

# Partial substitution of maize with soybean hulls in a concentrate supplement for grazing dairy cows

by

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## Abstract

Title: Partial substitution of maize with soybean hulls in a concentrate supplement for grazing dairy cows.

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Climate in the southern Cape region of South Africa permits dairy farmers to make use of cultivated pasture as their main nutrient and feed source for dairy cows. A commonly used pasture system in this region is kikuyu over-sown with ryegrass. Pasture has a limited supply of nutrients which necessitates the need to provide dietary supplementation in a concentrated form. Concentrates consists primarily of maize grain which is a highly priced product containing a high starch content. Including alternative feed ingredients with lower starch content, higher level of digestible fibre and possibly a lower cost in concentrates may improve milk production and milk composition. Soybean hulls are one of many by-products which are considered as an alternative to maize. The hulls are a by-product after processing of soybeans for oil and meal, and are high in energy, crude protein (CP) and fibre which make it a possible alternative to maize in dairy feed as it is digested more efficiently by ruminants. The aim of this study was to determine the effect of partial substitution of a maize in a dairy concentrate with soybean hulls, on milk production, milk composition, digestion of kikuyu-ryegrass pasture and rumen environment.

Fifty-one lactating Jersey cows from Outeniqua Research Farm were blocked according to mean milk yield, days in milk (DIM) and lactation number for the production study. Cows used were between  $127 \pm 50.5$  DIM. A complete randomised block design was used. Cows within each block were randomly allocated to one of the three treatments. Treatments were defined according to the level of soybean hulls included in the concentrate supplement: SH0, SH15 or SH30 containing respectively 0%, 15% or 30% soybean hulls. Cows were fed 6 kg/day (3 kg per milking session) concentrates. After each milking session the cows grazed fresh kikuyu-ryegrass pasture allocated at  $\pm 13$  kg dry matter (DM)/cow per day. There were no significant differences ( $P > 0.05$ ) in milk yield, 4% fat corrected milk (FCM) and energy corrected milk (ECM) between treatments. Milk fat tended ( $P = 0.06$ ) to increase when 15% soybean hulls were included. Milk protein and lactose percentages

increased significantly ( $P < 0.05$ ) when soybean hulls were included (15 and 30%) in the concentrates. Somatic cell count (SCC) did not differ significantly between treatments. The milk urea nitrogen (MUN) content (8.30 – 9.36 mg/dL) indicated that sufficient protein was supplied to cows on all treatments. Cows on all three treatments gained weight and improved in condition during the study. Live weight of cows did not differ between treatments. Body condition improved ( $P < 0.05$ ) when 15% soybean hulls were included indicating sufficient energy supply.

Nine ruminally cannulated cows from the Outeniqua Research Farm were used for the rumen study. A 3 x 3 Latin square design was used, where all cows were subjected to all three treatments. In each period, cows were randomly allocated to one of the three treatments. Cows were fed 6 kg/day (3 kg per milking session) concentrates. After each milking session the cows grazed together with the production study cows on fresh kikuyu-ryegrass pasture allocated at  $\pm 13$  kg DM/cow per day. There were no significant differences in the rumen pH among treatments. Acetate production showed no significant difference among treatments. Rumen propionate and butyrate concentration was lower ( $P \leq 0.05$ ) when 30% soybean hulls were included compared to the control. The ratio of acetate to propionate increased ( $P < 0.05$ ) when soybean hulls were included at 15 and 30%. Rumen ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) increased ( $P < 0.05$ ) when 30% soybean hulls were included. After 30 h of incubation the *in sacco* DM disappearance of ryegrass pasture was higher ( $P = 0.05$ ) when soybean hulls were included. The *in sacco* neutral detergent fibre (NDF) disappearance of kikuyu-ryegrass pasture after 30 h of incubation increased significantly when 15% soybean hulls were included, compared to 0% soybean hulls.

The study showed that milk production can be maintained when as much as 30% soybean hulls replaced maize in the concentrate. Replacing 15% of the maize tended to increase milk fat content and increased milk protein and lactose content significantly.

## Uittreksel

Titel:	Gedeeltelike vervanging van mielies met sojaboondoppe in 'n kragvoeraanvulling vir weidende melkkoeie.
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Klimaat in die suidelike Kaapstreek van Suid-Afrika laat suiwelboere toe om van aangeplante weiding as hul hoofvoedingstof en voerbron vir melkkoeie gebruik te maak. 'n Algemeen gebruikte weidingstelsel in hierdie streek is kikoejoe oor-gesaaï met raaigras. Weiding het 'n beperkte hoeveelheid voedingstowwe wat dit nodig maak om dieetaanvulling in 'n gekonsentreerde vorm te verskaf. Konsentrate bestaan hoofsaaklik uit mieliegraan wat 'n hoogs geprysde produk is wat 'n hoë styselinhoud bevat. Insluiting van alternatiewe voer bestanddele met laer styselinhoud, hoër vlak van verteerbare vesel en moontlike laer koste in konsentrate kan melkproduksie en melksamestelling verbeter. Sojaboondoppe is een van die vele neweprodukte wat as 'n alternatief vir mielies beskou word. Die doppe is 'n neweproduk na die verwerking van sojabone vir olie en meel, en is hoog in energie, ru-proteïen (RP) en vesel wat dit 'n moontlike alternatief vir mielies in suiwelvoedsel maak, aangesien dit meer doeltreffend deur herkouers verteer word. Die doel van hierdie studie was om die effek van gedeeltelike vervanging van 'n mielie-gebaseerde suiwelkonsentraat met sojaboondoppe op, melkproduksie, melksamestelling, vertering van kikoejoe-raaigras weiding en rumenomgewing te bepaal.

Een-en-vyftig lakterende Jersey-koeie van Outeniqua Navorsingsplaas was geblokkeer volgens gemiddelde melkopbrengs, dae in melk (DIM) en laktasienommer vir die produksiestudie. Koeie gebruik was tussen  $127 \pm 50.5$  DIM. 'n Volledige ewekansige blokontwerp was gebruik. Koeie binne elke blok was ewekansig toegeken aan een van die drie behandelings. Behandeling was gedefinieer volgens die vlak van sojaboondoppe wat ingesluit was in die konsentraat aanvulling: SH0, SH15 of SH30 wat onderskeidelik 0%, 15% of 30% sojaboondoppe bevat. Koeie was 6 kg/dag (3 kg per melk sessie) konsentraat gevoer. Na elke melksessie het die koeie vars kikoejoe-raaigras weiding gewei wat teen  $\pm 13$  kg droëmateriaal (DM)/koei per dag toegedien was. Daar was geen beduidende verskille ( $P > 0.05$ ) in melkopbrengs, 4% vet gekorrigeerde melk (FCM) en energie

gecorrigeerde melk (ECM) tussen behandelings nie. Bottervet het 'n neiging getoon ( $P = 0.06$ ) om toe te neem wanneer 15% sojaboondoppe ingesluit was. Melkproteïen en laktose persentasies het aansienlik toegeneem ( $P < 0.05$ ) wanneer sojaboondoppe (15 en 30%) in die konsentrate ingesluit was. Somatiesse seltelling (SCC) het nie beduidend verskil tussen behandelings nie. Die melkureumstikstof (MUN) inhoud (8.30 – 9.36 mg/dL) dui aan dat voldoende proteïen aan koeie op al die behandelings voorsien was. Koeie op al drie behandelings het gewig opgetel en in kondisie verbeter tydens die studie. Lewendige gewig van koeie verskil nie tussen behandelings nie. Liggaams-kondisie het verbeter ( $P < 0.05$ ) wanneer 15% sojaboondoppe ingesluit was, wat voldoende energievoorsiening aandui.

Nege rumen-gekannuleerde koeie van die Outeniqua Navorsingsplaas was vir die rumenstudie gebruik. 'n 3 x 3 Latynse vierkante ontwerp was gebruik, waar alle koeie aan al drie behandelings onderhewig was. In elke periode was koeie lukraak toegeken aan een van die drie behandelings. Koeie was 6 kg/dag (3 kg per melk sessie) konsentraat gevoer. Na elke melksessie het die koeie saam met die produksiestudie koeie vars kikoejoe-raaigras weiding gewei wat teen  $\pm 13$  kg DM/koeie per dag toegedien was. Daar was geen beduidende verskille in die rumen pH tussen behandelings nie. Asetaat produksie het geen beduidende verskil tussen behandelings getoon nie. Rumen propionaat en butyraat konsentrasie was laer ( $P \leq 0.05$ ) toe 30% sojaboondoppe ingesluit was in vergelyking met die kontrole. Die verhouding van asetaat tot propionaat het toegeneem ( $P < 0.05$ ) toe sojaboondoppe teen 15 en 30% ingesluit was. Rumen-ammoniakstikstof ( $\text{NH}_3\text{-N}$ ) het beduidend toegeneem ( $P < 0.05$ ) toe 30% sojaboondoppe ingesluit was. Na 30 uur van inkubasie was die *in sacco* DM verdwyning van kikoejoe-raaigras weiding hoër ( $P = 0.05$ ) toe sojaboondoppe ingesluit was. Die *in sacco* neutraalbestande vesel (NDF) verdwyning van kikoejoe-raaigras weiding na 30 uur van inkubasie het beduidend toegeneem toe 15% sojaboondoppe ingesluit was in vergelyking met 0% sojaboondoppe.

Die studie het getoon dat melkproduksie gehandhaaf kan word wanneer soveel as 30% sojaboondoppe die mielies in die konsentraat vervang. Die vervanging van 15% van die mielies het geneig om bottervetinhoud te verhoog en die melkproteïen en laktose-inhoud beduidend te verhoog.

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## Abbreviations

ADF	Acid detergent fibre
ADG	Average daily gain
ADIN	Acid detergent insoluble nitrogen
ADL	Acid detergent lignin
ANOVA	Analysis of variance
BC	Body condition
BCS	Body condition score
BUN	Blood urea nitrogen
Ca	Calcium
CF	Crude fibre
CHO	Carbohydrates
CP	Crude protein
DE	Digestible energy
DIM	Days in milk
DM	Dry matter
DMD	Dry matter disappearance
DMI	Dry matter intake
ECM	Energy corrected milk
EE	Ether extract
FCM	Fat corrected milk
GE	Gross energy
GLM	General linear model
IDF	Insoluble dietary fibre
IH	Insoluble hemicellulose

iNDF	Indigestible neutral detergent fibre
IPC	a Inductively Coupled Plasma Spectrometry
IVOMD	<i>In vitro</i> organic matter disappearance
IVTD	<i>In vitro</i> true digestibility
K	Potassium
LAN	Limestone ammonium nitrogen
LSD	Least significant differences
LW	Live weight
ME	Metabolisable energy
Mg	Magnesium
MUN	Milk urea nitrogen
N	Nitrogen
Na	Sodium
NDF	Neutral detergent fibre
NDFD	Neutral detergent fibre disappearance
NFC	Non-fibre carbohydrates
NFF	Non-forage fibre
NH <sub>3</sub> -N	Ammonia nitrogen
NSC	Non-structural carbohydrates
OM	Organic matter
P	Phosphorus
peNDF	Physically effective neutral detergent fibre
RP	Ru-proteïen
RPM	Rising plate meter
SCC	Somatic cell count

SEM	Standard error of the means
SDF	Soluble dietary fibre
TDF	Total dietary fibre
TDN	Total Digestion Nutrients
TiO <sub>2</sub>	Titanium dioxide
TMR	Total mixed ration
VFA	Volatile fatty acid

## Chapter 1 - Introduction

Milk is produced using different feeding systems on dairy farms in South Africa. The specific system used will be determined by the resources available to dairy farmers. Two systems, or a combination of the two, are widely implemented in South Africa. Dairy farmers either make use of 1) intensive total mixed ration (TMR) systems or 2) cultivated pasture-based systems. According to Delahoy *et al.* (2003) and Khalili & Sairanen (2000), it is possible for dairy farmers to produce milk at a lower cost when making use of cultivated pasture-based systems. The latter system is profitable as it makes use of less expensive feed sources to produce milk (Clark & Kanneganti, 1998; Peyraud & Delaby, 2001). Pasture as only feed source does not provide sufficient amounts of nutrients to meet the dietary requirements of a dairy cow. Therefore, lactating dairy cows need additional dietary supplementation, which is usually provided in a concentrated form and offered in the dairy parlour during milking.

Concentrate feed supplements for pasture based lactating dairy cows typically contain 70 – 80% maize grain, which supplies their high energy demand. As with maize grain, dietary components that contribute most to the energy content (metabolisable and digestible) of concentrates are mostly weather dependent crops. Due to variable weather conditions, the availability and price of raw materials used in concentrates are variable throughout the year. Therefore, cheaper alternative ingredients to maize are constantly being researched and considered for dairy concentrates. Partially replacement of maize with alternative high-fibre feed ingredients may improve milk production, milk composition and digestion of pasture (Lingnau, 2011; Steyn, 2012). Concentrates high in readily fermentable carbohydrates (CHO) and starch can decrease the rumen pH to pH 6.0 or lower due to starch being rapidly fermented to volatile fatty acids (VFA) (McDonald *et al.*, 2001). Consequently microbial activity and pasture digestion in the rumen are compromised, which may result in lowered dry matter intake (DMI) and milk production (Berzaghi *et al.*, 1996).

Supplementation of less expensive feed ingredients depends on availability of common ingredients used, as well as availability of the alternative ingredient. One alternative ingredient currently researched and considered is soybean hulls. Soybeans are used to extract the oil and to produce soybean meal, which is also used in dairy feed. During processing, soybean hulls are left as a by-product when the hull is separated from the bean during the extraction process. The hull can represent up to as much as 8% of the total weight of the bean (Barbosa *et al.*, 2008). Compared to most feed by-product sources available, soybean hulls are high in energy, crude protein (CP) as well as fibre which makes it a possible alternative to maize in dairy feed (Quicke *et al.*, 1959; Belyea *et al.*, 1989; NRC, 2001; Hopkins & Whitlow, 2002; Chee *et al.*, 2005; Jacela *et al.*, 2007; Barbosa *et al.*, 2008).

The demand for soybean meal and soybean oil used in poultry diets is increasing as the poultry industry is constantly growing. This increase in demand results in the industry having a surplus amount of soybean hulls which needs to be disposed of. Soybean hulls are easily acquired and instead of disposing the hulls, it can be incorporated into the diets of ruminants and specifically dairy cows. Soybean hulls are digested more efficiently by ruminants than by monogastrics (Barbosa *et al.*, 2008) making it an excellent raw material to use in ruminant feeds. By means of incorporating soybean hulls in the diets of ruminants, it can reduce the costs of hull disposal. Currently, some farmers already include up to 20% soybean hulls in their concentrates in the southern Cape region of South Africa (Meeske, 2017). The question, however, is would it be efficient to include as much as 30% soybean hulls in a maize grain based concentrate and still maintain acceptable production?

The aim of this study was to determine the effect of partial substitution of maize within a dairy cow concentrate with different inclusion levels of soybean hulls, on milk production, milk composition, digestion of kikuyu-ryegrass pasture and rumen environment.

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## Chapter 2 - Literature Review

### 2.1 Introduction

Worldwide, dairy farmers either make use of 1) intensive TMR systems, 2) cultivated pasture-based systems or 3) a mixture of the two systems. Pasture is a natural and less expensive high quality feed source (Clark & Kanneganti, 1998; Peyraud & Delaby, 2001), and is therefore preferred as basis for profitable milk production. The climate in the southern Cape region of South Africa permits dairy farmers to make use of cultivated pasture as their main nutrient and feed source. Pasture has a limited supply of nutrients which necessitates the dietary supplementation of a concentrate. Concentrates consist primarily of maize grain which is a highly priced product creating the opportunity to research and consider alternative ingredients to maize grain with the aim of lowering feed costs. Several high-fibre by-products like palm kernel expeller, bran, hominy chop, gluten, apple pomace, citrus waste and soybean hulls are available. Soybeans are processed for oil and meal and the hulls are left as a by-product high in energy, CP and fibre which makes it a great alternative to maize in dairy feed as it is digested more efficiently by ruminants (Quicke *et al.*, 1959; Belyea *et al.*, 1989; NRC, 2001; Hopkins & Whitlow, 2002; Chee *et al.*, 2005; Jacela *et al.*, 2007; Barbosa *et al.*, 2008).

### 2.2 Pasture-based systems

Pasture production is a key profit driver for pasture-based dairy farming and is highly dependent on the weather. Pasture-based dairy farmers focus on producing sufficient pasture with high nutritive values to optimise milk production per animal as well as per hectare (ha) (Marais, 2001; García *et al.*, 2014). In order to produce sufficient pasture throughout the year, it is best to have a mixture of forage species, instead of just a single species (Neal *et al.*, 2007). This will result in a more even fodder flow. In the southern Cape region of South Africa pasture-based dairy farming, using kikuyu (*Pennisetum clandestinum*) over-sown with ryegrass is common (Meeske *et al.*, 2006).

### 2.3 Kikuyu (*Pennisetum clandestinum*)

Kikuyu is classified as a perennial grass (Dickinson *et al.*, 2004) which is well adapted to the climatic conditions of the southern Cape region of South Africa (Botha, 2003; Botha *et al.*, 2008a). Kikuyu thrives under irrigation in the southern Cape region of South Africa between late spring and early autumn (high production). Kikuyu serves as a persistent and productive base for pasture (Bell *et al.*, 2011), but is dormant (low production) from autumn to late spring (Marais, 2001; Botha *et al.*, 2008b; García *et al.*, 2014). Pasture production is increased by over-sowing kikuyu with another grass species such as ryegrass during autumn. Botha *et al.* (2008a) and Botha (2003), found that successful over-sowing of ryegrass into kikuyu improved seasonal fodder availability and improved nutritional value (quality) of pasture. Nutritional quality of kikuyu changes depending on the leaf growth. Too high nitrogen (N) content in kikuyu reduces palatability and intake of pasture, resulting in reduced animal performance. Careful consideration needs to be taken when fertilizing as the N

content of the fertilizer can influence the N content of kikuyu (Reeves, 1997; Marais, 2001). Kikuyu is a common pasture for grazing cows due to its high DM yield and growth rates under favourable conditions. With well managed kikuyu pasture it is possible to support high stocking rates (3.5 – 4.94 cows/ha) and high milk production (20.68 – 25.90 kg/cow) per ha (Colman & Kaiser, 1974; Reeves, 1997). Despite the high DM yield, kikuyu is low in digestible energy content and its structural CHO are poorly digested (Reeves, 1997; Marais, 2001). Limitations of kikuyu are low metabolisable energy (ME) value, high NDF content, and low calcium (Ca) and sodium (Na) content (Joyce, 1974; Miles *et al.*, 1995; Reeves *et al.*, 1996a; Marais, 1998; Muller & Fales, 1998; Marais, 2001; Kolver, 2003). Calcium deficiency is due to oxalic acid found in kikuyu which binds Ca in kikuyu making it unavailable to grazing animals (Marais, 1998, 2001). Due to this, kikuyu tends to have imbalances of Ca:phosphorus (P) and potassium (K):Ca + magnesium (Mg) (Miles *et al.*, 1995; Botha, 2003). Over-sowing of kikuyu pasture with ryegrass is common practice in the southern Cape region of South Africa (Botha & Zulu, 2013).

## 2.4 Ryegrass (*Lolium spp.*)

Species selection to over-sow into kikuyu pastures should be considered carefully, as well as species effects on fodder flow and pasture availability. The purpose of over-sowing is to supplement already established pasture, instead of replacing it. The over-sowing species should therefore complement kikuyu pasture (Bartholomew, 2005). Clark (2010) believes it does not necessarily mean that milk/ha will increase if pasture production increases due to selection of alternative pasture species. Therefore alternative species over-sown into kikuyu should be evaluated to ensure its effect on production per animal, grazing capacity and if animal production per ha are quantifiable. Fulkerson & Slack (1993) stated that temperate pasture species such as ryegrass over-sown into kikuyu provides a cost-effective option to fill the forage gap during winter months and supplement during summer. Advantages of kikuyu-ryegrass pastures are ease of management, increased grazing capacity, greater seasonal production and more evenly distributed seasonal fodder flow (Botha *et al.*, 2008a). A more evenly distributed seasonal fodder flow decrease the variation in seasonal milk production and grazing capacity (Botha *et al.*, 2008b).

Ryegrass is a temperate grass and there are two common species that are predominantly being used in the southern Cape region of South Africa (Botha *et al.*, 2015). The first species is a perennial ryegrass called *Lolium perenne*. The second species is an annual ryegrass called *Lolium multiflorum*. There are two annual ryegrass types namely Italian (*Lolium multiflorum* var. *italicum*) and Westerwolds (*Lolium multiflorum* var. *westerwoldicum*). Annual species tends to be a hardier ryegrass with a faster growth rate compared to the perennial ryegrass (Dickinson *et al.*, 2004). Van der Colf *et al.* (2015a) showed that perennial ryegrass-kikuyu supported more cows per hectare and had a more even fodder flow than annual ryegrass-kikuyu pasture. Van der Colf *et al.* (2015b) found that Italian and Westerwolds ryegrass has similar growth rates during winter and highest growth rates respectively during spring (Italian) and summer (Westerwolds). Perennial ryegrass obtains its

highest growth rates from late spring to early summer (Botha & Zulu, 2013). Planting of Westerwolds results in the pasture having a higher kikuyu content during spring, summer, and autumn. Italian ryegrass which has a higher growth rate has a negative impact on the summer DM production. This is due to a delay in the commencement of growth during spring resulting in lower kikuyu density during summer (Van der Colf *et al.*, 2015b).

The ideal time to over-sow kikuyu with annual ryegrass is in autumn for Westerwolds and autumn or spring for Italian varieties (Archibald *et al.*, 2010). Italian ryegrass planted in spring is recommended as autumn plantings go to seed during spring, or summer and/or autumn when kikuyu is at low production (Goodenough *et al.*, 1984). It is advised to plant ryegrass no later than June, regardless of the variety, to avoid short productive periods (Botha *et al.*, 2015). Van der Colf *et al.* (2015b) found that during summer months in the southern Cape of South Africa, the ME content decreases as the DM and NDF content increases in the pasture. This is due to a decrease in ryegrass growth and an increase in kikuyu growth. Therefore, over-sowing of ryegrass during autumn will influence the production potential of kikuyu the following summer and autumn. If it is possible to maintain the ryegrass in kikuyu pastures during summer and winter, the nutritional value of pastures can be improved (Van der Colf *et al.*, 2015b). In order to maintain good quality pastures, the management of the pasture and grazing must be the main focus point.

## **2.5 Pasture management**

According to Trollope *et al.* (1990) fodder flow is the availability of fodder to livestock throughout the entire year expressed on a monthly basis. Fodder flow on dairy farms in the southern Cape region of South Africa with kikuyu-ryegrass pastures needs to be managed well as both kikuyu and ryegrass reaches a mutual low growth rate during winter (Marais, 2001; Van der Colf *et al.*, 2015b). This can be problematic as the low growth rates can restrict the production of the animals (Van Heerden *et al.*, 1989; Swanepoel *et al.*, 2014). Physiological characteristics of the selected grasses need to be taken into account when selecting for pasture composition. Pasture grazing management must not only focus on meeting the nutritive requirements of the animals. It must also focus on interaction between the grazing animal and the pasture, as well as management effects on pasture regrowth (Murphy, 1990; Fulkerson & Donaghy, 2001). The latter includes monitoring of the pasture and movement and monitoring of animals on pastures while maintaining minimal animal stress (Morley, 1966). This will help to minimize pasture loss due to wastage, decay, and maturation. This will also enable utilization of pasture in such a manner that pasture quality is sustained (Van Houtert & Sykes, 1999). The greatest challenges when making use of pasture-based systems is to ensure that pasture is grazed efficiently (Irvine *et al.*, 2010) and that optimal stocking rates are administered to avoid selective grazing (Van Houtert & Sykes, 1999).

### 2.5.1 Determination of pasture allowance

Correct pasture allocation according to stocking rate and estimated yield of available pasture on a daily basis to grazing animals is crucial to ensure efficient grazing (Parsons & Chapman, 2000; Fulkerson & Donaghy, 2001). Estimated pasture yield does not give an indication in which growth stage the pasture are after grazing or the effects grazing has on the pasture. Pasture must rather be allocated according to availability of pasture DM and the quality of the pasture, than according to a fixed pasture mass (Parsons & Chapman, 2000; Fulkerson *et al.*, 2005). To determine the growth stage of the pasture after grazing, it is best to look at the pastures' leaf-growth (Fulkerson & Donaghy, 2001). Kikuyu grazing is recommended to occur when kikuyu has approximately four to five leaves per tiller (Reeves *et al.*, 1996b; Fulkerson *et al.*, 1999). Ryegrass should be grazed when it has approximately three leaves per tiller (Cooper & Saeed, 1949; Fulkerson & Donaghy, 2001). Pasture maturity determination according to leaf growth for defoliation in the southern Cape region of South Africa are difficult as ryegrass is over-sown into kikuyu pastures. Maturity determination will then be according to the primary grass species in the specific season of grazing, as kikuyu and ryegrass have different growth rates and peak seasons. During summer months kikuyu is the primary grass, therefore maturity will be determined according to the leaf growth of the kikuyu. The same would apply for ryegrass during the winter months when ryegrass is the primary grass species then. Along with leaf growth, defoliation can also be determined by means of day rotations, sward surface height and/or pasture production mass (Sheath & Clark, 1996; Mayne *et al.*, 2000).

When making use of leaf growth, there is no benefit in letting pasture grow further than the recommended leaf growth for grazing. Pasture will reach a plateau as the sixth (kikuyu) or fourth (ryegrass) leaf starts to emerge, with the first leaf starting to decay (Fulkerson & Donaghy, 2001). Along with leaf growth, attention must also be given towards canopy cover. Too much canopy cover will prevent the sun from penetrating through the canopy. This will result in the first leaves of the pasture not getting sufficient sunlight and then starting to decay. Determining when to graze pasture can also be done by means of a rising plate meter (RPM) (Castle, 1976; Stockdale, 1984; Murphy, 1990). This method is based on the height of the pasture measured on the RPM. According to Fulkerson & Donaghy (2001) and Lee *et al.* (2008), the optimum post-grazing residual range of 40-60 mm will promote regrowth and persistence, as well as improve the quality of the pasture. It is thus best to incorporate both species and production related factors when determining pasture maturity (Steyn, 2012).

### 2.5.2 Grazing systems

Along with planning a fodder flow and selecting the right grass species to over-sow, the manner of grazing is also of importance and should be adapted according to local conditions (Walton *et al.*, 1981). Pasture can either be grazed continuously or rotationally. In the southern Cape region of South Africa it is common to over-sow kikuyu with ryegrass, which is why dairy farmers make use of strip grazing. Strip grazing is similar to rotational grazing in the sense of giving fresh pasture

frequently (Clark & Kanneganti, 1998). Difference being the camp is divided into strips and only a certain amount of strips are made available for grazing. This manner of grazing ensures that pasture is grazed evenly and prevents any unnecessary pasture wastage (Tainton, 2000). Compilation and condition of the pasture, available pasture, farm location and size, and stocking rate determines the grazing manner.

### **2.5.3 Stocking rate**

Stocking rate of a farm is one of the most important management factors (O'Reagain & Turner, 1992). It can be defined as a number of animals (of a particular class/breed) which can be supported by a unit area (ha) of the pasture for a specified time period. Stocking rate is determined by pasture availability, size of the cow and level of concentrate feeding. The stocking rate will have an influence on the interaction between the animals and pasture, as well as determine the production per animal, and animal production per ha (McMeekan, 1960; Macdonald *et al.*, 2008; McCarthy *et al.*, 2011). Stocking rate is also depended on factors such as pasture type and production, availability of pasture and seasonal changes. In general, pasture-based systems express stocking rate as animal numbers per unit land in ha (Tainton, 2000). Careful consideration must be taken when deciding on the stocking rate. With a too low stocking rate pasture is wasted even if milk production per cow is greater due to animals grazing more selectively. It does not necessarily mean that high stocking rates are better. Even though high stocking rates results in pasture being grazed more efficiently and productivity per unit area increases, production per animal will likely decrease (Colman & Kaiser, 1974; Van Houtert & Sykes, 1999; Macdonald *et al.*, 2008). Stocking rate must take grazing capacity and grazing period into consideration.

As seasons change and pasture growth rates either increase or decrease, the allocated strips for grazing must be adapted accordingly. More strips need to be allocated at the beginning of the growing season (growth rate still slow) to provide enough pasture, and fewer as the season changes and the growth rate increases. If the allocated strips are not decreased, time spent grazing should be increased to ensure efficient grazing to avoid any wastage (Clark & Kanneganti, 1998). Together with deciding on the number of strips allocated and time period spent grazing, pasture should be grazed preferably on a priority basis (Steyn, 2012). This is determined by the maturity of the pasture as mentioned in 2.5.1. Depending on the herd size, any excess pastures which are mature but not grazed should be ensiled. These ensiled pastures can be given to dry cows or to lactating cows during winter months when the growth rate of the pasture decrease. As mentioned in 2.5 both kikuyu and ryegrass reach a mutual low in growth rate during winter months. Therefore dairy cows grazing kikuyu-ryegrass pastures are supplemented with concentrates to improve milk production and to ensure optimum performance during winter months (Reeves, 1997; Marais, 2001; García *et al.*, 2014). The degree of supplementation is determined by the nutritional value as well as the seasonal variation in grazing capacity of the pastures.

## 2.6 Supplementation of grazing dairy cows

In the southern Cape region of South Africa, the first limiting factor for milk production of cows grazing on pasture is energy intake (Muller & Fales, 1998). It is not possible to meet the nutrient requirements of high producing dairy cows with pasture as the sole diet (Kolver & Muller, 1998; Dixon & Stockdale, 1999). Concentrates are supplemented to meet the nutrient requirements of cows, increase milk production and stocking rate, improve profitability and maintain body condition (BC) of cows (Kolver & Muller, 1998; Bargo *et al.*, 2003). Proper supplementation strategies need to be planned and implemented to meet these objectives in pasture-based systems (Delahoy *et al.*, 2003). Concentrates often contribute as much as 66% of the total feed costs in a pasture-based system (Meeske *et al.*, 2006). Therefore it is important to improve the production efficiency as well as to reduce the cost associated with supplemental concentrates (Van Wyngaard *et al.*, 2015).

The profitability of pasture-based systems is dependent on the concentrate level as well as the milk production response of grazing cows due to the concentrate provided. The milk response of grazing cows to concentrate is affected by pasture quality and allowance, nutritional value of the concentrate, level of concentrate fed and the genetic potential of the cow (Bargo *et al.*, 2003). Therefore determining the optimum level of concentrate feeding is of high importance (Meeske, 2006). Higher pasture allowance combined with higher levels of concentrate feeding may result in a reduced milk response per kg concentrate fed due to substitution of pasture by concentrate, as well as reduced fibre digestion (Grainger & Mathews, 1989; Robaina *et al.*, 1998). This also leads to lower stocking rates, poor pasture utilization, reduced profit per ha and a high substitution rate (Bargo, 2002). Concentrates should complement pasture and the composition of concentrates should be adjusted depending on the forage quality. When dairy cows consume forage of low-to-medium quality their energy intake may not be enough to sustain optimum milk production and then cows have a negative energy balance (Zervas *et al.*, 1998). Concentrates are therefore supplemented to increase energy supply and milk production and also maintain live weight (LW) and BC.

A typical concentrate supplement for lactating dairy cows often contains 700-800 g/kg maize grain. This necessitates the need to replace expensive energy sources with less expensive sources such as by-products (Van Wyngaard *et al.*, 2015). Not only energy and low DMI can limit milk production. Feed with high levels of highly degradable CP [in relation to non-structural carbohydrates (NSC)] will limit milk production due to an imbalance between protein and energy supply (Carruthers *et al.*, 1997). Using high-fibre by-products as an energy source has the ability to help maintain a normal rumen pH as well as an increase in DMI due to improved pasture digestion (Muller & Fales, 1998; Bargo *et al.*, 2003). Studies where high-fibre by-products such as hominy chop, wheat bran, gluten 20 and palm kernel expeller were included, has already been conducted by Lingnau (2011), Van Wyngaard *et al.* (2015) and Cawood (2016). It is possible in some scenarios to be profitable when replacing grain with a non-forage fibre (NFF) source such as soybean hulls (Bradford & Mullins, 2012). Alternative feed sources are sustainable if they are readily available, economically priced or

the traditional source are short in supply. As mentioned before, soybean hulls are currently being considered as an alternative energy source for dairy concentrates. However, recent research on using soybean hulls as a substitute for maize in concentrates of dairy cows is limited and most research is fairly outdated.

## 2.7 Soybean hulls as an alternative feed source

### 2.7.1 Composition

Soybeans are used to extract the oil and to produce soybean meal high in protein (48%), which is used in the feed of monogastric animals (Zervas *et al.*, 1998). During processing soybean hulls are left as a by-product when the cortex is separated from the bean. The hull can represent up to as much as 8% of the total weight of the bean (Barbosa *et al.*, 2008). Soybean hulls are high in NDF, acid detergent fibre (ADF) and energy but low in lignin, soluble CHO and protein. The hulls consist of high levels of readily fermentable polysaccharides and are very palatable for dairy cows. High fibre levels of the hull make it highly digestible in the rumen by micro-organisms (Quicke *et al.*, 1959; Belyea *et al.*, 1989). Hintz *et al.* (1964) states that soybean hulls can be considered as a bulky concentrate that is highly digestible, instead of being seen as roughage. Stern & Ziemer (1992) agree with Hintz *et al.* (1964) stating that soybean hulls cannot be regarded as a good roughage supplement for ruminants due to its low effective fibre content. In order to maintain normal milk fat percentages, it is essential to provide adequate amounts of dietary fibre (Balch *et al.*, 1955). Using soybean hulls as part of a dairy cows' concentrate the dietary fibre and energy levels can be maintained without decreasing ruminal acetate concentrations or milk fat percentages (Cunningham *et al.*, 1993). Several studies have been done where soybean hulls successfully replaced some or all of the grain in the diets of sheep (Hsu *et al.*, 1987; Anderson *et al.*, 1988; Boylan, 1993) and cows (MacGregor *et al.*, 1976; Owen *et al.*, 1984; Nakamura & Owen, 1989; Cunningham *et al.*, 1993).

Soybean hulls have a total digestible nutrient (TDN) value of 67.3%, 12 – 16% CP, 40 – 47% ADF, 57 – 67% NDF and 10.14 MJ/kg digestible energy (DE) on an as-is basis (NRC, 2001; Hopkins & Whitlow, 2002; Chee *et al.*, 2005; Jacela *et al.*, 2007; Barbosa *et al.*, 2008). The ME of soybean hulls is relatively low due to the high NDF levels (NRC, 2012), making soybean hulls an ideal source of energy for lactating dairy cows (Hintz *et al.*, 1964). Soybean hulls tend to have an energy value more or less equal to that of maize when incorporated into a pelleted concentrate (Nakamura & Owen, 1989). Composition of soybean hulls may differ to some extent due to the existence of different soybean cultivars (De Beer & Bronkhorst, 2016) but are also depended on the extrusion process of the bean. Dust *et al.* (2004) conducted a study to determine the effect of extrusion on the composition of various feed ingredients of which amongst others were soybean hulls. The extruder that was used was a single-screw extruder, and each condition had different screw profiles and temperatures (Table 2.1). During the study Dust *et al.* (2004) obtained the results given in Table 2.2 for the composition of soybean hulls as a percentage on a dry matter (DM) basis.

Dust *et al.* (2004) found that the DM content of the soybean hull decreased from 91% when unprocessed to 89.6% when extruded extremely, and decreased even more to 88.1% when extruded moderately. The organic matter (OM) content of the soybean hulls did not change much between unprocessed soybean hulls and soybean hulls extruded under different conditions. On the contrary, the CP content increased from 10.9% when unprocessed to 13.7% when extruded extremely. Unprocessed soybean hulls contained 67% NDF which is insoluble cell wall material and includes components such as insoluble hemicellulose, lignin, and cellulose. Acid detergent fibre consists of components like lignin and cellulose. With a decrease in ADF from 49.3% when unprocessed to 45.9% when extruded extremely, a decrease in cellulose content was expected. A decrease in cellulose content could be seen as 47% was present in unprocessed soybean hulls when compared to only 43.1% present when extruded extremely (Dust *et al.* 2004). Cellulose content of soybean hulls was reported as 40-50% on an air-dry basis by Quicke *et al.* (1959). Since acid detergent lignin (ADL) only consists of lignin, cellulose content can be obtained by subtracting ADL from ADF. The insoluble hemicellulose value of 17.7% was obtained by subtracting ADF from NDF (Dust *et al.* 2004). During a study on the *in vitro* digestibility of cellulose Quicke *et al.* (1959) found that soybean hulls contain 96.7% crude fibre (CF) as determined by the A.O.A.C. method.

Cunningham *et al.* (1993) found similar values than Dust *et al.* (2004) did for the unprocessed soybean hulls on DM and NDF, with only slight differences for OM (94.9%), CP (16.5%) and ADF (50.2%). According to Dust *et al.* (2004), the difference in NDF of at least 10% between unprocessed and extruded soybean hulls indicates conversion of insoluble fibre to soluble fibre when undergoing extrusion processes (Table 2.3). It is desirable to have more soluble fibre in feed as soluble fibre is highly fermentable and produce short-chain fatty acids, lactic acid, and gas when being digested in the large intestines of cattle (Dust *et al.*, 2004). When formulating a concentrate diet containing soybean hulls, the composition of the soybean hulls must be taken into consideration as the composition of soybean hulls vary due to different cultivars.

**Table 2.1:** Conditions of extrusion in the study conducted by Dust *et al.* (2004)

Extrusion condition	Screw profile	Temperature (°C)	Mechanical energy within extruder kJ/kg
Mild	One reverse lobe	80 – 90	75 – 329
Moderate	Three reverse lobes	100 – 110	93 – 383
Extreme	Five reverse lobes	120 - 130	145 – 613

**Table 2.2:** Composition of soybean hulls after different extrusion conditions (Dust *et al.* 2004)

Extrusion condition	Components (%) <sup>1</sup>							
	DM	OM	CP	NDF	ADF	ADL	IH <sup>2</sup>	Cellulose
Unprocessed	91.0	94.5	10.9	67.0	49.3	2.3	17.7	47.0

Mild	89.7	94.6	11.2	66.0	49.3	3.1	16.7	46.2
Moderate	88.1	94.5	11.2	67.6	49.4	3.0	18.2	46.4
Extreme	89.6	94.4	13.7	57.7	45.9	2.8	11.8	43.1

<sup>1</sup>DM = Dry matter; OM = Organic matter; CP = Crude protein; NDF = Neutral detergent fibre; ADF = Acid detergent fibre; ADL = Acid detergent lignin; IH = Insoluble hemicellulose.

**Table 2.3:** Total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) concentrations of soybean hulls on a dry matter basis (Dust *et al.* 2004)

Extrusion condition	TDF %	IDF %	SDF %	IDF:SDF %
Unprocessed	83.3	69.5	13.8	5.0
Mild	81.2	68.4	12.8	5.3
Moderate	80.5	67.2	13.3	5.1
Extreme	85.1	66.5	18.6	3.6

## 2.7.2 Effects of supplementation with soybean hulls on:

### 2.7.2.1 Digestion

In the feed industry, there are several recommendations for the nutrient requirements which formulated feed needs to adhere to. NRC (2001) recommends that 75% of NDF in a ration must originate from traditional forages. Soybean hulls contain a highly digestible NDF fraction, non-fibre carbohydrates (NFC) as well as a variety of energy substrates for ruminal microbes (Van Laar *et al.*, 1999; Miron *et al.*, 2001; Trater *et al.*, 2001). Therefore Sarwar *et al.* (1991, 1992) recommended that when high-fibre by-products such as soybean hulls are fed, the percentage of NDF originating from forage needs to be lower than NRC (2001) recommendation. It is known that due to the slower fermentation of NDF and longer retention time in the rumen, NDF content of the diet and DMI is negatively correlated. By adding more digestible fibre, intake can be stimulated to increase the passage rate (Robinson & Mcqueen, 1997).

Soybean hulls have the potential to be digested by ruminants, but according to Sarwar *et al.* (1992) and Mertens (1997), a large proportion of hulls can pass right through the rumen due to the small particle size. This passage happens before any extensive fermentation occurs (Cunningham *et al.*, 1993). Cunningham *et al.* (1993) however found that diets containing soybean hulls were digested similarly to the control diet. Ipharraguerre & Clark (2003) found no negative effects on the gastrointestinal nutrient fermentation or digestion of lactating dairy cows when replacing 30% DM of a maize-based concentrate with soybean hulls. Feeding of a soybean hull diet compared to a maize based diet to early lactation cows showed no significant differences ( $P > 0.05$ ) between diets for DMI and the digestibilities of the DM, CP and NDF components. However, for the soybean hull diet, there was a higher NDF intake (Mansfield & Stern, 1994; Ipharraguerre *et al.*, 2002). Sarwar *et al.* (1991) on the other hand found that ruminal NDF digestibility was lower with greater hindgut disappearance and total tract digestibility of NDF when soybean hulls were included to replace forage NDF. There

is a possibility of a reduction in energy originating from soybean hull fibre fermentation. This reduction in energy can be compensated for by adding fat to the diet (Zervas *et al.*, 1998). Digestion of soybean hulls can be influenced either by 1) adding a buffer or fat to a concentrate containing soybean hulls or 2) feeding a soybean hull concentrate along with hay or silage. Digestibility of soybean hulls can be decreased by the addition of fat making up more than 2 – 3% of the DM of the basal diet (Palmquist, 1988).

### **2.7.2.2 Milk production**

Milk production of dairy cows is limited by their genetic potential. Milk production and milk composition is affected by the cows' age and stage of lactation. Effects of concentrate diets containing soybean hulls as a partial substitution to maize grain on milk production vary between studies. Meijs (1986), Spörndly (1991) and Sayers (1999) found that adding a high-fibre by-product such as soybean hulls increased milk production and pasture DMI. Studies by Lingnau (2011), Van Wyngaard *et al.* (2015) and Cawood (2016) where maize was replaced with high-fibre by-products showed no difference in milk production between the treatments. Delahoy *et al.* (2003) who fed NFF-based supplements such as soybean hulls also found no difference in milk production or milk composition. Various authors found similar milk production between treatments (Bishop *et al.*, 1963; Firkins & Eastridge, 1992; Coomer *et al.*, 1993; Cunningham *et al.*, 1993; Mansfield & Stern, 1994; Ipharraguerre *et al.*, 2002). Miron *et al.* (2003) were able to maintain milk production by reducing the forage NDF content from 18 – 12% and supplementing more digestible NDF creating a more favourable environment for microbial cellulolysis in the rumen. Yet Nakamura & Owen (1989) found that by feeding maize-based concentrates, the cows produced more milk than cows fed a concentrate containing soybean hulls.

### **2.7.2.3 Milk fat content**

Dairy cows need sufficient acetate concentrations to produce milk fat, and the production thereof is depended on the acetate to propionate production ratio. Acetate and propionate are VFA's produced in the rumen, making their production depended on feed consumed (Meijs, 1986; Kennelly & Glimm, 1998; Bargo *et al.*, 2003; Sairanen *et al.*, 2006). Various studies where maize was partially substituted with soybean hulls and fed to dairy cows observed a positive linear effect for milk fat percentages (Coomer *et al.*, 1993; Mansfield & Stern, 1994; Ipharraguerre *et al.*, 2002). Delahoy *et al.* (2003) found that milk fat percentages had the tendency to increase ( $P = 0.08$ ). Miron *et al.* (2003) observed an increase in milk fat percentages when decreasing forage NDF content from 18 – 12% and supplementing more digestible NDF.

Studies by Van Wyngaard *et al.* (2015) and Cawood (2016), where maize was replaced with high-fibre by-products showed no difference in milk fat percentages between the treatments. However, Lingnau (2011), found an increase in milk fat percentages. Edionwe & Owen (1989), Nakamura & Owen (1989), Firkins & Eastridge (1992) and Cunningham *et al.* (1993) found similar milk fat

percentages between the treatments in their studies. Cunningham *et al.* (1993) observed a lower DMI with an increase in acetate production for the cows who received concentrates containing soybean hulls and concluded it was an indication of soybean hulls being fermented. Bishop *et al.* (1963) and Hawkins & Little (1967) found that maize-based pelleted concentrates in fact decreased milk fat percentages. It might be possible to limit a decrease in milk fat percentages by including soybean hulls in pelleted concentrates (Nakamura & Owen, 1989). Feeding dairy cows an NFF-based concentrate can prevent a sudden decrease in rumen pH, improve microbial activity, and increase acetate production by providing more fibre to rumen micro-organisms (Sayers *et al.*, 2003). Therefore, similar milk fat percentages can indicate that sufficient acetate concentrations can be obtained from fermentation of soybean hulls.

#### **2.7.2.4 Milk protein content**

In general milk protein percentages of dairy cows fed concentrates are higher than cows fed only forage due to a higher level of energy supply. Various studies found a decrease in milk protein percentages when NFF-based concentrates were fed to dairy cows (Nakamura & Owen, 1989; Kennelly & Glimm, 1998; Bargo *et al.*, 2002a; Sayers *et al.*, 2003; Cawood, 2016). Studies by Lingnau (2011) and Van Wyngaard *et al.* (2015) where maize was replaced with high-fibre by-products showed no difference in milk protein percentages between the treatments. Firkins & Eastridge (1992) who added fat to diets containing soybean hulls also found no difference in milk protein percentages between the diets. Delahoy *et al.* (2003) and Bishop *et al.* (1963) found an increase in milk protein percentages when CHO were supplemented, and pelleted concentrates were fed to dairy cows, respectively. Nakamura & Owen (1989) stated that milk fat and milk protein percentages are inversely related.

#### **2.7.2.5 Milk lactose content**

Depending on the protein concentrations and dairy breed (NRC, 2001) a slight variation in milk lactose percentages can be observed. Nevertheless dietary manipulation cannot easily change milk lactose percentages (Sutton, 1989; Kennelly & Glimm, 1998; Schwab *et al.*, 2008). Studies by Van Wyngaard *et al.* (2015) and Cawood (2016) where maize was replaced with high-fibre by-products showed a decrease in milk lactose percentages between the treatments. However, Lingnau (2011) found no difference in milk lactose percentages. Even though milk lactose percentages cannot easily be changed through dietary manipulation, the composition of concentrates does have an effect to some extent. Propionate which is a VFA produced in the rumen is converted in the liver into glucose through gluconeogenesis. The mammary gland then uses this glucose for lactose synthesis (Ørskov, 1986; Kennelly & Glimm, 1998; McDonald *et al.*, 2001; NRC, 2001). Therefore if insufficient propionate is produced, insufficient amounts of glucose are produced resulting in lower milk lactose percentages. This could explain the different results various authors observed.

### 2.7.2.6 Somatic cell count

Somatic cell count of dairy cows increase as their lactation number increases. Since SCC consist of leukocytes and epithelial cells of the udder itself, their concentrations increases due to wear and tear on the udder, irritation and injury of the udder. Genetics of dairy cows also contributes to their SCC (Kitchen, 1981; De Villiers *et al.*, 2000). During a study, SCC is not usually taken into account when selecting cows. It is often required that the selected cows are in early to mid-lactation, a healthy condition and averaging around 3<sup>rd</sup> to 5<sup>th</sup> lactation cycles. There is a possibility that the overall SCC per treatment will vary slightly (but not significantly) between treatments due to different lactation cycles of the cows in each treatment. Therefore studies by Lingnau (2011), Van Wyngaard *et al.* (2015) and Cawood (2016) where maize was replaced with high-fibre by-products showed no difference in SCC between the treatments. However SCC is usually not a result of a response to the treatments, but rather a result of the health of cows individually.

### 2.7.2.7 Milk urea nitrogen

Levels of MUN or blood urea nitrogen (BUN) indicate if protein feeding is sufficient. It is preferred to make use of MUN rather than BUN as BUN is a more invasive procedure and time-consuming (De Villiers *et al.*, 2000; Bucholtz & Johnson, 2007). If milk fat percentages of a cow are above 4.5% the reliability of MUN values decreases. It is advised to use MUN values only as a guideline for nutritional and management decisions (De Villiers *et al.*, 2000). Recommended MUN values for bulk tank collected milk samples averages from 8 – 12 mg/dL (Kohn, 2007). Low MUN levels (< 8 mg/dL) for bulk tank collected samples may indicate a protein deficiency in the diet. If the MUN levels of bulk tank collected samples are high (> 12 – 14 mg/dL) it may indicate that an excess amount of protein are supplied or that there is an imbalance between ruminal protein, protein fractions and energy (NSC). Difference in individual cow MUN values is due to its dependence on stage of lactation, milk production and body weight changes (Kohn, 2007). Delahoy *et al.* (2003) found a decrease in MUN indicating that N was utilised more efficiently which was confirmed by Carruthers & Neil (1997) and Sairanen *et al.* (2006). According to these two authors, grazing cows receiving balanced concentrate supplements had higher microbial activity, improved N utilisation, resulting in decreased MUN levels. Studies by Lingnau (2011), Van Wyngaard *et al.* (2015) and Cawood (2016) where maize was replaced with high-fibre by-products showed no difference in MUN between treatments. This was expected as diets were iso-nitrogenous.

### 2.7.2.8 Body condition score and live weight

Dairy cows receiving concentrate supplements mobilise less energy reserves than cows that do not receive concentrate supplements (Bargo *et al.*, 2002a). The age and lactation stage of a cow has an effect on the mobilisation of body reserves. Cows in the first lactation or during the early stage of lactation will mobilise more body reserves to ensure growth or high milk production resulting in a change in body condition score (BCS) (De Villiers *et al.*, 2000). It is best to make use of BC as an indication of achieving basal metabolic requirements than LW, as BC is more sensitive to changes

(Bargo *et al.*, 2002a). Due to nutritional research studies only spanning over a few months using multiparous cows, insignificant changes in LW are observed, encouraging to use BC instead of LW as an indicator of effects of treatments (Steyn, 2012). Studies by Lingnau (2011), Van Wyngaard *et al.* (2015) and Cawood (2016) where maize was replaced with high-fibre by-products, as well as Zambom *et al.* (2012), Delahoy *et al.* (2003) and Firkins & Eastridge (1992) showed no difference in BCS and LW between treatments.

### **2.7.2.9 Rumen pH**

Rumen health and functionality of ruminants can be determined by their ruminal pH. Genetic potential and physiological state of dairy cows determine the plateau their ruminal pH will reach as ruminal pH does not increase indefinitely (Mertens, 1997). According to studies conducted by Mertens (1997) a suggestion was made that in order to maintain a ruminal pH of at least pH 6.0 a concentration of 22.3% NDF of ration DM, or a physically effective NDF (peNDF) intake of 4.4 kg per day is needed. Fibre is digested more efficiently at a ruminal pH between pH 6.2 and pH 6.5 (Varga *et al.*, 1984; Shriver *et al.*, 1986). At a pH of pH 6.0 and below the digestion of NDF and the overall activity of micro-organisms decreases (Hoover, 1986). If the ruminal pH is maintained too low for extended periods the micro-organism population will start to decrease due to spending less energy on replication and more on maintenance and will increase once ruminal pH increases (Russell & Dombrowski, 1980).

Due to soybean hulls being high in NDF, the ME is relatively low (NRC, 2012) and can result in an increased ruminal pH which can enhance utilization and digestion of pasture as well as increased DMI (Bargo *et al.*, 2003). However, if starches are rapidly fermented to VFA's the ruminal pH can decrease to pH 6.0 or even less (McDonald *et al.*, 2001). Ruminal pH can also be decreased with concentrate supplements as it contains large portions of readily fermentable CHO, which are fermented rapidly (Carruthers & Neil, 1997; Bargo *et al.*, 2002a, 2003; Sayers *et al.*, 2003; Sairanen *et al.*, 2006). Ruminal pH can also be decreased if concentrates contain high NFC levels. Therefore by adding soybean hulls (which is an NFF source) to concentrates, the NFC content can be reduced and ruminal pH maintained (Ishler & Varga, 2001; Sayers *et al.*, 2003).

### **2.7.2.10 Rumen volatile fatty acids**

Carbohydrate fermentation in the rumen produces VFA's and of all the VFA's produced, acetate, butyrate and propionate are the three main end products. About 80 – 90% of VFA's produced get absorbed directly across the walls of the rumen, reticulum, and omasum. The remaining VFA's are either utilised by the rumen micro-organisms or passed on to the abomasum and small intestines (Kennelly & Glimm, 1998; McDonald *et al.*, 2001). Consuming concentrates high in NFC decreases rumen motility, reducing VFA absorption rate. Therefore by means of including soybean hulls in concentrates to decrease NFC levels, a reduction in VFA absorption rate can be prevented (Ishler & Varga, 2001). Cunningham *et al.* (1993) found similar VFA concentrations than the control treatment

when cows received treatments where portions of forage were replaced with soybean hulls. However, for cows who received treatments where portions of concentrate were replaced with soybean hulls, a quadratic response was observed with the reason for this response still being unclear.

Of the three major VFA's produced, acetate is the main VFA. Acetate is mainly found in the peripheral circulation and is mostly correlated to milk composition, especially milk fat (Ørskov, 1986; Kennelly & Glimm, 1998; McDonald *et al.*, 2001; Seymour *et al.*, 2005). During diffusion across the rumen wall butyrate is converted to  $\beta$ -3-hydroxybutyrate which is a ketone body and utilised by skeletal and heart muscles as an energy source, especially during early lactation when a cow is still in a negative energy balance (Ørskov, 1986; McDonald *et al.*, 2001). A portion of the propionate is converted to lactate when crossing the rumen wall. The remaining propionate is transported to the liver where it is utilised to produce glucose by means of gluconeogenesis (Ørskov, 1986; Kennelly & Glimm, 1998; McDonald *et al.*, 2001). Propionate is positively correlated to milk production but not milk composition (Kennelly & Glimm, 1998; Bargo *et al.*, 2002a; Seymour *et al.*, 2005). Seymour *et al.* (2005) stated that the ratio of acetate to propionate is positively correlated with milk composition resulting in an increased milk fat content if acetate concentration increases.

Volatile fatty acids are preferentially oxidised by the rumen epithelium cells in the order butyrate > propionate > acetate. By increasing butyrate concentration, the epithelium cells will oxidise less propionate to lactate meaning lower milk lactose percentages due to high propionate levels in the liver (Baldwin & McLeod, 2000; McDonald *et al.*, 2001). The VFA supply to peripheral tissues will also increase due to a decrease in acetate oxidation. An increase in milk production may be observed when the butyrate concentration increases. However, the increase in butyrate concentration was not the reason for increased milk production observed by Baldwin & McLeod (2000). It was; 1) lower oxidation rate of propionate to lactate and 2) increased supply of propionate to the liver that goes hand in hand with higher butyrate concentrations. The proportions of VFA's in the rumen can be altered by increasing the inclusion levels of concentrate supplements or by changing the composition of the diet. This can result in an increase in milk production, but a decrease in milk quality due to an increase in propionate production at the expense of acetate production (Carruthers & Neil, 1997; McDonald *et al.*, 2001; Sairanen *et al.*, 2005, 2006). Therefore it is quite interesting that Cunningham *et al.* (1993) found similar milk production and milk composition values with an increase in acetate in his study.

#### **2.7.2.11 Rumen ammonia nitrogen**

Depending on the type of supplement, pasture quality and time of the day; the rumen  $\text{NH}_3\text{-N}$  concentration levels change. Bargo *et al.* (2002b) observed rumen  $\text{NH}_3\text{-N}$  levels as low as 14.2 mg/dL and as high as 20.7 mg/dL. Rumen ammonia nitrogen concentrations increase and peak after milking sessions when cows return to pasture to graze and are at their lowest before milking sessions

(Khalili & Sairanen, 2000; Bargo *et al.*, 2002b). In studies conducted by Van Wyngaard *et al.* (2015) and Cawood (2016) where maize was replaced with high-fibre by-products, no significant differences in rumen  $\text{NH}_3\text{-N}$  concentrations were found between treatments. Yet Lingnau (2011) found an increase in rumen  $\text{NH}_3\text{-N}$  concentrations when high starch concentrates were replaced with low starch (high-fibre by-product) concentrates. This is due to a drastic drop in pH caused by rapid degradation of readily fermentable CHO.

If soybean hulls make up as much as 25% of the dietary DM, as much as 24% of total N intake comes from the soybean hulls (Cunningham *et al.*, 1993). Sarwar *et al.* (1992) observed a typical post-feeding decrease in rumen  $\text{NH}_3\text{-N}$  concentrations, but no significant differences ( $P > 0.05$ ) between treatments when forage NDF were replaced with soybean hull NDF and varying non-structural CHO concentrations. However, Cunningham *et al.* (1993) found a decrease in rumen  $\text{NH}_3\text{-N}$  concentrations when cows consumed diets where soybean hulls replaced portions of either concentrate or forage with the latter showing a linear decrease. Despite the fact that the intake of N was similar between the treatment and control groups, the microbial N flow of the treatment groups was lower than the control group. It was suggested that if the microbial N of the treatment groups is lower than the control group, the N in diets containing soybean hulls are utilised at a lower rate than diets without soybean hulls (Cunningham *et al.*, 1993). In contrast, Sarwar *et al.* (1991) observed a greater ruminal escape of N when diets contained soybean hulls and maize gluten feed due to 76.9% and 51.7% of the N in soybean hulls and corn gluten feed respectively being insoluble. Soybean hulls have varying effects on the quantity of available substrates needed for microbial growth in the rumen, therefore changes to ruminal characteristics and ruminal N metabolism occur (Cunningham *et al.*, 1993).

## 2.8 Conclusions

Research on supplementation of soybean hulls to Jersey cows grazing kikuyu-ryegrass pasture is limited. Concentrates high in starch may decrease pasture digestion due to a decrease in rumen pH when starches are rapidly fermented to VFA's. Therefore high-fibre by-products may improve pasture digestion due to maintaining a normal pH better. High-fibre by-products complement pasture well and more research is needed.

## 2.9 References

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## Chapter 3 - Materials and Methods

### 3.1 General information

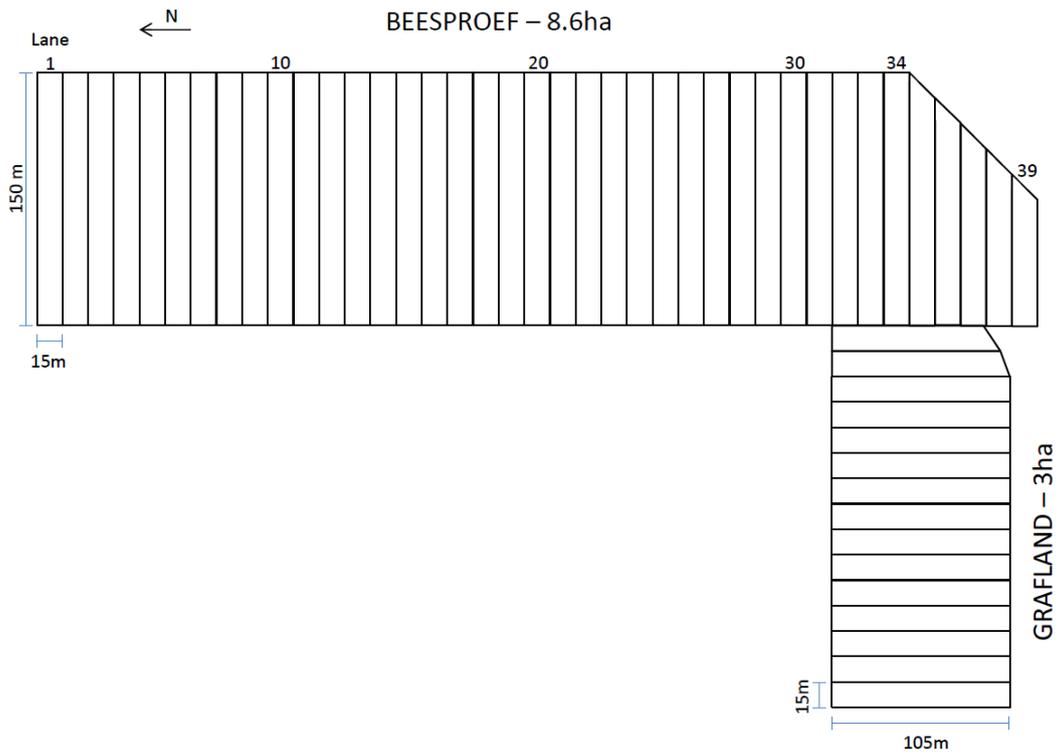
#### 3.1.1 Location and duration of the study

The study was carried out at Outeniqua Research Farm situated near George, Western Cape, South Africa (33°58'38" S and 22°25'16" E; altitude 210 m above sea level). George is known to have a temperate climate with a long-term (spanning over 50 years) mean annual precipitation of 730 mm. The study was carried out from 31 August 2017 to 2 November 2017 which was in total 64 days. The data collection period for the production study occurred 15 September 2017 to 2 November 2017. The rumen study consisted out of three data collection periods which occurred 15 to 20 September 2017, 6 to 11 October 2017 and 27 October to 1 November 2017. From 31 August 2017 until 14 August 2017 was the first adaptation period of 14 days.

#### 3.1.2 Pasture management and allocation

During the study, the cows grazed two camps. The first camp, *Beesproef* is approximately an 8.6 ha camp, which was divided into 39 pasture strips of approximately 150 m x 15 m. The second camp, *Grafland* is approximately a 3 ha camp, which was divided into 15 pasture strips of approximately 105 m x 15 m (Figure 3.1). Both camps have permanent irrigation and have Kikuyu (*Pennisetum clandestinum*) as a base and were over-sowed with an annual Italian ryegrass cv Yolande (*Lolium multiflorum*) during April 2017 and were sowed at a density of 24 kg/ha. After grazing, the pasture strips were top-dressed with 100 kg/ha limestone ammonium nitrate (LAN) as the cows moved on to the next fresh strip. The LAN contained 28% N, resulting in 28 kg N/ha that was put down on the pastures. The pastures were then thoroughly irrigated to initiate the washout period, whereafter the pasture was not suitable for grazing for at least 14 days.

Before and after each grazing, the pasture strips were measured by using the RPM method (Castle, 1976; Stockdale, 1984) on a daily basis. The measurements were done by walking in a zig-zag manner in each strip and taking 100 readings. The amount of available pasture (kg DM) per lane allocated to the cows was estimated with a linear regression equation and using the RPM measurements (Van der Colf, 2011). The linear regression equation used (Equation 3.1) during the study, was obtained from Van Wyngaard (2018) for a study done in September to November 2016. This equation was used to estimate pasture yield and allocate pasture since there was no linear regression equation yet for the period when the study was being conducted.



**Figure 3.1:** Layout of the two camps grazed during the study

**Equation 3.1:** Linear regression equation used during the study

$$Y = 102.99 \cdot H - 260.79$$

$$R^2 = 0.7282$$

Where: Y = DM yield

H = RPM reading

$R^2$  = Coefficient of correlation

During the study, pastures were sampled to construct a new linear regression equation for this study and are presented in Equation 3.2:

**Equation 3.2:** Linear regression equation determined during the study

$$Y = 81.054 \cdot H - 178.94$$

$$R^2 = 0.8313$$

Where: Y = DM yield

H = RPM reading

$R^2$  = Coefficient of correlation

### 3.1.3 Concentrate and pasture data collection

During the study, samples of the concentrate and pasture were taken once a week over seven weeks. Each week three representative concentrate samples were taken per treatment (3 samples x 3 treatments = 9 samples), three pasture samples for quality testing and nine pasture samples for a linear regression equation. The foil tray, in which the concentrate was sampled, was weighed before sampling, and then weighed again along with the concentrate, on a Sartorius BP 8100 scale (maximum = 8100 g;  $\pm 0.1$  g) which was tarred before each weighing. The foil trays with the concentrates were then dried for 72 h in a Labcon oven at 60 °C. After 72 h the three samples of each treatment were weighed again (scale was tarred) and then pooled (resulting in 1 sample per treatment = 3 samples per week). The weight of the foil tray was deducted from the before and after drying weight in order to determine the DM content of the concentrates. After completion of the study, two consecutive weeks for example week 1 & 2, and week 3 & 4 and so forth, were pooled per treatment. The pooled sample was then milled through a Scientec RSA Hammer mill Serie N 372 fitted with a 1 mm sieve at Department Animal Sciences, Stellenbosch University. After the samples were milled, they were stored in airtight plastic containers that were clearly marked and stored until further analyses could be done.

As mentioned, along with the concentrate samples, samples of the pasture were taken for quality analyses as well as for developing a linear regression equation for the study. The pasture samples were cut once a week by placing a metal ring with a diameter and height of 36 cm and 30 mm respectively, randomly on the pasture. The plant material that was within the diameter of the ring, was cut above the ring, which is approximately 30 mm above the ground. For the quality samples, three samples were cut per week and then placed in a marked brown paper bag. For the linear regression, pasture was sampled three times at three different heights (low, medium and high) with the difference in height depending on the pasture growth in the strip it was sampled from. Once the metal ring was placed on the pasture, the pasture was measured with an RPM before cutting the pasture. The pasture was then put in a marked brown paper bag for each height.

Each week the three quality and nine linear regression samples were weighed in brown paper bags before and after it was dried for 72 h in a Labcon oven at 60 °C. Before weighing the samples, an empty brown paper bag was put on the scale and tarred, to record the weight of the bag when samples were weighed. The brown paper bag that was used to tare the scale was dried with the samples and used again to tare the scale before weighing the dried samples. After weighing, the regression samples were discarded and the three quality samples of each week were pooled resulting in one sample per week. The pooled quality samples were then milled through a Wiley mill fitted with a 1 mm sieve at Outeniqua Research Farm, George. After milling, the samples were stored in airtight plastic containers that were clearly marked and stored until further analyses could be done on the samples.

### **3.1.4 Study overview**

The study comprised of a milk production component and a rumen study component. In total, 60 multiparous cows from the Outeniqua Research herd were used of which 51 lactating cows were in the production study and nine ruminally cannulated cows in the rumen study. A coloured tag was allocated to each treatment, and each cow within a treatment was marked with the allocated coloured tag, which had a number between 1 and 20 within each treatment. The coloured tags were used to identify and separate cows in the different treatment groups before each milking. Concentrates were fed separately to each treatment group during milking in the dairy parlour. All 60 cows grazed together as one group after each milking session. Cows were separated according to treatment before milking as will be discussed in sections 3.2.1 for the production study and 3.3.1 for the rumen study.

### **3.1.5 Animal welfare**

Animals were treated according to standard operating procedures for the Outeniqua Research Herd with ethical clearance DECRA R12/74.

## **3.2 Production study**

### **3.2.1 Experimental design**

Fifty-one multiparous Jersey cows from the Outeniqua Research herd that were  $127 \pm 50.5$  DIM were used for the study. The selected cows were first sorted according to their milk yield, DIM, then according to their lactation number. Three cows with similar milk yield, DIM and lactation number were grouped together to form a block. In total 17 blocks were formed and each of the three cows in a block were randomly allocated (Random number function, Microsoft Excel 2010) to a concentrate treatment. The variation in each treatment can be seen in Table 3.1. This resulted in 17 cows per treatment, having three treatments with different inclusion levels of soybean hulls (Table 3.2). The experimental design was a complete randomized block design. Reason for this type of design being that experimental error can then be relatively controlled as well as be reduced (Kuehl, 2000). A coloured tag (Figure 3.2) was allocated to each treatment, and each cow within a treatment was marked with the allocated coloured tag, which was numbered from 1-17 within each treatment. The coloured tag was attached to the cow with a 1 m chain and a 7.6 mm x 400 mm black nylon cable tie around its neck and can be seen in Figure 3.2. The chain was attached loosely to ensure that the cow had no restriction when eating, drinking or moving. The coloured tags were implemented to ensure the correct separation of the cows before each milking in order to receive the correct concentrate during milking.

**Table 3.1:** Mean ( $\pm$  SE) DIM, lactation number and milk yield of cows (n=17/treatment) in three concentrate treatments containing 0%, 15% or 30% soybean hulls fed at 6 kg (as is)

Parameter <sup>1</sup>	Treatments <sup>2</sup>		
	1 SH0	2 SH15	3 SH30
DIM	125 $\pm$ 50.9	131 $\pm$ 53.6	124 $\pm$ 46.3
Lactation no.	3.59 $\pm$ 1.375	4.59 $\pm$ 1.239	3.76 $\pm$ 1.767
Milk yield (kg/day)	19.7 $\pm$ 2.00	19.8 $\pm$ 2.61	19.8 $\pm$ 2.11

<sup>1</sup>DIM = days in milk.<sup>2</sup>Concentrate SH0, SH15 and SH30 containing respectively 0%, 15% and 30% soybean hulls.**Table 3.2:** The ingredients and calculated nutrient composition of three concentrate treatments containing respectively 0%, 15% or 30% soybean hulls fed at 6 kg (as is)

Parameter <sup>1</sup>	Treatments <sup>2</sup>		
	1 SH0	2 SH15	3 SH30
<b>Ingredients (g/kg DM)</b>			
Maize meal	800	650	500
Wheat bran	54.5	59.2	64.0
Molasses	60	60	60
Soybean oilcake	50	50	50
Soybean hull	0	150	300
Mono-calcium Phosphate	4.0	5.0	6.0
Feed lime	18	15	12
Salt	5.0	5.0	5.0
Magnesium Oxide	3.0	2.5	2.0
Urea	4.5	2.3	0
Premix	1.0	1.0	1.0
<b>Nutrient composition (g/kg DM)</b>			
Metabolisable energy (MJ/kg DM)	12.8	12.7	12.5
Crude protein	122	122	122
Ether extract	32.1	30.0	27.9
NDF	111	198	285
ADF	44.5	106	167
Starch	620	514	408
Calcium	8.5	8.6	8.7
Phosphorous	4.5	4.5	4.5
Magnesium	3.6	3.6	3.5

<sup>1</sup>DM = Dry matter; NDF = Neutral detergent fibre; ADF = Acid detergent fibre.<sup>2</sup>Concentrate SH0, SH15 and SH30 containing respectively 0%, 15% and 30% soybean hulls.



**Figure 3.2:** One of the cows with a tag and chain attached loosely around its neck that were used during the trial

### **3.2.2 Feeding and milking program**

The cows grazed together as one group and were separated before each milking session into their respective treatment group according to the coloured tag around their neck. The cows were milked twice a day at 05:30 and 13:30 and concentrates were fed to cows during milking at 6 kg/cow per day according to their treatment group (3 kg per milking). All three concentrates were mixed by NOVA Feeds, George, according to each treatment specifications. Each concentrate was manually and accurately weighed out on a Micro T7E scale (maximum = 30 kg;  $\pm 0.005$  kg) into plastic bags (400 x 600 mm; 70 micron) and each bag contained 3 kg concentrate to ensure each cow received exactly 3 kg concentrate during each milking session. Before each group entered the parlour the concentrate, according to cows' coloured tag, was placed manually into the troughs. Once the group was milked, the troughs were checked to ensure there was no residual concentrate left in the troughs. If there was any concentrate left, it was removed before feeding the next concentrate. After milking, the cows went back to the camp to graze before the next milking session.

### **3.2.3 Data collection**

#### **3.2.3.1 Milk production and milk samples**

Milk yields of each cow were electronically captured using Weigh All Dairy Master milk meters and the Dairy Master Computer software for each milking session for 50 consecutive days. The milking

parlour was equipped with a 20 point swing-over milking machine. During the trial period, proportional composite morning and afternoon milk samples were taken four times for each cow. The 4% FCM of each cow was calculated according to Gaines (1928) using Equation 3.3. The ECM of each cow was calculated using Equation 3.4. There are however more than one version of ECM formula with slightly different parameters used.

**Equation 3.3:** 4% Fat corrected milk (FCM)

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

**Equation 3.4:** Energy corrected milk (ECM)

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

These formulas are used in order to correct milk yield in such a manner, that it has a constant energy basis (Gaines, 1928; NRC, 2001). For each individual cow, their milk was sampled four times during the study to determine the composition of their milk. The samples were taken during both the morning and afternoon milking sessions in order to get a representative milk sample for each cow. The Dairy Master milking machine has a function where each time the milk meter has reached its capacity and empties the milk into the milking system, it releases a few drops of milk into a bottle attached to the milk meter. The bottle attached to the milk meter can be seen in Figure 3.3. Milk sampling bottles were detached from milk meters after milking. Milk was mixed gently to get a representative milk sample that was placed into a special pre-labeled bottle containing a preservative (Potassium Dichromate pellet).

### 3.2.3.2 Body conditioning score and live weight

On two consecutive days before the start of the study, each cow was weighed after the afternoon milking session. The average of the two weights for each individual cow was calculated. Live weight may vary due to differences in pasture intake, defaecation, urination or water drinking. The same procedure was followed on the last two days of the study, in order to determine if the cows gained or lost weight during the study. On the first and last day of the study, along with the weighing, each cow was given a BCS on a scale from one to five (1 – 5). The cows were scored according to appearance as well as by the tissue coverage over the prominent bones on the cows' back and hindquarters by means of palpations. The score is based on a five-point scale where 1 indicates that the cow is extremely thin, and five indicates that the cow is extremely fat (Wildman *et al.*, 1982; Edmonson *et al.*, 1989; Roche *et al.*, 2004).



**Figure 3.3:** Milk meter with a bottle which collects a representative milk sample

### **3.2.3.3 Faecal sampling for intake determination**

From the 51 cows that were part of the production study, 13 cows per treatment (13 cows x 3 treatments = 39 cows) were randomly selected (Random number function, Microsoft Excel 2010) for an intake study. Thirty-six of these cows were fitted with a halter to identify them to be dosed orally with a size 10 clear gelatine capsule that contained 3 g of Titanium dioxide ( $\text{TiO}_2$ ) (Figure 3.4). The  $\text{TiO}_2$  served as an inert external marker (Myers *et al.*, 2004). These cows were separated from the rest of the group after milking and secured into the crush. Once they were secured, each cow was dosed with a gelatine capsule orally. After the gelatine capsule was given, the cows joined the rest of the cows since they grazed together as one group. The remaining three cows that were not fitted with a halter served as background (control) cows and were not dosed with the gelatine capsule. The halters fitted on the 36 cows also served as an aid to hold the cows' head when the gelatine capsule was dosed. The 36 cows that received the gelatine capsules were marked on their back legs and tail with a red crayon for identification from behind. The remaining three cows that were not fitted with a halter were also marked on their back legs and tail, as well as on their head. The three background cows were marked on their head as well in order to identify them within the groups during faecal sampling, since their faecal samples had to be collected as well.



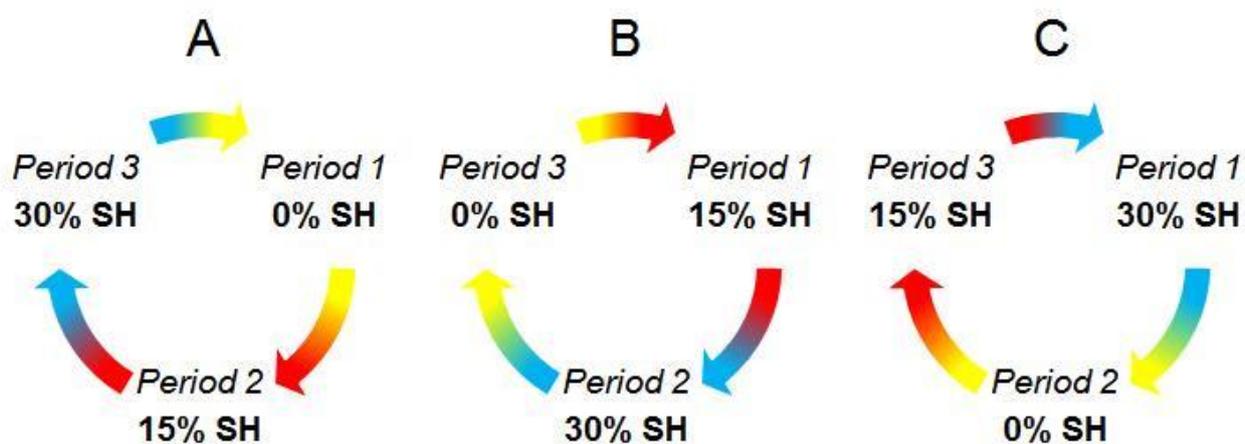
**Figure 3.4:** Filling of size 10 clear gelatine capsule that was given orally to cows, with Titanium dioxide ( $\text{TiO}_2$ ) which served as an inert marker

The 36 cows were given one gelatine capsule per cow, twice a day ( $6 \text{ g TiO}_2/\text{day}$ ), for 10 consecutive days. On days 1 to 5, the 36 cows were only given the gelatine capsules. During days 6 to 10, faecal grab samples of the 36 cows were collected twice a day along with giving the cows the gelatine capsules. Along with the 36 cows, the three control cows' faecal samples were also collected for the last five consecutive days. After each collection, the faecal samples were dried in a forced draft oven at  $70^\circ\text{C}$ . Due to fresh wet samples being inserted into the oven twice a day, all the faecal samples collected over the five days were left in the oven for four days after the last collection was inserted to ensure proper drying. Once the faecal samples were dry and taken out of the oven, the faecal samples of each cow were pooled and milled through a Wiley mill which was fitted with a 1mm sieve. After milling, the samples were stored in airtight plastic containers that were clearly marked and stored until further analyses could be done on the samples. Along with the faecal samples over the last five consecutive days, concentrate and pasture samples were taken. Each day grab samples of the concentrates were taken and stored in the same plastic bag until the fifth day to pool the samples. Pasture samples were taken the same way as the quality samples described in 3.1.3. The weighing, drying, milling, and storage of the concentrate and pasture samples were also done the same way as described in 3.1.3.

### 3.3 Rumen study

#### 3.3.1 Experimental design

Nine lactating ruminal cannulated cows were used to conduct the rumen study. All nine cows used were from the Outeniqua Research herd. Three of the cannulated cows were randomly allocated (Random number function, Microsoft Excel 2010) to a concentrate treatment. The rumen cannulated cows were marked the same way as in 3.2.1 according to the treatment group they were allocated to, numbered 18 – 20. The rumen study consisted of three periods as mentioned in 3.1.1, and each period consisted of 14 days adaptation and 6 days of data collection (14 + 6 = 20 days). On the last collection day of each period, the cannulated cows were separated from the rest after the afternoon milking session. The cows went into the crush where their coloured tags were removed and put on a different cow, ensuring that each cow was rotated to a new treatment each period. The three cows in a treatment group were rotated together and the rotation can be seen in Figure 3.5. The study was a 3 x 3 Latin Square design with three treatments and three periods.



**Figure 3.5:** Rotation of rumen cannulated cows between the three periods. SH refers to the soybean hull percentage inclusion in the different concentrates

Where: A – Cow 1, 2, 3;

B – Cow 4, 5, 6;

C – Cow 7, 8, 9

Treatments – 0% (yellow), 15% (red), 30% (blue) soybean hulls

#### 3.3.2 Feeding and milking program

The ruminally cannulated cows were milked along with the production study cows in their coloured group according to the colour tag around their neck. Therefore the feeding and milking program of the cannulated cows were the same as mentioned in 3.2.2 of the production study cows. Milk yield and milk composition for the cannulated cows were not included in the production study data.

### 3.3.3 Data collection

#### 3.3.3.1 Rumen pH

During each period, the rumen pH was recorded continuously for 80 h at 10 min intervals. The rumen pH was recorded by means of TruTrack pH Data Loggers (Model pH-HR mark 4, Intech Instruments LTD, New Zealand), and the loggers were fitted into a cannula plug. The assembling of the logger is described by Lingnau (2011) and the logger shown in Figure 3.6. The loggers were calibrated before the first period, by using the Omnilog Data Management Program, Version 1.64, and the calibration was valid for the whole study. A two point calibration procedure was done at pH 4.0 and pH 9.0. Following calibration, pH was checked at pH 7.0. The program was also used to download the recorded data from the logger. The loggers were connected to the computer shown in Figure 3.7. The computer had the Omnilog Data Management Program, Version 1.64, installed and was used to calibrate the loggers, start and stop the loggers recording data as well as to download the recorded data.

During each period, the loggers were inserted on a Friday morning and were removed the following Monday afternoon resulting in 80 h of recording. On the Thursday before insertion, the loggers were connected to the computer, turned on to start recording and placed together in a bucket with distilled water. On the morning of insertion (Friday), the ruminally cannulated cows were separated from the groups after milking and secured in a crush. The cannula plug was removed and placed into a bucket with warm water to ensure easy cleaning of the plugs and to keep them soft for later replacement. The plugs with the embedded loggers were then inserted into the rumen cannulated cows and remained there for 80 h. Over the weekend while the loggers were recording, the cows were checked before the morning and afternoon milking sessions to ensure that the loggers were still in place.



**Figure 3.6:** The TruTrack pH Data Loggers (Model pH-HR mark 4, Intech Instruments LTD, NZ) used during the trial to log rumen pH

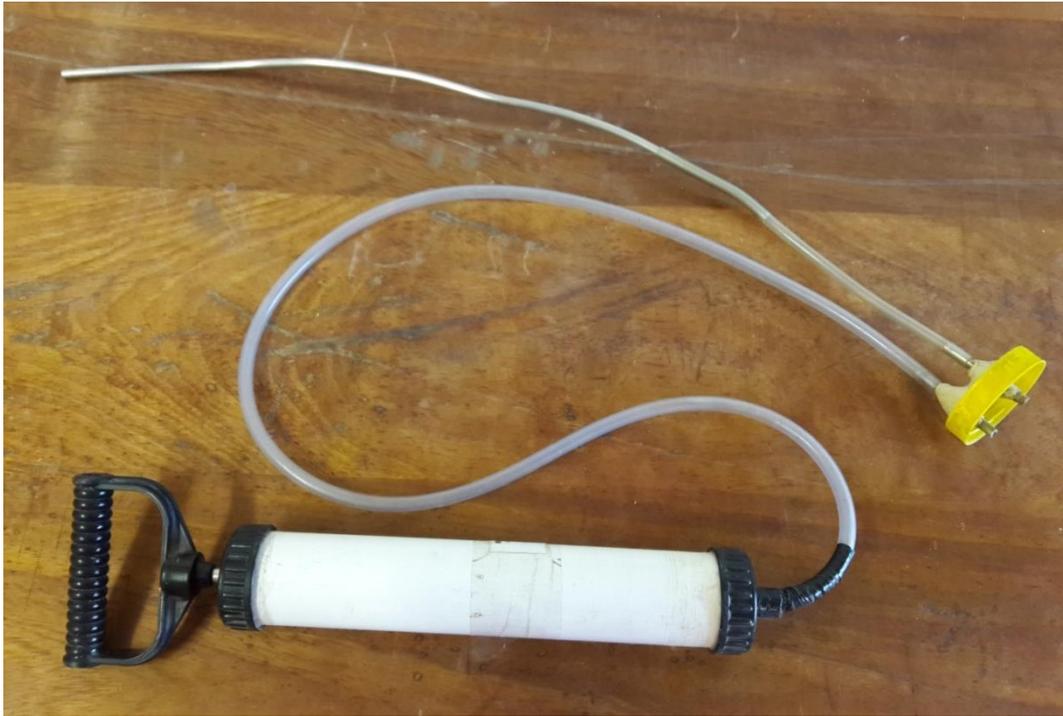


**Figure 3.7:** TruTrack pH Data Logger (Model pH-HR mark 4, Intech Instruments LTD, NZ) connected to a laptop which has the Omnilog Data Management Program, Version 1.64 installed in order to download data from and calibrate the logger

When a logger was not in place, the specific cow was taken to the crush to correct the fault. A logger that was out of place was usually found at the bottom of the rumen and then returned. In each period, following the afternoon milking on Mondays, the cannulated cows were again taken to the crush to remove and rinse the loggers. The original cannula plug was then replaced and the cows returned to the herd. The recorded pH data were downloaded and exported to an Excel file for later processing. The size of the data set was reduced by calculating average 30 min interval values. In order to reduce variation, the same pH logger that was allocated to a specific cow during the first period was again allocated to the corresponding cow during the second and third periods.

### 3.3.3.2 Rumen fluid samples

Rumen fluid samples of each cow were collected in each period on a Monday at three different times throughout the day, viz. 06:00, 14:00 and 21:00. The first two samples were collected after the milking sessions while the third sample was collected at night in the camp where the cows grazed. With the aid of headlights the cannulated cows were quietly and calmly separated from the rest of the group. A barrier was made with rope to make a small enclosure in order to work with the cows. The cows were kept calm by preventing rapid movements and loud talking. In order to collect the rumen fluid, a modified hand pump was used as shown in Figure 3.8. The pump consisted of a 29 cm drain pump to which a rubber tube of 1 m was attached. The other end of the tube was connected to the lid of the plastic container via a 5 cm stainless steel nozzle. A similar



**Figure 3.8:** A modified hand pump used to collect rumen fluid from rumen cannulated cows

nozzle connected to the container lid to a second tube (25 cm) to which a stainless steel pipe with a length of 55 cm was attached. A clearly marked plastic container that identified the cow, was screwed to the lid.

When collecting the rumen fluid, the stainless steel pipe was inserted into the rumen through a small hole in the cannula plug, just big enough for the pipe to pass through. When the pipe was inserted, care was taken not to insert the pipe straight down or too deep in order to prevent damaging of the rumen wall. After collection the hole was closed with either a plastic or a stainless steel screw to prevent air entering the rumen. With the stainless steel pipe inserted one person operated the hand pump, while another moved the stainless steel pipe slowly up and down to collect fluid into the plastic containers. After collection the pH was recorded immediately, using a portable pH logger (WTW pH 340i pH meter with a WTW Sentix 41 pH electrode; Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Back in the lab, the rumen fluid of each cow was filtered through four layers of cheese cloth to remove solid material. Figure 3.9 shows the Erlenmeyer flasks and plastic funnels used to process the rumen fluid samples. The filtered rumen fluid was then split in two and 25 ml was transferred into two smaller clearly marked plastic containers.



**Figure 3.9:** Nine Erlenmeyer flask placed in a straight line with a funnel, four layers of cheesecloth on top and two clearly marked containers in front (left) and a close up (right)

After the first rumen fluid sampling at 06:00, the smaller containers were placed in a freezer and removed again once the next samples were brought to the laboratory for filtering. The same procedures were followed with the 14:00 and 21:00 samples which were then added to the 06:00 sample. Thus, for each cow there were two containers, each containing a pooled 75 ml rumen fluid sample, resulting in 18 containers (2 containers x 9 cows = 18 containers) per period. The samples of all three periods thus obtained (54 in total) were kept at -18 °C until analysed. All the samples were analysed for VFA and rumen NH<sub>3</sub>-N, therefore the samples were taken in duplicate (27 samples for VFA analyses and 27 samples for rumen NH<sub>3</sub>-N analyses).

### 3.3.3.3 *In sacco* dacron bag study

Cruywagen (2006) described a technique to be used when retrieving dacron bags during an *in sacco* study and the technique was used during this study. Before the onset of the study, a representative pasture sample was collected from the camp in which the cows would graze. The pasture was sampled in the same manner as described in 3.1.3 when pasture was sampled for quality analyses. The grass was dried for 72 h at 60 °C in a Labcon oven whereafter it was cut into pieces of 5 – 10 mm in length and stored in a zip-sealed plastic bag. The dacron bags that were used for the study, were clearly numbered beforehand with a permanent marker. Eighty-three bags (number of bags used per period) at a time were dried for 72 h at 60 °C. After the 72 h, the bags were taken out and weighed immediately one at a time on a Sartorius L420P scale (maximum = 420 g; ± 0.001 g). This was done two more times for the second and third period resulting in 249 bags being dried and weighed. The person weighing the bags wore nitrile gloves to prevent moisture transmission from the skin onto the bags to avoid inaccurate weighing.

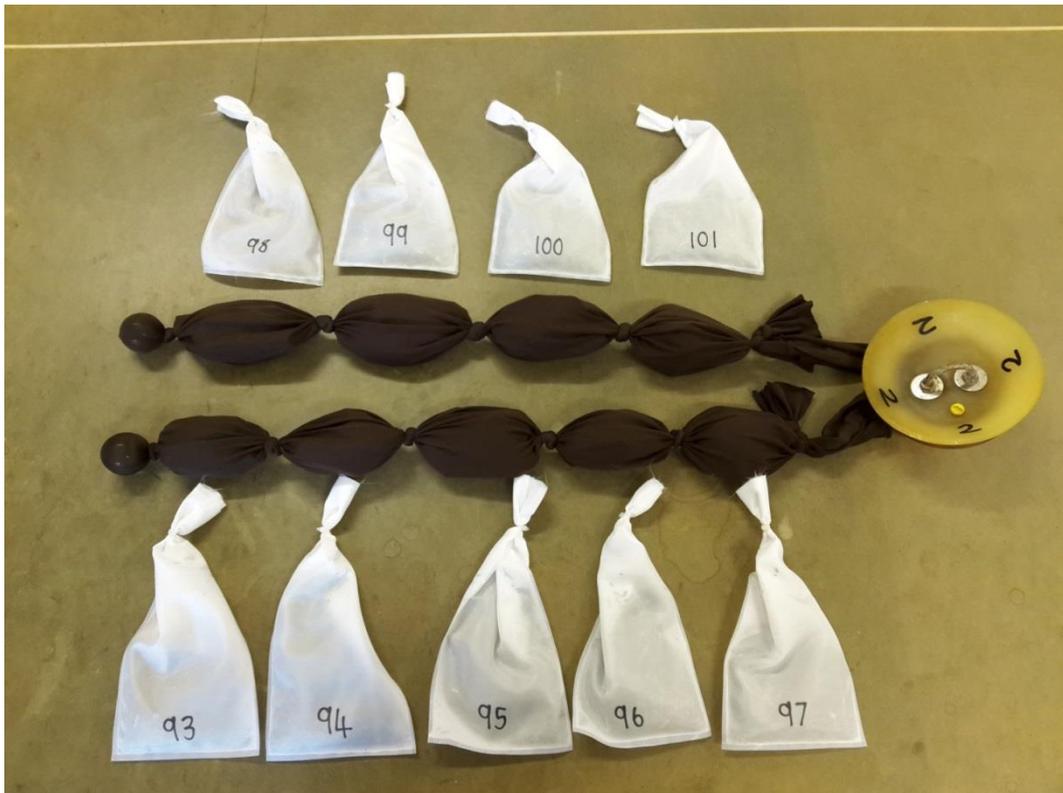
One week before the *in sacco* study for the first period took place, 83 nylon Dacron bags were filled with the dried grass. Each bag was placed individually on a Sartorius L420P scale (maximum = 420 g; ± 0.001 g) and the scale was then tared before filling the bag. Each bag was filled with 5.5 g ± 0.002 of dried grass, closed with a 2.5 mm x 100 mm white nylon cable tie and weighed again



**Figure 3.10:** Dacron bag being filled with 5.5 g  $\pm$  0.002 of dried grass and closed with a 2.5 mm x 100 mm white nylon cable tie and weighed again

(Figure 3.10). For each cow, nine bags were prepared with two extra bags (blanks) per period. Therefore 83 bags were prepared for each period. For each cow, one pair of 44 decitex ladies stocking was used to hold the bags. A glass marble of 20 mm in diameter (for weight) was placed inside the foot of each leg and secured with a knot. The marble was to ensure that the nylon bags in stockings would be weighed down into the rumen contents. Each leg was filled respectively with four and five bags. In each leg, the bags were evenly placed and separated from one another with a knot (Figure 3.11). A stainless steel U-bolt was embedded on the inside of the cannula plug to which the legs were fastened.

In each period on the Monday after the afternoon milking session, the cannulated cows were taken to a crush. First, the pH data logger was removed from the cannula after which the cannula plug with the stockings attached, were inserted. Bags were incubated for 6, 18 and 30 h. To ensure sufficient residue for NDF determination, two, three and four bags were incubated in each cow per incubation time mentioned, respectively. At all three bag removal times, the cows were in the pasture camp and the protocol described in 3.3.3.2 was applied. After retrieval the bags were taken to the laboratory on the farm and rinsed four times with clean water to remove rumen fluid and to stop further fermentation. After the four rinses, most water was removed and the bags were placed inside a zip-sealed plastic bag and frozen. At 18 h and 30 h, the same process was followed.



**Figure 3.11:** A 44 decitex stocking filled with nine nylon Dacron bags which were filled with dried pasture, and a glass marble in the foot of each leg for weight

During the second and third periods, the same procedures were followed. At the end of the study, all the frozen bags from all three periods were removed from the zip-sealed plastic bags. Along with the frozen bags, the two extra bags per period (2 extra bags x 3 periods = 6 bags) were placed together in cold water in a Defy Twinmaid washing machine. The bags were left in the water for 20 – 30 min to allow the frozen bags to defrost completely. Once the bags were defrosted, they were washed five times for three minutes, replacing the water between each wash. After the bags were washed, about 50 bags at a time were spun for 1 min to remove the excess water before it was laid out on a drying rack. Once all the bags were laid out on drying racks, the racks along with the bags were placed in an oven and the bags were dried for 72 h at 60 °C. After 72 h the bags were weighed back. Nitrile gloves were worn while weighing.

After weighing the dried bags were sorted according to period number, cow and removal time. The bags for each removal time were pooled for each cow per period. The cable tie was carefully cut and the residue of each bag was transferred to a clearly marked airtight plastic container according to the period, cow and removal time. The plastic containers were stored until further analyses.

### 3.4 Analytical procedures

#### 3.4.1 Milk samples

Milk samples were sent to Mérieux NutriSciences, Jeffreys Bay and analysed for milk fat, protein, lactose, SCC and MUN. The CombiFoss FT+ (Foss Allé 1, 3400 Hillerød, Denmark) on which the

samples were analysed consists of two sub-parts namely Fossomatic FC and MilkoScan FT+. The Fossomatic FC analysed the samples for SCC, and the analysis is based on flow cytometry technologies. The NIR based MilkoScan FT+ analysed the samples for fat, protein, lactose, and MUN.

### 3.4.2 Concentrate and pasture samples

All the pasture and concentrate samples were sent to Elsenburg, Stellenbosch for proximate analyses. All the samples were analysed in duplicate for DM (AOAC, 2012; method 934.01), ash (AOAC, 2012; method 942.05), CP (AOAC, 2012; method 990.03) using the Leco N analyser, model FP 528, ether extract (EE) (AOAC, 2012; method 2003.06), NDF and ADF (Van Soest *et al.*, 1991) using the ANKOM 200/220 Fiber Analyser (ANKOM Technology Corporation, New York, USA), gross energy (GE) (MC 1000 Modular Calorimeter, Energy Instrumentation, Sandton, South Africa, 2146), and acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN) determined by analysing the residue of the samples obtained from the ADF and NDF analysis respectively with the Leco N analyser, model FP 528. The *in vitro* true digestibility (IVTD) of the concentrates and pasture were analysed using the Daisy<sup>II</sup> incubator (ANKOM Technology Corporation, New York, USA) using the IVTD procedure provided by ANKOM Technology. The metabolisable energy was calculated according to Equation 3.5 as obtained from McDonald *et al.* (2001).

**Equation 3.5:** Metabolisable energy (ME)

$$ME = GE \times IVOMD \times C$$

Where: GE = Gross energy

IVOMD = *in vitro* organic matter disappearance

C = 0.81 (for concentrates) or 0.84 (for pasture)

### 3.4.3 Rumen fluid samples

The rumen fluid samples were analysed for NH<sub>3</sub>-N and VFA's at the Department of Animal Sciences, Stellenbosch University. Rumen fluid was prepared for analysis for NH<sub>3</sub>-N according to the procedure described by Broderick & Kang (1980) and was analysed on the SPECTROStar Nano, BMG Labtech, Germany spectrophotometer at 630 nanometers (nm). As for the VFA analysis, the rumen fluid was prepared according to the procedure as described by Siegfried *et al.* (1984). The rumen fluid samples were analysed on an Agilent 6890 N (G1530N) GC-FID, Agilent Technologies, machine via gas-liquid chromatography (GC-FID). The machine is the property of the GC-MS lab at the Stellenbosch University's Central Analytical Facility (CAF). During preparation of the rumen fluid, the rumen fluid undergoes a 'clean-up' procedure where the fluid is deprotonated and all the sugars

removed. The 'clean-up' procedure ensures that the rumen fluid sample is fairly clean and only consists of fermentation products.

#### **3.4.4 Faecal samples for intake study**

Faecal samples were sent to Bemlab, Strand, to analyse for total titanium content. One gram (1 g) of the faecal samples were dissolved in 20 ml nitric acid and 5 ml peroxide. The samples were then evaporated in a sand bath, rinsed with 10 ml distilled water and measured in an Inductively Coupled Plasma Spectrometer (IPC). The faecal samples along with concentrate and pasture samples that were taken during the same faecal sampling period were analysed for indigestible NDF (iNDF) by incubating them for 120 h. The samples were analysed for DM digestibility by means of *in vitro* incubation in a Daisy<sup>II</sup> incubator (ANKOM Technology Corporation, New York, USA) as described by Mabweesh *et al.* (2000) and Tilley & Terry (1963). Instead of 48 h the samples incubated for 120 h and the residue of each sample was then analysed for NDF (Van Soest *et al.*, 1991) using the ANKOM 200/220 Fiber Analyser (ANKOM Technology Corporation, New York, USA).

#### **3.4.5 Dacron bag study**

As mentioned in 3.3.3.3 two, three and four bags were removed at 6 h, 18 h and 30 h respectively. The bag residues were pooled per time per cow for analyses. The pooled residues were used for the determination of DM, ash and NDF concentration. The samples were sent to Elsenburg, Stellenbosch, therefore the samples were analysed according to the same procedures as mentioned in 3.4.2.

#### **3.4.6 Statistical analyses**

The experimental designs of the production study and rumen study were respectively a complete randomized block design and a 3 x 3 Latin Square design. The production study data (milk yield, milk composition, LW and BCS) was analysed using main effects analysis of variance (ANOVA) where only differences between treatment groups were relevant since the other factor(s) were blocked for. Co-variates were not included in the ANOVA, as cows were blocked before the study according to milk production, DIM and lactation number. Therefore, the data were analysed with a main effects ANOVA. Levene's Test for Homogeneity of Variance was used to test for homogeneity of the variances between treatment means. Differences between the treatment means were investigated with either 1) least significant differences (LSD) when Levene's test for homogeneity of variances could not be rejected or 2) Games-Howell multiple comparisons if there were significant non-homogeneity among the variance of the treatment means. Rumen study data (pH, NH<sub>3</sub>-N, VFA and *in sacco* Dacron bag study) were analysed similarly using a main effects ANOVA in a Latin Square design. Levene's Test for Homogeneity of Variance was also used to test for homogeneity of the variances between the treatment groups, with either LSD or Games-Howell multiple comparisons according to the outcome of Levene's test as described above. All analyses were done using the General Linear Model (GLM) procedure of Statistica (data analysis software system),

Version 13 (TIBCO Software Inc., 2017). The null hypothesis of equal treatment means and equal treatment groups was rejected when  $P < 0.05$ . For all analyses Shapiro-Wilk tests were used to test for normality of the residuals.

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## Chapter 4 - Results and Discussion

### 4.1 Production study

Milk yield and milk composition are presented in Table 4.1.

**Table 4.1:** Mean milk yield and milk composition of cows (n=17/treatment) receiving concentrates containing 0%, 15% or 30% soybean hulls as partial replacement of maize. Cows grazed on kikuyu/ryegrass pasture during spring and received 6 kg (as is) of concentrate per day

Parameter <sup>1</sup>	Treatments <sup>2</sup>			SEM <sup>3</sup>	Contrasts <sup>4</sup>		
	1 SH0	2 SH15	3 SH30		1 vs 2	1 vs 3	2 vs 3
Milk yield (kg/cow per day)	19.3	19.4	19.2	0.44	0.98	0.79	0.78
4% FCM (kg/cow per day)	22.4	23.5	23.0	0.61	0.23	0.51	0.58
ECM (kg/cow per day)	23.9	25.0	24.5	0.60	0.21	0.45	0.61
Fat (%)	5.12	5.48	5.33	0.127	0.06	0.26	0.41
Protein (%)	3.67	3.81	3.82	0.046	0.04	0.03	0.82
Lactose (%)	4.55	4.74	4.75	0.036	0.00	0.00	0.79
SCC (x 1000/mL)	164	205	201	47.6	0.55	0.58	0.96
MUN (mg/dL)	8.30	8.54	9.36	0.256	0.50	0.01	0.03

<sup>1</sup>FCM = Fat corrected milk; ECM = Energy corrected milk; SCC = Somatic cell count; MUN = Milk urea nitrogen.

<sup>2</sup>Concentrate SH0, SH15 and SH30 containing respectively 0%, 15% and 30% soybean hulls.

<sup>3</sup>SEM = Standard error of the mean.

<sup>4</sup> $P \leq 0.05$  = significant difference;  $P > 0.05$  = not significant difference.

#### 4.1.1 Milk yield

The average milk yield of cows did not differ ( $P > 0.05$ ) when they received either 0%, 15% or 30% soybean hulls in the concentrate (Table 4.1). Meijs (1986) found an increase in milk yield when soybean hulls were included in concentrates of pasture based cows, whereas others found no difference when soybean hulls partially replaced maize silage (Firkins & Eastridge, 1992; Cunningham *et al.*, 1993; Weidner & Grant, 1994a). Meijs (1986) compared a high starch concentrate to concentrates containing 20% or 30% soybean hulls where Friesian cows received concentrates at 7 kg/day and grazed perennial ryegrass. The studies were conducted in the Netherlands during the summer months. The higher level of concentrate feeding, season and different breed may explain the increase in milk production. In the study of Firkins & Eastridge (1992) soybean hulls replaced 7.0% of maize silage. The energy content of maize silage and soybean hulls is similar which may explain the lack of response. Cunningham *et al.* (1993) replaced approximately 27% and 50% of maize silage or concentrate in the diet of Holstein cows with soybean hulls and found no differences in milk yield. Weidner & Grant (1994a) used Friesian cows, but fed a TMR where 25 or 42% of the maize silage were replaced with soybean hulls. Milk yield was maintained as soybean hull inclusion increased. Studies where high-fibre by-products such as bran, hominy chop and gluten 20 partially replaced maize grain in concentrates also showed no differences in milk

yield (Lingnau, 2011; Van Wyngaard *et al.*, 2015; Cawood, 2016). All three latter authors fed concentrates at 6 kg/cow daily, with cows grazing either kikuyu (Cawood, 2016) or kikuyu-ryegrass (Lingnau, 2011; Van Wyngaard *et al.*, 2015) pastures. Cawood (2016) observed a mean daily milk production of 18.7 kg/cow for cows grazing on kikuyu pastures. Lingnau (2011) and Van Wyngaard *et al.* (2015) found mean daily milk productions of 19.7 kg/cow and 21.1 kg/cow, respectively, for cows grazing kikuyu-ryegrass pastures. Milk production in the current study is very similar to that of Cawood (2016). Due to similar ME values (Table 4.4) of the three concentrates in the current study, similar milk yield between the diets were expected and were obtained, indicating that soybean hulls can successfully replace at least 30% of the maize in a pasture concentrate. The DM and NDF digestibility of ryegrass increased when soybean hulls was included (Table 4.11 and Table 4.12). This may have resulted in increased pasture intake.

#### 4.1.2 Milk fat

Milk fat percentage tended ( $P = 0.06$ ) to be higher on the SH15 treatment compared to the control (SH0). The SH30 treatment did, however, not differ from the SH0 and the SH15 treatment in terms of milk fat content (Table 4.1). It is unclear why the SH15 treatment increased milk fat, but the SH30 treatment did not. Coomer *et al.* (1993), Mansfield & Stern (1994) and Ipharraguerre *et al.* (2002) also found an increase in milk fat when partially substituting maize with soybean hulls. Soybean hulls substituted 2.0, 6.9 and 12.6%; 28 and 30.1%; and 10, 20, 30 and 40% of maize in studies by Coomer *et al.* (1993), Mansfield & Stern (1994) and Ipharraguerre *et al.* (2002) respectively. Due to a higher NDF content for the SH15 and SH30 concentrates, an increase in milk fat was expected. High-fibre by-product studies by Van Wyngaard *et al.* (2015) and Cawood (2016) showed no difference in milk fat percentages, but Lingnau (2011) on the contrary observed an increase. In the studies of Lingnau (2011) and Van Wyngaard *et al.* (2015) cows grazed ryegrass dominant pastures in spring. However in the study of Lingnau (2011) maize was replaced with hominy chop, bran and gluten 20 while Van Wyngaard *et al.* (2015) replaced maize with palm kernel expeller. High-fibre by-products differ in fibre composition and digestibility as well as fat content and therefore have different effects on fibre digestion and milk fat content. Cawood (2016) replaced maize with hominy chop, bran and gluten 20 in the concentrate for cows grazing kikuyu pasture in summer. It can be expected that milk fat would be less affected when concentrates high in fibre are fed to cows grazing kikuyu with a higher NDF content than when cows graze high quality ryegrass pasture with a lower NDF.

#### 4.1.3 Milk protein content

Including soybean hulls in the concentrates increased the protein content of milk. There was a higher milk protein content ( $P < 0.05$ ) for the SH15 and SH30 treatments compared to the SH0 treatment. Increasing the soybean hull content from 15% to 30% did, however, not result in a further increase in milk protein. Various authors found a decrease in milk protein percentages when soybean hulls replaced as much as 95% of maize grain in the concentrates (Nakamura & Owen, 1989); soybean hulls replaced 30.1% of the concentrate DM of a diet consisting of 32% maize silage, 19.8% alfalfa-

grass hay and 48.2% concentrate (Mansfield & Stern, 1994); a NFF-based supplement consisting of 35% ground maize, 18% beet pulp, 18% soybean hulls and 8% wheat middlings plus protein, mineral, and vitamins for supplementing carbohydrates of cows on pasture (Delahoy *et al.*, 2003); bran, hominy chop and gluten 20 replaced maize in the concentrate of cows on pasture (Cawood, 2016). A milk protein decrease in the studies may be due to lower energy supply when maize was replaced with lower energy by-products. In the current study, digestion of pasture DM and NDF was improved (Table 4.11 and Table 4.12) when 15% of maize was replaced with soybean hulls and less starch and more digestible fibre was fed to cows. This may have increased energy available for milk protein synthesis.

#### **4.1.4 Milk lactose content**

Milk lactose content increased significantly ( $P < 0.01$ ) when soybean hulls were included in the concentrates. Improved energy supply may result in increased milk lactose content (Thomas, 1983). In contrast, Van Wyngaard *et al.* (2015) and Cawood (2016) found a decrease while Lingnau (2011) found no difference when substituting maize with high-fibre by-products such as bran, hominy chop and gluten 20. Nakamura & Owen (1989) also found milk lactose percentages of cows that received concentrates containing 95.3% soybean hulls, did not differ from that of cows fed only maize in their concentrate. Mansfield & Stern (1994) found a lower lactose content for cows that received diets containing soybean hulls and ascribed the decrease to glucose deficiency. However, lactose content does not only depend on the diet or breed of the dairy cow and should average between 4.7 – 4.8% (Gibson, 1989; NRC, 2001). Lactose in milk can also be depressed by high milk SCC (Kitchen, 1981; Welper & Freeman, 1992).

#### **4.1.5 Somatic cell count**

In order for milk to be safe for human consumption, the SCC has to be below 500 000 cells/ml milk. However, a SCC above 300 000 cells/ml milk can be an indication of mastitis in the herd (De Villiers *et al.*, 2000). The SCC did not differ between treatments and all three the concentrate treatments had an average SCC around or below 200 000 cells/ml milk indicating good udder health. Similar results were found by Lingnau (2011), Van Wyngaard *et al.* (2015) and Cawood (2016) in their high-fibre by-product studies. As mentioned before, the SCC is usually not a result of a response to the treatments, but rather a reflect on the health of cows.

#### **4.1.6 Milk urea nitrogen**

During milk sample collection from a bulk tank, the recommended value for MUN should average between 8 – 12 mg/dL (Kohn, 2007). The MUN values in all three treatments were within the recommended range, indicating that protein and energy in the total diets were sufficient. The MUN level of the SH30 treatment was significantly ( $P < 0.05$ ) higher than that of the SH0 and SH15 treatment. The differences in MUN were, however, small and could be explained by the higher protein content of the SH30 concentrate (Table 4.4) as well as possible higher pasture intake.

Studies by Lingnau (2011), Van Wyngaard *et al.* (2015) and Cawood (2016) showed no difference in MUN when high-fibre by-products were included in the concentrates.

#### 4.1.7 Live weight and body condition score

The LW (kg) and BCS (scale of 1 – 5) of cows were measured before and after the trial (Table 4.2). The LW before and after the trial did not differ among treatments, but LW of cows on all three treatments increased over the duration of the study. The highest LW change of 37.6 kg was observed for cows on the SH15 treatment. Cows in the SH15 treatment tended ( $P = 0.06$ ) to have a higher LW change and average daily gain (ADG) than cows on the SH30 treatments. The BCS of cows did not differ among treatments at the start or the end of the study. There were, however, a significant difference ( $P = 0.02$ ) in terms of BCS change. Cows on the SH15 treatment gained more condition than cows on the SH0 and SH30 treatments, but it is unclear why.

**Table 4.2:** Mean live weight and body condition score before and after the production study of cows receiving concentrates containing 0%, 15% or 30% soybean hulls (n=17/treatment) fed at 6 kg (as is)

Parameter <sup>1</sup>	Treatments <sup>2</sup>			SEM <sup>3</sup>	Contrasts <sup>4</sup>		
	1 SH0	2 SH15	3 SH30		1 vs 2	1 vs 3	2 vs 3
LW before (kg)	386	389	389	10.1	0.86	0.88	0.98
LW after (kg)	422	426	419	10.5	0.75	0.84	0.60
LW change (kg)	+35.4	+37.6	+30.1	2.64	0.56	0.17	0.06
ADG (kg/day)	0.55	0.59	0.47	0.041	0.56	0.17	0.06
BCS before	2.15	2.09	2.18	0.037	0.28	0.58	0.11
BCS after	2.24	2.31	2.26	0.039	0.19	0.60	0.43
BCS change	0.09	0.22	0.09	0.038	0.02	1.00	0.02

<sup>1</sup>LW = Live weight; ADG = Average daily gain; BCS = Body condition score.

<sup>2</sup>Concentrates SH0, SH15 and SH30 containing respectively 0%, 15% and 30% soybean hulls.

<sup>3</sup>SEM = Standard error of the mean.

<sup>4</sup> $P \leq 0.05$  = significant difference;  $P > 0.05$  = not significant difference.

#### 4.1.8 Concentrate intake and nutrient composition

The daily fed and the actual intake of the three concentrates, are given in Table 4.3, averaging at 5.51 kg/day on a DM basis. The feed samples that were collected over seven weeks during the trial, were analysed for the chemical composition and results are presented in Table 4.4. The nutrient composition of the ingredients for the concentrates mixed by NOVA Feeds, according to which the concentrates were formulated, is given in Table 3.2. The analysed chemical composition results correspond well to the estimated chemical composition. When comparing the chemical composition of the three different concentrates with the three different concentrates used by Van Wyngaard *et al.* (2015), similarity between the chemical compositions of the concentrates can be observed as the high-fibre by-products in both studies increased. Since the three different concentrates were formulated to have similar ME and CP levels, it was expected to be similar between the treatments and it was observed. However the calculated ME values (using Equation 3.5) of the three

concentrate treatments were higher than the estimated values from NOVA (13.5, 13.4 and 13.3 MJ/kg vs. 12.8, 12.7 and 12.5 MJ/kg). The analysed CP values of the three concentrate treatments were on the other hand lower than the estimated values from NOVA (109, 109 and 113 g/kg vs. 122, 122 and 122 g/kg). As expected the NDF and ADF levels within the treatments increased as the inclusion level of soybean hulls in the concentrates increased, since soybean hulls are higher in NDF and ADF than maize.

**Table 4.3:** Concentrate fed and actual dry matter intake throughout the study period for concentrates containing 0%, 15% or 30% soybean hulls fed at 6 kg (as is)

Parameter <sup>1</sup>	Treatments <sup>2</sup>		
	1 SH0	2 SH15	3 SH30
Fed (kg/day as is)	6	6	6
Actual intake (kg DM/day)	5.53	5.51	5.50

<sup>1</sup>DM = Dry matter.

<sup>2</sup>Concentrates SH0, SH15 and SH30 containing respectively 0%, 15% and 30% soybean hulls.

**Table 4.4:** The analysed chemical composition (Mean  $\pm$  SD) of the concentrates containing 0%, 15% or 30% soybean hulls, as collected over a seven week period, expressed on a dry matter basis

Parameter <sup>1</sup> (g/kg DM)	Treatments <sup>2</sup>		
	1 SH0	2 SH15	3 SH30
Dry matter	921 $\pm$ 0.2	918 $\pm$ 0.3	917 $\pm$ 0.5
Ash	50.2 $\pm$ 1.00	51.8 $\pm$ 1.27	56.8 $\pm$ 2.68
Metabolisable energy (MJ/kg)	13.5 $\pm$ 0.04	13.4 $\pm$ 0.05	13.3 $\pm$ 0.06
Crude protein	109 $\pm$ 0.9	109 $\pm$ 2.3	113 $\pm$ 3.4
NDF	80.5 $\pm$ 1.68	163 $\pm$ 3.4	246 $\pm$ 3.2
NDIN	5.72 $\pm$ 0.282	9.29 $\pm$ 4.149	11.5 $\pm$ 3.33
ADF	31.0 $\pm$ 1.31	103 $\pm$ 2.5	172 $\pm$ 2.9
ADIN	13.0 $\pm$ 0.33	6.35 $\pm$ 0.602	4.39 $\pm$ 0.676
ADL	5.15 $\pm$ 0.403	7.11 $\pm$ 1.336	10.9 $\pm$ 1.36
IVTD	957 $\pm$ 2.2	954 $\pm$ 1.2	947 $\pm$ 3.3
Calcium	8.26 $\pm$ 0.274	7.69 $\pm$ 0.051	8.85 $\pm$ 0.108
Phosphorous	3.86 $\pm$ 0.144	3.92 $\pm$ 0.071	4.08 $\pm$ 0.089
Magnesium	3.52 $\pm$ 0.098	3.53 $\pm$ 0.084	3.64 $\pm$ 0.081
Potassium	8.08 $\pm$ 0.196	10.0 $\pm$ 0.11	11.5 $\pm$ 0.19
Sodium	2.37 $\pm$ 0.059	2.53 $\pm$ 0.137	2.49 $\pm$ 0.086

<sup>1</sup>NDF = Neutral detergent fibre; NDIN = Neutral detergent insoluble nitrogen; ADF = Acid detergent fibre; ADIN = Acid detergent insoluble nitrogen; IVTD = *In vitro* true digestibility.

<sup>2</sup>Concentrates SH0, SH15 and SH30 containing respectively 0%, 15% and 30% soybean hulls.

## 4.1.9 Pasture samples

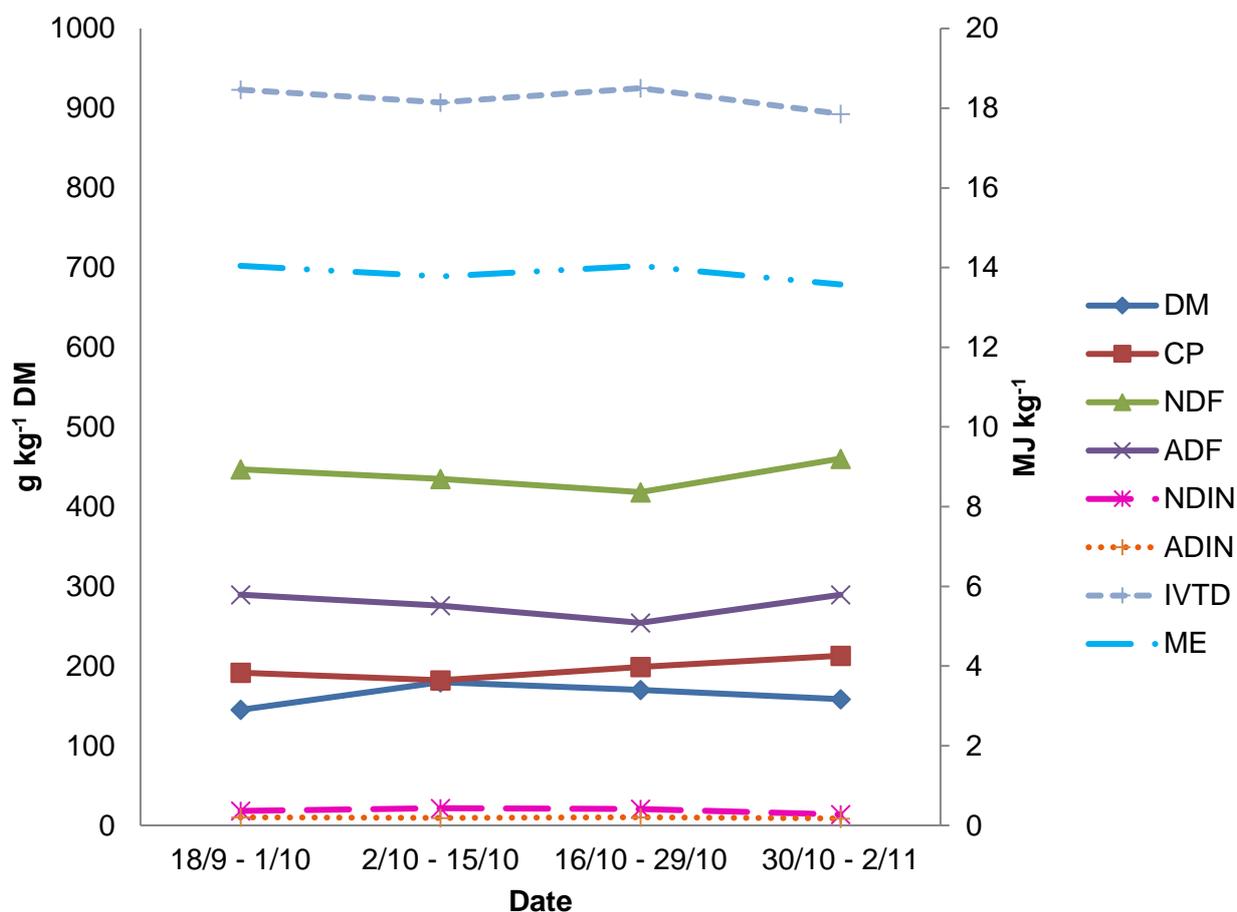
### 4.1.9.1 Pasture nutrient composition

The quality of the pasture grazed by the cows throughout the trial is presented in Table 4.5. Pasture quality, measured in a trial by Meeske *et al.* (2006) were very similar to those obtained in the current trial, with slight differences in the NDF (437 g/kg vs. 568 g/kg) and ME (13.9 MJ/kg vs. 10.0 MJ/kg) levels. The high ME value appears to be unrealistic and is probably an overestimation based on laboratory analysis. However, a high value was expected as the ryegrass reached its peak quality and growth rate during late spring and early summer. If pasture quality are compared to the analysed composition of the three concentrates (Table 4.4), the pasture was higher in CP, NDF, ADF and potassium but lower in calcium and similar in phosphorous and magnesium. The change in the nutritional composition of the pasture as the season started to change from early spring to late spring are graphically presented in Figure 4.1. The data points were obtained by combining the pasture samples that were taken within a specific two week period. As the season changed, from early spring to late spring, the IVTD, ME, NDIN and ADIN decreased. On the contrary the NDF, ADF and CP decreased slightly during the first three quarters of the trial, and increased again during the last quarter of the trial. The DM in fact increased in the first quarter of the trial and decreased during the last three quarters of the trial. This may be due to grazing management and differences in pasture maturity.

**Table 4.5:** Mean ( $\pm$  SD) quality of kikuyu-ryegrass pasture samples collected over a seven week period from 18 September 2017 to 2 November 2017 on a dry matter basis (n=7)

Parameter	g/kg DM <sup>1</sup>
Dry matter	164 $\pm$ 2.8
Ash	103 $\pm$ 7.1
Metabolisable energy (MJ/kg)	13.9 $\pm$ 0.19
Crude protein	194 $\pm$ 12.6
Neutral detergent fibre	437 $\pm$ 14.7
Neutral detergent insoluble fibre	19.3 $\pm$ 5.50
Acid detergent fibre	276 $\pm$ 15.4
Acid detergent insoluble fibre	9.80 $\pm$ 2.214
Acid detergent lignin	53.9 $\pm$ 19.18
<i>In vitro</i> true digestibility	915 $\pm$ 12.3
Calcium	4.55 $\pm$ 0.291
Phosphorous	4.06 $\pm$ 1.079
Magnesium	3.99 $\pm$ 0.374
Potassium	22.4 $\pm$ 7.32
Sodium	18.0 $\pm$ 3.52

<sup>1</sup> DM = Dry matter.



**Figure 4.1:** The response of pasture quality parameters to the changing of the season from early spring to late spring of samples collected over a seven week period during the study. (DM = Dry matter; CP = Crude protein; NDF = Neutral detergent fibre; ADF = Acid detergent fibre; NDIN = Neutral detergent insoluble nitrogen; ADIN = Acid detergent insoluble fibre; IVTD = *In vitro* true digestibility; ME = Metabolisable energy)

#### 4.1.9.2 Pasture allocation and intake

Parameters according to which pasture were allocated to the cows are presented in Table 4.6. Pasture height were obtained by means of the RPM method and pasture yield before and after grazing calculated by means of a regression equation. The regression equation used during the study was obtained by Van Wyngaard (2018):  $Y = 102.99 * H - 260.79$ , where  $Y = \text{DM yield}$  and  $H = \text{RPM reading}$ . The pasture had an average yield of 1990 kg DM/ha before grazing and was allocated at 13.2 kg DM/cow per day, resulting in an average yield of 612 kg DM/ha after grazing. An average of 1378 kg DM/ha pasture was removed by the cows resulting in an average intake of 9.11 kg DM/cow per day. In the current study a new regression equation was obtained from a series of cuttings taken once a week for seven weeks:  $Y = 81.054 * H - 178.94$ , where  $Y = \text{DM yield}$  and  $H = \text{RPM reading}$ . According to the new regression equation the pasture had an average yield of 1592 kg DM/ha before grazing and was allocated at 10.5 kg DM/cow per day resulting in an average yield of 508 kg DM/ha after grazing. An average of 1084 kg DM/ha pasture was removed by the cows resulting in an average intake of 7.17 kg DM/cow per day. Despite the difference in pasture allocation and intake the cows received enough pasture and it can be confirmed by an increase in their body

weight and body condition as seen in Table 4.2. Regression equations are never completely accurate but measuring pasture height and estimating pasture yield is useful to allocate pasture. The regression equation obtained during the current study should be more accurate as indicated by the  $R^2$ -value of 0.8313.

**Table 4.6:** Mean rising plate meter reading and pasture yield before and after grazing of Jersey cows grazing kikuyu over-sown with ryegrass

Parameter <sup>1</sup>	Regression equations <sup>2</sup>	
	$Y = 102.99 * H - 260.79^3$	$Y = 81.054 * H - 178.94^4$
<b>Before grazing</b>		
RPM reading	21.9	21.9
Pasture yield (kg DM/ha)	1990	1592
Allocated pasture (kg DM/cow per day)	13.2	10.5
<b>After grazing</b>		
RPM reading	8.47	8.47
Pasture yield (kg DM/ha)	612	508
Pasture intake (kg DM/cow per day)	9.11	7.17
Pasture removed (kg DM/ha)	1378	1084
<b>Coefficient of determination (<math>R^2</math>)</b>	0.7282	0.8313

<sup>1</sup>RPM = Rising plate meter; DM = Dry matter.

<sup>2</sup>Y = Dry matter yield; H = Rising plate meter height.

<sup>3</sup>Regression obtained from Van Wyngaard (2018) and used in current study.

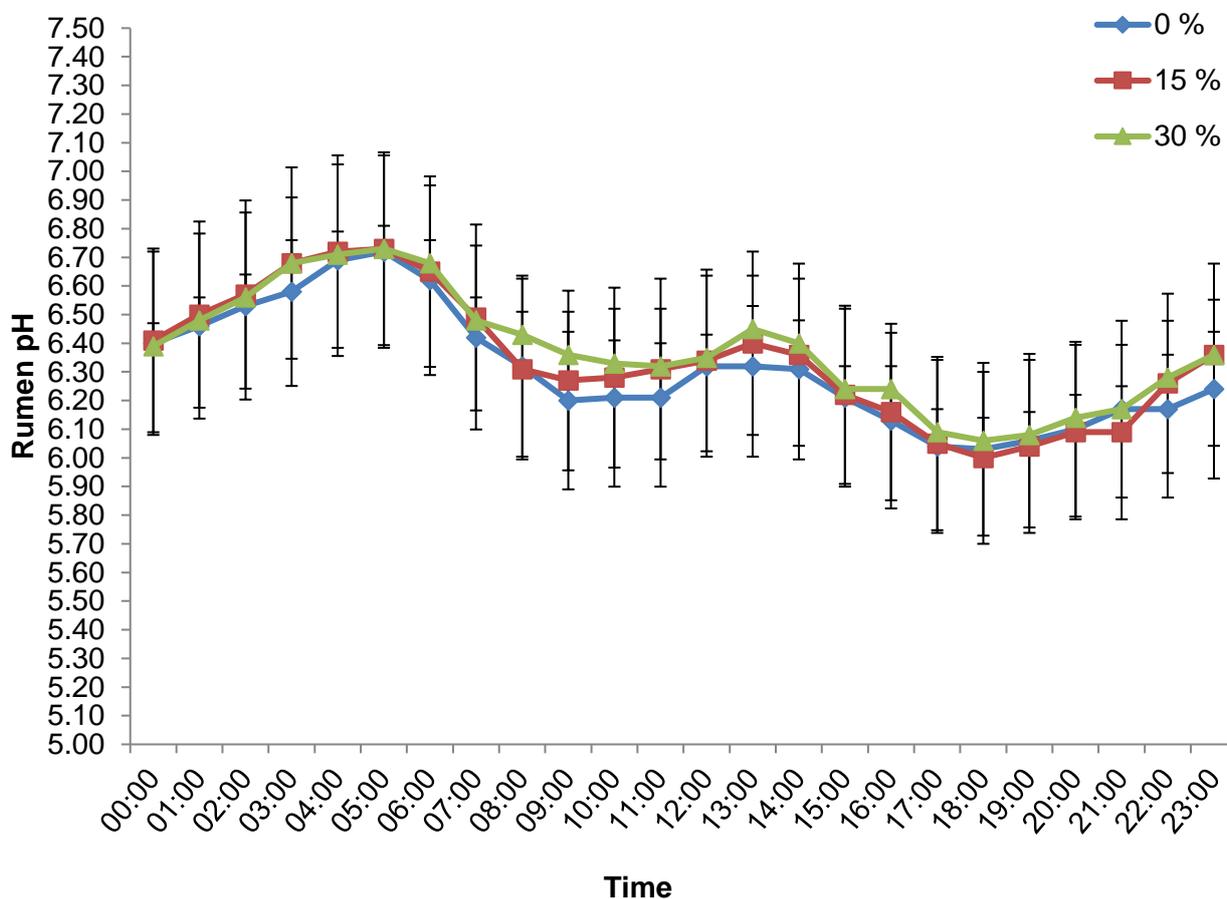
<sup>4</sup>Regression obtained during the current study.

## 4.2 Rumen study

### 4.2.1 Rumen pH profiles

Rumen pH of the cannulated cows was recorded during the rumen study and is presented in Figure 4.2. It can be seen in Figure 4.2 that the pH of the cows on the three different concentrates was very similar. The curve seen in Figure 4.2 are very similar than found by Lingnau (2011) and Van Wyngaard *et al.* (2015). The standard error of the means (SEM) is included as error bars in the graph and therefore cannot be seen as an indication of significance. A distinctive drop in the pH of all three treatments can be seen at 06:00 and 14:00 which corresponds to the milking times reported in Chapter 3 (3.2.2) during which the cows were fed the concentrates in the milking parlour. Rumen pH values were the highest in the morning just before the morning milking session and the lowest late afternoon after the afternoon milking session. Rumen functionality especially fibre digestion, microbial activity and certain bacterial species growth starts to be compromised when the rumen pH drops below pH 6.0, and is optimal when the rumen pH reaches a pH 6.2 (Hoover, 1986; Shriver *et al.*, 1986). Therefore, the number of hours at which the rumen pH was below pH 6.2, pH 6.0 and pH 5.8 were determined for each individual cow and then averaged for each treatment. The results are presented in Table 4.7. As shown in the table, the effect of treatment on these pH values was not

significant, because of one or two cows that were inclined to have a lower ruminal pH. It can also be seen in Figure 4.2 that the treatment means never declined below 6.0 at any time during a 24 h period. As already mentioned rumen functionality, especially fibre digestion, microbial activity and certain bacterial species growth starts to be compromised when the rumen pH drops below pH 6.0, and is optimal when the rumen pH reaches a pH 6.2 (Hoover, 1986; Shriver *et al.*, 1986).



**Figure 4.2:** Diurnal fluctuations of the ruminal pH (mean  $\pm$  SEM) of cows ( $n=9$ /treatment) in three concentrate treatments containing 0%, 15% or 30% soybean hulls fed at 6 kg (as is)

**Table 4.7:** Mean time (hours) that the rumen spent below a specific pH (6.2, 6.0 and 5.8) of cows ( $n=9$ /treatment) in three concentrate treatments containing 0%, 15% or 30% soybean hulls fed at 6 kg (as is)

Parameter	Treatments <sup>1</sup>			SEM <sup>2</sup>	Contrasts <sup>3</sup>		
	1 SH0	2 SH15	3 SH30		1 vs 2	1 vs 3	2 vs 3
< 6.2	8.56	7.61	5.44	1.602	0.68	0.18	0.35
< 6.0	2.50	2.17	1.61	1.065	0.83	0.56	0.72
< 5.8	1.67	0.17	0.61	0.977	0.29	0.45	0.75

<sup>1</sup>Concentrates SH0, SH15 and SH30 containing respectively 0%, 15% and 30% soybean hulls.

<sup>2</sup>SEM = Standard error of the mean.

<sup>3</sup> $P \leq 0.05$  = significant difference;  $P > 0.05$  = not significant difference.

## 4.2.2 Rumen fluid samples

### 4.2.2.1 Volatile fatty acid profiles

The mean ruminal VFA concentrations (mM/L) as well as molar proportions are presented in Table 4.8. Total VFA and especially acetate values for all three treatments obtained in the current study were much lower than found in literature and in previous studies done at Outeniqua Research Farm. Propionate values for SH0 were similar than those reported in other literature, but values for SH15 and SH30 were lower. Butyrate values for all three treatments were comparable with values found in literature. Total VFA, acetate, propionate and butyrate in literature averaged 100 – 156 mM/L, 62.6 – 100 mM/L, 15.4 – 29.6 mM/L and 9.23 – 20.8 mM/L respectively (Cunningham *et al.*, 1993; Mansfield & Stern, 1994; Weidner & Grant, 1994b; Lingnau, 2011; Steyn, 2012; Van Wyngaard *et al.*, 2015; Cawood, 2016). The reason for such a difference in acetate and consequently total VFA from other literature may be due to analyses error, or due to the fact that the fatty acids are volatile and might have been lost. However, the reason is unclear. Cows on SH0 treatment had a higher total VFA than cows on SH15 or SH30 treatment, with a significant difference ( $P < 0.05$ ) between SH0 and SH30 treatments. As mentioned in Chapter 2 (2.7.2.10) acetate and the ratio of acetate to propionate are mostly correlated to milk composition, especially milk fat (Ørskov, 1986; Kennelly & Glimm, 1998; McDonald *et al.*, 2001; Seymour *et al.*, 2005). The acetate concentration of all three treatments was similar with no significant differences between the three treatments. The acetate to propionate ratio of rumen contents was higher ( $P < 0.05$ ) for cows on the soybean hull treatments compared to the control. The acetate did not differ significantly ( $P > 0.05$ ) between treatments which coincide with the milk fat composition in Table 4.1. Propionate, which is correlated to milk yield, but not to milk fat, was significantly ( $P < 0.01$ ) higher for the SH0 treatment compared to SH15 and SH30 treatment. The decrease in propionate production as the inclusion level of soybean hulls increased, can explain the significant difference ( $P < 0.01$ ) in the ratio of acetate to propionate. Due to the small, although significant difference ( $P < 0.01$ ) in propionate, a significant difference in milk yield was not expected among treatments (Table 4.1). The butyrate content of rumen fluid of cows on SH0 treatment was higher ( $P = 0.05$ ) than that of cows on the SH30 treatment. When butyrate concentration increases, less propionate gets oxidised to lactate resulting in lower milk lactose percentages due to higher propionate levels in the liver (Baldwin & McLeod, 2000; McDonald *et al.*, 2001). This coincides with the tendency for milk fat to increase when 15% soybean hulls were included in the concentrate (Table 4.1). Since butyrate is converted to a ketone body which is utilised by skeletal and heart muscles as an energy source (Ørskov, 1986; McDonald *et al.*, 2001), more of the energy consumed by means of the concentrate are utilised for maintenance and milk production. This could be an explanation for why there was no significant difference in milk yield among the treatments. However butyrate only makes up as much as 9 – 14% of the total VFA profile (Church, 1983), making small changes in butyrate concentrations less important than acetate or propionate concentration changes. An increase in the ratio of acetate to propionate was expected for SH15 and SH30 treatments due to the higher NDF content in the concentrates (McDonald *et al.*, 2001; Sairanen

*et al.*, 2005), and it was in fact observed in the current study. Lingnau (2011) and Cawood (2016) did not observe an increase, but Van Wyngaard *et al.* (2015) found similar results to the current study. What is interesting is that in the current study the acetate and propionate concentrations were much lower than what Lingnau (2011) (82.6 mM/L acetate and 17.3 mM/L propionate), Van Wyngaard *et al.* (2015) (75.9 mM/L acetate and 22.8 mM/L propionate) and Cawood (2016) (100.7 mM/L acetate and 26.84 mM/L propionate) obtained in their studies, resulting in a much lower ratio in the current study. Their studies would imply that a higher acetate to propionate ratio would result in higher milk fat content, when in fact lower ratios obtained in the current study resulted in higher milk fat content than in their studies. There was a significant difference ( $P < 0.05$ ) between cows on SH0 treatment and cows on SH15 and SH30 for valerate, iso-butyrate and iso-valerate with SH0 treatment having the highest levels. Molar % of acetate, propionate, valerate, iso-butyrate and iso-valerate were significant different ( $P \leq 0.05$ ) between cows on SH0 treatment and cows on SH15 or SH30 treatment. Levels for acetate were highest for SH30 treatment, and for propionate, valerate, iso-butyrate and iso-valerate highest for SH0 treatment.

**Table 4.8:** Mean ruminal volatile fatty acid concentrations (mM/L) of cows (n=9/treatment) in three concentrate treatments containing 0%, 15% or 30% soybean hulls fed at 6 kg (as is)

Volatile fatty acid	Treatments <sup>1</sup>			SEM <sup>2</sup>	Contrasts <sup>3</sup>		
	1 SH0	2 SH15	3 SH30		1 vs 2	1 vs 3	2 vs 3
<b>Total VFA (mM/L)</b>	48.5	46.6	46.2	0.78	0.10	0.05	0.69
Acetate	15.3	15.8	16.0	0.34	0.34	0.16	0.62
Propionate	16.0	14.7	14.2	0.34	0.01	0.00	0.34
Butyrate	12.6	12.2	12.1	0.16	0.10	0.05	0.71
Valerate	2.23	1.92	1.87	0.060	0.00	0.00	0.57
Iso-Butyrate	1.35	1.19	1.14	0.047	0.03	0.01	0.49
Iso-Valerate	1.07	0.89	0.85	0.053	0.02	0.01	0.61
<b>Acetate:Propionate</b>	0.96	1.08	1.13	0.006	0.00	0.00	0.14
<b>Total VFA molar%</b>							
Acetate	31.4	33.7	34.6	0.36	0.00	0.00	0.10
Propionate	32.9	31.5	30.7	0.44	0.03	0.00	0.23
Butyrate	26.1	26.2	26.3	0.35	0.81	0.65	0.83
Valerate	4.60	4.12	4.03	0.078	0.00	0.00	0.45
Iso-Butyrate	2.79	2.56	2.48	0.078	0.05	0.01	0.51
Iso-Valerate	2.21	1.90	1.84	0.090	0.02	0.01	0.65

<sup>1</sup>Concentrates SH0, SH15 and SH30 containing respectively 0%, 15% and 30% soybean hulls.

<sup>2</sup>SEM = Standard error of the mean.

<sup>3</sup> $P \leq 0.05$  = significant difference;  $P > 0.05$  = not significant difference.

#### 4.2.2.2 Ammonia nitrogen profiles

The mean rumen NH<sub>3</sub>-N concentration (mg/dL) measured during the rumen study are presented in Table 4.9. Including 30% soybean hulls in the concentrate decreased rumen NH<sub>3</sub>-N concentration

significantly ( $P = 0.02$ ). Including 15% soybean hulls in the concentrate did not affect ( $P > 0.05$ ) the rumen  $\text{NH}_3\text{-N}$  concentration when compared to the control. According to Satter & Slyter (1974) the assumption that ruminal and abomasal  $\text{NH}_3\text{-N}$  concentrations are similar can be made. This assumption is made after Hume *et al.* (1970) found similar concentrations for ruminal  $\text{NH}_3\text{-N}$  than Ørskov *et al.* (1972) for abomasal  $\text{NH}_3\text{-N}$ . These authors found that maximum rumen bacteria growth occurred between 4 – 8 mg/dL rumen  $\text{NH}_3\text{-N}$  concentrations. Several other studies found that rumen  $\text{NH}_3\text{-N}$  concentration for optimum growth increases if the primary diet contains limited available protein, but high fermentable CHO (Belasco, 1954; Hume *et al.*, 1970; Allen & Miller, 1976; Okorie *et al.*, 1977). Results recorded are similar than found by Cunningham *et al.* (1993) (11.7 – 12.8 mg/dL) and Mansfield & Stern (1994) (11.6 – 17.2 mg/dL) that fed soybean hulls. In other studies that fed high-fibre by-products rumen  $\text{NH}_3\text{-N}$  varied between 14.6 – 24.82 mg/dL (Lingnau, 2011; Steyn, 2012; Van Wyngaard *et al.*, 2015; Cawood, 2016). Bargo *et al.* (2003) found similar values for cows on pasture and indicates efficient utilization of N originating from pasture (Kolver, 2003).

**Table 4.9:** Mean rumen ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) concentration (mg/dL) of cows ( $n=9/\text{treatment}$ ) in three concentrate treatments containing 0%, 15% or 30% soybean hulls fed at 6 kg (as is)

Parameter <sup>1</sup>	Treatments <sup>2</sup>			SEM <sup>3</sup>	Contrasts <sup>4</sup>		
	1 SH0	2 SH15	3 SH30		1 vs 2	1 vs 3	2 vs 3
$\text{NH}_3\text{-N}$ (mg/dL)	15.5	16.7	11.3	1.13	0.47	0.02	0.00

<sup>1</sup> $\text{NH}_3\text{-N}$  = Ammonia nitrogen.

<sup>2</sup>Concentrates SH0, SH15 and SH30 containing respectively 0%, 15% and 30% soybean hulls.

<sup>3</sup>SEM = Standard error of the mean.

<sup>4</sup> $P \leq 0.05$  = significant difference;  $P > 0.05$  = not significant difference.

#### 4.2.2.3 pH

The mean rumen pH values measured during the study are presented in Table 4.10. The pH was measured for three time intervals by means of a portable pH logger during rumen fluid sampling, as well as by means of TruTrack pH Data Loggers as described in Chapter 3 (3.3.3). Ruminal pH measurements with the hand held logger for time intervals 14:00 and 21:00 were similar between the treatments, except for pH measurements at time interval 06:00. At 06:00 there were significant differences ( $P < 0.05$ ) between the pH measurements of cows on SH0 treatment and cows on SH15 or SH30 treatment. Ruminal pH measurements with the TruTrack pH Data Loggers were similar and with no significant differences ( $P > 0.05$ ) between the three treatments were found. Ruminal pH of cows can be maintained, even if the NFC content of the diet is low. When including soybean hulls in dairy concentrates, NFC content is reduced due to soybean hulls being an NFF source (Ishler & Varga, 2001; Sayers *et al.*, 2003). This may be a reason why the average ruminal pH of cows on SH15 and SH30 treatments were similar to cows on SH0 treatment. However, Figure 4.2 is a more accurate representation of the pH profile for each treatment.

**Table 4.10:** Mean ruminal pH values measured at three time intervals, using a portable pH logger, and pH values measured with TruTrack pH Data Loggers of cows (n=9/treatment) in three concentrate treatments containing 0%, 15% or 30% soybean hulls fed at 6 kg (as is)

Time	Treatments <sup>1</sup>			SEM <sup>2</sup>	Contrasts <sup>3</sup>		
	1 SH0	2 SH15	3 SH30		1 vs 2	1 vs 3	2 vs 3
<b>Hand held logger</b>							
06:00	6.37	6.23	6.21	0.049	0.05	0.03	0.79
14:00	5.83	5.70	5.84	0.054	0.11	0.93	0.09
21:00	5.55	5.64	5.65	0.068	0.37	0.33	0.94
<b>Data logger</b>	6.31	6.34	6.37	0.048	0.62	0.38	0.70

<sup>1</sup>Concentrates SH0, SH15 and SH30 containing respectively 0%, 15% and 30% soybean hulls.

<sup>2</sup>SEM = Standard error of the mean.

<sup>3</sup> $P \leq 0.05$  = significant difference;  $P > 0.05$  = not significant difference.

### 4.2.3 *In sacco* Dacron bag study

#### 4.2.3.1 Dry matter disappearance (DMD)

Dry matter disappearance (%) of dried pasture samples in Dacron bags that were incubated in the rumen for 6 h, 18 h and 30 h are presented in Table 4.11. After 6 h of incubation there were no significant differences ( $P > 0.05$ ) in DMD recorded between the three treatments. Including soybean hulls in the concentrate increased DMD of pasture significantly ( $P \leq 0.05$ ) after 30 h of incubation in the rumen. The lower DMD for SH0 treatment may be partly due to lower pH values of one or two cows with a pH below pH 6.2 (Table 4.7) that may have resulted in reduced microbial activity when the pH is below pH 6.2. High-fibre by-products have the ability to help maintain normal rumen pH and increase DMI due to improved pasture digestion (Muller & Fales, 1998; Bargo *et al.*, 2003).

**Table 4.11:** Mean % of dry matter disappearance of pasture at 6, 18 and 30 hours of incubation within the rumen of cows (n=9/treatment) in three concentrate treatments containing 0%, 15% or 30% soybean hulls fed at 6 kg (as is)

Removal time	Treatments <sup>1</sup>			SEM <sup>2</sup>	Contrasts <sup>3</sup>		
	1 SH0	2 SH15	3 SH30		1 vs 2	1 vs 3	2 vs 3
6 hours	46.1	46.9	46.7	1.11	0.64	0.73	0.90
18 hours	70.9	74.3	73.2	1.00	0.03	0.11	0.47
30 hours	81.8	84.3	84.4	0.86	0.05	0.05	0.93

<sup>1</sup>Concentrates SH0, SH15 and SH30 containing respectively 0%, 15% and 30% soybean hulls.

<sup>2</sup>SEM = Standard error of the mean.

<sup>3</sup> $P \leq 0.05$  = significant difference;  $P > 0.05$  = not significant difference.

#### 4.2.3.2 Neutral detergent fibre disappearance (NDFD)

Neutral detergent fibre disappearance (%) of dried pasture samples in Dacron bags that were incubated in the rumen for 6 h, 18 h and 30 h are presented in Table 4.12. After 6 h of incubation there were no significant differences ( $P > 0.05$ ) in NDFD recorded between the three treatments.

The pasture NDFD of the SH15 treatment was significantly ( $P < 0.05$ ) higher after 30 h of incubation in the rumen than that of the SH0 treatment. The lower NDFD for SH0 treatment may be partly due to possible lower pH values of one or two cows with a pH below pH 6.2 (Table 4.7). Microbial activity may be reduced when rumen pH is below pH 6.2. High-fibre by-products have the ability to help maintain normal rumen pH and increase DMI due to improved pasture digestion (Muller & Fales, 1998; Bargo *et al.*, 2003). Therefore cows on SH15 or SH30 treatment were possibly able to maintain a ruminal pH above pH 6.2 for longer after 30 h. Reducing starch and increasing digestible fibre, improved fibre digestion of ryegrass.

**Table 4.12:** Mean % of neutral detergent fibre (NDF) disappearance of pasture at 6, 18 and 30 hours of incubation within the rumen of cows (n=9/treatment) in three concentrate treatments containing 0%, 15% or 30% soybean hulls fed at 6 kg (as is)

Removal time	Treatments <sup>1</sup>			SEM <sup>2</sup>	Contrasts <sup>3</sup>		
	1 SH0	2 SH15	3 SH30		1 vs 2	1 vs 3	2 vs 3
6 hours	8.53	11.8	11.1	1.80	0.21	0.33	0.78
18 hours	48.2	54.4	52.3	1.78	0.02	0.12	0.41
30 hours	67.9	72.6	71.7	1.99	0.05	0.14	0.71

<sup>1</sup>Concentrates SH0, SH15 and SH30 containing respectively 0%, 15% and 30% soybean hulls.

<sup>2</sup>SEM = Standard error of the mean.

<sup>3</sup> $P \leq 0.05$  = significant difference;  $P > 0.05$  = not significant difference.

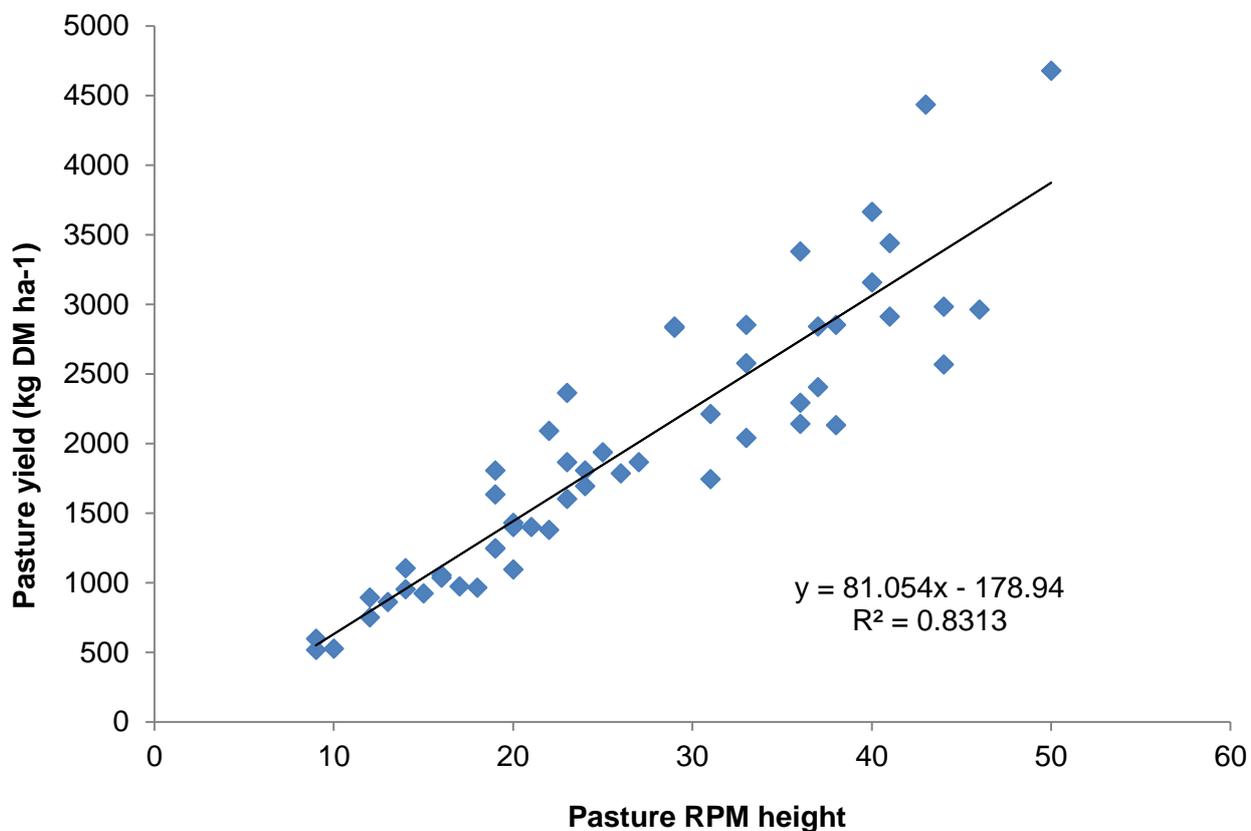
#### 4.2.4 Intake study

Faecal samples were analysed for Titanium dioxide (TiO<sub>2</sub>), but the values obtained were not useable. Samples were re-analysed, but the results did not change. There is a possibility that an error could have occurred during sampling as cows that were not dosed with TiO<sub>2</sub> had higher TiO<sub>2</sub> levels than some cows that were dosed.

### 4.3 Regression

The regression equation which was obtained throughout the duration of the study by means of grass cuttings generated the following linear regression equation:  $Y = 81.054 * H - 178.94$ , where Y = DM yield and H = RPM reading. Grass cuttings for a regression were cut weekly on the pasture which was grazed by the cows as one group from all three treatments. The regression is graphically presented in Figure 4.3, but only when the study was completed, could the regression equation be used to increase the accuracy of indication of the pasture intake of the cows as the regression equation refers directly to the grazed pasture during the study. The equation explains that 83.1% of the variation was experienced through sampling, but is still a reasonable fit. The regression equation used to allocate pasture to the cows during the study was obtained by Van Wyngaard (2018). The regression equation of Van Wyngaard (2018) was the most accurate regression equation available at the onset of the current study as it was determined the previous year (2016) during the same season and on the same camp than as the current study. Estimating pasture intake using the RPM

will result in some degree of inaccuracy. Regression equations are affected by a specific camp, pasture type and season of the year (Sanderson *et al.*, 2001). Regardless of the regression equation used, the optimum post-grazing height remains at 10 – 12 RPM. This will ensure that pasture is not under or over utilized.



**Figure 4.3:** The relationship between the rising plate meter (RPM) reading and the pasture yield (kg DM/ha) of kikuyu over-sown with ryegrass pasture used throughout the study

#### 4.4 Conclusion

Cows consuming a maize based concentrate containing 15 or 30% soybean hulls were able to maintain similar milk production, 4% FCM and ECM than cows consuming a maize based concentrate containing 0% soybean hulls. Milk fat % showed a tendency to increase when 15% soybean hulls were included in the concentrate. Milk protein and lactose content increased when either 15 or 30% soybean hulls were included in the concentrates. This can have a positive effect on the milk price if the milk buyer pays according to milk solids. Milk urea nitrogen indicated that protein supply was sufficient to cows on all treatments. Somatic cell counts are within the acceptable range for human consumption of milk, indicating good udder health. All the cows gained weight and improved in body condition during the study. Body condition improved more in cows on SH15 treatment. Rumen pH below pH 6.2 inhibits microbial activity, resulting in inefficient digestion. Rumen of cows on SH15 and SH30 treatments spent less time below pH 6.2, pH 6.0 and pH 5.8 than cows on SH0 treatment. Therefore feed were digested more efficiently by cows on SH15 and SH30 treatments. This coincides with the DM disappearance after 30 h of incubation being higher

for cows on SH15 and SH30 treatments, and with NDF disappearance after 30 h of incubation being higher for cows on SH30 treatment. These pH, DM and NDF disappearance findings can possibly explain why milk production was maintained for cows on SH15 and SH30 treatments as microbial activity decreases when rumen pH is below pH 6.2. Yet over a 24 h period the average pH of all the cows were similar indicating rumen health was not compromised. Kikuyu-ryegrass pasture was digested more efficiently when soybean hulls were included in the diet. This might have increased pasture intake of cows on SH15 and SH30 treatments, resulting in similar milk yield than cows on SH0 treatment. Including soybean hulls affected acetate concentration, and 30% soybean hull inclusion increased rumen  $\text{NH}_3\text{-N}$  concentration significantly ( $P < 0.05$ ). Including up to 30% soybean hulls in the concentrates did not have a negative effect on milk yield, milk composition, rumen environment or cow health. No long-term effects could be concluded as the duration of the study was short, but monitoring of the rumen parameters did indicate that the cows were healthy. Replacing 15% of maize with soybean hulls in the concentrate for dairy cows improved milk solids and digestibility of the pasture.

## 4.5 References

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## Chapter 5 - Economic Impact

The economic impact of partial replacement of maize with soybean hulls is substantial. The price for milk of cows fed a concentrate containing 15% soybean hulls was 20 cents per kg higher than that of cows on the control maize concentrate. Milk prices were obtained in October 2018 from Nestlé for the different treatments as shown in Table 5.1. Nestlé base their milk price on the milk composition and milk volume. The economic implications were calculated for a herd of 400 Jersey cows fed concentrate at 6 kg/cow per day. It was assumed that pasture DMI did not differ between treatments and that the cost of soybean hulls and maize were the same. Economic impact is given in Table 5.1. The average milk production per treatment as obtained in the study were used to calculate the milk production of 400 cows for 30 days and multiplied with the milk price. Feeding a concentrate containing 15% soybean hulls would increase the monthly profit by R52 812 compared to cows fed a concentrate containing 0% soybean hulls.

**Table 5.1:** Milk price, milk yield and monthly profit increase due to inclusion of 0%, 15% or 30% soybean hulls in the concentrate for Jersey cows grazing high quality ryegrass pasture

Parameter	Treatments <sup>1</sup>		
	SH0	SH15	SH30
Milk price R/kg	R5.21	R5.41	R5.39
Milk production kg/cow per day	19.3	19.4	19.2
Milk income R/month	R1 206 636	R1 259 448	R1 241 856
Milk profit/month increase	-	R52 812	R35 220

<sup>1</sup>Concentrates SH0, SH15 and SH30 containing respectively 0%, 15% and 30% soybean hulls.

## **Chapter 6 - General conclusion**

The study showed that including up to 30% soybean hulls in the concentrates did not have a negative effect on milk yield, milk composition, rumen environment or cow health. Kikuyu-ryegrass pasture was digested more efficiently when soybean hulls were included in the diet. Replacing 15% of the maize tended to increase milk fat content and increased milk protein and lactose content significantly. With the difference in milk price for milk with similar compositions than cows on SH0 and SH15 treatments from the study, a monthly profit can be made. It was assumed that the prices for maize and soybean hulls are the same. No long-term effects could be concluded as the duration of the study was short, but monitoring of the rumen parameters did indicate that the cows were healthy.

## Chapter 7 - Critical evaluation

Milk sampling: The milking machine did not have the ability to siphon off the same and correct amount of milk from the cows, resulting in variation in milk sampling data. A new milking system was installed after the study was completed. It would be interesting to see if the new milking system decreases the amount of variation.

Faecal sampling: The cows whose faeces were collected grazed along with the rest of the cows and were collected between the other cows. Contamination of the samples could have occurred while sampling, resulting in the inconclusive results regarding the TiO<sub>2</sub> analyses. An error also might have occurred when dried samples were pooled per cow. Less errors and variation may occur if the cows are kept in separate stalls receiving fresh cut pasture from the same camp grazed by the rest of the herd.

Trial period: The trial was only for 64 days in total, therefore no long-term effects were detected. It may be of interest to do a long-term study to see the effect of the SH15 and SH30 on the LW and BC of the cows, as well as on their conception rate.