 2013). The broadleaf nature of plantain increases bitesize, which in turn increases chewing time. This, along with low physical effective fibre of plantain is associated with smaller particles supplied to the rumen, with high rumen fermentation rates (Gregorini *et al*., 2013).

In conclusion, low rumen pH can lower DMI, fibre digestion and microbial yield which in turn reduces milk yield and milk fat production. Neutral detergent fibre originating from forage sources typically have lower degradation rates compared to non-forage NDF. This leads to more rumination time and higher buffering capacity (Allen, 1997).

2.7.2 Volatile fatty acids

Volatile fatty acids are created in the rumen during the fermentation of organic matter and account for around 75% of the ruminant's energy supply (Bergman, 1990). Propionate, acetate, and butyrate are the primary VFAs, and their concentrations are diet and time dependent (Aluwong *et al.,* 2010). Highest production normally takes place 2-4 h post-feeding. Propionate goes through gluconeogenesis and is the primary precursor to produce glucose in ruminant animals (Wiltrout & Satter, 1972; Bergman, 1990). Increasing the supply of propionate positively effects milk yield and milk protein yield. Lactose production is mostly dependent on the production of propionate and microbial protein synthesis is dependent on available energy (Wiltrout & Satter, 1972). Live weight gains are also associated with the energy supply form propionate (Moller *et al*., 2015).

Milk fat content however is negatively impacted when the acetate: propionate ratio (A:P) is decreased (Dijkstra, 1993). Acetate is the most common VFA generated in the rumen (50-60%) and is strongly linked to milk fat synthesis (Ørskov, 1986; Ishler *et al*., 1996; Aluwong *et al.,* 2010).

Fermentation of non-structural carbohydrates such as starch produces more propionate. Structural carbohydrates (NDF) are responsible for acetate production (Dijkstra, 1993). Butyrate (11- to 12%) also produces milk fat and is the main VFA produced when the` fermentation of water-soluble carbohydrates (WSC) occurs in the rumen (Fang *et al.,* 2018). Theoretically, a fibre-based diet and pasture with higher NDF will have a higher A:P compared to starch-based diets and pasture with lower NDF values. Plantain's low NDF value is likely to cause a lower A:P and subsequently this can be problematic in terms of milk fat production.

Studies comparing the effect of high- and low-starch concentrates found total VFA of 120- to 156.1 mmol/L and 113- to 149 mmol/L for starch- and fibre-based concentrates respectively. Acetate: propionate ratios for these studies ranged between 2.16-4.62 and 2.86-4.77 for starch and fibre-based concentrates respectively (Khalili & Sairanen, 2000; Sayers *et al.,* 2003; Lingnau, 2011; Van Wyngaard *et al.,* 2015; Cawood, 2016). Bargo *et al.* (2003) reported total rumen VFA production ranging between 90.3- to 151.4mmol/L over 10 studies where cows were grazing pasture and received concentrate. Lingnau (2011) found significant differences between starch and fibre-based concentrates. The total amount of VFA as well as propionate, acetate and butyrate individually had higher values for the starch-based diets. Various studies (Khalili & Sairanen, 2000; Sayers *et al.,* 2003; Van Wyngaard *et al.,* 2015; Cawood, 2016) showed no differences for starch- vs fibre-based diets in terms of total and individual VFA concentrations. Higher A:P were found for fibre-based diets (Sayers *et al.,* 2003; Lingnau, 2011; Van Wyngaard *et al.,* 2015). Conversely, studies done by Cawood (2016) and Khalili & Sairanen (2000) found higher A:P for the starch-based diets. Fang *et al*. (2018) compared a ryegrass-white clover pasture to a plantain pasture and discovered that cows feeding plantain had greater total VFA. The ryegrass-white clover pasture as expected had a higher A:P. Kara *et al*. (2016) found a total VFA production of 133 mmol/L with an A:P of 2.91 when cows were fed plantain. In the same study plantain showed a tendency for significantly higher acetate production (60.6 mmol/L) compared to lucerne (*Medicago sativa*) (47.4 mmol/L) even though crude fibre (CF) of lucerne was significantly higher compared to plantain.

2.7.3 Rumen ammonia-nitrogen

Protein levels and quality along with sufficient dietary energy are the main determinants for microbial population growth within the rumen (Hoover & Stokes, 1991). Microbial populations in turn determine energy supply to the ruminant through the action of carbohydrate fermentation. Free rumen ammonia is utilised by microbes for their own growth (Maeng *et al.,* 1976). Without means of microbial growth, carbohydrate fermentation and energy supply to the ruminant is low (Hoover, 1986). Excess protein is excreted as urine nitrogen which contributes to water pollution

(Dalley *et al.,* 2017). Balancing the ration to ensure utilisation of high percentages of protein in turn can reduce the cost of feed and increase profit margins (Moller *et al.,* 2015).

Over 10 experiments where dairy cows received energy supplementation, Bargo *et al*. (2003) reported an average rumen NH3-N concentration of 18.3 mg/dl (range: 8.7 to 32.2 mg/dl). Feedstuff with higher DM or OM digestibility leads to higher concentrations of rumen NH_3-N (Erdman *et al.,* 1988). Pasture normally has protein with higher digestibility compared to concentrates which in theory means that pasture will contribute more to rumen NH_3-N (Van Vuuren *et al*., 1986). With increase in concentrate supplementation, decrease in grazing time is observed, thus decreasing utilisation of pasture (Bargo *et al.,* 2003). Cajarville *et al*. (2006) discovered that the highest rumen NH3-N corresponds to the lowest pH, which occurs 2-5 hours after concentrate consumption.

The minimal rumen NH3-N for maximal microbial development, is 5mg/dl rumen fluid (Satter & Roffler, 1974). According to Slyter *et al*. (1979) rumen NH3-N concentrations greater than 4.5 mg/dl rumen fluid had no effect on VFA synthesis in steers. Compared to lucerne and atriplex (*Atriplex patula*), cows eating plantain had considerably less rumen ammonia levels (Kara *et al*., 2016). The values were 4.79-, 4.80- and 3.65 mg/dl for alfalfa, atriplex and plantain respectively. Secondary compounds (aucubin, catapol and verbascoside) can lower rumen NH_3-N concentration (Navarette *et al*., 2016).

Mean rumen NH_3-N values of studies conducted by Khalili & Sairanen (2000), Lingnau (2011), Sayers *et al.* (2003) and Van Wyngaard *et al.* (2015) where high- and low-starch treatments were compared to one another are shown in Table 2.4. Khalili & Sairanen (2000) and Lingnau (2011) reported that on a daily average, the high-starch treatment had greater rumen NH_3-N ($P<0.05$). Van Wyngaard *et al.* (2015) found that at 6:30, the high-starch treatment group had greater rumen NH_3-N concentrations ($P<0.05$), while at 20:30, the low-starch treatment group had higher rumen NH_3-N concentrations ($P<0.05$). Sayers *et al.* (2003) reported no effect on rumen NH_3N when high- and low-starch treatments were compared to each other.

2.7.4 Pasture *in situ* degradability

The primary purpose of *in situ* degradability is to quantify DM and NDF disappearances by incubating bags containing the feedstuff under examination within the rumen (Dong *et al.,* 2017). The removal of DM from the rumen is described by the equation P = a + b (1 – e-ct). In the equation, *P* equals to the DM disappearance in *t* (time in h). Rapidly and potentially degradable fractions are expressed as *a* and *b*, whereas *c* indicates the degradation rate for *t* (Ørskov & MacDonald, 1979).

According to Sayers *et al*. (2003), starch-based concentrates increased the rapidly degradable fraction (P<0.05) and degradation rate (P<0.05) of DM while decreasing the potentially degradable fraction, resulting in significantly higher overall DM degradability for the high-starch treatment compared to the high-fibre concentrate. The rumen degradation of DM and NDF of ryegrass had little effect (Sayers *et al*., 2003). In studies by Lingnau (2011) and Van Wyngaard *et al*. (2015), when starch-based concentrates were replaced with fibre-based concentrates, no difference was observed for the *in situ* rumen digestion of ryegrass. Bargo *et al.* (2003) discovered that high quantities of concentrate supplementation reduced the rate of pasture *in situ* degradation. Increasing dietary fibre creates a rumen environment where capacity for forage digestion increases, consequently increasing DM and NDF disappearance as well as degradation rate (Beauchemin, 1991).

It is suggested that plantain's biologically active compounds can interfere with the rumen microflora in such a way as to slow down the breakdown of material. This in turn can interfere with rumen degradation dynamics (Labreveux *et al.,* 2006). Minnee' *et al.* (2017) found that degradation rate as measured by *in situ* analysis was faster in plantain compared to ryegrass.

Table 2.4 Summary of the effect of starch- and fibre-based concentrates on ruminal parameters

Reference	Cow Breed	Pasture[1]		Concentrate[2]		Rumen pH	VFA[3]					NH_3-N
		Type	Intake	Type	Intake		Total (mmol/L)	Acetate	Propionate	Butyrate	A: P[4]	(mg/dl)[5]
Lingnau (2011)[6]	Jersey	K/AR	12.9	S (Maize)	6.0	6.05	122.0^{a}	87.7^{a}	19.0^{a}	11.9^{a}	4.90	21.2^{a}
				F(HC/WB)	6.0	6.08	113.0^{b}	82.6^{b}	17.3^{b}	10.4^{b}	4.99	18.8^{b}
Cawood (2016)[6]	Jersey	K	10	S (Maize)	6.0	5.96	156.1	104.8	27.0	17.2	3.91	-
				F (HC, WB, G20)	6.0	5.98	149.0	100.7	26.8	15.1	3.81	-
Van Wyngaard *et al.* (2015)[6]	Jersey	K/AR	10	S (Maize)	6.0	6.42	120.7	76.6	24.2	17.3	3.22^{a}	13.8
				F (PKE)	6.0	6.33	118.3	75.9	22.8	16.5	3.40^{b}	14.6
Van der Vyver *et al.* (2019)[6]	Jersey	K/AR	9	S (Maize)	6.0	6.31	-	-	-	-	-	-
				SF (SBH)	6.0	6.34	-	-	-	-	-	-
				F (SBH)	6.0	6.37	-	-	-	-	-	-
Khalili & Sairanen (2000)	Holstein Friesian	PTG/MFG	40*	S (B)	4.0	6.17^{a}	121.6	81.4	24.3	15.9	-	32.2^{a}
				F (O, BP)	4.0	6.01^{b}	122.5	84.5	27.1	15.3	-	21.8^{b}
Sayers *et al.* (2003)	-	PR	23*	S (B, W, Maize)	5 - 10	5.80	121.6	68.1^{a}	31.6^{a}	17^{a}	2.26^{a}	12.0
				F (BP, CIP)	5 - 10	5.96	122.5	73.5^{b}	25.7^{b}	18.4^{b}	2.94^{b}	13.6

[1] K/AR - Kikuyu (*Pennisetum clandestinum)* and annual ryegrass (*Lolium multiflorum*); K – kikuyu; PTG/MFG – Perennial timothy grass (*Pleum pratense*) and meadow fescue grass (*Festuca pratensis*); PR - perennial ryegrass (*Lolium perenne*)
[2]F – Fibre-based concentrate; S – starch-based concentrate; SF – intermediate fibre- and starch-based; HC – hominy chop; WB – wheat bran; G20 – gluten 20; PKE – palm kernel expeller; SBH – soybean hulls; B – barley; O – oats; BP –beet pulp; W – wheat; CIP – citrus pulp
[3]VFA – volatile fatty acids
[4]A: P – acetate to propionate ratio
[5]NH_3-N –ammonia nitrogen
[6]Concentrate level on 'as is` basis
[a, b] Means in the same column with different superscripts differ ($P<0.05$)
[*]Pasture allowance

2.8 Conclusion

Producing milk with pasture based dairy production systems lowers feed cost when compared to total mixed ration systems. Providing cows with a pasture only diet on the other hand can be counterproductive, as nutrient requirements aren't met, especially energy. Cows won't meet their genetic potential for milk production without supplementation. Plantain is more often being used as a pasture option. The energy and protein levels are comparable to that of ryegrass with higher levels of minerals. Plantain has a high biomass yield potential and is palatable. Low NDF levels of plantain increases the need for fiber in the total diet of cows grazing it. Raw materials used in supplementation are volatile and to be economical as a dairy farm you need to consider alternative sources to use. By-products of the maize milling industry has proven to be successful when partially replacing maize. Additionally, these by-products contain less readily fermentable carbohydrates which increases the safety margin for metabolic disorders such as rumen acidosis.

CHAPTER 3

Materials and methods

3.1 Introduction

The effects of various quantities of starch in dairy concentrates fed to Jersey cows grazing plantain and ryegrass on production and rumen parameters were investigated in this study. In the production study, 51 multiparous Jersey cows were divided into three groups based on milk production, days in milk (DIM), and lactation number. In a randomised complete block design, the cows (n=17) were randomly assigned to three *isonitrogenous* treatments with varied starch levels. The treatments were high-starch (HS), containing 80% maize, medium-starch (MS), containing 50% maize, and low-starch (LS), containing 20% maize. Six more rumen-cannulated cows were randomly assigned to the HS or LS groups in a two-period cross-over design for the rumen study.

3.2 General information

3.2.1 Location, climate and soil

The research was carried out at the Outeniqua Experimental Farm, which is located near George in the Republic of South Africa's Western Cape province (RSA). The climate in the area is described as temperate, with an average long-term rainfall of 730mm (over 50 years). The altitude, latitude and longitude are 204 metres (m) above sea level, 33°58’38’’S and 22°25’16’’E respectively. The research trial took place in the spring (12 September- to 29 October of 2019).

3.3 Pasture management

3.3.1 Paddock layout

Two paddocks were used during the study. The first paddock was approximately 8.55 ha and divided into 39 strips of perennial ryegrass-based pasture with electrically charged poly wire. Each strip was 150m long and 15m wide. Nine evenly spaced sprayer heads on each side of an individual strip made it possible to divide the strips into 10 equal spaces except for strips 35 to 39 which had less spaces (see figure 3.1). Each one of the spaces was 225m^2. At one end of the camp, automated drinking troughs were placed, and the cows had unlimited access to fresh water. The second camp was 5ha big and had the same structure as the perennial ryegrass paddock, but instead of 39 strips of 10 spaces each, it had 15 strips of 15 spaces each (225m^2 per space) (see figure 3.1). Both paddocks were under permanent irrigation.

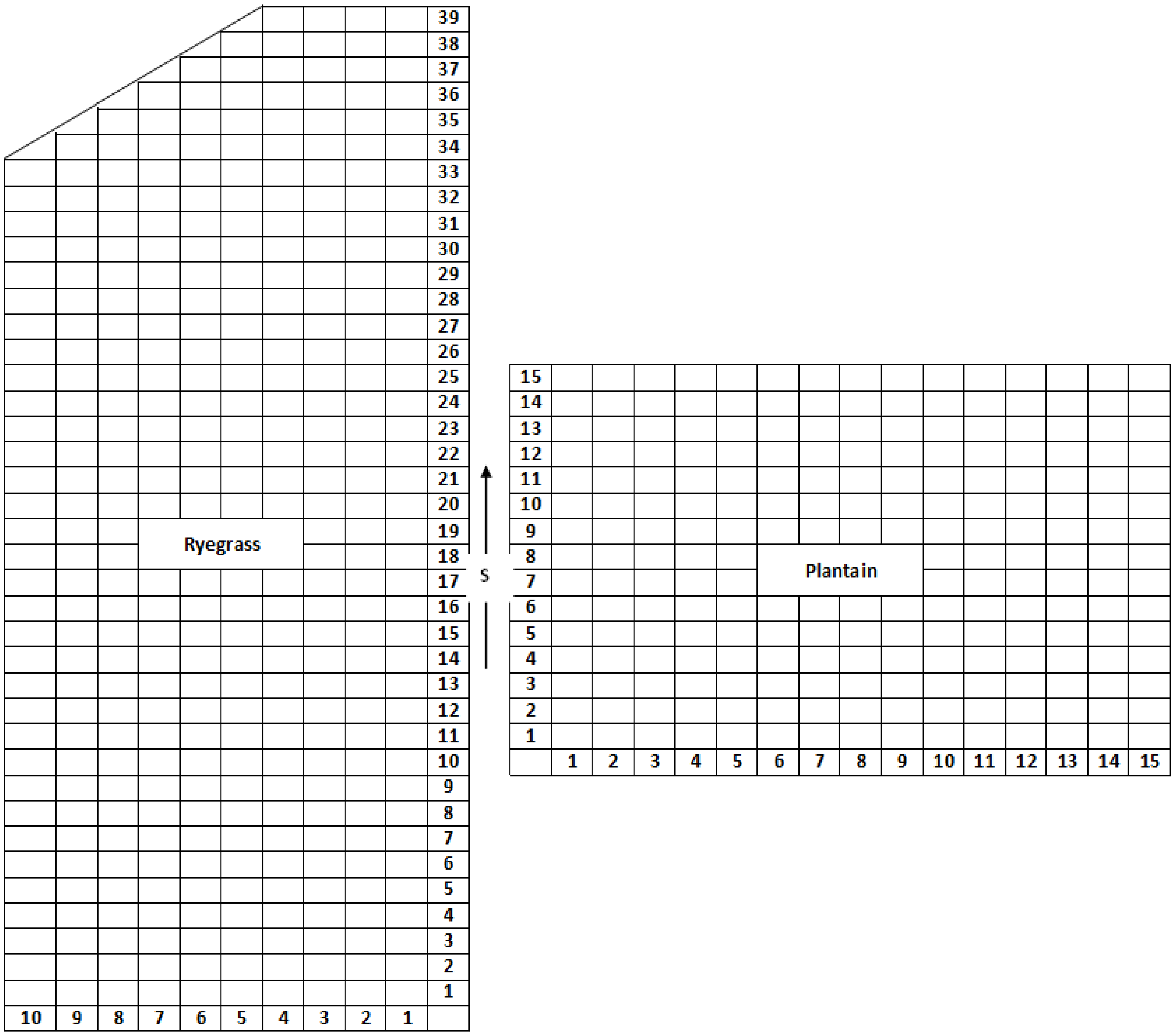

Figure 3.1 Perennial ryegrass (8.55ha) and plantain (5ha) paddock layout

3.3.2 Pasture establishment

In April of 2019, the Perennial ryegrass (Lolium perenne) cv. 24/7 was oversown at 20 kg/ha into a kikuyu-based pasture (Pennisetum clandestinum). Kikuyu's pasture contribution was minor and for purposes of discussion. The ryegrass dominant pasture will be referred to as ryegrass pasture throughout the dissertation. Plantain (*Plantago lanceolate*) cv. Agritonic was established at 9 kg/ha in April 2019 as a pure stand.

3.3.3 Fertilisation, irrigation and weed control

Both the plantain and ryegrass' post-grazing strips were top dressed with 42 kg N/ ha using 150kg/ha limestone ammonium nitrate containing 28% nitrogen (LAN, 28% N). After the fertiliser was applied, it was irrigated for 5-10 minutes to reduce nitrogen losses. The irrigation was timed using manual tensiometers. Irrigation began at a pressure of -25 kPa and stopped at a pressure of -10 kPa (Botha, 2002). Weed control was only necessary within the plantain paddock. There was an infestation of gousblom (*Arctotis*) under the sprayer heads in all 15 of the strips. Gousblom has a creeping growth habit, and it was moving inward toward the plantain which it outcompeted. Basagran was applied to these areas, without any of the herbicide contacting plantain

Figure 3.2 Structure of plantain (*Plantago lanceolate*)

3.3.4 Pasture allocation determination

A rising plate meter (Filip's folding plate pasture meter, Jenquip, Rd 5, Fielding, New Zealand) was used to estimate pasture yield. Rising plate meter (RPM) height is regressed with dry matter yield to calibrate RPM (kg DM. Ha-1). Regression sampling took place over eight weeks (2019/09/15 to 2019/10/30). Strips to be grazed next were used on sampling days. On each sampling day, nine samples were taken by picking three areas with high, three areas with intermediate, and three areas with low growth heights. These areas were located all along the strip, considering the spread of growth. Over eight weeks a total of seventy-two samples were taken. Samples were taken 30 mm

(RPM reading of six) from the ground which adds a safety margin. The safety margin avoids over-estimation, as cows rarely remove herbage below this height.

Before taking a sample, a metal ring with the exact diameter (35.4 cm) of the RPM's plate was placed over the section of pasture. An RPM height (1 unit= 5mm) was then recorded directly above the metal ring. Sharp scissors were then used to remove all the herbage within the metal ring, 30 mm above soil level. Subsequently, samples were oven-dried for 72 h at 60°C (Botha, 2003) and weighed (Sartorius BP8100, weighing accurately to 0.1g). A blank bag was taken along during sampling to match moisture uptake from other bags. The blank bag was weighed as reference weight of other bags. Weight of herbage for each individual bag was then recorded. After the study the seventy-two samples were used to calculate a linear regression equation (Y=aH + b, where Y= Pasture mass in kg DM/ha, a= gradient, H= RPM reading and b= intercept value) using the LINEST function in Microsoft Excel. Pre-defined regression equations developed by similar studies conducted at the Outeniqua research farm were used for pasture management during the study. For ryegrass, the regression equation developed during the study by Van Wyngaard (2018): 102.99* H – 260.79 was used and for plantain 66.75* H – 391.79 was developed by Janke van der Colf from a study she was busy conducting at the Outeniqua Experimental Farm.

Pre-and post-grazing heights were taken individually for every strip of ryegrass and plantain pasture (with dimensions as stated in section 3.3.1). Uniformity of growth within a pasture differs. By taking multiple RPM readings throughout the pasture, a better spread of data is obtained, and accuracy of measurement is improved (Haultain *et al*, 2014). In each strip of pasture, hundred readings were taken for ryegrass and hundred and fifty readings were taken for plantain (10 readings per 225m^2 space). The average was used to determine pasture yield (kg. ha^{-1}) by inserting RPM height into the pre-defined regression equations.

Pre-grazing yield was used for pasture allocation (stocking rate) and post grazing yield was used to determine wastage or shortage. Pre-grazing and post-grazing heights for ryegrass aimed to stay within RPM readings of 20-25 (100-125mm) and 10-12 (50 – 60mm) respectively to avoid over- and under grazing and to minimise cow intake as a limiting factor. Plantain's recommended pre- and post-grazing RPM heights are not well defined (Haultain *et al.,* 2014). As extra precaution the cows were visited three hours after they started a grazing to ensure that enough herbage was available.

Figure 3.3 Rising plate meter used to measure pre- and post-grazing heights and to determine seasonal regression equations

3.3.5 Pasture fractioning

Pasture fractioning was done in the ryegrass paddock to determine the relative species composition. A total of seven composite samples, each consisting of three high, three medium and three low heights were taken over two grazing strips that has yet to be grazed. The seven composite samples were pooled together, thus giving a reference sample containing pasture cut over a wide range within the pasture. Out of this reference sample, species were divided and placed into paper bags. The bags were weighed (Sartorius BP8100, weighing accurately to 0.1g) to give the weight per species and subsequently the percentage composition on an as-is basis. The bags were then oven dried at 60°C for 72 h (Botha, 2003). The weight and species composition were then determined on a DM basis.

3.3.6 Pasture grazing

The cows grazed the plantain after morning milking at approximately 6h00. They were then removed from the plantain for afternoon milking at 13h00. After, afternoon milking (14h00), they were placed on the ryegrass pasture until 5h00 the next morning. This gave the herd of sixty cows, seven hours to graze plantain in the morning and fifteen hours to graze ryegrass. The cows strip grazed both the ryegrass and plantain pastures as a group for equal pasture allocation and normal social and behavioural needs.

3.4 Pasture and concentrate sampling and analytical methods

3.4.1 Pasture sampling

Pasture samples for quality analysis were taken twice a week in the same manner for both ryegrass and plantain. At each sampling day, four samples were taken at random in the strip the cows would be grazing from next. A 35.4 cm ring was placed on the pasture and all the grass within the ring was chopped and put into paper bags. Using the ring gives more even contribution of samples when they are pooled. At the base of the ring, 30mm legs were attached to make it possible to take pasture samples 30mm from the ground. If samples were taken to ground level, it would not have represented the quality of the actual intake of the cows. The ring was randomly placed so other pasture species present would also be included in the analysis. The pasture samples were weighed (Sartorius BP8100, weighing accurately to 0.1g) before and after it was dried for 72 h at 60°C to determine the DM (Botha, 2003). All eight samples per week were pooled together to end up with eight ryegrass samples and eight plantain samples after eight weeks.

3.4.2 Concentrate sampling

Feed came in the form of 40kg bags from NOVA feeds. Before 3kg was weighed out for each cow a couple of 40kg bags bags were thrown into large tubs to ease the fractioning process. Random grab concentrate samples were taken from these tubs. Sampling took place weekly for each experimental treatment. These samples were pooled for every two weeks (14 days) resulting in four samples for each treatment (twelve concentrate samples in total at the end of the study). The random samples taken each week were dried at 60°C for 72 h to determine the dry matter (Botha, 2003) before they were pooled.

3.4.3 Pasture and concentrate analytical procedures

The dried samples were milled through a 1mm screen using a Retsch GmbH5657 Laboratory mill and put in airtight containers for laboratory analysis at UP Nutrilab (Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria), Elsenburg (Animal Science Feed and Plant Production Laboratories, Western Cape Department of Agriculture, Elsenburg) and Cal labs (Division of Astral Operations Ltd. Roodepoort, Johannesburg).

All the samples were individually analysed in duplicate for DM (AOAC, 2012; 934.01) CP (AOAC, 2012; 990.03) using Leco N analyser, model FP 528, NDF and ADF (Van Soest *et al*., 1991) using ANKOM 200/220 fibre analyser (ANKOM Technology Corporation, New York, USA), crude fibre (CF) was analysed using the method of Goering & Van Soest (1970) making use of the ANKOM A200 Fibre Analyser (ANKOM Technology Corporation, New York, USA), starch (AACC, method 76-11), *in vitro* organic matter digestibility (IVOMD) (two stage rumen fluid-pepsin technique by Tilley & Terry, (1963), ether extract (EE) (AOAC, 2012: method 2003.06)) and ash (AOAC, 2012, method 942.05). Mineral fractions (calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), manganese (Mn), copper (Cu), iron (Fe) and zinc (Zn)) were determined using (ALASA, 1998: method 6.1.1). Equation 3.1 (NRC, 2001), 3.2, 3.3 (Ensminger *et al*., 1990) and 3.4 (McDonald *et al*. (2001) shows how NFC, nitrogen free extract (NFE), total digestible nutrients (TDN) and metabolizable energy (ME) were calculated respectively.

Equation 3.1 Non fibre carbohydrates (NFC)

NFC (%) = [100 (%) - (NDF (%) + ASH (%) + CP (%) + EE (%))] (NRC, 2001)

Equation 3.2 Nitrogen free extract (NFE)

NFE (%) = 100 (%) - ((100 - DM (%) + ASH (%) + CP (%) + Crude fibre (CF) (%) + Ether extract (EE) (%))

Equation 3.3 Total digestible nutrients (TDN)

TDN (%) = (0.8 x CP (%)) + (0.4 x CF (%)) + (0.9 x NFE) + (0.9 x 2.25 x EE (%))

Equation 3.4 Metabolisable energy (ME)

ME = (TDN (%) x 14.99)/100

3.5 Production study

3.5.1 Introduction

The estimation of pasture quality by methods explained in section 3.3.3 only gives an indication of its value as forage. It ignores how efficiently it is utilised by an animal. Therefore, animal feeding trials are necessary (Waghorn & Clark 2004).

The research trial consisted of a lactation production study (fifty-one Jersey cows) and rumen fermentation study (six Jersey cows). These trials were carried out at the same time throughout the trial and had no confounding effects on one another. This section will explain the methodologies used during the production lactation study. The purpose of the production lactation study was to see how changing starch levels in concentrate feed influenced the effects of cows grazing plantain from 06h00 to 13h00 and perennial ryegrass-based pasture from 14h00 to 5h00. The parameters measured during the lactation production study were milk yield, milk components, BCS and body weight (BW).

3.5.2 Animal welfare

Ethical clearance was received through the Western Cape Department of Agriculture. DECRA approval number: R19/131.

3.5.3 Duration of the study

The study commenced on 12 September 2019 with its fourteen-day adaptation period. Sampling took place from 26 September 2019 until 29 October 2019. Data collection for the study occurred over a period of 34 days.

3.5.4 Grouping of cows

Fifty-one multiparous Jersey cows from the Western Cape agricultural research trust were selected for the production study in a complete randomised block design. The cows were blocked according to: Mean milk production three weeks prior to study commencement (2 August 2019 – 23 August 2019), lactation no. and DIM (from 23 August 2019) as shown in Table 3.1. Cows were then randomly allocated to three groups totalling 17 cows per group. Each group received different concentrate feeds which acted as the experimental treatments. The experimental groups consisted of a high-starch (HS) group (80% maize), medium-starch (MS) group (50% maize) and low-starch (LS) group (20% maize). From the HS to LS groups, maize was replaced with alternative high fibre by-products, as shown in Table 3.2.

The milking parlour had a 20-point switch over design. To ensure twenty cows per group, the rumen cannulated cows (three in HS and three in LS groups) were milk simultaneously with the production study cows. Three non-participating cows were put in the MS group. This was done to ease the flow of cows within the milking parlour so time would not be wasted, because the cows participating in the study were part of a larger commercial herd.

Primiparous cows and cows not within 20-165 DIM were excluded as they would have been a source of extra variation. NOVA feeds (Nova feeds George, Industrial area, George, Western Cape, RSA) prepared and pelleted all the concentrates on the same day. They added the premix to the three concentrate batches, and it was received on 11 September 2019. Before the cows entered the milking parlour, they were divided into the three respective groups with the help of coloured tags which were hung around their necks with a thin metal chain and a cable tie. Each tag contained a number which represented the specific treatment the cow was in. This separated the cows so that each one got the correct concentrate in the milking parlour. The sixty cows grazed the perennial ryegrass-based and plantain pastures as a group. They were fetched at 5h00 for morning milking and 13h00 for afternoon milking. After cows were milked, they were herded calmly to a waiting area until all three groups were finished milking.

Table 3.1 Mean and standard deviation values for milk yield (mean for previous 3 weeks), 4% fat corrected milk, milk fat, days in milk (DIM), lactation number and live weight of Jersey cows within blocks (n=17) before the commencement of the study

Parameters[1]	Treatment concentrate[2]		
	HS	MS	LS
Milk yield (kg/cow/day)	21.1 ± 2.86	21.2 ± 3.18	21.1 ± 2.93
FCM (kg/cow/day)	23.3 ± 1.78	24.7 ± 3.7	23.5 ± 3.02
Milk fat (%)	4.69 ± 0.26	4.69 ± 0.34	4.77 ± 0.28
DIM (d)	119 ± 58.5	114 ± 54.3	118 ± 47.6
Lactation no.	4.00 ± 2.06	3.76 ± 1.78	4.23 ± 2.32
BW (kg)	396 ± 35.6	389 ± 30.2	400 ± 42

[1]FCM – 4% fat corrected milk; DIM – days in milk; BW – body weight
[2]HS – high-starch concentrate; MS – medium-starch concentrate; LS – low-starch concentrate

Figure 3.4 Coloured tags for identification of cows and treatments

3.5.5 Concentrate feed allocation

The three different experimental treatments were labelled to eliminate any confusion. For each milking time 20 x 3kg feed was weighed for each treatment group using a Micro T7E scale (maximum = 30kg; ± 0.005). The 3kg weighed feed rations were put into separate plastic bags (400 x 600mm; 70 micron) to enable manual feeding. The concentrates were placed in individual feeding bowls in the milking parlour, ensuring that each cow received the identical amount of the experimental concentrate each time. During milking time, all the cows received 3kg of concentrate in the morning and 3 kg in the afternoon, for a total of 6 kg of concentrate per cow per day "as is."

Table 3.2 Ingredients and nutrient composition of the three different concentrate feeds (experimental treatments) manufactured by NOVA feeds[1]

Ingredient[2] (g/kg)	Concentrate treatment[5]		
	HS	**MS**	**LS**
Maize	800	500	200
Hominy chop	0	175	350
Wheat bran	50	115	180
Soybean hulls	0	90	180
Soybean oilcake	77	49	21
Molasse	40	40	40
Feed lime	20	20	20
MCP	4	2	0
Salt	5	5	5
MgO	3	3	3
Vitamin and mineral premix[3]	1	1	1
Nutrient[4](g/kg DM, or as stated)			
DM	890	890	890
CP	121	122	122
ME (MJ/kg)	12.9	12.7	11
NDF	113	213	278
Hemicelluloses	67	112	180
Starch	620	459	300
Ca	9.2	9.4	9.7
P	4.7	4.8	5
Ca:P ratio	1.95	1.96	1.94

[1]NOVA feeds George, George, Western Cape, RSA
[2]MCP – mono-calcium phosphate; MgO - magnesium oxide
[3]Outeniqua dairy premix – (per kg of premix) 127g Ca; 166.6g Mg; 33g S; 10g Cu; 40g Mn; 46,7g Zn 333mg I; 66.7mg Co; 200mg Se; 2 million IU vitamin A; 100 000 IU vitamin D; 3 333 IU vitamin E

[4] DM – dry matter; CP – crude protein; ME – metabolizable energy; NDF – neutral detergent fibber; Ca – calcium; P – phosphorus
[5]HS – high-starch concentrate; MS – medium-starch concentrate; LS – low-starch concentrates

3.5.6 Milking procedure

Cows were milked twice a day (morning and afternoon). Before each milking, cows were fetched as a group and had an average walking distance of 800m. Cows were assigned to one of three treatment groups when they arrived at the milking parlour, using color-coded tags around their necks. Each group consisting of twenty Jersey cows each took turns entering the milking parlour. Before cows entered, the allocated concentrates were manually added to twenty individual bowls situated in front of the cows as they entered the milking parlour. A pre-milking teat dip was used to sterilise the teats before the milking clusters were attached. The cows were milked using a twenty-point Dairy Master swing over milking parlour with electronic milk meters (Total Pipeline Industries, 33 Van Riebeeck Street, Heidelberg, 6665). The milking system and management procedures followed standard dairy principles and practices. When the flow of milk decreases below a minimum flow speed the milking clusters automatically detached and the cow was considered fully milked. A post-milking teat dip was applied to all the cows after cluster detachment. Cows then exited the milking parlour and proceeded to the waiting area until all sixty cows were milked, then cows were herded back to the paddock for grazing.

3.5.7 Milk production and milk sampling

Using the Afikim milk meter and management system, daily milk production was recorded during each milking. This system keeps track of all the cows' milking history, allowing you to compare data and see any milk yield trends.

At each milk sampling day (30 September, 15 October and 30 October), composite milk samples (16ml in the morning and 8ml in the afternoon) were obtained for each cow participating in the production study. Sampling bottles attached to the main milk line allowed milk to be sampled without mixing with milk from other cows. Post milking, sampling bottles were removed and gently tilted three times to distribute milk solids and subsequently transferred to 24 ml containers containing bronopol for sample preservation. The 16 ml milk samples were held in the fridge after morning sampling until afternoon sampling, when the 8 ml afternoon sample was added.

The fifty-one individual milk samples were sent to Merieux Nutriscience Pty (Ltd)., Jeffrey's Bay, South Africa to analyse for milk fat, milk protein, milk lactose and milk urea nitrogen (MUN) using NIR based MilkoScan FT+ (Rhine Ruhr Process Equipment (PTY) Ltd., Johannesburg, South

Africa). The Fossomatic FC analyser (Rhine Ruhr Process Equipment (PTY) Ltd., Johannesburg, South Africa) determined SCC content of the milk. Daily milk composition (fat, protein and lactose) was also determined using the Afilab system for management purposes. Daily 4% fat corrected milk (4% FCM) was calculated using equation 3.5 (Gaines, 1928). Energy corrected milk (ECM) were calculated as shown below using equation 3.6 (NRC, 2001).

Equation 3.5 4% fat corrected milk (4% FCM)

$$4\%\ \text{FCM} = (0.4 \times \text{kg milk}) + (15 \times \text{kg fat})$$

Equation 3.6 Energy corrected milk (ECM)

$$\text{ECM} = (0.3246 \times \text{kg milk}) + (12.86 \times \text{kg fat}) + (7.04 \times \text{kg protein})$$

3.5.8 Live weight and body condition score

The BW and BCS of the cows were measured at the beginning and end of the study. These measurements were taken on two consecutive days and averaged to consider variations in pasture intake, water intake and excretion behaviour of individual cows. Weighing was performed each time after morning milking to ensure empty udders. The same scale was used (Tru-Test EziWeigh version 1.0; 0.5kg accuracy, Auckland, New Zealand) at each weighing. The BCS was determined according to (Wildman *et al*., 1982) using a 1-5 scale. The scoring system is based on appearance and palpation of the back and hindquarters. Each time the measurements were done by the same technician (Pieter Cronje; Jersey herd manager at the Outeniqua Research Farm) to eliminate bias, because BCS is a subjective measure.

Figure 3.5 Twenty-point Waikato Afikim electronic swing over milking parlour with electronic milk meters and cluster removal.

3.5.9 Statistical analysis

Data for the production study was statistically analysed as a complete randomised block design with three treatments randomly allocated (random function in Microsoft Excel, 2010) to 17 blocks. Analysis of average affects were done using the Proc Mixed model (Statistical Analysis System, 2020). Means and standard error were calculated and significance of difference ($P<0.05$) between means was determined by Fischer's test (Samuels, 1989). Tendency for difference was declared at $P < 0.10$. The linear mix model is described by the following equation:

$Y_{ij} = \mu + T_i + B_j + e_{ij}$

Where:

Y_{ij} = variable studied during the period

μ = overall mean of the population

T_i = effect of the i^{th} treatment

B_j = effect of the j^{th} block

e_{ij} = error associated with each Y

3.6 Rumen study

3.6.1 Introduction

A rumen fermentation study was carried out to assess rumen parameters (pH, VFA, and rumen NH_3-N production), and also an *in situ* degradability study (dry matter disappearance (DMd), neutral detergent fibre degradability (NDFd), and neutral detergent fibre degradability rate (NDFkd)). Only the HS and LS treatment groups were included in the rumen study. Cows participating in the rumen fermentation study formed part of the herd dynamics of the fifty-one production study animals.

3.6.2 Grouping of cows

A two-period crossover design was used for the rumen fermentation study, where six rumen cannulated Jersey cows from the Western Cape Agricultural research trust were randomly assigned (using the random function in Microsoft Excel 2010) to either the HS or LS groups. This grouping gave rise to three cannulated animals for each group. Colour coded tags were hung around their necks that placed the 6 rumen study cows in either the HS or LS group. All the cannulated cows grazed alongside the production study cows and were milked in their respective groups. Production study data obtained from the cannulated cows were dismissed. The cannulated cows switched groups for the second period, exposing cows to both concentrate treatments at the conclusion of the study to account for individual variance.

3.6.3 Duration of study

The rumen fermentation study consisted of two nineteen-day adaption periods and two ten-day sampling periods. Rumen pH was assessed with indwelling rumen pH loggers and a handheld rumen pH meter during the rumen fermentation study. Rumen fluid was sampled for determination of rumen NH_3-N and VFA production. The *in situ* technique was used to determine pasture DM_d, NDF_d and NDF_{kd} in the rumen. The 10-day period began on September 30th, when the indwelling pH loggers were put into the rumens of the cannulated cows (during afternoon milking) for a 3-day period (removed 3 October at afternoon milking time). Sampling of rumen fluid took place on 3 October. *In situ* degradability started on 8 October and the last Dacron bags were removed the evening of 9 October. The cannulated cows were given a fourteen-day adaption time to their new concentrate feed after the switch-over on October 10th. After the adaptation period for the second round a 6-day period (25 October to 30 October) was used to complete the three tasks associated with the rumen study. This 6-day period was considerably shorter than the 10-day period used in the first round, as the preparation and methods were refined since the first period. Rumen loggers were inserted the morning of 25 October and were removed the morning of 28 October. Rumen fluid sampling took place on 28 October. The *in situ* pasture degradability started 29 October just before afternoon milking time and ended the evening of 30 October.

3.6.4 Rumen pH logging system

Rumen pH was measured using pH-HR, pH temperature Tru-Test loggers (TruTrack Data logger, www.intech.co.zn). These logging devices can measure the pH and temperature of any substance that the electrode is exposed to. The logging device itself was placed into a watertight capsule to avoid the low pH and liquid constitution of the rumen to damage any of the electronics. The airtight capsule was permanently installed onto a cannula plug, thus allowing it to be put into the rumen and mounted on the cannula so it does not move around or pop out.

Before the loggers could be put into the rumen, it had to be calibrated using the Omnilog Data Management Program with pH buffers of 4, 9 and 7. The calibration process starts by connecting the logger to a computer and opening the Omnilog data management program. The logger was started via the program and it logs the pH every 10 seconds giving an average after 10 minutes. The electrode was placed into a standard pH solution of 4 until the pH of the logger stabilised. The stabilised pH was then assigned pH 4. The same was done with pH 9. After stabilisation at pH 9, the electrode was put into a standard solution with pH of 7 to test if the calibration was successful. If the pH stabilised at any value beyond 7.05 and below 6.95 it was considered unsuccessful and the whole process needed to be repeated. The electrode of the logger was rinsed with distilled water when moved from one standardised solution to the other to avoid any contamination. When the logger was successfully calibrated, a lid filled with 3 molar potassium chloride (KCl) was placed onto the electrode to avoid losing the calibration. Before the insertion of a logger into the rumen, the loggers were all started and placed into a bucket filled with water at around 40°C for 2 h to test any difference in pH determination. The pH values obtained every 10 minutes were averaged for all the loggers and the differences between them were adjusted.

Loggers were placed into the cannulated cows for 72 h after which they were removed and again connected to the Omnilog Data Management program. The data obtained showed pH values for every 10 minutes on exactly the time and date that it was logged. The large volume of data was reduced by combining three 10-minute periods into 30-minute intervals by averaging the pH readings taken before, during, and after each 30-minute interval. The mean for each cow was finally calculated using the 30-minute interval pH readings. The 72-hour period was reduced to period of 24-hours.

Figure 3.6 Example of an Indwelling pH logger

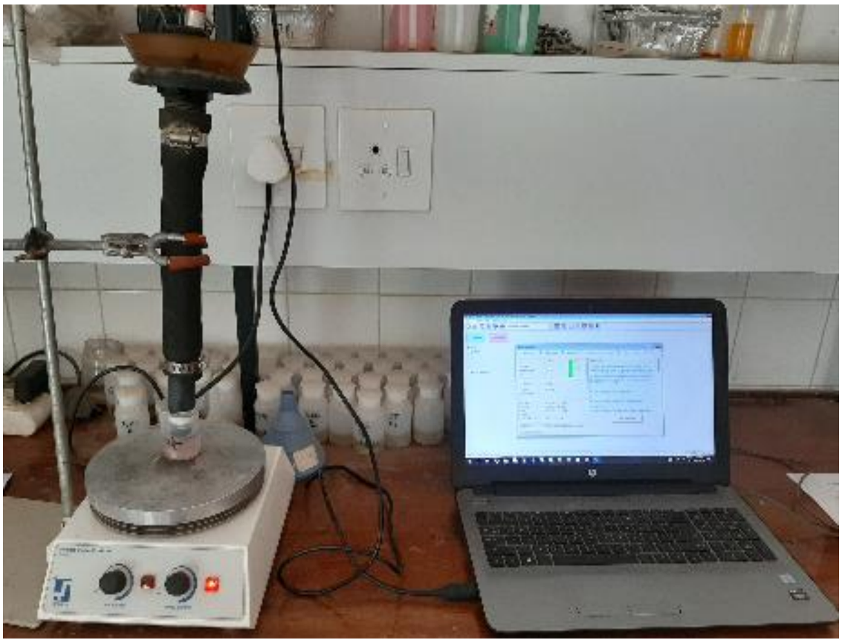

Figure 3.7 Indwelling pH logger calibration with Omnilog data management program

3.6.5 Rumen fluid sampling and analysis

Rumen fluid was extracted from all the cannulated cows in the HS and LS groups during the two rumen sampling weeks as mentioned in section 3.4.3. The samples were taken with a modified handheld pump which created a suction effect. A thin translucent pipe of approximately 1m was connected to the pump on one end and on a sample bottle with two holes at the other end. Another piece of pipe with the same dimensions was connected to the other hole on the sample bottle and at the end of the pipe a thin steel rod of approximately 50cm long and 5mm in diameter was connected. Tiny holes were drilled into the rumen cannula plugs which allowed the steel rod to be inserted into the rumen without removing the plugs. The tiny holes were sealed when not in use with screws fitting the dimensions of the holes. The thin steel rod was pushed through these holes into the rumen and the suction effect of the pump created a negative vacuum inside the sampling bottle, allowing rumen fluid to flow into the bottle (about 100ml per sample). An illustration of the sampling device is shown in Figure 3.10.

On both sampling occasions the rumen fluid extraction process was done three times in one day at 6h00, 12h00 and 20h00. The sample bottles were marked before the process was done to avoid confusion. Directly after rumen fluid was extracted, a manual pH reading was taken and recorded using a handheld pH meter (WTW pH240i pH meter/data logger attached with a WTW Sentix 41 pH electrode). After the pH reading of a specific sample was taken, the sample bottle was quickly closed to minimise exposure to air and risk volatilisation of compounds. After the process was completed the rumen fluid was filtered through four layers of cheesecloth to remove solid particles. The rumen fluid that remained from each 100ml sample was transferred into two clearly marked, sealable 25ml bottles and frozen at -20 °C, pending analysis. For each of the two sampling days 18 x 100ml (6 cows x 3 sampling times) samples were taken, and each 100 ml sample was split into two 25 ml bottles. The one half of the 25ml bottles was sent to the Department of Biotechnology, University of Free state main campus, Bloemfontein to analyse for VFA content using the Gas Chromatographic method (Broderick & Kang, 1980). The other half was sent to Nutrilab, University of Pretoria for analysis of rumen NH_3-N content using the catalysed phenol-hypochlorite and ninhydrin colorimetric procedures (Broderick & Kang, 1980).

Figure 3.8 Handheld pH meter (WTW pH240i pH meter/data logger attached with a WTW Sentix 41 pH electrode)

Figure 3.9 Rumen cannulated cow

Figure 3.10 Customised hand pump used for rumen fluid collection via the rumen cannula

Figure 3.11 Rumen fluid collected, filtered through four layers of cheesecloth and divided into two 25ml sample bottles for VFA and ruminal ammonia nitrogen analysis respectively

3.6.6 *In situ* ryegrass pasture degradability

An *in situ* nylon bag study was carried out to measure the DM_d, NDF_d and NDF k_d of perennial ryegrass for cows grazing plantain and ryegrass while receiving either the HS or LS concentrate treatments.

A 10kg representative sample of perennial ryegrass pasture was cut 3cm from the ground using scissors. The sample was placed into paper bags and oven dried for 72 h at 60°C (Botha 2003). After removal from the oven, the dry matter was calculated and all the grass from the paper bags were pooled. Using scissors, the dried grass samples were chopped into 5-10 mm lengths.

Nylon bags with 53 µm pore size and inner size of 10 x 20 cm were numbered from 1-57 for the first round and 58-114 for round two. All 114 bags were oven-dried for 12 h at a temperature of 55 °C and then weighed using a Sartorius L420P scale (maximum = 420g; ± 0.001g). Nylon bags were individually prepared by weighing approximately 5g of dried and cut perennial ryegrass into them.

The bags were sealed with a cable tie and weighed again. This allowed the weight of the bag, grass sample and cable tie to be known, making it possible to determine grass weight difference after removal from the rumen.

The all in-gradually out system was used for this study as explained by Dong *et* al. (2017). Opaque leg stockings were used to house the nylon bags within the rumen. The stockings were cut in half and within each leg four or five prepared bags were inserted giving a total of nine bags for both legs. For each removal time, three duplicate bags were used to address variation in degradation characteristics. A glass marble weighing ± 48 g was held in place at the bottom of the stockings to act as an anchor. If the bags are not anchored, they might float on top of the rumen contents and not be submerged within the rumen fluid. The rumen plugs were fitted with small metal rings where the opaque tights were tied to. This prevented it from getting lost within the rumen. The bags were separated from each other by making knots between them. Incubation occurred at 12h00, just before afternoon milking. The three duplicate nylon bags were removed from each cow 6-, 18- and 3 h after incubation respectively. Bags 55-57 for round one and bags 112-114 for round two were used to represent the zero time and were not inserted into a rumen. After removal of bags at the different periods, bags were rinsed under cool water for one minute to remove excess rumen fluid and stop the degradation process. The bags were sealed in zip lock bags and stored in a freezer at – 20°C.

After the second round of the fermentation study, bags were removed from the fridge and thawed. The bags were then placed into a washing machine with clean water and gently washed for three cycles of three-minute intervals. After each cycle, the dirty water was drained, and clean water was added. After all the bags had been washed and the water in the washing machine remained clear, the bags were put in the oven for 72 h at 55°C. The DM weight of grass that remained after incubation was determined by weighing each bag individually on the same scale. Only one bag was removed from the oven at a time to prevent moisture to be absorbed. The blank bags were treated in the same way as the rumen-incubated bags.

Before NDF analysis could be conducted, the three bags removed at each time period were pooled and ground through a 1mm screen using a Retsch GmbH5657 Laboratory mill. Neutral detergent fibre analysis of residuals was done for each individual bag at UP Nutrilab (Department of Animal and Wildlife Sciences, University of Pretoria) using the filter bag technique (ANKOM technology method 13: Filter bag technique, ANKOM^{2000} fibre analyser). The DM_d and NDF_d was calculated using equation 3.7 and 3.8 respectively (Tilley & Terry, 1963).

Equation 3.7 Dry matter disappearance (DM_d)

DM_d = 100 – (Grass DM after incubation/Grass DM before incubation) x 100

Equation 3.8 Neutral detergent fibre disappearance (NDF_d)

NDF_d = 100 – ((Grass DM after insertion x % NDF after insertion/100)/ (Grass DM before insertion x % NDF before insertion/100)) x 100

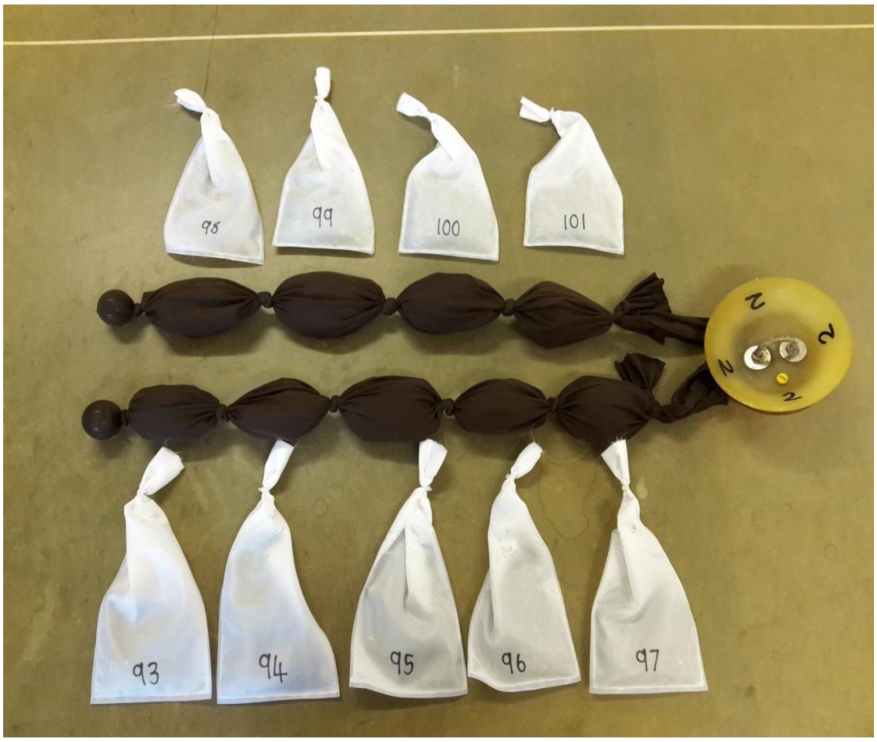

Figure 3.12 Opaque leg stockings containing nine nylon bags and a marble at the base, anchored to metal ring on cannula plug

3.6.7 Statistical analysis

The rumen fermentation data was statistically analysed using a cross-over design to ensure that both treatments were represented in both time periods. The Proc Mixed model was used to analyse mean effects (Statistical Analysis System, 2020). Means and standard error were calculated and significance of difference ($P<0.05$) between means was determined by Fischer's test (Samuels, 1989). Tendency for significant difference was declared at ($P<0.10$). The following equation describes the linear mixed model:

$Y_{ij} = \mu + T_i + P_j + TP_{ij} + e_{ij}$

Where:

Y_{ij} = variable studied during the period

μ = overall mean of the population

T_i = effect of the i^{th} treatment

P_j = effect of the j^{th} block

TP_{ij} = effect of the ij^{th} interaction between treatment and period

e_{ij} = error associated with each Y

3.6.8 Economical evaluation

Based on the price of the concentrate, cost of pastures, milk price and milk production an economic evaluation was done to see if there was any advantage in feeding either the HS, MS or LS treatments to cows grazing plantain and ryegrass.

CHAPTER 4

Results and discussion

4.1 Pasture management and quality

4.1.1 Pasture allocation

The regression equations to predict DM yield for plantain and ryegrass developed during the study are depicted in figures 4.1 and 4.2 respectively. The equations follow a linear trend (Y=mx + c, where x= RPM height and y= dry matter yield in kg DM/ha). These figures show how much herbage was available while the pasture was at a certain RPM height (1 unit = 5mm). When the regressions from the study were applied, pre-grazing yields for ryegrass and plantain were determined to be 2203 kg DM/ha and 1868 kg DM/ha, respectively. Regressions developed during the study obtained post-grazing yield values of 1293kg DM/ha and 739kg DM/ha for ryegrass and plantain respectively. Ryegrass's regression developed during the study yielded a R-square value of 0.46, meaning that only 46% of the variation was explained by the model. Plantain's regression developed during the study found a R-squared value of 0.659 (65.9%). These low R-squared values indicates that intake might have differed from predicted values. Weight gain by cows during the trial however suggests that intake was sufficient.

Average pre-grazing heights show that plantain had a 2.44 higher RPM reading (12.2 mm) than ryegrass, but ryegrass had 335 kg DM ha^{-1} higher pre-grazing yield. This can be attributed to the reproductive stem development of plantain, lowering plant density and over-estimating DM yield (Waghorn & Clark, 2004). Plantain also showed high variation in pre-grazing height and yield between measuring days. Towards the end of the study, plantain struggled to regrow due to a short final grazing cycle and reproductive stem development. As a result, the study period was reduced to 47 days to avoid variation in quality over both plantain's grazing cycles. Plantain showed lower post-grazing heights compared to ryegrass, which can be explained by the ease of harvesting of plantain by animals (Barre *et al.,* 2006). Ryegrass fully recovered to the recommended pre-grazing heights of 100-125mm (20-25 RPM height) as specified by Stockdale (2000) during its two 30-day grazing intervals. Overgrown strips of ryegrass were not grazed to maintain uniformity of grazing quality during the study.

Pasture allocation allowed cows to each consume on average 4.9kg DM plantain and 7.16 Kg DM ryegrass per day (average=12.06 kg DM pasture intake/cow/day). Trampled and soiled pasture caused under-estimation of available herbage. Plantain's erect, rosette forming growth habit lowers its sward density and increases open patches within the stand (Stewart, 1996; Rumball *et al.,* 1997). The accuracy of pasture measurement is lowered under these circumstances and being unbiased during RPM measurements is especially important. Including other pasture species with plantain might increase total stand density.

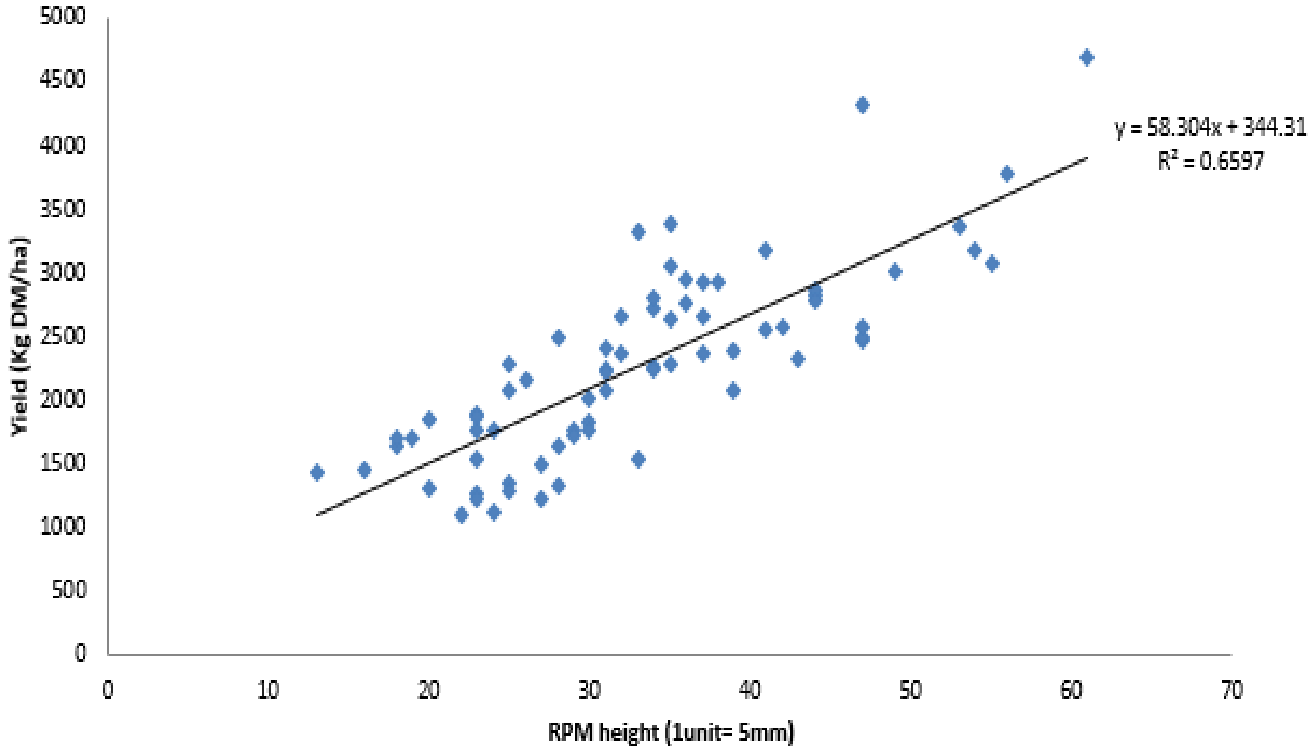

Figure 4.1 The seasonal regression indicating the relationship between rising plate meter (RPM) reading and the corresponding pasture yield (kg DM/ha) for plantain developed during the study

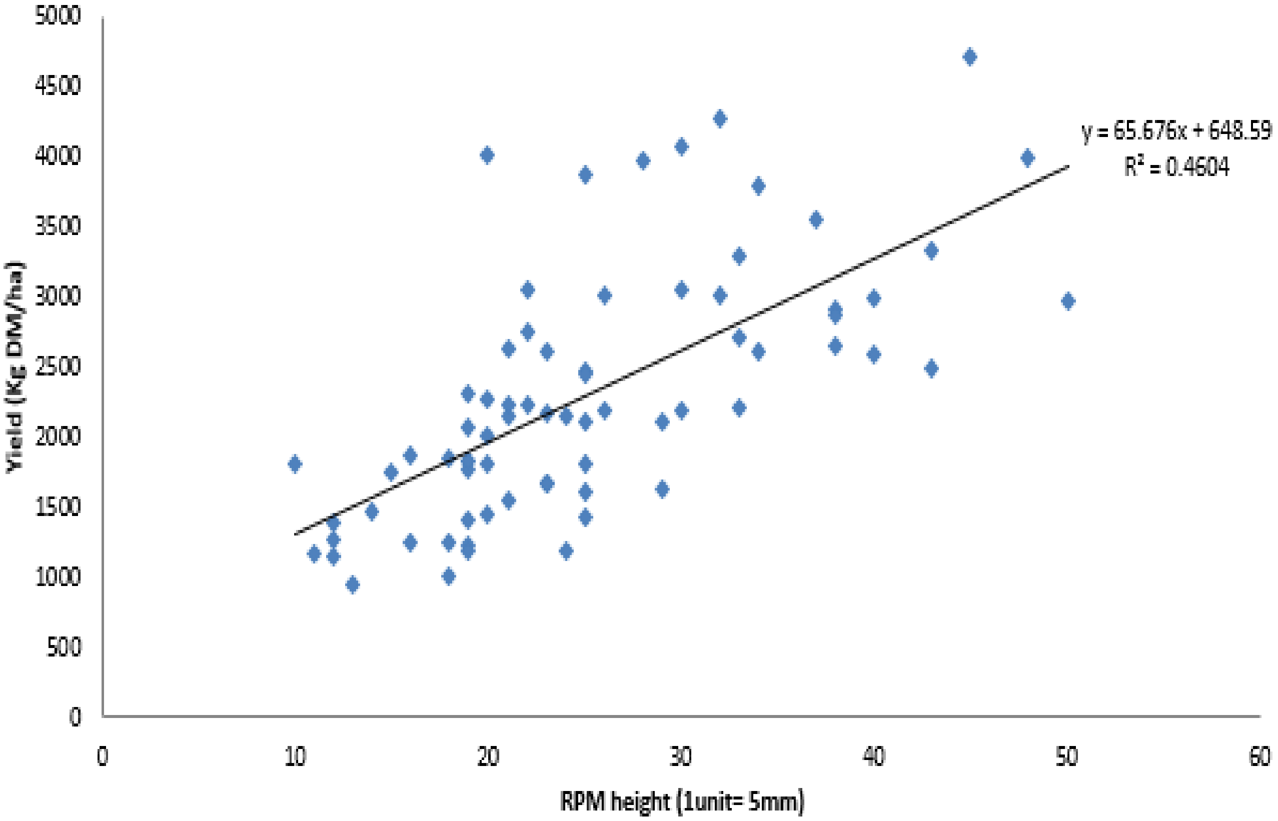

Figure 4.2 The seasonal regression indicating the relationship between rising plate meter (RPM) reading and the corresponding pasture yield (kg DM/ha) for perennial ryegrass dominant pasture developed during the study

Table 4.1 Ryegrass and plantain's mean and standard deviation values for pre- and post-grazing parameters using regression equations developed during the study: Y=65.68 x H + 648.6 and 58.30 x H + 344.3 for ryegrass and plantain respectively

Parameter[1]	Ryegrass (Night grazing)	Plantain (Day grazing)
Pre-grazing		
RPM reading	23.7 ± 3.74	26.1 ± 4.34
Yield (kg DM/ha)	2203 ± 246	1868 ± 253
Pasture allowance (kg DM/cow/day)	7.84 ± 0.87	4.92 ± 0.82
Pasture intake (kg DM/cow/day)	5.9 ± 0.82	4.62 ± 0.83
Post-grazing		
RPM reading	9.82 ± 1.73	6.98 ± 1.07
Yield (kg DM/ha)	1293 ± 114	739 ± 62.6
Pasture removed (kg DM/ha)	910 ± 216	1129 ± 234

[1]RPM – rising plate meter; DM – dry matter; ha – hectare
± - mean and standard deviation

4.1.2 Pasture quality

The perennial ryegrass paddock utilized in the study was pasture fractioned, and the composition revealed that perennial ryegrass (Lolium perenne) was the dominant species in the sward (Table 4.2). Kikuyu (*Pennisetum clandestinum)* had a low contribution which coincides with García *et al.* (2014) and Pearcy & Ehleringer (1984) stating that kikuyu is mostly dormant during winter and early spring. Brome grass (*Bromus intermis*) proved to be the second largest contributor within the perennial ryegrass dominant pasture. Brome grass is an early maturing pasture species, increasing the fibre content of a mature sward (Prichard *et al.,* 1963).

Table 4.2 Pasture composition of the perennial ryegrass dominant paddock showing the dry matter (DM) contribution of each species during the study

	Pasture species[1]					
	Perennial ryegrass	**Brome grass**	**Kikuyu grass**	**Clovers**	**Vasey grass**	**Weeds**
% of DM[2]	70.2	16.7	7.36	2.88	2.14	0.54

[1]Perennial ryegrass – *Lolium perenne;* Brome grass – *Bromus catharticus*; Kikuyu grass – *Pennisetum clandestinum*; clovers – *Trifolium repens and Trifolium pratense*; Vasey grass – *Paspalum urvillei*
[2] DM – dry matter

The chemical compositions of plantain and perennial ryegrass used in the study are shown in Table 4.3. Plantain's dry matter content is similar to that of Box *et al*. (2017), who recorded a DM of 98 g/kg in their study. According to research by Box *et al*. (2017), Mangwe *et al*. (2020), Pembleton *et al*. (2016), and Waghorn & Clark (2004) the ME content of plantain was between 9.2- and 12.2 MJ/kg DM. In the present study we found relatively low IVOMD values for plantain. Published studies generally show high IVOMD values (76.5- to 87.2%) (Labreveux *et al.,* 2006; Cheng *et al.,* 2017; Mangwe *et al.,* 2020). Søegaard *et al.* (2008) found an IVOMD value of 63% but had a much higher NDF value (426 g/kg DM) than the present study (322 g/kg DM). Lee *et al.* (2015) had a NDF value of 304 g/kg DM and corresponding IVOMD value of 70.2% which relates more to the present study. From these comparisons it is likely that the low IVOMD found in the current study can be related to different growth stages when sampling took place between studies. The high ADF: NDF ratio of plantain indicated that a high portion of the fibre was indigestible. Another possibility could be the presence of biologically active compounds with antimicrobial properties, inhibiting microbes in the rumen digesta used during the procedure (Stewart, 1996; Navarrete *et al*., 2016). Plantain's crude protein levels vary widely between research, with the present study being similar to that of Mangwe *et al.* (2020) who found a CP 194 g/kg DM. The NDF value of plantain is generally low. Box *et al.* (2017), Cheng *et al.* (2017), Fang *et al.* (2018), Mangwe *et al.* (2020) and Waghorn & Clark (2004) found NDF values ranging from 212- to 299 g/kg DM. These values are also variable as studies by Labreveux. *et al.* (2006), Pembleton *et al* (2016), Sanderson *et al.* (2003) and Søegaard *et al.* (2008) found NDF values ranging between 374- and 426 g/kg DM for plantain. The present study shows that plantain's NDF value (332 g/kg DM) falls within the recommended range (250- to 330 g/kg DM) published by the NRC (2001). Starch and sugar content were found to be low, even though fibre was low. It is likely that the inorganic portion of plantain dilutes the NFC content. The mineral (ash) fraction of plantain showed high values across studies (Sanderson *et al.,* 2003; Box *et al.,* 2017) and is similar to the present study.

The perennial ryegrass sward utilised throughout the study shows values comparable to Meeske *et al.* (2006). The study, however, had a higher mean DM value (188- vs 145 g/kg). Values for CP, ME, NDF and ADF were 207g/ kg DM, 10.6 MJ ME/kg, 437 g/kg DM and 285 g/kg DM respectively (Meeske *et al.,* 2006). The IVOMD of the present study agrees with Pembleton *et al.* (2016) who found a value of 71.9%, although having a higher NDF value (521 g/kg DM) than the present study (438 g/kg DM).

Figure 4.3 and 4.4 illustrates the nutrient changes during the study for plantain and perennial ryegrass pastures respectively. The final grazing cycle of plantain was too short to reach complete maturation and consequently leaving younger forage herbs with a slightly higher CP and IVOMD value. Fibre concentration remained constant, with a slight decline towards the end. Ryegrass showed much less variability and nutrient composition remained relatively constant throughout the experimental period.

Table 4.3 Mean and standard deviation values for the chemical composition of ryegrass and plantain pasture (n=8) grazed by Jersey cows during the study period

Nutrient[1] (g/kg DM, or as stated)	Plantain pasture	Ryegrass pasture
DM	103 ± 10.2	188 ± 1.71
IVOMD (%)	62.8 ± 1.26	70.7 ± 0.72
ME (MJ/kg DM)	9.8 ± 0.29	10.2 ± 0.13
CP	190 ± 16.6	221 ± 6.62
CP:ME ratio	2.05 ± 0.178	1.83 ± 0.295
NDF	322 ± 25.8	438 ± 14.4
ADF	245 ± 23.4	266 ± 7.23
Starch*	39.1 ± 7.48	50.2 ± 5.20
Sugar (WSC)*	14.2 ± 3.92	32.8 ± 5.69
NFC*	357 ± 12.7	260 ± 6.32
EE*	28.3 ± 1.06	40.6 ± 0.611
Ash	177 ± 21.1	111 ± 7.09
Ca	18.1 ± 0.906	4.69 ± 0.373
P	3.06 ± 0.534	3.39 ± 0.241
Ca:P ratio	6.45 ± 1.19	1.46 ± 0.088
Mg	4.96 ± 0.507	3.55 ± 0.208
K	22 ± 4.12	28.5 ± 3.85
Na	21 ± 1.88	8.08 ± 1.85
Mn (mg/kg)	36.7 ± 15.2	54.2 ± 18.9
Cu (mg/kg)	12.8 ± 2.28	6.15 ± 0.449
Fe (mg/kg)	178 ± 43.4	151 ± 38.4
Zn (mg/kg)	75 ± 14.5	48.7 ± 5.90

[1]DM – dry matter; CP – crude protein; ME – metabolizable energy; IVOMD – in vitro organic matter digestibility; NDF – neutral detergent fibre; ADF – acid detergent fibre; EE – ether extract; ADIN – acid detergent insoluble nitrogen; Ash – mineral fraction; Ca – calcium; P – phosphorus; Ca:P – calcium to phosphorus ratio; Mg – magnesium; K – potassium; Na – sodium; Mn – manganese; Cu – copper; Fe – iron; Zn – zinc
± Mean and standard deviation
*Analysis done by Cal labs

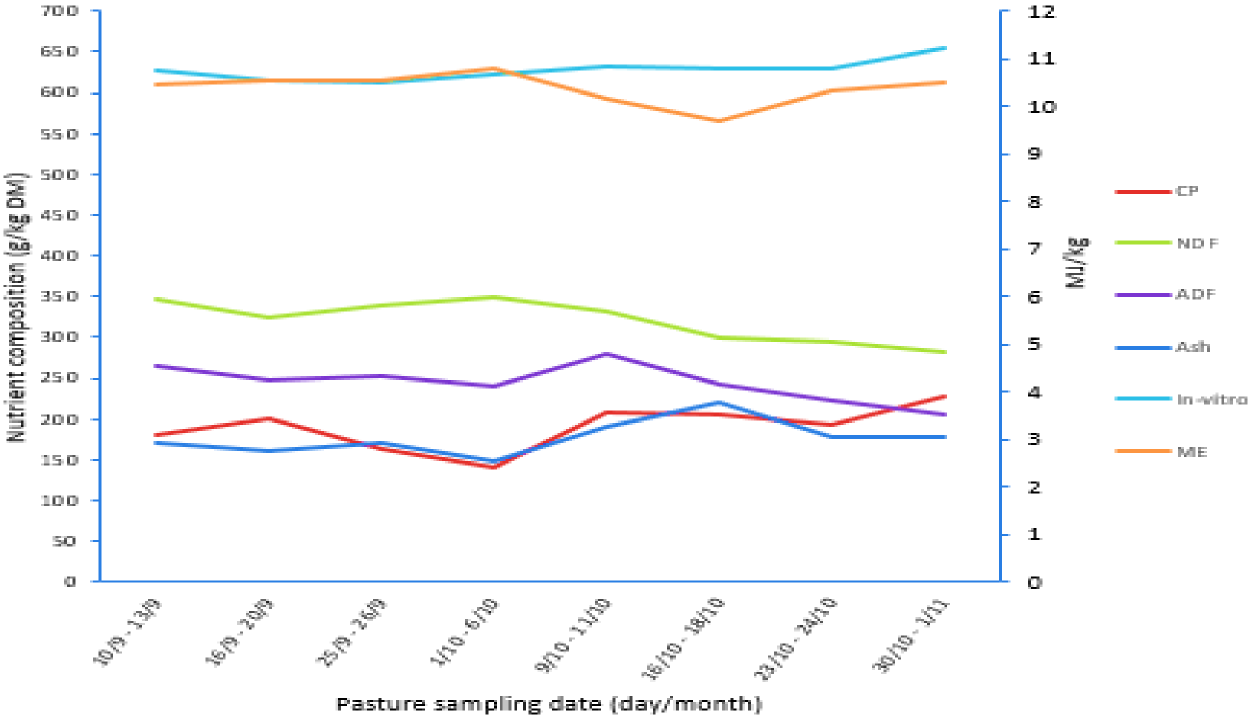

Figure 4.3 Plantain pasture quality parameters affected by the progression from early- to late-spring of samples collected over an eight-week period during the study

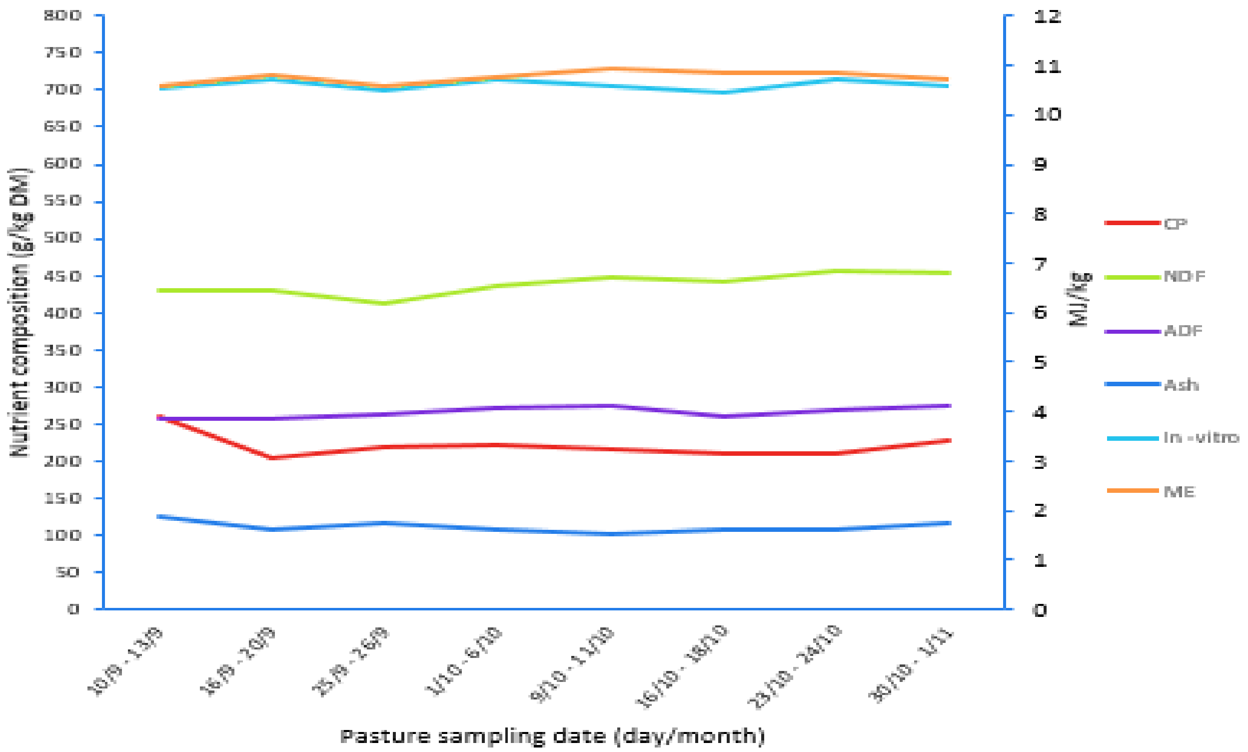

Figure 4.4 Perennial ryegrass pasture quality parameters affected by the progression from early- to late-spring of samples collected over an eight-week period during the study

4.2 Concentrate treatment nutrient composition

The analysed concentrate nutrient composition for the three different treatments differing in starch and NDF content is shown in Table 4.4. Table 3.1 of chapter 3 only shows estimated values provided by the Nova feeds database. The low-starch (LS) group had a higher analysed value (11.9 MJ/kg DM) compared to the estimated value (11 MJ/kg DM) and can be explained by the much higher analysed starch value.

The feeds were formulated to be *iso*-nitrogenous, as proven by the measured CP values. Lower analysed values were seen for NDF, but an upwards trend from HS to LS was still observed. The fat content of by-products is highly variable and is reflected in the increase in fat content with higher inclusion levels of by-products. Ash contributions between treatments stayed relatively constant with only slight increase from HS to LS treatments.

Table 4.4 Mean and standard deviation of the nutrient composition of the three respective concentrate treatments (n=4) fed to the Jersey cows during the production and rumen fermentation studies

Nutrient[1](g/kg DM, or as stated)	Concentrate treatments[2]		
	HS	MS	LS
DM	923 ± 2.06	912 ± 0.25	899 ± 2.68
IVOMD (%)	85.7 ± 1.42	81.8 ± 1.65	81 ± 0.896
ME (MJ/kg DM)	12.4 ± 0.011	12.2 ± 0.032	11.9 ± 0.039
CP	116 ± 0.513	120 ± 1.28	123 ± 1.89
NDF	75.1 ± 1.35	136 ± 0.98	199 ± 5.68
ADF	26.8 ± 1.90	66.6 ± 3.34	115 ± 4.24
Starch*	610 ± 4.60	518 ± 5.94	431 ± 6.86
NFC*	728 ± 17.3	658 ± 8.63	622 ± 5.80
EE*	29.7 ± 1.13	35.1 ± 0.07	40.1 ± 0.14
Ash	58.2 ± 2.05	61.4 ± 0.427	63.9 ± 0.443
Ca	11.8 ± 0.330	12.1 ± 0.377	11.9 ± 0.761
P	4.76 ± 0.05	4.95 ± 0.05	4.90 ± 0.05
Ca:P ratio	2.54 ± 0.08	2.51 ± 0.07	2.5 ± 0.165
Mg	3.86 ± 0.054	4.06 ± 0.056	4.33 ± 0.153
K	9.32 ± 0.388	9.81 ± 0.135	11.7 ±1.25
Na	2.54 ± 0.185	2.21 ± 0.129	2.43 ± 0.335
Mn (mg/kg)	137 ± 7.87	156 ± 11.2	173 ± 7.0
Cu (mg/kg)	32.3 ± 3.38	35.3 ± 3.41	39 ± 3.19
Fe (mg/kg)	226 ± 12.5	255 ± 9.10	305 ± 2.49
Zn (mg/kg)	162 ± 2.20	193 ± 4.77	211 ± 7.76

[1]DM – dry matter; ME – metabolizable energy; IVOMD – in vitro organic matter digestibility; CP – crude protein; NDF – neutral detergent fibre; ADF – acid detergent fibre; EE – ether extract; Ash – mineral fraction; Ca – calcium; P – phosphorus; Ca:P – calcium to phosphorus ratio; Mg – magnesium; K – potassium; Na – sodium; Mn – manganese; Cu – copper; Fe – iron; Zn – zinc; GE – gross energy; IVOMD – in vitro organic matter digestibility; CP: ME ratio – crude protein to metabolizable energy
[2]HS – high-starch concentrate; MS – medium-starch concentrate; LS – low-starch concentrate
± Mean and standard deviation
*Analysis done by Cal labs

4.3 Effect of different levels of starch supplementation on production performance

4.3.1 Milk yield

Table 4.5 shows the average milk yield, 4% FCM, and ECM values. There were no differences in these parameters across the HS, MS, and LS treatments (P>0.05). Tendencies for increased milk production were found for the MS group when compared to the HS (P=0.10) and LS (P=0.07) groups. On average the DMI of plantain was 4.62 kg DM/cow/day and for ryegrass it was 5.90 kg DM/cow/day. Plantain had a ME content of 9.8 MJ/kg DM and perennial ryegrass 10.2 MJ/kg DM with a mean calculated ME intake of 106 MJ ME intake from pasture per cow per day. Intake from HS, MS and LS were on average 5.54-, 5.47- and 5.39 kg DM/cow/day, thus a total energy intake of 175- ,173- and 170 ME/cow/day respectively. According to (NRC, 2001) energy was not a limiting factor. *In vitro* organic matter digestibility values were similar between MS and LS treatments and the HS group as expected had a higher IVOMD. Differences in ME intake and IVOMD were relatively minor which explains the lack of differences in milk.

Similar studies observed no differences in milk yield between treatments when maize was partially replaced with high-fiber by-products (Delahoy *et al*., 2003; Lingnau, 2011; Cawood, 2016; Van Wyngaard *et al.,* 2015; Van der Vyver *et al*., 2019). These studies differed from the present study in terms of ME content between concentrate treatments. Van Wyngaard *et al.* (2015) reported a decrease in ME content from 13.2- to 12. 2 MJ ME/kg DM when maize was partially replaced with palm kernel expeller (PKE). Cawood (2016) reported a decrease from 14.4- to 13.4 MJ ME/kg DM when high- and low-starch treatments were compared. Lingnau (2011) found a decrease from 12.04- to 10.95 MJ ME/kg DM for the high-starch and low-starch treatments respectively. Van der Vyver *et al* (2019) compared three treatment groups where soybean hulls replaced 0-, 15- and 30% of maize. Metabolizable energy values between treatments were very similar as is the case in the present study. Van Wyngaard *et al.* (2015) suggested that cows fed the low-starch treatment consumed more pasture, leading to similar ME intakes and milk yield responses compared to lower starch treatments.

4.3.2 Milk solid production

The influence of high-, medium-, and low-starch concentrates on milk yield and composition is illustrated in Table 4.5.

4.3.2.1 Milk fat

There were no differences between treatments in terms of average milk fat percentage (%) (P>0.05). The total neutral detergent fibre for the HS, MS and LS treatments were 27.0-, 29.2- and 31.4% respectively when the intake of ryegrass and plantain is taken into consideration. The amount of NDF from forage for the HS, MS and LS treatments were 90.4-, 84.1- and 78.5% respectively. The minimum recommended total NDF intake is 25-33% of the total diet with 75% originating from forage (NRC, 2001). The NDF levels in the present study fell well within these ranges. It was hypothesized that as NDF content increased from the HS to the LS treatments, milk fat content would rise as well (Zebeli *et al*., 2008). Higher % NDF originating from pasture trended from HS to LS. This could explain the lack of difference in milk fat between treatments, because NDF from forage is more effective (NRC, 2001) The VFA acetate, butyrate and the A:P however also showed no difference, supporting the lack of significance for milk fat between treatments (Table 4.9a).

Previous studies conducted by Cawood (2016); Delahoy *et al.* (2003); Meijs (1986); Van der Vyver *et al.* (2019) and Van Wyngaard *et al.* (2015) also found no difference in milk fat % when high-fibre by-products partially replaced maize. Conversely Lingnau (2011) and Meeske *et al.* (2009) reported higher milk fat % when maize was replaced with high fibre by-products (P<0.05). Cawood (2016); Lingnau (2011) and Meeske *et al*. (2009) included hominy chop and wheat bran as by-products to replace maize. Kikuyu pasture used in the study by Cawood (2016) had a higher NDF content when compared to studies reported by Lingnau (2011) and Meeske *et al.* (2009) that used perennial ryegrass dominant pasture.

High fibre concentrates are less effective to increase milk fat content supplemented to cows grazing lower quality pasture as it contains more effective fibre and lack of energy is usually a constraint. The digestibility of different by-products, as well as lipid and NDF concentrations, cause changes in rumen fermentation dynamics. The present study as well as studies by Cawood (2016); Lingnau (2011); Meeske *et al.* (2009); Van der Vyver *et al.* (2019) and Van Wyngaard *et al.* (2015) used Jersey cows, thus explaining the higher milk fat contents compared to studies by Delahoy *et al.* (2003) and Mejis (1986) that used Holstein- and Dutch Friesian cows respectively.

4.3.2.2 Milk protein

Mean milk protein % did not differ between treatments (P>0.05). Numerous other studies also support our results in that no differences between starch- and fibre-based concentrates fed to cows grazing pasture were found (Meijs, 1986; Khalili & Sairanen, 2000; Meeske *et al.,* 2009; Lingnau, 2011; Van Wyngaard *et al.,* 2015). Like the present study, Lingnau (2011) and Meeske *et al.* (2009)

replaced maize with hominy chop and wheat bran, results lacked difference between treatments (P>0.05). In contrast, Cawood (2016) also replaced maize with hominy chop and wheat bran and reported higher milk protein % in the high-starch treatment group (P<0.05), suggesting it's due to the high-starch treatment's higher energy value (Schwartz *et al.,* 1995). Van der Vyver *et al.* (2019) reported higher milk protein % when soybean hulls were increased from 0- to 15% and 0- to 30% of total DM respectively with reasoning being increased digestion of pasture DM and NDF when soybean hulls were included (P<0.05). The concentrate treatments in the present study were formulated to be *Iso*-nitrogenous and sufficient concentrate of NH_3-N were available for microbial protein synthesis (Table 4.9b) (Satter & Roffler, 1974). Minor differences in ME and CP between treatments might explain the lack of difference for milk protein % in the present study.

4.3.2.3 Milk lactose

Milk lactose % lacked difference between treatments (P>0.05). This agrees with the study by Lingnau (2011) where maize was partially replaced with hominy chop and wheat bran. According to Sayers et al. (2003), milk lactose % increases as the degree of concentrate feeding increases. Box *et al.,* (2017) found that milk lactose % increased when cows grazed 50-50 ryegrass-plantain pasture compared to ryegrass pasture alone. These increases in milk lactose are likely because of increase in milk yield. Cawood (2016) and Van Wyngaard *et al.* (2015) reported that when maize was replaced with high-fiber by-products, milk lactose percent decreased unexpectedly without a loss in milk production.

Welper & Freeman, (1992) stated that milk lactose content differs little across breeds and the present study is close to the average of 4.85%, reported by the NRC (2001). Sutton *et al.* (1987) stated that milk lactose content does not respond readily to changes in diet composition. Severe feeding conditions and poor udder health may be causes of differences in milk lactose % (Kitchen, 1981; Jenkins & McGuire, 2006). In the present study, good management practises and low SCC contributed to similar milk lactose values.

4.3.2.4 Milk urea nitrogen

The LS group had a greater milk urea nitrogen (MUN) level than the MS group (P<0.05). Kohn (2007) recommended a range of 8- to 12 mg/dl and the values found in the current study were within this range. Metabolizable energy values of the concentrate feeds were high which could have made nitrogen use efficiency optimal. This is especially true when considering that high pasture intakes contribute to increase MUN levels (Hwang *et al.,* 2000; Bargo *et al.,* 2003). Van der Vyver *et al*. (2019) found similar values (8.30-, 8.54- and 9.36 mg/dl for high-, medium- and low-starch treatments respectively) when maize was partially replaced with soybean hulls, supporting our results. Delahoy *et al.* (2003) found a significantly higher MUN value for the high-fibre treatment suggesting the cows on the high-starch treatment (ground corn) had higher nitrogen use efficiency. Even though there were substantial variances in the present study, the range of MUN values was within the acceptable range of 8- to 12 mg/dl, therefore these differences are biologically insignificant. In general, literature shows no difference for MUN between fibre- and starch-based concentrates (Meeske *et al.,* 2009; Lingnau, 2011; Van Wyngaard *et al.,* 2015; Cawood, 2016). Khalili & Sairanen (2000) observed that cows on a pasture-only diet had greater MUN levels than cows fed a high- or low-starch diet. This could be explained by lower energy values and higher amounts of degradable protein content associated with the pasture only diet.

4.3.2.5 Somatic cell count

Average SCC values did not differ between treatment groups (P<0.05). This result was expected, considering that SCC is generally not diet dependent, but is more a measure of individual cow health and good management practises (Van der Vyver *et al.,* 2015). The values for each group are less than 200 X 103 cells/ml of milk, showing that the cows in this study had healthy udders (Skryzpek *et al.,* 2004). Milk produced during the study was safe for human consumption as SCC values were under 500 X 10^3 cells/ml milk (Skryzpek *et al.,* 2004).

Table 4.5 Mean milk production and milk composition of Jersey cows grazing perennial ryegrass and plantain pasture supplemented with 6kg (as is) of high-, medium- or low-starch concentrates per day during the study

Parameter[1]	Treatment concnetrates[2]			SEM[3]	Contrast P-values		
	HS	MS	LS		HS vs MS	HS vs LS	LS vs MS
Milk yield (kg/cow/day)	20.9	21.9	20.8	0.42	0.10	0.87	0.07
FCM (kg/cow/day)	23.5	24.7	23.5	0.52	0.11	0.87	0.11
ECM (kg/cow/day)	25.4	26.8	25.4	0.53	0.08	0.99	0.08
Milk composition							
Milk fat (%)	4.88	4.91	4.90	0.12	0.85	0.89	0.96
Milk protein (%)	3.89	3.92	3.89	0.06	0.71	1.0	0.71
Milk lactose (%)	4.72	4.69	4.69	0.03	0.47	0.47	1.0
MUN (mg/dl)	8.89	8.22a	9.97b	0.46	0.30	0.10	0.01
SCC (x 10^3/ml)	120	190	185	40.5	0.23	0.26	0.94

[1]FCM – 4 % fat corrected milk; ECM – energy corrected milk; MUN – Milk urea nitrogen; SCC – somatic cell count
[2]HS – high-starch concentrate; MS – medium-starch concentrate; LS – low-starch concentrate
[3]SEM – standard error of means
a,b – means in the same row with different superscripts differ (P<0.05)

4.3.3 Body weight and body condition score

The average BW and BCS at the start and end of the study, as well as variations throughout its duration, are shown in Table 4.6. There were no variations in BW between treatments at the start and end of the study, as well as changes throughout its duration (P>0.05). However, all treatment groups experienced a rise in BW from the start to the end of the study. This was to be expected, given that most cows had reached peak production. The LS treatment had a higher BCS at the end of the study compared to the HS and MS treatments. The baseline BCS and the change in BCS over the study period were not different between treatment groups (P>0.05). The findings reveal that cows did not use bodily reserves to maintain production, indicating that the diets supplied enough nutrients. Previous studies that provided cows with similar feeding regimes showed similar results (Meeske *et al.,* 2009; Lingnau 2011; Cawood, 2016).

Table 4.6 Mean initial and end liveweight (LW) and change in LW and body condition score (BCS) of Jersey cows grazing plantain and ryegrass pasture supplemented with 6kg of high-, medium- or low-starch concentrates

Parameter[1]	Treatment concnetrates[2]			SEM[3]	Contrast P-values		
	HS	MS	LS		HS vs MS	HS vs LS	MS vs LS
Live weight							
BW before (kg)	396	389	400	6.62	0.45	0.71	0.26
BW after (kg)	419	415	422	6.87	0.69	0.79	0.50
BW change (kg)	+23	+26.2	+22.1	1.79	0.22	0.73	0.12
Body condition score							
BCS before (scale 1-5)	2.31	2.28	2.43	0.047	0.66	0.087	0.035
BCS After (scale 1-5)	2.28	2.29	2.46	0.044	0.81	0.007	0.014
BCS change (scale 1-5)	-0.03	0.01	0.03	0.039	0.43	0.30	0.79

[1]BW – body weight; BCS – body condition score
[2]HS– high-starch concentrate; MS – medium-starch concentrate; LS – low-starch concentrate
[3]SEM – standard error of means

4.4 Effect of different levels of starch supplementation on rumen parameters

4.4.1 Rumen pH

Table 4.7 shows the mean rumen pH values obtained by TruTrack indwelling loggers and a handheld pH meter respectively. No differences in pH were found between the HS and LS treatments for either of the two pH meter reading methods (P>0.05). Higher mean pH values at 6h00, 12h00 and 20h00 were found when TruTrack indwelling loggers were used, compared to handheld pH values. Handheld pH meter readings were measured during rumen fluid sampling when fluid was exposed to air and other external factors which might explain the difference between measuring techniques. Another possible explanation might be the difference in location of rumen fluid measured by the different apparatus (Cawood, 2016). Cows were given their respective concentrate treatments at approximately 6h00 and 13h00 each day. Figure 4.5 illustrates the diurnal pattern of the rumen pH as measured using the pH loggers. The highest pH values were between 05h00 and 06h00. At this time cows spent the longest time without consuming concentrate. A gradual decline in rumen pH after concentrate feeding was evident. The lowest pH values were recorded at 11h00 and 7h00-8h00 which is comparable to the results of Cajarville *et al.* (2006) who stated that lower pH values will be observed 2-5 h after feeding. When concentrate is fed to cows, readily fermentable carbohydrates are quickly fermented to VFA which lowers rumen pH (Dixon &

Stockdale, 1999). The HS and LS treatments showed similar diurnal patterns with an exception between 19h00 to 22h00 when using the pH indwelling loggers. Greater variation was observed during this time with the HS treatment measurements being approximately 0.2 units higher than the LS treatment at 19h30 and 20h30. This difference was likely due to cow and logger variation. Between 2h30 and 7h30 the LS treatment was higher than the range (5.8-6.4) suggested by Banakar *et al*. (2018) for optimal rumen health and function. The HS treatment showed even higher values during this time.

In the present study, cows on the HS treatment were expected to result in lower rumen pH values compared to the LS treatment group, because of lower fibre content (Erdman, 1988). It could be that physical effectiveness of fibre remained constant between groups. Allen (1997) stated that rumen pH responds more to the physical effectiveness of fibre compared to the absolute concentration in the diet. The starch and NFC levels of the total diet, however, were within the recommended ranges of the NRC (2001) explaining the lack of treatment effects. Minnee' *et al*. (2017) found that if plantain is included into a perennial ryegrass sward, that the rate of pH decline was faster compared to perennial ryegrass.

Cawood (2016), Lingnau (2011), Van der Vyver *et al.* (2019) and Van Wyngaard *et al.* (2015) conducted similar studies where high- and low-starch concentrate treatments were given to the experimental units. In these studies, cows grazed kikuyu/ryegrass-based pastures which had higher effective neutral detergent fibre (eNDF) content compared to the present study where plantain was also included as herbage. No difference was found in pH between the high- and low-starch treatments which is in accord with the present study ($P>0.05$). The time rumen fluid pH was below a pH of 6.2, 6.0 and 5.8 is depicted in Table 4.8. No differences were found between treatments ($P>0.05$). The study conducted by Van der Vyver *et al*. (2019) found that rumen fluid spent less time under a pH of 6.2, but time spent under pH 6.0 and 5.8 is very comparable to the present study with no difference between treatments. Lingnau (2011) found more time was spent under pH 6.2, 6.0 and 5.8 compared to the present study with no difference between treatments ($P>005$). This shows that by adding plantain to ryegrass pasture and consequently lowering the peNDF value, the rumen pH of cows spends more time under 6.2, 6.0 and 5.8. Feeding animals higher levels of fibre in the concentrate, however, does not impact rumen pH. Owens *et al.* (2011) stated that the benchmark values for sub-acute and chronic rumen acidosis is 5.6 and 5.2 respectively. According to these values, none of the cows suffered from this metabolic disorder during the present study.

Table 4.7 Mean pH values for the high- and low-starch treatments at 6h00, 12h00, 20h00 and overall mean as measured using a handheld pH meter and the mean pH as measured by indwelling loggers of cannulated cows fed 6kg concentrate/day (as is)

Time	Treatment[1]		SEM[2]	P-value[3]
	HS	LS		
6:00	6.42	6.42	0.047	0.98
12:00	5.88	5.80	0.040	0.19
20:00	5.85	5.83	0.033	0.78
Handheld pH average	6.04	6.02	0.018	0.24
pH logger average	6.28	6.21	0.053	0.34

[1] HS – high-starch concentrate; LS – low-starch concentrate
[2]SEM – standard error of means

Table 4.8 Mean time (hours) of rumen pH below 6.2, 6.0 and 5.8 in cannulated cows using indwelling loggers ((n=6/treatment fed 6kg (as is) per day)) fed either high- or low-starch concentrate treatments

Parameter	Treatment[1]		SEM[2]	P-value[3]
	HS	LS		
< 6.2	10.2	11.2	2.01	0.73
< 6.0	2.50	5.83	1.69	0.20
< 5.8	0.08	1.5	0.89	0.29

[1] HS – high-starch concentrate (80% maize); LS – low-starch concentrate (20% maize)
[2]SEM – standard error of means

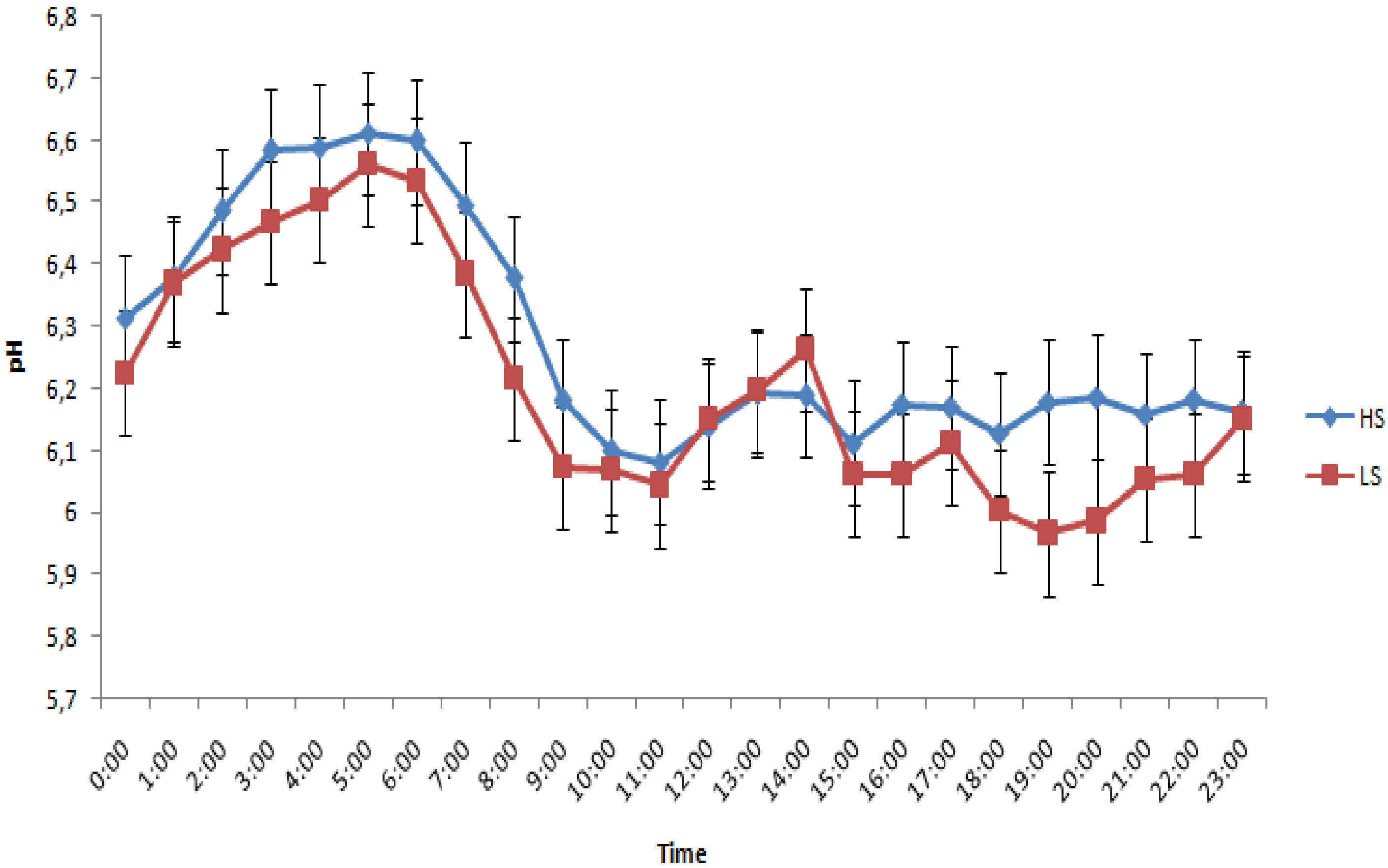

Figure 4.5 Diurnal fluctuations of the ruminal pH (mean ± SEM) of cannulated cows (n=6/treatment) in the high- and low-starch concentrate treatment groups as measured by indwelling loggers

4.4.2 Volatile fatty acids

Mean values for rumen VFA (mmol/L) at 6h00, 12h00 and 20h00 as well as overall means are depicted in Table 4.9a and 4.9b. Previous published studies with similar treatments showed total VFA in the range of 120-156mmol/L for high-starch treatments and 113-149 mmol/L for high-fibre treatments (Khalili & Sairanen, 2000; Sayers *et al.,* 2003; Lingnau, 2011; Van Wyngaard *et al.,* 2015; Cawood, 2016). An additional 10 studies investigating grazing cows receiving supplementation was summarized by Bargo *et al*., 2003. The summary showed a range of 90.3- to 151.4 mmol/L with a mean of 120.9 mmol/L for total VFA concentration in the rumen. In the present study study total VFA values of 123- and 125 mmol/L were found for HS and LS treatments respectively with a tendency to differ between them (P=0.067). These values are comparable to those found by Khalili & Sairanen, (2000) and Sayers *et al.* (2003) where starch-based concentrates were also replaced with fibre-based concentrates. Both these studies found 121.6- and 122.5 mmol/L for starch- and fibre-based concentrates respectively with no difference between them (P>0.05). Lingnau, (2011), however, found a difference with the starch-based concentrate yielding an average of 122.0 mmol/L total VFA and fibre-based concentrate yielding 113.0 mmol/L (P<0.05). The tendency for higher total VFA in the LS group is supported by lower pH observed for this treatment. Similar results were reported by Seymour *et al.* (2005) who found that rumen pH and VFA production is inversely proportionate. Variation in VFA concentrations were observed between different sampling times, with 20h00 being highest and 12h00 being lowest. The same pattern was found for acetate and propionate concentration. This corresponds to findings by (Bergman, 1990) who stated that the highest concentration VFA can be expected to be present 2-4 h after feeding.

No differences were found for individual VFA's between the HS and LS treatments (P>0.05). Mean acetate concentration for the present study agrees with the study done by Van Wyngaard *et al.* (2015) (76.6- and 75.9 mmol/L for high- and low-starch treatments respectively with no difference between them) where PKE replaced maize in concentrate supplements for cows grazing ryegrass dominant pasture. Cawood (2016) investigated variations in VFA concentrations in cows grazing ryegrass dominant pasture when maize was partially replaced with hominy chop, wheat bran, and gluten 20. When higher concentrations of maize (high-starch) were fed, acetate levels increased (P<0.05). When barley, wheat and maize were replaced with beet pulp and citrus pulp, Sayers *et al*. (2003) discovered a decrease in acetate (P<0.05). Acetate is a substantial contributor to the synthesis of milk fat (Ørskov, 1986). The lack of changes in milk fat across treatments in the present study (P>0.05) is supported by the lack of differences in acetate between treatments (P>0.05).

Propionate is the major VFA contributing to milk yield and milk lactose production (Moller *et al*., 2015). The fact that no differences were found in milk yield is supported by propionate concentration being constant between treatments (P>0.05). Results from other studies considering differences between high- and low-starch treatments are variable. Khalili & Sairanen (2000) and Van Wyngaard *et al.* (2015) found similar values for propionate concentration compared to the present study. Lingnau (2011) and Sayers *et al* (2003) reported that when cows grazing ryegrass pasture were fed a high-starch diet apposed a low-starch diet, propionate levels increased. Because the ratio of acetate and propionate is a major driver of milk yield and milk component yield, it must be examined. The present study's mean ratios of 3.34 and 3.41 for high- and low-starch respectively (P>0.05) are comparable to results from Cawood (2016) who found 3.91 and 3.81 for high- and low-starch treatments respectively (P>0.05). The study by Lingnau (2011) resulted in higher A:P (4.90 and 4.99), but values did not differ (P>0.05). Sayers *et al.* (2003) and Van Wyngaard *et al.* (2015) found that when high-starch components were replaced with high-fibre by-products, A:P increased significantly (P<0.05). Cawood (2016) and Lingnau (2011) found no differences in A:P which agrees with the present study as they found no difference in milk yield and milk fat % ($P>0.05$). Van Wyngaard *et al.,* (2015) and Sayers *et al* (2003) also found no differences in milk yield and milk fat yield even though the A:P was different ($P<0.05$).

Butyrate concentrations are variable amongst studies comparing high- and low-starch diets. Lingnau (2011) found an increase in butyrate ($P<0.05$), where Sayers *et al.* (2003) detected a decrease when high-starch ingredients were replaced with high fibre by-products. Cawood (2016), Khalili & Sairanen (2000) and Van Wyngaard *et al.* (2015) found no difference in butyrate between high- and low-starch treatments which agrees with the present study ($P>0.05$). Valeric acid, *iso*-butyric acid and *iso*- valeric acid are minor VFA and in the present study they showed no differences between treatments ($P>0.05$).

4.4.3 Rumen ammonia-nitrogen

Mean rumen NH_3-N values measured at 6h00, 12h00 and 20h00 as well as the overall daily mean are presented in Table 4.9b. No differences were found between the HS and LS treatment groups at any of the sampling times or on the daily mean. The values fall in the range (8.7-32.2 mg/dl) compiled over 10 studies by Bargo *et al.* (2003) and are well above the minimum of 5 mg/dl for maximum microbial growth suggested by Satter & Roffler (1974). In section 4.2, Table 4.4 the CP levels for the HS and LS group were 109- and 116 g/kg DM respectively. *In vitro* dry matter digestibility values were 80.8- and 76.8% of DM for the HS and LS treatment groups respectively. The HS concentrate, although having lower CP content, had higher digestibility compared to the LS treatment group. According to Hoover (1986), feeds with higher digestibility will have a higher contribution to rumen NH_3-N. The ME content of the concentrate plays a role in subsequent pasture intake. In the case of the present study, ME values were very similar, suggesting that cows had similar pasture intake. With higher digestibility, cows in the HS group were expected to have higher rumen NH_3-N. It might be that the higher CP value associated with the LS group balanced out supply of ammonia to the rumen. The present study agrees with Cajarville *et al*. (2006) who found that maximum rumen NH_3-N is associated with minimum rumen pH. In the present study, this was found at 20h00. At 6h00 pH was at its highest and at 12h00 pH increased again (Figure 4.5).

The present study agrees with Sayers *et al.* (2003) who showed average values of 12.0- and 13.6 mg/dl for high- and low-starch treatments respectively with no difference between them ($P>0.05$). Van Wyngaard *et al*. (2015) also reported similar values (13.8 and 14.6 mg/dl) for high- and low-starch treatments respectively) with differences at 6h30 and 20h30 ($P<0.05$), but not at 13h30 or as daily mean ($P>0.05$). The higher value at 6h30 was found for the high-starch group and the opposite occurred at 20h30 where the low-starch treatment had a higher value. It was suggested that these discrepancies occurred, because of differences in CP degradability between treatment groups. This is in contrast with Khalili & Sairaanen (2000) and Lingnau (2011) who found higher rumen NH_3-N for the high-starch treatment ($P<0.05$). Both these studies found comparatively higher rumen NH_3-N values than the present study. Higher values found by Khalili & Sairanen (2000) could be, because cows received lower levels of concentrate which increased their pasture intake and supplied more degradable protein (Hoover, 1986). Lingnau (2011) attributed the higher rumen NH_3-N in the high-starch group to lower energy available in the low-starch group for proteolysis.

Table 4.9a Mean concentrations of major volatile fatty acids (VFA) obtained at three-time intervals from rumen fluid collections of Jersey cows (n=6/treatment) fed 6 kg/day (as is) of either high- or low-starch concentrate treatments

Parameter[1]	Time	Treatment[2]		SEM[3]	P-value
		HS	LS		
Total VFA (mmol/L)	6:00	124	132	2.78	0.19
	12:00	108	109	2.12	0.71
	20:00	137	137	1.50	0.97
	Ave	123	125	0.46	0.067
Acetic acid (mmol/L)	6:00	77.5	82.5	2.58	0.32
	12:00	68.5	69.6	1.48	0.60
	20:00	83.5	85.1	2.12	0.61
	Ave	76.5	78.6	1.02	0.18
Propionic acid (mmol/L)	6:00	21.7	24.3	0.95	0.11
	12:00	18.6	19.3	0.57	0.41
	20:00	28.6	27.8	1.16	0.64
	Ave	23	23.5	0.58	0.56
A:P	6:00	3.59	3.43	0.15	0.43
	12:00	3.70	3.64	0.098	0.70
	20:00	2.92	3.17	0.10	0.23
	Ave	3.34	3.41	0.076	0.53
Butyric acid (mmol/L)	6:00	21.2	22.4	0.52	0.30
	12:00	17.4	17.4	0.45	0.89
	20:00	21.4	20.7	0.66	0.50
	Ave	20	20	0.37	0.97

[1]VFA – volatile fatty acids; A:P – Acetate to propionate ratio
[2]HS – high-starch (80% maize); LS – low-starch (20% maize)
[3]SEM – standard error of means

Table 4.9b Mean concentrations of minor volatile fatty acids (VFA) and ruminal ammonia nitrogen obtained at three-time intervals from rumen fluid collections of cannulated cows (n=6/treatment) fed 6kg/day (as is) of either high- or low-starch concentrate treatments

Parameter[1]	**Time**	**Treatment**[2]		**SEM**[3]	**P-value**
		HS	**LS**		
Iso-Butyric acid (mmol/L)	6:00	0.083	0.053	0.010	0.25
	12:00	0.24	0.30	0.023	0.15
	20:00	0.071	0.066	0.007	0.56
	Ave	0.13	0.15	0.009	0.25
Valeric acid (mmol/L)	6:00	1.53	1.41	0.094	0.31
	12:00	2.37	2.29	0.11	0.64
	20:00	2.19	2.16	0.081	0.78
	Ave	2.03	1.98	0.038	0.41
Iso-Valeric acid (mmol/L)	6:00	1.80	1.41	0.16	0.19
	12:00	0.94	0.75	0.076	0.21
	20:00	1.49	1.32	0.084	0.19
	Ave	1.41	1.20	0.088	0.12
NH3-N (mg/dl)	6:00	11.8	12.2	1.05	0.79
	12:00	10.4	11.1	0.92	0.59
	20:00	17.1	18.2	0.89	0.41
	Ave	13.1	13.8	0.82	0.54

[1]NH_3-N – Ammonia nitrogen
[2]HS – high-starch (80% maize); LS – low-starch (20% maize)
[3]SEM – standard error of means

4.4.4 *In situ* pasture degradability

The *in situ* DM_d, NDF_d and NDF k_d of ryegrass pasture for cows receiving HS or LS concentrate treatments are depicted in Table 4.10. No differences were observed for these parameters at any of the incubation periods (6 h, 18 h and 30 h) between the HS and LS treatments (P>0.05).

The high DM_d levels can be attributed to the ryegrass pasture's superior quality. Ruminal pH is a major determinant of degradability as microbes thrive within a certain pH range (Banakar *et al.,* 2018). Lowering the amount of readily fermentable carbohydrates, decreases the rate of production of VFA and creates a rumen environment that is more favourable for fibre digestion (Meijs, 1986;

Beauchemin, 1991). In the present study the pH for the HS and LS treatments were similar which is in accord with degradability parameters showing no difference (P>0.05). The nylon bags were incubated at 12h00. Figure 4.5 shows the diurnal pattern of pH, indicating that the pH ranged between 6.26 and 5.96 from 12h00 to 18h00, which was the first incubation period of 6 h. This could explain the low NDF_d, because according to Shriver *et al.* (1986) and Varga *et al.* (1984) optimal activity of cellulolytic bacteria is observed between pH 6.2 and 6.5. Cows in the HS group was expected to have higher NDF_d after 6 h as higher pH was experienced during this time (12h00-18h00) but was not the case. Lingnau, (2011), Sayers *et al.* (2003), and Van Wyngaard *et al.* (2015) also found no difference for *in situ* pasture degradability parameters when comparing starch- and fibre-based concentrates (P>0.05).

Dry matter intake is improved when higher degradability of dry matter and NDF is observed (Oba & Allen, 1999). With no differences between treatments for degradability parameters the results suggest that DMI between cows were the same for the HS and LS treatments which partly explains the lack of difference for production parameters between them (P>0.05).

Table 4.10 Mean values for dry matter disappearance (DM_d), neutral detergent fibre disappearance (NDF_d) and neutral detergent fibre rate of disappearance (NDF_{kd}) for perennial ryegrass pasture at three rumen incubation periods for Jersey cows (n=6) receiving either high-or low-starch concentrate treatment at 6 kg/day (as is)

Parameter[1]	Incubation period (h)	Concentrate treatments[2]		SEM[3]	P-value
		HS	LS		
DM_d (%)	6	40.8	40.7	0.99	0.94
	18	72.1	71.5	1.54	0.77
	30	83.3	84.1	0.84	0.54
NDF_d (%)	6	0.91	2.13	0.90	0.37
	18	52.2	52.1	2.60	0.98
	30	71.6	70.8	1.13	0.62
$NDFk_d$ (%/h)	6	0.18	0.42	0.18	0.37
	18	7.63	7.65	0.63	0.98
	30	7.23	7.11	0.32	0.79

[1]DM_d – dry matter disappearance; ND_d – neutral detergent fibre disappearance; NDF k_d – rate of neutral detergent fibre disappearance
[2]HS – high-starch (80% maize); LS – low-starch (20% maize)
[3]SEM – standard error of mean

4.4.5 Economical evaluation

The economic evaluation for the present study is summarised in Table 5.1. Only the margin over feed cost is presented which does not include labour and other farm related costs. Because milk fat and protein content did not differ between treatments, the price of milk was assumed to be identical between treatment groups (Table 4.5 in chapter 4). Milk price was obtained from Nestle' in September 2019 for milk with similar milk fat, milk protein and SCC (parameters affecting milk price). Pasture intake (5.90 and 4.62 kg DM/cow/day for ryegrass and plantain respectively) and cost (R1.50/kg pasture) was assumed to be equal amongst treatments, making the cost of concentrate the only variable. Cost of concentrate was obtained from Nova feeds (September 2019) and pasture cost was obtained from Outeniqua Research Farm (September 2019).

There was a tendency for increased milk production for the medium-starch treatment group compared to the low- and high-starch groups (Table 4.5). When this is taken into consideration the extra litre of milk produced by the medium-starch group increases milk production of a 400-cow herd with 400 litres per day. At R 5.50, it adds up to R 2200 per day. It is important to notice that this statement is made based on a tendency and not significant differences. Repeats of the trial is needed to make it concrete. The ratio between maize and by-products influenced the price of the respective feeds, with increased amounts of by-products, decreasing the cost per ton in this situation. The margin over feed cost for the medium- and low-starch groups increased with R 2 831.00 and R 1 191.00 respectively per day for a 400-cow unit relative to the high-starch group. Replacing maize with high-fibre-by-products showed potential for increased profit margins.

Table 5.1 Economic implications of feeding 6kg (as-is) high-, medium- or low-starch concentrates to a dairy herd of 400 cows in milk, grazing ryegrass and plantain during spring

Parameter[1]	Concentrate treatments[2]		
	HS	MS	LS
Milk Yield (L/cow/day)	20.9	21.9	20.9
Milk yield (L/herd/day)	8360	8760	8360
Milk price (R/L)	R 5.50	R 5.50	R 5.50
Milk income (R/cow/day)	R 114.95	R 120.45	R 114.95
Milk income (R/herd/day)	R 45 980.00	R 48 180.00	R 45 980.00
Increase in daily income (R/herd/day)	R 0.00	R 2200	R 0.00
Concentrate price (R/ton)	R 4 905.90	R 4 670.15	R 4 436.70
Concentrate cost (R/cow/day)	R 29.44	R 28.02	R 26.62
Concentrate cost (R/herd/day)	R 11 776.00	R 11 208.00	R 10 648.08
Decrease in daily concentrate cost (R/herd/day)	R 0.00	R 568.00	R 1092.00
Pasture cost (R/kg)	R 1.50	R 1.50	R 1.50
Pasture cost (R/cow/day)	R 15.78	R 15.78	R 15.78
Pasture cost (R/herd/day)	R 6 312.00	R 6 312.00	R 6 312.00
Increase in daily input cost (R/herd/day)	R 0.00	R 0.00	R 0.00
Margin over feed cost (R/cow/day)	R 69.78	R 76.65	R 72.55
Margin over feed cost (R/herd/day)	R 27 829.00	R 30 660.00	R 29 020.00
Margin over feed cost (R/herd/month)	R 834 870.00	R 919 800.00	R 870 600.00
Increased margin over feed cost compared to HS (R/herd/day)	R 0.00	R 2 831.00	R 1 191.00
Increased margin over feed cost compared to HS (R/herd/month)	R 0.00	R 84 930.00	R 35 730.00

[1]Herd – 400 cows; R – South African Rand
[2]HS – high-starch treatment containing 80% maize; MS – medium-starch treatment containing 50% maize; LS – low-starch treatment containing 20% maize

4.5 Conclusion

For the production study, 51 Jersey cows, grazing plantain, and ryegrass were evenly divided into three groups, each receiving 6kg "as is" of either a high-starch (80% maize), medium-starch (50%) or low-starch (20%) concentrate treatment. The high- and low-starch concentrate treatment groups did not differ in terms of milk yield, % FCM, or ECM (P>0.05). Milk yield and FCM tended to be higher for the medium-starch treatment group when compared to the high- and low-starch groups (P=0.07). Milk fat %, milk protein % and milk lactose % were consistent amongst the three treatments, leading to equal milk price per litre between them. Milk urea nitrogen values for all the treatments fell within recommended ranges, with the low-starch concentrate treatment having a higher value than the medium-starch group (P<0.05). The somatic cell counts were lower than accepted ranges, indicating that milk produced during the study was safe for human consumption. Because the milk parameters determined in both the high- and low-starch treatment groups were consistent, the decision to substitute maize with high-fibre by-products could be made based on raw material costs. The tendency for higher milk yield (P= 0.07) and 4% FCM (P=0.08) in the medium-starch group, further increases the potential for higher profit margins. The high quality of ryegrass and plantain in the spring, reduces need for maize in the dairy meal decreasing the likelihood of rumen health issues. Body weight and body condition increased from the onset to the termination of the trial, with no difference between treatments, indicating that energy supply was sufficient (P>0.05).

In a two-period cross-over design, six rumen-cannulated cows grazing plantain and ryegrass were allocated equally into the high- or low-starch concentrate treatments. Mean ruminal pH and the time spent below pH 5.8, 6.0 and 6.2 showed no difference between treatments. Even though it was likely that cows receiving the low-starch treatment had increased pasture intake, the lack of effective fibre made no difference in the rumen pH. Mean values for individual volatile fatty acids were similar between the high- and low-starch treatments, which supports the lack of difference in milk fat % and milk yield. Treatments were formulated to be *iso*-nitrogenous which is supported by the similarities in rumen NH_3-N, which were similar between treatments and was higher than the minimum recommended for maximum microbial growth. The digestibility (DM_d, NDF_d, and $NDFk_d$) of ryegrass was unaffected by varying quantities of starch in the concentrate, which coincided with the lack of difference in pH between the high- and low-starch treatments. Long term effects on rumen health could not be established, as the trial period was only 48 days in length. Rumen pH values, however, were within ranges defining a healthy rumen environment throughout the course of the trial.

Plantain and ryegrass had a high energy value, which made it possible to reduce starch inclusion in the concentrate supplements. The effective fibre content of plantain was low, but did not adversely affect rumen parameters, which was especially important in the high-starch treatment group. Ruminants grazing only plantain might show larger variation in rumen fermentation parameters with the lack of fibre likely to cause lower pH values. Differences in productivity and rumen parameters could have been more apparent if concentrate supplementation had been supplied at higher levels, but because to the high quality of forage in the spring, this was not commercially viable. Towards the end of the study, plantain's regrowth was slow. This was likely because of the reproductive phase partitioning energy towards the growth of reproduction stems. The post-grazing heights of plantain were very low and lengthening the grazing cycle will allow for proper regrowth and increase pasture yield.

In conclusion, replacing maize with high-fibre by-products did not affect milk production and composition or rumen fermentation parameters. The inclusion of more high-fibre by-products has the potential to lower feed cost and improve margin over feed cost.

CHAPTER 6

Critical evaluation

Study duration: The study was shorter than expected (47 days) with the reason being plantain's low recovery rate during the second grazing cycle. Before plantain was grazed at various lengths between days the study was terminated. This led to only three milk samples taken over the study period. With more milk samples the results would have been more definitive.

Pasture measurement: Yield and pasture intake was determined with a rising plate meter (RPM). The RPM is relatively inaccurate for yield and intake determination and is more a management tool for stocking rate. Herd average intake was calculated with likely over- or under-estimation. Individual intake, leading to the determination of intake variation between treatments is not included in this technique. Methods to estimate individual intake will give more accurate results.

Plantain reproductive stem: By measuring plantain with an RPM during its reproductive stage further decreases accuracy. The reproductive stems are rigid and sticks past the plantain leaves. When the disc is placed on the canopy, it cannot move to the appropriate height for accurate estimation of the leaves and available herbage is over-estimated.

Plantain digestibility: The *in vitro* organic matter digestibly of plantain for the present study was low compared to published literature. Growth stage of plants or error during lab analysis could have caused this.

Rumen fluid sampling times: Only three rumen collection times were included for each of the two periods (6h00, 12h00 and 20h00). A clearer picture of the effect of concentrate on VFA and rumen NH_3-N production would have been achieved if an extra one or two sampling times were included.

Dacron bag study: Ryegrass was harvested and manually cut with scissors to try and achieves uniform size of 5 mm pieces. With the use of a mill and sieve of appropriate pore size the grass pieces within the dacron bag would have been more uniform.

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