UNIVERSITEIT VAN PRETORIA
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Faculty of Natural and Agricultural Sciences

Effect of rumen-specific live yeast supplementation on *in situ* ruminal degradation of forages differing in nutritive quality

by

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Submitted in partial fulfilment of the requirements for the degree

MSc (Agric) Animal Science: Animal Nutrition

In the Faculty of Natural and Agricultural Sciences

University of Pretoria

Pretoria

2019

INDEX

DECLARATION

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

JW van Niekerk

Pretoria

July 2019

Signature_____

ACKNOWLEDGEMENTS

This dissertation is dedicated to my late grandfather, Dr Johan van Niekerk. He would have been very proud of this accomplishment. He is the inspiration behind my love for animals.

First I would like to thank the Lord for giving me the strength and perseverance to be able to complete my studies.

This dissertation would not have been possible if it wasn't for the following people:

A very big thank you to my parents, Danie and Louise van Niekerk, and the rest of my family for always believing in me, for the financial support and most importantly the emotional support.

A special thanks to my wife, Sulize, for never losing faith and always supporting my late nights of work to complete this dissertation. Your love and support carried me through the toughest of times.

To my supervisor Prof Erasmus, thank you for all your advice and guidance, but especially thank you for your patience.

To Prof Meeske, thank you for all your support during my stay in George. You were never too busy to assist me with this project.

A massive thank you to Lallemand for the funding of the trial and especially to Eric Chevaux for the support before, during and after the trial.

To Josef van Wyngaard and Zanmari Moller, thank you for always being prepared to help with sampling and giving general advice on the farm. You made my stay in George worthwhile.

To Bertus, Pieter, Denver and the rest of the staff on the Outeniqua research farm, thank you for all the help with my trial.

Last but not least, thank you Oom Roelf Coertze for all your help with the statistical analysis. This would definitely not have been possible without you.

For everyone involved but not specifically mentioned here, thank you for being part of my journey!

SUMMARY

Effect of rumen-specific live yeast supplementation on *in situ* ruminal degradation of forages differing in nutritive quality

by

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Interest in alternative rumen fermentation modifiers has increased significantly since the ban of antibiotic growth promoters in 2006 by the European Union. The use of live yeast in ruminant feed is nothing new, but the mode of action has only recently been described. Inconsistent results in efficacy have sparked a new interest in the role of different feedstuffs on the effectiveness of the live yeast as rumen fermentation modifier. We investigated the effect of a live yeast supplement on *in situ* ruminal degradation of four different forages, each varying in quality within forage species.

The experimental design was a crossover study using eight rumen cannulated Jersey cows all being fed a lucerne based total mixed ration (TMR). Four cows received a control diet with no live yeast supplemented, and the other four received the control diet supplemented with 0.5g grams live yeast per cow per day. The live yeast was supplemented directly into the rumen via the rumen cannula. The adaptation to each treatment was 21 days and the sampling period was 10 days thereafter. Four forages (Eragrostis hay, lucerne hay, kikuyu and rye grass pasture) with 3 qualities within each forage (Low, medium and high) were ruminally incubated for 12, 24 and 36h to measure *in situ* NDFom and dry matter disappearance. Rumen fermentation parameters were also measured.

Live yeast supplementation did not affect the performance of the cows or any of the rumen fermentation patterns measured. Within forages the live yeast treatment did not affect ruminal NDFom disappearance in the high quality forage category. For lucerne hay the live yeast treatment appeared to increase *in situ* NDFom disappearance in the medium quality category, and also the overall average that included all qualities. For kikuyu and rye grass, the live yeast treatment decreased the *in situ* NDFom disappearance in the lower quality category as well as the overall average. For the *Eragrostis curvula* hay the live yeast treatment did not have any effect on *in situ* NDFom disappearance. Further research are needed to better understand the interaction between forage species, forage quality and the efficacy of live yeast under different dietary scenarios.

LIST OF ABBREVIATIONS

µm	Micro meter
ADG	Average daily gain
ADL	Acid detergent lignin
NDFom	Neutral detergent fibre organic matter corrected
BW	Body weight
℃	Degrees Celsius
Ca	Calcium
Ca:P	Calcium to Phosphorus ratio
$CaCo_3$	Calcium carbonate (Limestone)
CFU	Colony forming unit
CGF	Corn gluten feed
cm	Centimetres
CP	Crude protein
DFM	Direct fed microbials
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
FAO	Food and Agriculture Organization of the United Nations
g	Gram
GE	Gross energy
h	hours
IVOMD	In vitro organic matter digestibility
Kg	Kilograms
L	Litres
LYC	Live yeast culture
m	Meter
ME	Metabolizable energy
MgO	Magnesium oxide

Min	Minutes
mL	Millilitres
Mm	Millimetres
Mmol	Milimoles
mV	Millivolts
N	Nitrogen
NDF	Neutral detergent fibre
NH_3-N	Ammonia nitrogen
NPN	Non-protein nitrogen
Nr	Number
ORP	Oxidation reduction potentiometer
pH	The negative logarithm to the base 10 of the hydrogen ion activity in the solution
P	Phosphorus
PSFPS	Penn State forage particle separator
RNA	Ribonucleic acid
SEM	Standard error of the mean
Sol CP	Soluble crude protein
TMR	Total mixed ration
UN	United Nations
VFA	Volatile fatty acid

LIST OF TABLES

CHAPTER 3

CHAPTER 4

LIST OF FIGURES

CHAPTER 3

CHAPTER 4

CHAPTER 1

INTRODUCTION

The UN projects that by the year 2050, the world human population will more than 9 Billion people and that would require food production to double, on virtually the same area of available land as today. The FAO states that 70% of these additional food supplies must come from an increase in production efficiency. The biggest portion of this growth in production is expected to happen in developing countries (Webb and Erasmus, 2013). The modern producer also faces challenges such as ever-increasing feed costs, fluctuating markets and lower profit margins. This emphasizes the need to be more efficient on the farm.

The concept of precision farming is based on the presence of in-field variation. This is due to the fact that animals within a herd may vary with regards to age, weight, sex, production stage and production potential and that implicates vast differences in nutrient requirements (Pomar *et al*., 2009). With feed costs averaging around 70% of total costs of a dairy operation, it has the greatest potential to be manipulated and reducing total production costs. Precision feeding is regarded by many as the best option to increase production efficiency and lowering environmental nutrient losses. According to Professor Willem van Niekerk from the University of Pretoria, “precision feeding can be described as feeding the right amount of feed at the correct nutrient ratio at the right stage of production, ensuring consistent animal health and optimal production with the lowest environmental impact.” It basically implicates doing the right thing at the right time in the right place and in the correct manner. In livestock production systems, the implementation of precision feeding would require 1) a proper nutritional analysis of the individual feed raw materials, 2) accurate evaluation of nutritional requirements of the animal, 3) formulation of balanced diets that would meet the requirements during the different production stages of the animals and 4) the dynamic, parallel alteration of the feed supply and concentration of nutrients to each individual animal in the herd (Pomar *et al*., 2009).

Feed additives play a pivotal role in precision feeding of ruminants by increasing feed efficiency and reducing the environmental impact per unit of animal product (Reddy and Krishna, 2009). A major concern in the animal production industry involved the use of antibiotics and other growth promoters as means of increasing production efficiency. This concern has sparked a renewed interest in the use of probiotics (Krehbiel *et al*, 2003). In addition, consumers’ demand for safe, high quality nutritious food, has stimulated the use of natural feed additives (Reddy and Krishna, 2009).

Yeast products such as *Saccharomyces cerevisiae* and Aspergillus oryzae are preferred over antibiotics and appear to be more useful in manipulating rumen metabolism. As a result, the use of *S. cerevisiae* as a microbial feed additive has significantly increased during the past 20 years (Patra, 2012).

Yeast products are used more frequently in dairy cattle diets not only to improve animal performance and feed efficiency, but also play a role in preventing health disorders (Chaucheyras-Durand *et al*, 2008). One of the key functions of live yeast additives is to increase the rate of degradation of fibrous feeds and thereby increasing dry matter intake. Results, however, are inconsistent and it has been reported that there is an interaction between the quality of a specific roughage and the magnitude of the response caused by the yeast. The nature of the dietary ingredients, especially regarding NDF degradation, has a great influence in the rumen's response to yeast (Chaucheyras-Durand *et al*, 2012).

From the review of Robinson (2002), it appears that animal responses to live yeast products are independent of the diet. However, experimental support for uniform effects of *Saccharomyces cerevisiae* on rumen fibre degradation, regardless of the digestibility or quality of the basal diet, is inconsistent (Krehbiel *et al*, 2006). According to Chaucheyras-Durand *et al*, (2012) the type of dietary ingredient, thereby implicating quality as well, has a great influence on ruminal response to yeast probiotics, especially for fibre degradation.

In our study we investigated the effect of rumen specific live yeast supplementation on in situ NDF degradation and rumen fermentation characteristics of 4 forages differing in quality between and within forages. The following hypotheses have been investigated:

i) H_0 : The effect of live yeast supplementation on ruminal NDF degradation and rumen fermentation characteristics is not affected by the type of forage.

   H_1 : The effect of live yeast supplementation on ruminal NDF degradation and rumen fermentation characteristics is affected by the type of forage.

ii) H_0 : The effect of live yeast supplementation on ruminal NDF degradation and rumen fermentation characteristics is not affected by differences in forage quality, within forage species.

   H_1 : The effect of live yeast supplementation on ruminal NDF degradation and rumen fermentation characteristics is affected by differences in forage quality, within forage species.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Milk producers worldwide are constantly looking for ways to improve the efficiency of milk production due to the challenging economic situation facing milk producers. Increasing feed costs and fluctuating markets make it very difficult for milk producers with small herds to stay competitive in the industry. Profit margins are small and the need for enhanced efficiency is now more important than ever. The main goal is to reduce input costs without sacrificing milk yield. In addition to increase productivity, the modern approach to supplement feed additives is to also reduce the risk of ruminants carrying pathogens that pose a risk to human health as well as limiting the excretion of nitrogen and methane into the environment (Fonty and Chaucheyras-Durand, 2006).

Ruminant animals, a key component of livestock production systems, are very unique mammals in a sense that they have a symbiotic relationship with microbes that are able to utilize the most abundant nutrient source on earth in the form of complex carbohydrates. Grass and other plant material, depending on stage of maturity, consist mainly of complex carbohydrates which are not digestible by mammalian enzymes. The ruminant four-compartment stomach is the perfect environment and acts as the host for many different micro-organisms. The main digestive compartment called the rumen, acts as a fermentation chamber and contains over 200 different species of microbes. This complex symbiotic mixture of microbiota, composed of anaerobic bacteria, ciliate protozoa and fungi, have the ability to break down these complex carbohydrates. Efficacy, extent and rate of digestion depend on many factors including the nature of the plant material, physiological characteristics of the microbes and physio-chemical conditions within the rumen (Mertens, 1977). Microbial interactions may also contribute to the dynamics of the digestion process. When the ruminal conditions are favourable, these organisms colonise, hydrolyse and ferment forage cell wall polysaccharides and subsequently release volatile fatty acids as fermentation end products (Krause *et al*., 2003). This volatile fatty acids in substrate-dependant ratios, serve as the primary energy source to the host animal. As the microbes move through the digestive tract, they get exposed to various mammalian digestive enzymes and ultimately serve as a major source of protein to the ruminant animal.

The provision of forage fibre in dairy cattle diets is an important factor for optimising milk and milk component production as well as maintaining rumen health. The NRC (2001)

recommends a minimum of 28% neutral detergent fibre (NDF) in total diet dry matter (DM), of which 75% should be supplied by forage. A major component of the dairy diet consists of forage which generally varies between 40% and 60% of a lactating cow diet (Hutjens, 2008). By increasing the digestibility of the roughage component of the diet, the whole process of milk production becomes more efficient.

Alexander Flemming is regarded as the father of antibiotics and was awarded a Nobel Prize for the discovery thereof. However, he did warn about the danger of antimicrobial resistance when under-dosing for prolonged periods. The animal production industry soon realised the growth promoting advantages of the sub-therapeutic use of antibiotics. This did result in antibiotic resistant gut microflora and the practice was criticized by many after concerns were raised with regards to human health. The use of antibiotics as growth promotants in animal feeds is prohibited in the European countries due to potential human health risks such antibiotic resistance and the spread of these resistant genes (Hong *et al*., 2005). The public consumer is putting pressure on authorities by demanding safer production chains from farm to plate. This will speed up the inevitable ban of any sub therapeutic use of any antibiotic product as growth promotant. This sparked a renewed interest in the field of probiotics (Krehbiel *et al*, 2003).

Over the past 3 decades, nutritionists have shown a lot of interest in the manipulation of the rumen fermentation process with the use of probiotics. Feeding microbes to animals to improve production is not a new concept at all. The term “Probiotics” have been widely used, however, this term implies “curing”, and that’s not necessarily the case. Therefore, the term “Direct fed microbial” (DFM) has been established to describe the act of feeding organisms to the animal to alter metabolic reactions in the gut with the goal of enhancing production efficiency (Denev *et al*, 2007). Direct fed microbials can be categorized as either bacterial- or fungal DFM, or a combination of the two.

The exact mode of action of various combinations of DFM’s will depend on factors such as strain, dose and management practices such as time and frequency of feeding. The target area in the digestive tract will differ between products. Some will be effective in the rumen and others lower down the small- or large intestines (Mona *et al*, 2014). Most commonly used Bacterial DFM strains are classified as lactic acid utilizing bacteria, lactic acid producing bacteria or other microorganisms. These include, but are not limited to, *Lactobacillus*, *Propionibacterium, Bifidobacterium*, *Enterococcus*, *Streptococcus*, *Bacillus, Megasphaera elsdenii* and *Prevotella bryantii* (Kung 2006; Seo *et al.* 2010). Fungal DFM’s are commonly used in the ruminant industry and is known for its beneficial effects on

performance and stabilizing rumen fermentation. *Saccharomyces cerevisiae* and Aspergillus oryzae are well researched and also the most widely used fungal DFM's (Mona *et al*, 2014).

2.2 Yeast products

2.2.1 Introduction

Yeast can be described as microscopic, single-celled plant-like organisms forming part of the phylogenetic family of fungi. Many different strains exist and can be found in any non-sterile environment. Cell size of yeasts (5 x 10µm) are bigger than that of bacteria (0.5 x 5µm). Yeasts have genetic resistance against antibiotics and can be used in combination with anti-microbial products (Balzi and Goffeau, 1995).

Fungal DFM's can be divided into 3 categories. The first category consists of products containing live cultures of *Saccharomyces cerevisiae* with a guaranteed number of colony forming units (CFU's), also referred to as live yeast cultures (LYC). The second category consists of additives that contain culture extracts and/or *S. cerevisiae* but cannot give any guarantee of any live organisms although some products contain some live organisms. The last category is additives which contain fermentation end products of *Aspergillus oryzae* and *Aspergillus niger* and do not guarantee any number of live organisms (Denev *et al*, 2007).

Effects of live yeast supplementation in dairy cows are inconsistent and these differences could be ascribed to the differences in strains of live yeasts used in the past few decades of research, as well as different experimental designs of the studies and type of diet fed. An in vitro experiment by Lynch and Martin (2002) showed live yeast to increase the pH and dead live yeast to decrease pH when soluble starch or lucerne hay was incubated. Chaucheyras-Durand *et al* (2008) suggested that the magnitude of effect elicited by live yeasts depend on biotic and abiotic factors. Biotic factors include the strain of live yeast as well as the viability. Abiotic factors such as management and nature of the diet play a major role in the degree of response by live yeast supplementation. Consequently, the need for more focussed research arises which can ultimately lead to scientifically proven answers as to which strain and form of live yeast is most effective (if at all) under specific dietary conditions and the use of specific raw materials.

Live yeast products have been fed to dairy cows with varied and inconsistent responses (Chaucheyras-Durand *et al*, 2008). Improvements in DMI and milk yield (Erasmus *et al*, 1992, Robinson and Garrett, 1999, Desnoyers *et al*, 2009), stabilisation of rumen pH

(Bach *et al*, 2007, De Ondarza *et al*, 2012) and increased NDF digestibility (Carro *et al*, 1992, Guedes *et al*, 2008) have been reported. In other studies, however, no beneficial responses were found (Arambel and Kent, 1990, Swartz *et al*, 1994). Milk composition is generally not or only slightly affected by live yeast supplementation (Chaucheyras-Durand *et al*, 2012). There is sufficient proof that live yeast supplementation improves rumen fibre digestion in vivo (Plata *et al*, 1994, Guedes *et al*, 2008, Chaucheyras-Durand *et al*, 2010), although it has not always been observed (Angeles *et al*, 1998). These results show that forage quality plays a role since a positive result was observed in higher quality forages (lucerne hay, maize silage) with no response in low quality forage such as maize stover (Angeles *et al*, 1998). In contrast, live yeast supplementation increased NDF digestibility in oat straw, which is similar to maize stover in terms of quality. Results therefore suggest that both forage quality and forage species have an impact on the effect of live yeast supplementation on the efficiency of utilization of forages by ruminants.

Very little information is available on the effect of live yeast supplementation on the ruminal rate and extent of NDF degradation of forages that vary in quality between and within forage species. More research is therefore needed to expand an efficacy database for different diets and raw materials, differing in quality. This is especially true for roughages that forms the major part of dairy cattle diets. In addition, feed additives are costly and should be strategically supplemented throughout the lactation, taking into account the type of forage and the quality forage.

2.2.2 Modes of action

For the past two decades the mode of action of live yeast products has been intensively researched to understand the mode of action (Newbold *et al*, 1996). By understanding the mode of action, the live yeast can be strategically implemented in intensive production systems. Modelling systems can also be used to predict the effect of live yeast supplementation if the mode of action is understood and backed up by data from trials that are designed to highlight these effects.

Many mechanisms have been hypothesized to explain differences in ruminal fermentation dynamics and higher production responses when dairy diets have been supplemented with live yeasts. It is difficult to link increased performance to a specific action by the live yeast as the mode of action is very complex. The live yeast causes various changes in the rumen environment including changes to the microbial ecosystem and also changes to the electro-chemical environment. These changes brought about by live yeast leads to secondary effects and different combinations of these primary and secondary

effects lead to measurable performance changes. As described by Chaucheyras- Durand *et al* (2008), live yeast products exert three main outcomes (whether it be primary or secondary): 1) improved microbial establishment leading to better rumen maturation, 2) stabilization of rumen pH by interacting with lactate utilizing bacteria and 3) increased fibre degradation and interactions with plant-cell wall degrading microorganisms.

2.2.2.1 Microbial Stimulation

With improving molecular technology based on ribosomal RNA sequencing, it has recently come to light that the rumen microbial population harbours a far more diverse community than previously thought (Fonty and Chaucheyras-Durand, 2006). Microbes in the rumen can be classified into 3 main groups; Fungi, Bacteria and Protozoa.

2.2.2.1.1 Fungi

Fungi's contribution to cell wall degradation in the rumen is by far the greatest when compared to bacteria and protozoa (Lee *et al*, 2000). By supplementing a live yeast to a ruminant animal, one could expect an increased fungal colonization of plant cell walls. This was demonstrated under in vitro conditions by Chaucheyras *et al*. (1995b) and results suggest that this may be due to an increased supply of thiamine. Thiamine is an important vitamin required by fungi to reproduce via zoosporogenesis. The fungal numbers of *Neocallimastix frontalis* was stimulated by the addition of live yeast (Chaucheyras-Durand *et al*., 2008). These interactions can lead to increased fibre digestion, discussed later in this chapter

2.2.2.1.2 Bacteria

One of the most consistent observations in yeast research over the past three decades would be the increase in number of bacterial cells (Bach *et al*., 2007). Supplementation of *S. cerevisiae* significantly increases the amount of viable bacterial cells that can be recovered from the rumen (Newbold *et al*., 1996). In gnotoxenic lambs, animals reared in a sterile environment, and conventional lambs fed live *S. cerevisiae* cells daily, the rate of establishment of the cellulolytic bacterial population was increased and this population was more stable than in non-supplemented lambs (Chaucheyras-Durand and Fonty, 2001). It is suggested that *S. cerevisiae* may provide growth factors, like organic acids and vitamins, thereby, stimulating the rumen populations of cellulolytic bacteria (Chaucheyras *et al*., 1995a). Sousa *et al.* (2018) reported significant increases in *R. flavefaciens* populations when grazing steers were supplemented with live yeast.

2.2.2.1.3 Protozoa

Protozoa is an important microbial group in the rumen and can account for up to half of the total biomass. The actual numbers are far less than that of bacteria and fungi, but due to the size of the organisms it accounts for such a high percentage of the biomass. Protozoa replicate at a much slower rate (15-24h) as opposed to that of bacteria (as fast as 13 min). When low roughage diets are fed, the protozoa leave the rumen faster than they can regenerate and that causes a decrease in protozoa numbers (Dayyani *et al*., 2013). The effect of protozoa on pH regulation will be discussed later in this chapter. Brossard *et al*. (2004) detected an increase in protozoa population in sheep fed the same live yeast strain used in this study. This was confirmed by Chaucheyras-Durand *et al*. (2008) who reported an increase in Entodiniomorphid protozoa numbers when live yeast was supplemented.

2.2.2.2 Oxygen Sequestration (Redox Potential)

The majority of cellulytic bacteria in the rumen is considered as strictly anaerobic microorganisms. Oxygen enters the rumen when feed is chewed and swallowed. This process is inevitable and as much as 16L of oxygen can enter an ovine rumen daily during feed and water intake, rumination or salivation (Newbold *et al*, 1995).

Live yeasts possess the ability to remove oxygen on the surfaces of freshly ingested feed in order to maintain metabolic activity in the rumen (Newbold *et al.* 1996) and keep the rumen an anaerobic chamber. This was tested by comparing respiratory-deficient mutants of *S. cerevisiae* to wild-type parent strains. The mutant strain was unable to stimulate bacterial numbers whereas the parent strains stimulated bacterial activity effectively (Newbold *et al.* 1996). This ability of live yeast causes a decrease in the redox potential in the rumen (Jouany, 1999) which provides a better environment for the growth of strict anaerobic cellulolytic bacteria and also stimulates their attachment to forage particles (Roger *et al.* 1990) and increases the initial rate of cellulolysis.

Redox potential of rumen fluid has been decreased in studies using lambs (Chaucheyras-Durand and Fonty, 2002) and sheep (Jouany *et al*., 1998), by supplementing a live yeast. This research suggests the supplementation of a live yeast to ruminant animals may induce a reduction of oxygen and ultimately create a more favourable condition for the proliferation of the anaerobic autochthonous microbiota.

2.2.3 Secondary ruminal effects of yeast supplementation

2.2.3.1 Rumen Maturation

For the first few hours in the life of a neo-natal calf, the rumen is considered to be sterile which means it does not have the necessary microbes to ferment any feed and essentially functions as a monogastric animal. Not long after birth, the curious young animal will sniff, touch and nibble on vegetation and also come in contact with the mother's saliva or even faecal matter on the ground, resulting in the ingestion of microbes that would ultimately colonize in the rumen. This is essential for the inoculation of the rumen with microbes ultimately to develop the rumen microbial system, a very complex microbial population inducing a favourable rumen environment for fermentation of ingested feeds (Hobson, 1997). A few hours after birth, the immature rumen will contain strictly anaerobic organisms. Two to four days after birth, methanogenic archaeons and cellulytic bacteria will be present and ciliate protozoa only 2 weeks after birth (Fonty and Chaucheyras-Durand, 2006).

In the majority of modern dairy production systems, the cow-calf interaction is very limited due to early separation of the cow and calf. The transition from milk to solid feeds usually occurs even before the rumen colonization is complete, a state called "climax community" (Fonty *et al*., 1983, 1987). This practice often leads to imbalances in the microbial population and would lead to digestive disturbances, higher mortality and ultimately economic losses (Collado and Sanz, 2007).

It is important for dairy farmers to speed up rumen development with the aim to wean a calf early. The extent of early rumen development is of great importance to the dairy farmer as it will determine the functionality of the rumen (*e.g.*, absorption ability and digestion efficiency). Early developments of the rumen also lead to timely advances in the calf's own immune system and overall gut health (Hooper *et al*., 2001). This correlates to the findings from Zanton & Heinrichs (2005) that explains the correlation between certain target weights at specific ages and the production potential in the first lactation of the animal. These suggested target weights can only be reached if the rumen development is optimal. A study by Lesmeister *et al*. (2004) explored the effects of live yeast supplementation on the performance of calves and found that the addition of the live yeast not only increased the DMI and ADG, but also the hip weight and hip width. These improvements could be correlated to an improvement in rumen development parameters such as papillae length and width, and rumen wall thickness.

Chaucheyras-Durand and Fonty, (2002) conducted a study on the rumen development of lambs and found that the establishment of cellulolytic bacterial population was earlier and more stable in the lambs supplemented with live *S. cerevisiae* cells, than in

control lambs. The supplementation of a live yeast also stimulated a faster colonization of ciliate protozoa in the rumen and Fonty *et al* (1988) stated that this can only happen when bacterial communities have been established in the rumen.

2.2.3.2 Rumen pH stabilization

Modern dairy farms feed highly fermentable diets which can put the animals to risk with various metabolic disorders. The ruminal pH is probably the most important parameter with regards to rumen health. The microorganisms in the rumen produce a wide variety of organic acids. When compounds such as lactic acid accumulate, the rumen pH drops below 6 and can have detrimental effects on rumen function and cow health, and a condition called rumen acidosis can develop. This decrease in pH is caused by diets consisting of high levels of rapidly fermentable carbohydrates. Acidosis (sub-acute or acute) are associated with low feed intake, milk fat depression, laminitis, diarrhoea and liver abscesses (Bach *et al*., 2007). To control acidosis, you have to control lactate concentrations by either reducing production of lactate or increase the rate of removing lactate from the rumen.

Supplementation of live yeast products and the effects on rumen fermentation have been researched thoroughly and it has been found that live yeast may help to buffer the rumen against excess lactic acid production when ruminants are fed high concentrate diets (Williams *et al*., 1991). The positive effects *S. cerevisiae* is likely due to both stimulation of lactate utilizing bacteria and inhibition of lactate producing bacteria, causing a combined effect on rumen pH (De Ondarza *et al*., 2010). Supplementation of live yeast cultures may increase the use of lactate by *Selenomonas ruminantium* (Nisbet and Martin, 1990; Rossi *et al*., 2004) and *Megaspheara elsdenii* (Waldrip and Martin, 1993; Chaucheyras *et al*., 1996) by providing a source of dicarboxcylic acids (e.g., malic acid) and other growth factors (Martin and Streeter 1995; Kung 2006). In vitro experiments showed the same effect using mixed ruminal microorganisms (Lila *et al*., 2004; Lynch and Martin, 2002).

Other research also concluded that the live yeast reduced lactate levels in the rumen but suggested another possible mode of action. Chaucheyras *et al*., (1996) suggested that this is due to the fact that one strain of *S. cerevisiae* will outnumber the *Streptococcus bovis* with regards to the utilization of rapidly fermentable sugars, ultimately resulting in lower concentrations of lactate in the rumen. Under in vitro conditions, the bacterial counts of *S. bovis,* a lactic acid producing bacteria, were reduced by 47-fold when live yeast was supplemented (Chaucheyras-Durand *et al*., 2005). The author suggested this was due to the competition for glucose uptake.

As mentioned above, live yeast supplementation can result in microbial stimulation. Another microbe that is stimulated by *S. cerevisiae* is Entodiniomorphid protozoa and can stabilize pH. These microorganisms are known to engulf starch granules (Abbou-Akkada and Howard, 1961), competing with amylolytic bacteria for their substrate. This means the starch is not fermented by amylolytic bacteria but rather by protozoa at a much slower rate than the bacteria. Some of the lactate will also be utilized by the entodiniomorphs and reduce lactate accumulation (Newbold *et al*., 1987). However, it is important to remember that the effect of buffering is subtle and that live yeast supplementation cannot always prevent lactic acidosis in diets with high level of fermentable carbohydrates (Dawson and Hopkins 1991; Aslan *et al.* 1995)

2.2.3.3 NDF digestion

Neutral detergent fibre is the insoluble fibre in feeds that is either indigestible or slowly digested, and occupies space in the digestive tract of animals (Mertens, 2002). Cellulose and hemicelluloses constitute 15–70% of most ruminant diets. These polymers make up the bulk of the plant cell wall and are insoluble. The complexity of the structures is the main limiting factor with regards to the breakdown of the polymers (Nagaraja *et al*., 1997; Forsberg *et al*., 2000).

The main factors influencing fibre digestion include animal characteristics, diet composition and structure of the fibre, physio-chemical properties of the rumen environment, physiology of fibrolytic microorganisms and microbial interactions. Differences in physico-chemical composition of the feedstuffs are likely to influence the colonization process by the rumen microbial populations and subsequently the efficiency of fibre digestion (Chaucheyras-Durand *et al*., 2012, 2016). This was also proven to be correct in a study by Noel *et al*. (2017).

Supplementation of live yeast products have also been investigated as a method of increasing fibre degradation in the rumen and positive responses have been reported in studies where high starch diets were fed (Ding *et al*. 2014) and several mechanisms have been suggested to account for such a response (Wallace, 1994). High starch diets can induce ruminal acidosis with detrimental impacts on rumen microbiota balance (Fernando *et al*. 2010; Petri *et al*. 2013), and in that case live yeasts may indirectly promote microbial fibre degradation by stabilizing the rumen pH (Chaucheyras-Durand *et al*. 2008; Marden *et al*. 2008).

It appears that the main mechanism by which *Saccharomyces cerevisiae* supplementation improves digestion of fibre is by promoting substrate colonisation by rumen bacteria and fungi, and also significant increases the total number of bacteria in the rumen, mainly the fibrolytic bacteria (Newbold *et al.,* 1995; Chaucheyras-Durand and Fonty, 2001; Mosoni *et al*., 2007). As mentioned previously, microbial stimulation will also be one of the mechanisms. By increasing nutrient and vitamin supply to the fibrolytic populations within their microhabitat, a healthy balance in rumen microbiota exists which leads to an environment fit for efficient fibre digestion.

Chaucheyras Durand *et al* (2012) reported that feedstuffs with highest levels of lignin and thereby with less easily accessible digestible carbohydrates were better degraded in the presence of live yeast, suggesting a particularly marked impact on the microbial breakdown of ligninpolysaccharide linkages. Such effects could explain the improvement in ruminal fibre degradation often reported in vivo (Wiedmeier *et al*., 1987, Erasmus *et al*., 1992, Plata *et al*., 1994; Wohlt *et al*., 1998; Chaucheyras-Durand and Fonty, 2001; Marden *et al*., 2008;), although some other authors could not see the same effect (Angeles *et al*., 1998; Corona *et al*., 1999).

Guedes *et al*. (2008) found that live yeast increased fibre digestion by 24% with low-quality maize silages; however, with high-quality silages, there was no improvement in fibre digestion. The authors concluded that initial digestibility of silages, in essence the feed quality, and also the level of live yeast inclusion would affect animal response.

In summary, the mechanisms underlying these positive effects on fibre digestibility are linked to the metabolic activities of the live yeast. The microbial stimulation, pH regulation and oxygen scavenging all create a more favourable environment for better fibre digestion. The supplemented live yeast organisms also elicit a mechanical breakdown of the lignin barrier that enables fibre digesting bacteria to attach and digest the fibre faster and more efficiently. This should lead to increased feed efficiency and optimised animal health.

2.2.3.4 Fermentation parameters

2.2.3.4.1 Volatile Fatty Acid concentrations and ratios

The addition of *Saccharomyces cerevisiae* to ruminant animals has resulted in increased number of fibre digesting bacteria (Fonty & Chaucheyras-Durand, 2006). A reasonable assumption would be that the change in microbial population would lead to changes in the end products of rumen fermentation. Increased acetenogenesis from hydrogen should result in increased acetate to propionate ratio when live yeast is

supplemented (Chaucheyras *et al*., 1995a). This theory is based upon the assumption of greater fibre digestibility due to increased fibrolytic bacterial numbers when cows are supplemented with live yeast products (Wiedmeier *et al*., 1987; Wohlt *et al*., 1998).

However, this theory is not yet backed by consistent results in live yeast based trials. Responses to ruminal VFA concentrations varies among authors (Erasmus *et al*., 1992; Erasmus *et al*., 2005; Doreau and Jouany, 1998; Piva *et al*., 1993; Wiedmeier *et al*., 1987; Williams *et al*., 1991) and no clear conclusion can be made as to what effect live yeast would have when used in dairy cattle diets. A study by Doreau and Jouany (1998) found no effect on fermentation profile when using the same strain as used in our study. The same conclusion was made by Bitencourt *et al*. (2011) also using the same strain and dose. Supplementing a live yeast culture also had no effect on VFA concentrations in other studies (Putnam *et al*., 1997; Biricik and Yavuz., 2001 and Alshaikh *et al*., 2002). In contrast, supplementation of live yeast increased concentrations of VFA and altered the ratios in some studies (Brydl *et al*., 1995; Doreau and Jouany, 1998; Pestevsek *et al*., 1998; Sullivan and Martin, 1999; Kamra *et al*., 2002)

The strain of live yeast used plays a major role in the ability to cause changes in ruminal fermentation patterns (Newbold *et al*., 1995). Other authors suggest the response depends on the qualitative and quantitative composition of the experimental diet (Vrzgula *et al*., 1990; Bíreš *et al*., 2000).

2.2.3.4.2 Ammonia nitrogen concentrations

Over the past few years animal production has come under the spotlight with regards to the environmental impact of intensive animal production. Excretion of excess nitrogen is a concern with regards to global warming. This sparks great interest in finding ways to increase the efficiency of nitrogen use in animal production systems.

The current parameter used to assess the effect of additives on efficiency of nitrogen metabolism is ammonia nitrogen concentration in the rumen fluid. This parameter can be highly variable and depend on many diet and animal related factors (Chaucheyras-Durand *et al*., 2008)

Some research suggests that live yeast supplementation can decrease ammonia concentration (Chaucheyras-Durand and Fonty., 2001; 2002; Erasmus *et al*., 1992; Kumar *et al*., 1994), increase bacterial nitrogen production, and increase efficiency of microbial protein synthesis (Moya *et al*., 2007). This could be due to live yeast inhibiting proteolytic bacteria (Chaucheyras-Durand *et al*., 2008) and increased efficiency in the conversion of ammonia to microbial protein that supports the theory of microbial stimulation (Williams and Newbold,

1990). However, many authors found no effect of live yeast supplementation on ammonia concentrations (Enjalbert at al., 1999; Newbold *et al*., 1996; Thrune *et al*., 2009; Yoon and Stern., 1996). The different outcomes underline the fact that there are many biotic and abiotic factors that may play a role in the effect of live yeast supplementation on ammonia concentrations in the rumen and that more research is needed to understand the concept better.

2.2.4 Effect of yeast supplementation on performance

Improved performance due to live yeast supplementation can be regarded as a secondary effect. The primary or direct effect of live yeast supplementation is increasing efficiency and altering fermentation as discussed earlier in the chapter. Studies over the last 3 decades have shown variable results when comparing dairy cow performance parameters. However, there are sufficient studies that reported positive effects on performance to be able to conclude that live yeast can increase performance, but the specific environment in which you can expect responses is still an area to be clearly defined. Factors influencing efficiency of live yeast supplementation include, but are not limited to diet, animal physiology, yeast strain and environment.

Increased dry matter intake (DMI) has been observed in some cases (Ali-Haimoud-Lekhal *et al*., 1999; Desnoyers *et al*., 2009; Erasmus *et al*., 1992; Wohlt *et al*., 1998), while a meta-analysis reported no effect (Sauvant *et al*., 2004; De Ondarza *et al*., 2010) on dry matter intake when dairy cows were supplemented with live yeast. The observed increase in DMI can be linked to the increased fibre digestion (Marden *et al*., 2008) based on findings from Oba and Allen (1999) showing clear correlation between NDF digestibility and DMI. The increase in fibre digestibility and DMI leads to higher milk production (Oba and Allen., 1999). In the study done by Ali-Haimoud-Lekhal *et al* (1999), higher feed intakes also contributed to an increase in yield in cows supplemented with live yeast.

A meta-analysis by De Ondarza *et al* (2010) reviewed the effect of yeast supplementation to dairy cows on production parameters including milk yield, milk component content and yield and also feed efficiency. The study used data from 14 research trials and found that live yeast improved ($P<0.0001$) 3.5% fat corrected milk yield (35.5 vs. 34.6kg/d for live yeast and control respectively) and feed efficiency (kg 3.5% FCM/kg DMI) was improved ($P<0.001$) with 1.75 for live yeast vs 1.70 for the control. The author suggested that the increased milk yield was a consequence of overall improved rumen function.

Considering the bulk of research on the effect of yeast on dairy cows, the results tend to be inconclusive (Poppy *et al*., 2012). Significant effects on milk production have been observed by some (Harrison *et al*., 1988; Hippen *et al*., 2007; Lehloenya *et al*., 2008; Ramsing *et al*., 2009), while others only noted tendencies towards increased production (Williams *et al*., 1999; Dann *et al*., 2000; Wang *et al*., 2001), or no differences were reported (Robinson, 1997; Schingoethe *et al*., 2004). General consensus among the researchers is that live yeast does not have an effect or have very little effect on milk composition, with the exception of milk fat content that is generally increased by live yeast supplementation (Ali-Haimoud-Lekhal *et al*., 1999; Desnoyers *et al*., 2009; Robinson, 2002; Sauvant *et al*., 2004).

2.3 Bottom line

Many different responses, as well as no response have been observed when cows were supplemented with yeast products and the inconsistent responses are probably due to differing trial conditions and designs, different strains of yeast used and different diets fed. The influence of the yeast on rumen fermentation decreases with decreasing level of concentrates and also with decreasing dry matter intakes (Gurbuz 2007; Desnoyers *et al*. 2009). The same diminishing effect of the yeast was observed in later stages of lactation (Shaver & Garrett., 1997; Moallem *et al*., 2009), with other feed additives (sodium bicarbonate; Swartz *et al*., 1994; Marden *et al*., 2008; Ferraretto *et al*., 2012) and with different management strategies (Bach *et al*., 2007; Chaucheyras-Durand *et al*., 2008; AlZahal *et al*., 2014; De Vries & Chevaux., 2014). To accurately predict the effect of yeast supplementation, more research is needed. It is difficult to directly correlate a single production response to a single mode of action. Observed results may be due to a collective effort and combination of direct and indirect mechanisms. The effects of differing qualities of forage in the diet must be evaluated to predict production response of yeast supplementation. This trial was developed to demonstrate the effect of live yeast supplementation on forage digestibility of different roughages, that also differed in quality within roughages.

Apart from inconsistencies in results between published research, there are still areas where limited information is available. One such area is the supplementation time needed before a response to live yeast can be expected. Another area is the effect of live yeast on NDFom degradability of high, medium and for low quality forages, within the same forage species. The latter is the topic of this study. In the next chapter the materials and methods will be discussed followed by the results and discussion and ended off with a conclusion chapter.

CHAPTER 3

MATERIALS AND METHODS

This research study was approved by the ethics committee of the University of Pretoria, project code: EC032-15.

3.1 Location

This study was conducted at the Outeniqua Research Farm (Western Cape Department of Agriculture) near George (33° 58' 38'' S, 22° 25' 16'' E) in the Western Cape of South Africa. The altitude of the farm is 201m above sea level with a temperate coastal climate. The average rainfall is 725mm per year. The average minimum and maximum temperatures, range between 7-15°C and 18-25°C, respectively (ARC-ISCW, 2010).

3.2 Duration

The trial was done over a 10-week period. There was a 10-day feed adaptation period (from pastures to a TMR) and 2 experimental periods each four weeks long. Each experimental period consisted of a 21-day adaptation period and an 8-day sampling period.

3.3 Animals

Eight rumen cannulated, lactating Jersey cows, differing from early to late lactation were used (Table 3.1). They were all disease free and healthy and were selected based on days-in-milk and parity.

Table 3.1 Description of the cows used in this study.

Cow nr.	Cow Name	Bodyweight	Body Condition	Lactation nr.	Days in milk
1	DORA 159	443	2.3	5	162
2	FIREFLY 52	381	2.2	7	115
3	DORA 187	412	2	3	66
4	MARTA 232	410	2.5	2	63
5	LIN 42	411	2.5	3	219
6	DORA 107	465	3	10	513
7	BERTA 132	406	2.3	2	81
8	MARTA 239	376	2	2	71

3.4 Pens

The cows were kept in eight individual camps of 6m x 6m with water troughs and feeders (Figure 3.1). The ground surface was covered with wood chips.

Figure 3.1 Illustration of the pens used in this study.

3.5 Experimental design and treatments

The experimental design was a crossover design with two four-week experimental periods. The eight cows were blocked into 4 pairs based on days-in-milk and parity, and each pair was randomly assigned to one of the squares in Table 3.2. Blocking cows in squares 1 to 4 allowed any differences in treatment effect due to production level or physiological status of cows to be equalized.

Table 3.2 Experimental design and assignment of different treatments to each cow during each experimental period

Square – Group (cow)	Pre-Trial	Period 1	Period 2
1 – A (Cow 1)	Control	Control	Treatment
1 – B (Cow 2)	Control	Treatment	Control
2 – A (Cow 3)	Control	Control	Treatment
2 – B (Cow 4)	Control	Treatment	Control
3 – A (Cow 5)	Control	Control	Treatment
3 – B (Cow 6)	Control	Treatment	Control
4 – A (Cow 7)	Control	Control	Treatment
4 – B (Cow 8)	Control	Treatment	Control
Week:	1	2,3,4,5	6,7,8,9

Before the start of the two experimental periods there was an adaptation phase of one week (week 1) where all cows received the control diet. Period 1 started with 3 weeks adaptation (week 2, 3 and 4) to the experimental diets before the in-situ incubations and rumen sampling commenced (week 5). Period 2 started directly after week 5, with 3 weeks adaptation (week 6, 7, and 8) to the experimental diet followed by sampling and in situ incubations during week 9.

All cows received the same diet (Table 3.3) throughout the trial. There were two different treatments, a control and a yeast treatment. The control diet was a TMR consisting of a roughage fraction (lucerne and *Eragrostis curvula* hay) and a concentrate fraction. The concentrates (all ingredients in Table 3.3, except the lucerne and *Eragrostis curvula* hay) have been pelleted by Nova Feeds George (Industrial area, George, Western Cape, South Africa). The NDS program was used to formulate the TMR using the raw material database of Nova feeds.

Table 3.3 Ingredient composition of the control diet.

Ingredient	**g/kg DM**
Lucerne hay	334
Eragrostis curvula hay	155
Maize	300
Hominy chop	70
Wheat bran	45
Soya oilcake	80
Urea	2
Mono calcium phosphate	3
$CaCO_3$	2
Salt	5
MgO	2
Premix	1

Chemical composition[1]	
DM (g/kg)	887.5
Starch (g/kg DM)	277.4
CP (g/kg DM)	164.4
NPN (g/kg DM)	11.7
Sol CP (g/kg DM)	73.2
NDFom (g/kg DM)	327.1
ADL (g/kg DM)	58.6
EE (g/kg DM)	35.7
Ash (g/kg DM)	72.7
Ca (g/kg DM)	7.0
P (g/kg DM)	3.5
Ca : P	2.1

[1]DM = Dry Matter; CP = Crude Protein; NDFom = Neutral Detergent Fibre determined on Organic Matter basis; EE = Ether Extract; Ca = Calcium; P = Phosphorus

The premix was a standard dairy premix and was added according to the manufacturer's specifications (1kg per ton of feed). The composition of the premix is specified in Table 3.5.

Table 3.4 Composition of the premix based on one ton of feed

Ingredient	g/ton TMR
Vitamin E	10
Vitamin A	6
Vitamin B12	0.02
Manganese	86
Zinc	120
Copper	30
Iodine	2
Selenium	0.3
Ferrous	80
Cobalt	1.5
Magnesium	250

The cows were fed ad lib and received feed twice daily at 08:30 and 16:30 just after milking. Before the 08:30 feeding, the residual feed was collected and weighed back to adjust the next feeding accordingly. Feed allocations were adjusted to be approximately 5 - 10% more than the previous day's intake.

3.6 Yeast

The live yeast that was used in the trial was supplied by Lallemand (19 rue des Briquetiers, 31702 Blagnac cedex, France). The product is commercially known as Levucell SC20. It is a *Saccharomyces cerevisiae* strain with the registration number CNCM I-1077, registered at the Pasteur Institute collection in France. The recommended dose is 0.5g/cow per day which results in a minimum dose of $1x10^{10}$ CFU's per day of live yeast.

The live yeast was administered directly into the rumen via the rumen cannula to the cows in the yeast treatment group. Firstly the 0.5g of live yeast was weighed off, then dissolved into 20ml of distilled water. The live yeast solution was then injected into a small hole in the rumen cannula and the hole was then plugged with a plastic screw to avoid oxygen entering the rumen. Control cows were handled the same by administering 20ml of distilled water as a placebo. By using this application method, we ensured that each cow received the correct dose of live yeast throughout the duration of the trial.

3.7 Mixing of feed

A new batch of feed was mixed on a weekly basis using a Seko Samurai 5 mixing wagon (90 Via Gorizia, 35010 Curtarolo, Italy) with electronic mixing capacity. The *Eragrostis curvula* hay was weighed off and then turned in the mixer for 15 minutes to break the hay into smaller pieces. The lucerne hay was then added and the combination was mixed for an additional 5 minutes. After the hay was chopped to a satisfactory length, the concentrate was added and mixed thoroughly for another 10 minutes. The homogenous TMR mix was then weighed off into 20kg feed bags and stored for daily use.

3.8 Sampling

3.8.1 Milking and Milk samples

The cows were milked twice daily at 08:00 and 16:30 respectively. Milk production was recorded of each milking using the Waikato milking system. Milk samples were taken once a week (every Tuesday) for three consecutive weeks (Two weeks prior to, and including sampling week) of each of the two periods. Two samples were taken, one in the afternoon and one in the morning milking to get a representative sample of both milking sessions. Milk samples were sent to Deltamune (Oudshoorn, Western Cape) for analysis. Analysis included fat percentage, protein percentage, milk urea nitrogen (MUN), lactose percentage and somatic cell count.

3.8.2 Feed samples

3.8.2.1 Roughage samples

Each bale of lucerne and *Eragrostis curvula* were sampled using a core sampler. All lucerne samples were pooled into one composite sample representing the lucerne used for the TMR fed for the duration of the trial. The same was done with the *Eragrostis curvula* bales and the composite samples were sealed in an air tight container and sent to Nutrilab (University of Pretoria, Dept. of Animal and Wildlife Sciences, Hatfield, Pretoria, 0083) for CP, NDFom, ADF, and moisture analysis.

3.8.2.2 Concentrate samples

Every week at the time of feed mixing, a grab sample of the concentrate used for the TMR was stored in an air tight container. After the trial, all concentrate samples were pooled. The pooled sample was sent to Nutrilab for a full proximate analysis. Detail of the analysis is described in section 3.9.

3.8.2.3 TMR samples

Weekly TMR samples were taken just after mixing and stored in a freezer. After the trial, all samples were pooled and a sub-sample was taken, using the quartering technique, to represent the basal diet fed to all 8 cows during the trial. The sub-sample was sent to Nutrilab just after the trial for chemical analysis as described in section 3.9.

3.8.2.4 Penn State Forage Particle Separator

After the TMR sub-sample was taken from the composite TMR sample mentioned in 3.8.2.3, the remaining sample was sieved through the Pen State Forage Particle Separator (PSFPS) to evaluate the different size fractions of the TMR (Heinrichs and Kononoff, 2002).

The PSFPS consists of 3 sieves and a bottom pan. The top sieve has a pore size of 1.91cm, the second sieve 0.79cm and the third sieve 0.13cm. The pores in the 3rd sieve are square, so largest opening is the diagonal, which is 0.18cm. This is the reason the largest particles that can pass through the lower sieve are 18mm in length (Heinrichs and Kononoff, 2002).

The 4 plastic boxes (3 sieves and bottom pan) were stacked and then the forage is added to the top sieve. Jud Heinrichs and Paul Kononoff (Department of Dairy and Animal Science, Pennsylvania State University, 324 Henning Building, University Park, PA 16802) recommends approximately 3 x 500ml jugs full of TMR to be added to the separator. Then on a flat surface, the sieves were shaken in one direction 5 times then the separator box was turned one-quarter turn. There should be no vertical motion during shaking. This process was repeated 7 times, for a total of 8 sets or 40 shakes, rotating the separator after each set of 5 shakes. After shaking was complete, the material on each sieve and on the bottom pan was weighed. Note that the particle size of the material on the upper sieve was greater than 1.91cm long, material on the middle sieve is between 0.78cm and 1.91cm, material on the third sieve was between 0.18cm and 0.78cm, and material in the bottom pan was less than 0.18cm. The weight of each sieve's content was recorded and then expressed as a percentage of the total weight added.

3.8.3 Rumen fluid samples

After the 3-week adaption phase of each period, an 8-day sampling period commenced. On the 1st day of each sampling period, rumen fluid was collected at 09:00; 12:00; 15:00; 18:00 and 21:00. This was done by removing rumen contents via the cannula and pressing the fluid into a bottle by hand (Figure 3.2).

Directly after the fluid was obtained, the pH and redox potential was measured and recorded using handheld meters for each (pH meter: WTW pH 340i pH meter; Redox potential meter: Lutron ORP-213 Pen type ORP meter) (Figure 3.3). In figure 4.4 is illustrated how the rumen fluid was strained through cheesecloth in order to remove solid particles. The bottles were then closed until all the animals were sampled and then taken to the lab. At the lab, the fluid was filtered through four layers of cheesecloth and divided into two sub-samples of 50ml each (Figure 3.4) and immediately frozen at -20℃. The bottles were clearly marked to identify exactly which cow, what period, treatment and sampling time it was obtained from. The two sub-samples were analysed for NH_3-N and volatile fatty acid concentrations.

Figure 3.2 Pressing rumen fluid from the rumen contents.

Figure 3.3 Measuring pH and Redox potential.

Figure 3.4 Straining the rumen fluid through the cheesecloth and making sub-samples.

3.8.4 *In sacco* study

The *in sacco* technique as developed originally by Ørskov *et al*. (1980) was used during this study. The *in sacco* ruminal degradation of four different forages (lucerne hay, kikuyu pasture, rye grass pasture and *Eragrostis curvula* hay) and 3 different qualities within each forage (High, Medium, and Low), as shown in Table 3.6 were determined. There were therefore 12 test forages (4 forages and 3 qualities of each). The lucerne hay was obtained from Lubern Feeds (Hartswater, Northern Cape, 8570), the kikuyu and rye grass pasture were cut at an early, mid and late stage of growth at the experimental farm in George where the trial was conducted and the Eragrostis hay was sampled from a farm near Middelburg (Mpumalanga, South Africa).

The kikuyu and rye grass were oven dried at 50℃ for 48h before milling. The lucerne and Eragrostis hay were dry enough to be milled. All 12 test forages were then milled using a Retsch ultra-centrifugal mill with a 4 mm screen at Nutrilab (University of Pretoria).

For the *in sacco* study there was 3kg of milled forage available for each of the 12 samples. The Dacron bags (Ankom) that were used was 10 x 20cm with a pore size of 53 microns. Bags were allocated an individual number and dried for 48h at 50℃. The bags were then weighed to the accuracy of 3 decimals using a Sartorius L420P scale and the weight recorded. Approximately 8g of forage was weighed and placed into the bags and sealed using a cable tie. After the cable tie the total weight was recorded and then placed into an air tight plastic bag until used for incubation. The method of incubation is described by Cruywagen (2006) and all the different forage samples were incubated for 12h, 24h and 36h respectively.

Each forage type was incubated separately. The bags were incubated for 36 hours, 3 replicate bags per quality (3 x High, 3 x medium and 3 x low quality) (n = 9), were randomly selected and separated into two stockings, one containing four bags, the other containing five. Three bags per quality were incubated and pooled after weigh back, in order to ensure enough material was left after 36h for lab analysis. For the 12 and 24h incubations, 2 replicate bags were enough to ensure sufficient material for analysis. The six bags of forage (2 bags x 3 qualities) that was used for the 24h incubation, was added in random order into one stocking. The same was done with the bags used for the 12h incubation.

The two stockings containing the bags destined for the 36h incubation, were attached to the cannula plug at 9pm on the 1st day of the sampling week. Twelve hours later the bags

destined for the 24h incubation were attached to the cannula plug and inserted into the rumen. The same routine was followed for the stocking used for the 12h incubation. The stockings were evidently added in reverse order; first the 36h incubation followed by the 24 and 12h incubations respectively. The bags were then removed from the rumen, all at once at 09:00 am in the morning. The samples were then taken to the lab on the farm were excess rumen contents on the bag surface was removed by rinsing under running tap water until a clear stream of water ran from the bags. Excess water was drained, and the samples were placed in the freezer at -20℃ to stop any microbial degradation. Twelve hours after the bags were removed, the same incubation procedure followed for the next forage type, until all incubations were done.

Once all the incubations were finished, the frozen forage bags were thawed and washed five times for three minutes. This procedure ensured the rumen bacteria was washed out of the bag since its presence can affect chemical analysis of the remaining forage in the bag. The bags were placed on a drying rack to get rid of excess water. After that, the bags were placed in the drying oven for 72 hours at 55℃. Three unincubated bags of each quality of each forage were also washed with the incubated bags to get the zero-hour incubation value. After drying, the bags were then weighed on the same scale as before incubation and values were recorded. The full process was repeated for the second incubation period. In total 1396 bags were used.

Table 3.5 Protein and NDFom analysis (DM) of the twelve forages used in the *in sacco* study

Forage	**Quality**	**Crude Protein %**	**NDFom %**
	High	23.14	40.70
Lucerne	Medium	20.30	43.20
	Low	22.21	49.54
	High	23.14	61.10
Kikuyu	Medium	21.79	60.62
	Low	19.56	61.29
	High	24.80	44.89
Rye Grass	Medium	19.83	50.34
	Low	19.72	51.67
	High	10.60	75.76
Eragrostis curvula	Medium	7.77	78.52
	Low	5.84	79.82

3.9 Laboratory analysis

Total mixed rations and raw material samples were sent to Nutrilab (Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria) for analysis. Before analysis the samples were milled with a Retsch ultra-centrifugal mill using a 1mm screen. The following procedures were used during analysis: DM (AOAC, 2000, procedure 934.01), Ash (AOAC 2000, procedure 942.05), CP (Leco TruMac N determinator, model FP-428, Leco Corporation, St Joseph, MI, USA was used to determine N (CP calculated from N x 6.25 (AOAC, 2000, procedure 968.06))), NDF (Ankom 2000 fibre analyser, Ankom Technologies, Macedon, NY, USA; Robertson and Van Soest, 1981), EE (crude fat; AOAC, 2000, procedure 920.39), Ca (AOAC, 2000, procedure 965.09), P (AOAC, 2000, procedure 965.17) and Starch (MaCrae and Armstrong, 1968; Faichney and White, 1983; AOAC, 1984).

3.9.1 *In sacco* residues

The twelve forages used in the *in sacco* study were analysed for DM, CP and NDFom before the trial to establish the basal values used in the determination of DM disappearance and NDFom disappearance. The bags were then put into small plastic bottles for analysis. Samples that contained the same forage, was incubated for the same amount of hours in the same period and in the same cow, were pooled together for analysis. There were eventually 600 bottles with samples. Eight cows, twelve forages, two periods, and three incubation intervals (12h, 24h and 36h). Added to that were twelve pooled samples for the twelve zero hour incubation values, for each of the two sampling periods. All *in sacco* samples were analysed for DM and NDFom.

3.9.2 Rumen ammonia nitrogen and volatile fatty acids

One hundred and sixty bottles (50ml) of rumen fluid was collected (Eighty for NH_3-N and eighty for VFA). Ammonia nitrogen was analysed at Nutrilab (Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria) using Catalyzed phenol-hypochlorite and ninhydrin colorimetric procedures (Broderick and Kang, 1980). The ruminal VFA concentrations were analysed at the University of the Free State (Bloemfontein) using a Gas Chromatographic method (Webb, 1994).

3.10 Statistical analysis

Data were analysed statistically as a crossover design with the GLM model (Statistical Analysis system, 2017) for the average effects over time. Repeated measures Analysis of variance with the GLM procedure were used for repeated measures. Means and standard error were calculated and significance of difference ($P<0.05$) and tendencies for differences ($P< 0.10$) between means was determined by Fischer's test (Samuels, 1989).

The linear model used is described by the following equation:

$$Yij = \mu + Ti + Cj + Pk + eijk$$

Where Y = variable studied during the period
μ = overall mean of the population
T = effect of the ith treatment
C = effect of the jth cow
Pk = Effect of the kth period
e = error associated with each Y

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Feed analysis

The chemical analysis of the TMR is presented in Table 4.1. All cows received the same basal diet; the only difference between the two treatments (Control vs Live yeast) was the addition of 0.5g of live yeast directly via the cannula, once daily.

Table 4.1 Chemical analysis of the control TMR used during the trial.

Nutrient[1]	
DM (g/kg)	924
Starch (g/kg DM)	228
CP (g/kg DM)	135
NDFom (g/kg DM)	392
EE (g/kg DM)	31.9
Ash (g/kg DM)	72
Ca (g/kg DM)	3.9
P (g/kg DM)	3.1
Ca:P	1.2:1

[1]DM = Dry Matter; CP = Crude Protein; NDFom = Neutral Detergent Fibre determined on Organic Matter basis; EE = Ether Extract; Ca = Calcium; P = Phosphorus

4.1.1 Total mixed ration

Although the nutrient content (Starch, CP, EE) of the analysed TMR are somewhat lower than the feed database calculated analysis (Table 3.4) it did not affect the outcome of the results since the criteria was that an average local TMR fed to mid-lactation cows should be the control diet. In addition, the focus of the study was NDF degradability and rumen fermentation parameters and not production performance as such. The analysis of the roughages and concentrate pellets are shown in Table 4.2. The chemical analysis of the lucerne hay and the *Eragrostis curvula* hay are in agreement with other published results (Meissner and Paulsmeier, 1995; Codron *et al*., 2007)

Table 4.2 Chemical analysis of the lucerne hay, Eragrostis hay and concentrate pellets that were used to mix the TMR for the duration of the trial.

	Ingredient		
Parameters[1]	**Lucerne hay**	***Eragrostis curvula* hay**	**Concentrate Pellets**
CP (g/kg DM)	177.40	57.20	157.80
NDFom (g/kg DM)	487.70	779.80	133.70
Starch (g/kg DM)	22.80	26.80	541.30
Ca (g/kg DM)	10.00	1.60	3.60
P (g/kg DM)	1.72	1.20	5.10
Ca:P	5.81	1.33	0.71

[1]CP = Crude Protein; Ca = Calcium; P = Phosphorus; NDFom = Neutral Detergent Fibre determined on Organic Matter basis

4.1.2 TMR particle size analysis

The particle size distribution was measured using a Penn State Forage Particle Separator. Table 4.3 contains the average percentages of particle sizes of the TMR used in this trial. General recommendation by Heinrichs and Kononoff (2002) suggest the top sieve to contain a maximum of 8% of the total weight sieved, the second sieve 30-50%, the third sieve also 30-50% and the bottom pan a maximum of 20%. Our results suggest the TMR was relatively well mixed, except for the first sieve containing too many particles longer than 1.91cm. This could make the TMR more prone to sorting of the TMR by the cows during feedings. In our study sorting was minimal since the cows left very little orts.

Table 4.3 Average percentage particle size distribution of the TMR measured by the Penn state forage particle separator.

Sieve	**Percentage distribution**	**Target**
First	17%	≤8%
Second	12%	30-50%
Third	54%	30-50%
Bottom pan	18%	≤20%

4.2 Milk Production and cow performance data

The milk production and milk composition data of cows that received either a control diet or a control diet supplemented with the live yeast are shown in Table 4.4.

Table 4.4 Milk production and milk composition of cows receiving a control or yeast supplemented diet.

	Treatments[1]		
Parameters	**Control**	**Live yeast**	**SEM[2]**
Milk Production (kg/day)	18.78	18.44	0.51
Fat %	5.47	5.13	0.15
Protein %	3.93[a]	3.84[b]	0.02
Lactose %	4.69	4.72	0.04
MUN (mg/dL)	12.25	13.04	0.52

[1]Live yeast: Cows received 0.5g of live yeast per cow per day directly through the cannula

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd] Means the same row without a common superscript tends to differ (P<0.10)

4.2.1 Milk yield

No treatment effect was observed with regards to daily milk production for the sampling weeks (P>0.05). This contradicts the findings of a meta-analysis done by De Ondarza *et al*. (2010) where 14 different studies were included and a mean increase of 1.15kg/day was found in cows supplemented with the same strain of live yeast that was used in this study. The average number of cows per treatment (n = 45) and average days on treatment (n = 63) were far greater than in our study and could have had an impact on the significance of the dataset. Poppy *et al* (2012) mentioned the lack of sample size as a reason for lower statistical power and hence the inability to pick up any significance. Various other authors (Harrison *et al*., 1988; Shaver *et al*., 1997; Ali-Haimoud-Lekhal *et al*.,1999; Hippen *et al*., 2007; Lehloenya *et al*., 2008; Ramsing *et al*., 2009; and Salvati *et al*., 2015) also found a significant increase in milk production when supplementing live yeast products to dairy cows. A comprehensive meta-analysis by Desnoyers *et al*., (2009) which included 110 research papers, also found a significant increase in milk yield (+1.2g/kg BW) as a result of live yeast supplementation. However there are studies that concurred with our finding that

live yeast supplementation had little or no effect on milk production (Robinson, 1997; Schingoethe *et al*., 2004).

4.2.2 Milk Composition

In the meta-analysis by Desnoyers *et al* (2009) the authors found live yeast to have very little influence milk composition. Only milk fat content tended to be increased by live yeast supplementation. This correlates with other studies showing very little or inconsistent effects of live yeast supplementation on milk composition (Ali-Haimoud-Lekhal *et al*., 1999; Robinson, 2002; Sauvant *et al*., 2004).

4.2.2.1 Milk Fat

The milk fat percentages obtained in this study showed no evidence of live yeast being able to increase milk fat percentage ($P>0.05$). De Ondarza (2010) also found numerical decreases in milk fat percentages, but overall increase in milk yield due to a milk yield response, which was not the case with this study. It remains uncertain whether the lack of a pH challenge in the rumen played a part in yeast having no effect on milk fat. The absence of a ruminal pH challenge will be discussed later in the chapter.

4.2.2.2 Milk Protein

The only significant effect that live yeast had on milk composition was on milk protein content. The protein percentage of milk was decreased ($P<0.05$) and contradicts the findings of Poppy *et al*., (2012). However Desnoyers *et al* (2009) found no influence of yeast on milk protein percentage. Shaver and Garrett (1997) also found a slight reduction in milk protein percentage and suggested that a decrease in milk protein percentage could be due to the dilution effect of an increase in milk yield; however this was not the case in our study. It remains unclear as to why the milk protein percentage was reduced in this study.

4.2.2.3 Milk lactose

There was no response in milk lactose content between the two treatments ($P>0.05$). No literature indicated any lactose response when cows received live yeast supplementation. This supports the statement by Sutton (1989) that dietary changes usually don't affect milk lactose content. It is in the nature of the process of milk let down, that the amount of lactose molecules will be in proportion to the amount of water molecules. Lactose is a function of milk yield and concentration of lactose should be relatively constant.

4.2.2.4 Milk Urea Nitrogen (MUN)

In this study, live yeast treatment did not affect the milk urea nitrogen concentrations (P>0.05). Chaucheyras-Durand *et al* (2008) also found no live yeast-treatment effect on MUN concentrations. However, a study by Zhu *et al* (2016) showed a linear response to live yeast supplementation. Milk urea nitrogen concentrations decreased (P<0.01) as live yeast dose increased. It is important to mention that in the study by Zhu *et al* (2016), the live yeast product was a fermentation product, and not a live yeast. However in the same study, it was mentioned that there was no difference in conversion of dietary nitrogen to milk nitrogen. The suggestion is that the lower MUN values observed by Zhu *et al* (2016) can indicate higher amino acid utilization for productive uses.

4.3 Rumen fermentation parameters

4.3.1 Rumen pH

The pH was measured every three hours, starting 9am and the last measurement at 9pm, using a handheld meter. The mean values are presented in table 4.5. No differences (P>0.05) were observed between the two treatments. Figure 4.1 shows the mean pH values over the sampling period of both treatments. Although it was not intended, the diet did not pose much of a pH challenge in the rumen. The lowest pH was observed at 21:00, measuring 5.60 for the live yeast group and 5.65 for the control group. The highest pH was observed at 09:00 for both control and live yeast groups. This is in line with Guedes *et al*., (2008) stating that the lowest pH should be around four hours after the afternoon feeding, and at its highest just before the morning feeding.

In contrast to our findings, numerous other studies concluded that live yeast supplementation stabilised, increased rumen pH, or even reduced variation (Michalet-Doreau and Morand, 1996; Nocek *et al*., 2002; Bach *et al*., 2007; Moya *et al*., 2007; Thrune *et al*., 2007; Chaucheyras Durand *et al*., 2008 and Guedes *et al*., 2008). A review by Robinson (2002; 14 experiments) and a meta-analysis by Desnoyers *et al*., (2009; 157 experiments) also found live yeast supplementation to increase ruminal pH significantly.

However this is not the overall trend. After reviewing 40 research papers, Lescoat *et al*. (2000) failed to find any effect of live yeast supplementation on rumen pH. In addition, Sauvant *et al*. (2008) reviewed 78 experiments and did not observe any influence of live yeast supplementation on rumen pH. It still remains unclear as to why the live yeast would exert a significant effect under some scenarios, but not in others. The effects of live yeast

supplementation on pH are modest since yeast cannot prevent acute acidosis if the rumen is overloaded with starch from a diet rich in fermentable carbohydrates (Dawson and Hopkins 1991; Aslan *et al*. 1995). Thrune *et al*. (2009) speculated that stage of lactation, source/type of live yeast and dietary factors could be the main sources of variation in results. It would be worthwhile to conduct a meta-analysis where not all yeast products are included but only live yeast products with guaranteed CFU's.

Table 4.5 Effect of Live yeast supplementation on pH of rumen fluid of cows fed a total mixed ration (n=8)

	Treatments[1]		
Time	**Control**	**Live yeast**	**SEM[2]**
09:00	6.66	6.71	0.11
12:00	6.21	6.16	0.06
15:00	5.96	5.82	0.05
18:00	5.74	5.78	0.06
21:00	5.65	5.60	0.06
Mean	5.78	5.73	0.05

[1]Live yeast: Cows received 0.5g of live yeast per cow per day directly through the cannula

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd] Means the same row without a common superscript tends to differ (P<0.10)

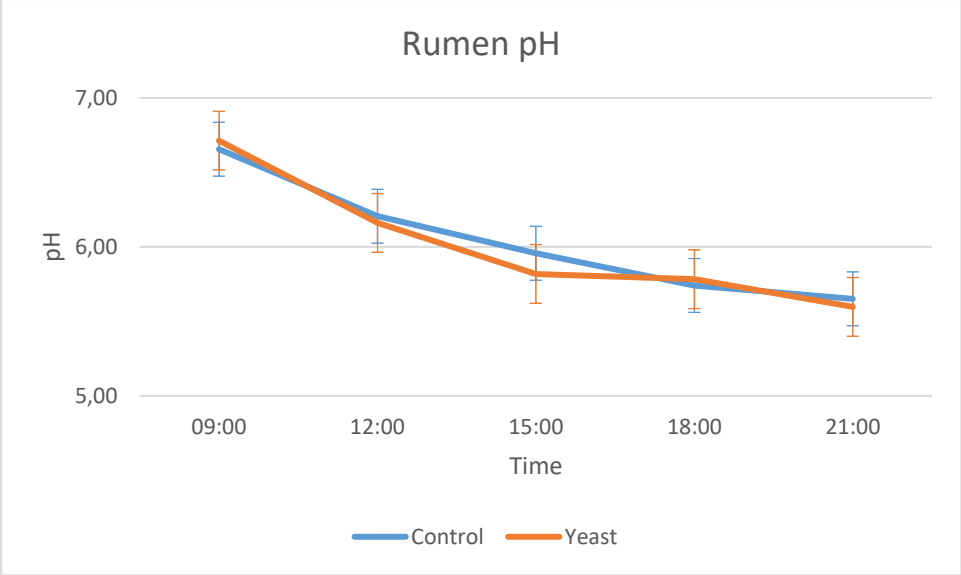

Figure 4.1 The effect of live yeast supplementation on ruminal pH over a 12-hour period measured using a handheld pH meter at 3 hourly intervals

4.3.2 Redox potential

Redox potential was measured at the same times as rumen pH, using a handheld meter. The mean values for each sampling time are shown in table 4.6. The values found in literature differ considerably, and ranges between -150mV and -260mV (Broberg, 1957; Barry *et al*., 1977). The values we found are in range and yeast supplementation had no effect on redox potential (P>0.05). Marden *et al*., (2013) reported that the supplementation of live yeast did reduce redox potential by an average of 20mV (from -176.5mV to -196.5mV). The difference between the trial by Marden *et al*, (2013) and our trial was the method of measuring redox potential. Marden *et al*., (2013) made use of a complete anaerobic system of retrieving rumen fluid, measuring in a thermostatic vessel and then recycling the fluid. We, on the other hand pressed rumen fluid from the rumen content and measured the redox potential from a bottle.

In Fig 4.2 is shown a graph of the mean redox potential values and it follows a pattern very similar to the rumen pH graph. Results from Marden (2013) are in agreement with our data.

Table 4.6 Effect of live yeast supplementation on redox potential of rumen fluid of cows fed a total mixed ration (n = 8)

	Treatments[1]		
Time	**Control**	**Live yeast**	**SEM[2]**
09:00	-287.75	-284.25	20.940
12:00	-214.13	-230.13	8.614
15:00	-193.88	-192.38	9.548
18:00	-208.75	-214.50	7.107
21:00	-175.38	-173.88	5.094
Mean	-192.67	-193.58	4.04

[1]Live yeast: Cows received 0.5g of live yeast per cow per day directly through the cannula

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd] Means the same row without a common superscript tends to differ (P<0.10)

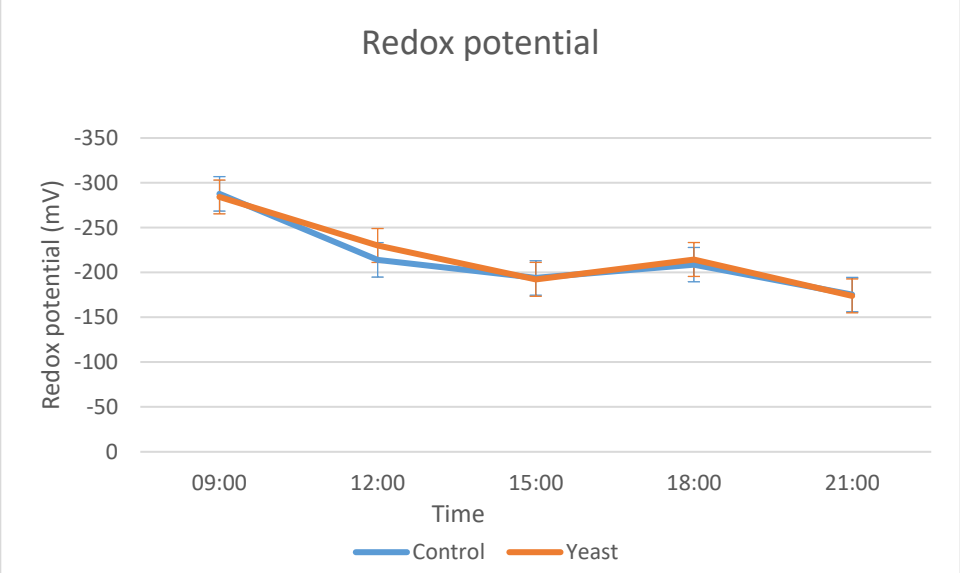

Figure 4.2 The effect of live yeast supplementation on ruminal redox potential over a 12-hour period measured using a handheld meter at an interval of every 3 hours

4.3.3 Volatile fatty acids

In Table 4.7 is shown the mean concentrations of the different VFA's as well as total VFA concentrations (mmol/L) and molar percentages. Rumen fluid was sampled at 3h intervals starting at 09:00 and ending at 21:00 and the mean at the different time intervals are shown in Table 4.8.

Table 4.7 Effect of live yeast supplementation on the concentrations and ratios of different VFA's in the rumen fluid of cows fed a total mixed ration (n = 8)

	Treatments[1]		
Parameters	**Control**	**Live yeast**	**SEM[2]**
Total VFA (mmol/L)	68.08	65.35	3.87
Acetic acid (mmol/L)	47.32	45.39	2.35
Propionic acid (mmol/L)	10.14	10.01	0.92
Butyric acid (mmol/L)	8.85	8.34	0.62
Iso butyric acid (mmol/L)	0.34	0.31	0.02
Valeric acid (mmol/L)	0.78	0.73	0.06
Iso Valeric acid (mmol/L)	0.44	0.40	0.01
VFA Molar%			
Acetic acid %	69.89	69.62	0.69
Propionic acid %	14.66	15.20	0.63
Butyric acid %	12.83	12.72	0.25
Iso butyric acid %	0.52	0.47	0.05
Valeric acid %	1.12	1.10	0.03
Iso Valeric acid %	0.67	0.62	0.05
Acetic:Propionic	4.84	4.65	0.26
NH3-N (mg/dL)	12.45	12.63	0.45

[1]Live yeast: Cows received 0.5g of live yeast per cow per day directly through the cannula

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd] Means the same row without a common superscript tends to differ (P<0.10)

The total VFA concentrations for both treatments are lower than the expected concentrations. Sakkers *et al*., (2012) conducted a trial in the same herd with Jersey cows receiving a similar TMR than the diet fed in our study. The total VFA concentrations from that study ranged from 116 to 139mmol/L with comparable intakes to our study. The obtained values are approx. half of what is normal. There is no obvious explanation for the low values but could be due to sampling method or sample handling during transport to the lab. The molar percentages, however are comparable to what can be expected and is in the same range as results reported by Thrune *et al* (2009) where the same yeast as in our study was supplemented. A study by Doreau and Jouany (1998) found no effect of yeast

supplementation on fermentation profile when using the same yeast strain as used in our study. In support, Bittencourt *et al*. (2011) also using the same strain and dose found no effect of yeast supplementation on ruminal VFA production.

Table 4.8 Effect of live yeast supplementation on total volatile fatty acid concentrations of rumen fluid at different times of the day in cows fed a total mixed ration (n = 8)

	Treatments[1]		
Time	**Control**	**Live yeast**	**SEM[2]**
09:00	53.35	49.75	4.98
12:00	71.82	50.16	11.11
15:00	67.97	68.08	10.74
18:00	75.32	75.45	8.82
21:00	71.95	83.32	7.90
Mean	68.08	65.35	3.87

[1]Live yeast: Cows received 0.5g of live yeast per cow per day directly through the cannula

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd] Means the same row without a common superscript tends to differ (P<0.10)

When considering all yeast products, many researchers reported no effect of live yeast supplementation on total VFA concentrations in the rumen (Dawson *et al*., 1990; Erasmus *et al*., 1992; Carro *et al*., 1992; Piva *et al*., 1993; Plata *et al*., 1994; Yoon and Stern, 1996; Doreau and Jouany, 1998; Enjalbert *et al*., 1999). In addition, the review by Sauvant *et al*. (2004, including 78 experiments) also found no effect on total VFA concentrations. In contrast, findings by other authors (Brydl *et al*., 1995; Doreau and Jouany, 1998; Pestevsek *et al*., 1998; Sullivan and Martin, 1999; Kamra *et al*., 2002; Lescoat *et al*., 2000; Robinson, 2002) reported a significant increase in VFA due to live yeast supplementation. This is also supported by the meta-analysis conducted by Desnoyers *et al*, (2009).

No treatment effect was observed on the acetic acid concentration. A reasonable assumption would be that the change in microbial population would lead to changes in the fermentation parameters. Increased acetenogenesis from hydrogen should result in increased acetate to propionate ratio when live yeast is supplemented (Chaucheyras *et al*., 1995a). This theory is based upon the assumption of greater fibre digestibility due to increased fibrolytic bacterial numbers when cows are supplemented with live yeast products

(Wiedmeier *et al*., 1987; Wohlt *et al*., 1998). The results from this trial do not support this theory.

No effect on acetic acid concentrations was reported by Newbold *et al* (1995), Putnam *et al* (1997) and Thrune *et al* (2009). This study showed no effect of live yeast supplementation on the acetate to propionate ratio. Considering the theories of stimulation of fibrolytic bacteria, higher ruminal pH, oxygen sequestration and increased fibre digestion, it would be reasonable to expect higher acetate to propionate ratios in animals supplemented with live yeast. However research have shown the opposite (Erasmus *et al*., 1992; Erasmus *et al*., 2005; Marden *et al*., 2008; Williams *et al*., 1991). The decreased acetate to propionate ratio might be due to better conversion of lactate into propionate by bacteria stimulated by live yeast supplementation (Bitencourt *et al*; 2011). A meta-analysis by Sauvant *et al*. (2004) determined live yeast supplementation does not influence the acetic acid to Propionic acid ratio and is coherent with the findings of Desnoyers *et al*. (2009).

These contrasting results highlight the uncertainty about the effect of live yeast supplementation on the fermentation parameters under different scenarios. This emphasizes the possible interactions between diet composition, stage of lactation and live yeast used.

The pattern of total VFA production over time (Table 4.8) was as expected with values increasing after the morning feeding and declining from around 21h00. Similar results were reported by Muller (2012) from a study with jersey cows.

4.3.4 Ammonia Nitrogen

The mean rumen NH_3-N values were 12.45 and 12.63mg/dL for the control and the yeast treatments respectively (Table 4.7). Thrune *et al* (2009) and Wang *et al* (2009) reported mean NH_3-N values of 12.1 and 13.6mg/dL from TMR studies with dairy cows and is in the same range as our study. Most research on live yeast supplementation suggests either a reduction (Erasmus *et al*., 1992; Kumar *et al*., 1994; Chaucheyras-Durand and Fonty., 2001, 2002; Miller- Webster *et al*., 2002; Moya *et al*., 2007; De Ondarza *et al*., 2010) or no effect on rumen ammonia nitrogen concentrations (Yoon and Stern., 1995; Newbold *et al*., 1996; Enjalbert *et al*., 1999; Guedes *et al*., 2008; Thrune *et al*., 2009 and Dehghan-Banadaky *et al*., 2013). Results from this study support the majority of research having observed no treatment effect. The decreased ammonia nitrogen levels in the rumen when diets are supplemented with live yeast could be due to the assimilation of nitrogen by the increased microbial numbers (Harrison *et al*., 1988; Williams *et al*., 1990). Another theory

contradicts that statement and suggests the decrease in peptidase activities seen in the presence of live yeast in the rumen fluid (Chaucheyras-Durand *et al*., 2008; Putnam *et al*., 1997). The pattern observed in Figure 4.3 shows an increase in ruminal ammonia nitrogen approximately 2-3 hours after the morning feeding time and then a slight increase again after the afternoon feeding, as was expected.

Table 4.9 Effect of live yeast supplementation the ammonia nitrogen concentrations of rumen fluid during different times of the day for cows fed a total mixed ration (n = 8)

	Treatments[1]		
Time	**Control**	**Live yeast**	**SEM[2]**
09:00	12.24	11.51	0.73
12:00	15.74	17.29	1.24
15:00	9.75	11.17	1.19
18:00	12.58	12.37	0.74
21:00	11.91	10.80	1.44
Mean	12.45	12.63	0.45

[1]Live yeast: Cows received 0.5g of live yeast per cow per day directly through the cannula

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd] Means the same row without a common superscript tends to differ (P<0.10)

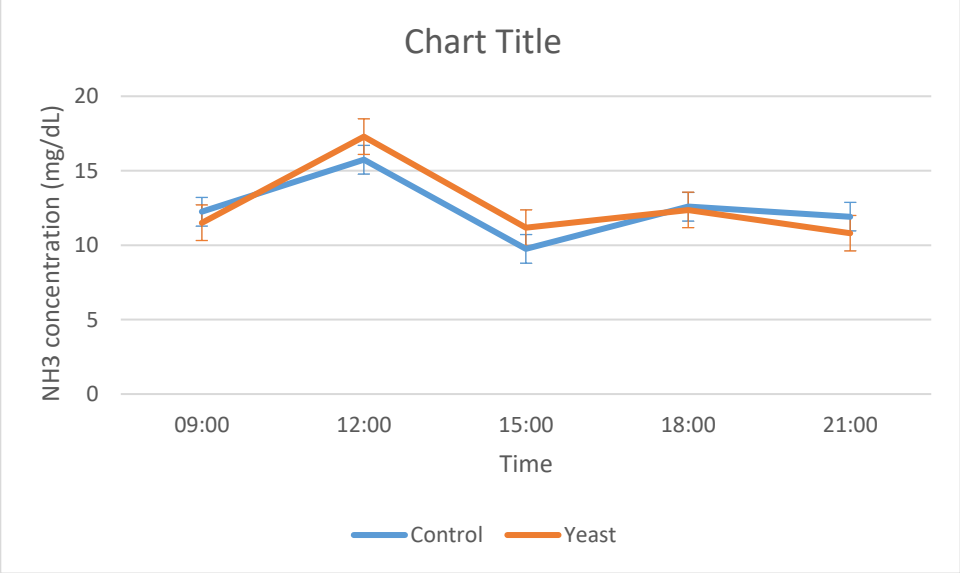

Figure 4.3 The effect of live yeast supplementation on ruminal NH_3-N concentration over a 12-hour period, measured every 3 hours.

4.4 *In sacco* study

4.4.1 Dry matter disappearance

Studying the effect of live yeast supplementation on fibre degradation has resulted in many different responses and conclusions. One of the major factors affecting DM and NDFom disappearance appears to be diet composition, or more specifically initial fibre degradability (forage quality). In Table 4.10 is shown the effect of live yeast supplementation on *in sacco* DM disappearance of the high quality samples of each different forage.

Table 4.10 Effect of live yeast on dry matter disappearance of different forages in the high quality category after being incubated *in sacco* for 12, 24 and 36h respectively for cows fed a TMR (n = 8)

		Treatments[1]		
Forage	**Incubation Period**	**Live yeast**	**Control**	**SEM[2]**
Lucerne	12	54.06	55.54	1.06
	24	65.34	62.25	1.48
	36	70.42	68.94	1.48
Kikuyu	12	30.36	30.17	1.06
	24	38.28	35.88	1.48
	36	46.55	49.57	1.48
Rye Grass	12	58.12	59.95	1.06
	24	71.24	72.71	1.48
	36	80.85	82.19	1.48
Eragrostis	12	20.02[d]	22.69[c]	1.06
	24	27.00	27.35	1.48
	36	33.50	34.46	1.48

[1]Live yeast: Cows received 0.5g of live yeast per cow per day directly through the cannula

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd] Means the same row without a common superscript tends to differ (P<0.10)

The live yeast treatment tended (P<0.10) to decrease DM disappearance after 12h incubation but only for the high quality *Eragrostis curvula* hay. Considering the four different forages used in this study, Eragrostis is by far the lowest quality. Even the high quality Eragrostis has a much higher NDFom value (79.8%) than the other three forage types. Considering the research of Guedes *et al*. (2008), it should be expected that the lower quality forages would show the greatest response to live yeast supplementation. Results from this study showed the opposite, with the yeast tending to decrease DM disappearance.

Table 4.11 Effect of live yeast on dry matter disappearance of forages in the medium quality category after being incubated *in sacco* for 12, 24 and 36h respectively for cows fed a TMR (n = 8)

Forage	Incubation Period	Treatments[1]		SEM[2]
		Live yeast	Control	
Lucerne	12	48.90	49.49	1.06
	24	58.74	57.66	1.48
	36	64.83	61.37	1.48
Kikuyu	12	32.74	31.56	1.06
	24	42.58	40.71	1.48
	36	51.94[d]	55.68[c]	1.48
Rye Grass	12	49.28	48.70	1.06
	24	59.81	62.44	1.48
	36	69.86	71.84	1.48
Eragrostis	12	16.92	18.06	1.06
	24	24.00	26.80	1.48
	36	31.15	32.41	1.48

[1]Live yeast: Cows received 0.5g of live yeast per cow per day directly through the cannula

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd] Means the same row without a common superscript tends to differ (P<0.10)

Evaluating the effect of live yeast supplementation on the medium quality category forages, only kikuyu tended to have a lower *in sacco* DM disappearance (P<0.10) when the live yeast was added, and the rest of the forages showed no difference throughout the 36h incubation (P>0.05). Comparing the high and medium category forages, the same tendencies was observed, but in this instance a different forage and a different incubation time.

Table 4.12 Effect of live yeast on dry matter disappearance of forages in the low quality category after being incubated *in sacco* for 12, 24 and 36h respectively for cows fed a TMR (n = 8)

Forage	Incubation Period	Treatments[1]		SEM[2]
		Live yeast	Control	
Lucerne	12	46.15	48.01	1.06
	24	56.50	54.89	1.48
	36	65.22	62.28	1.48
Kikuyu	12	30.31	28.48	1.06
	24	36.31	37.73	1.48
	36	47.61[b]	52.79[a]	1.48
Rye Grass	12	47.57	47.87	1.06
	24	56.96	58.50	1.48
	36	66.12[b]	71.29[a]	1.48
Eragrostis	12	16.09	16.94	1.06
	24	21.69	22.49	1.48
	36	26.77	27.17	1.48

[1]Live yeast: Cows received 0,5g of live yeast per cow per day directly through the cannula

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd]Means the same row without a common superscript tends to differ (P<0.10)

After 36h of incubating the lower quality roughages, the DM disappearance of kikuyu and rye grass was reduced (P<0.05) by the live yeast treatment. Differences in physico-chemical composition of the feedstuffs are likely to influence the colonization process by the rumen microbial populations and subsequently the efficiency of fibre digestion (Chaucheyras-Durand *et al*., 2012, 2016). The fact that there was no kikuyu or rye grass part of the basal diet could have affected the composition of the microbial population and thereby contributing to the yeast not being able to elicit a positive response. The NRC (2001) for example, in their description of the *in sacco* procedure, recommends that the feedstuffs being evaluated, should also be incubated as an ingredient in the basal diet fed. The reason why yeast in this instance had a negative impact on DM disappearance remains unknown.

Taking the forage quality factor out of the equation, Table 4.13 shows the effect of live yeast supplementation on *in sacco* DM disappearance of the 4 roughages irrespective of quality. After being incubated for 36h, DM disappearance of lucerne was increased (P<0.05) by the live yeast treatment, and the kikuyu and rye grass decreased (P<0.05). At the 12h incubation DM disappearance tended to be lower for the yeast treatment.

Table 4.13 Effect of live yeast on dry matter disappearance of different forages, irrespective of quality, after being incubated *in sacco* for 12, 24 and 36h respectively for cows fed a TMR (n = 8)

Forage	Incubation Period	Treatments[1]		SEM[2]
		Live yeast	Control	
Lucerne	12	49.70	51.01	0.61
	24	60.19	58.27	0.85
	36	66.82[a]	64.19[b]	0.86
Kikuyu	12	31.14	30.07	0.61
	24	39.06	38.11	0.85
	36	48.70[b]	52.68[a]	0.86
Rye Grass	12	51.66	52.17	0.61
	24	62.67	64.55	0.85
	36	72.27[b]	75.11[a]	0.86
Eragrostis	12	17.68[d]	19.23[c]	0.61
	24	24.23	25.55	0.85
	36	30.48	31.35	0.86

[1]Live yeast: Cows received 0.5g of live yeast per cow per day directly through the cannula; Control: Cows did not receive any live yeast.

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd] Means the same row without a common superscript tends to differ (P<0.10)

In Table 4.14 is shown the overall effect of live yeast on the DM disappearance of the forages used in this trial, irrespective of roughage type or quality. The live yeast decreased (P<0.05) DM disappearance after 36h. To our knowledge, this is the first trial where live yeast supplementation decreased DM disappearance of roughages. Chaucheyras-Durand *et al* (2015) found the rate of degradation of corn gluten feed (CGF) and wheat bran to be decreased by live yeast, but not the final amount degraded. Both the CGF and wheat bran are much lower in NDF values than any of the roughages used in this study. Therefore the higher quality products were degraded at a slower rate, but in our study the ultimate amount of the lower quality roughages that disappeared were negatively influenced by the live yeast.

Table 4.14 Overall effect of live yeast on the percentage dry matter disappearance, irrespective of forage type or quality, after being incubated *in sacco* for 12, 24 and 36h respectively for cows fed a TMR (n = 8)

	Treatments[1]		
Incubation Period	**Live yeast**	**Control**	**SEM[2]**
12	37.54	38.12	0.31
24	46.54	46.62	0.43
36	54.57[b]	55.83[a]	0.43

[1]Live yeast: Cows received 0.5g of live yeast per cow per day directly through the cannula;

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd] Means the same row without a common superscript tends to differ (P<0.10)

In Table 4.15 the four high quality samples of the four different forages were combined into one high quality category, irrespective of forage species. The same were done for the medium and low quality categories. In the high quality category at the 12h incubation the yeast tended to decrease (P<0.10) the DM disappearance with a similar tendency for the 36h incubation of the low quality forage categories.

Table 4.15 Effect of live yeast on dry matter disappearance of different qualities of forages, irrespective of forage species, after being incubated *in sacco* for 12, 24 and 36h respectively for cows fed a TMR (n = 8)

Forage Quality	Incubation Period	Treatments[1]		SEM[2]
		Live yeast	Control	
High	12	40.64[d]	42.09[c]	0.53
	24	50.46	49.55	0.74
	36	57.83	58.79	0.74
Medium	12	36.96	36.95	0.53
	24	46.28	46.90	0.74
	36	54.44	55.32	0.74
Low	12	35.03	35.32	0.53
	24	42.87	43.40	0.74
	36	51.43[d]	53.38[c]	0.74

[1]Live yeast: Cows received 0.5g of live yeast per cow per day directly through the cannula

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd] Means the same row without a common superscript tends to differ (P<0.10)

The main purpose of the trial however, was to evaluate the effect of live yeast on the NDF disappearance of different forage qualities within forages. This is discussed in the following section of this dissertation.

4.4.2 NDFom disappearance

In Table 4.16 is shown the effect of yeast supplementation on *in sacco* NDFom disappearance of high quality samples from four different forages. Irrespective of incubation time, yeast did not have any effect on NDFom disappearance. The overall trend is very similar to the results of the dry matter disappearance discussed earlier in this chapter. The results on the high quality forage partly agrees with the findings of Guedes *et al*., (2008) where they concluded that live yeast would have no impact on feedstuffs with high initial degradability. However compared to the higher quality silages used in their study, our Eragrostis samples used, even in the high quality Eragrostis category, are of much lower quality than the silages and disappearance should theoretically be expected to increase. The argument is perhaps more relevant regarding the high quality grasses and lucerne.

Table 4.16 Effect of live yeast supplementation on percentage neutral detergent fibre (NDFom) disappearance of different high quality forages after being incubated *in sacco* for 12, 24 and 36h respectively for cows fed a TMR (n = 8)

Forage	Incubation Period	Treatments[1]		SEM[2]
		Live yeast	Control	
Lucerne	12	25.52	24.73	1.80
	24	35.00	32.72	2.05
	36	42.14	42.00	2.19
Kikuyu	12	11.38	11.51	1.80
	24	23.34	22.06	2.05
	36	35.04	38.77	2.19
Rye Grass	12	37.64	41.82	1.80
	24	57.07	61.62	2.05
	36	72.06	75.05	2.19
Eragrostis	12	9.70	11.50	1.80
	24	18.44	19.84	2.05
	36	27.46	28.60	2.19

[1]Live yeast: Cows received 0.5g of live yeast per cow per day directly through the cannula; Control: Cows did not receive any live yeast.

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd] Means the same row without a common superscript tends to differ (P<0.10)

Results on the medium forage quality category are represented in Table 4.17. Lucerne did show an increase (P<0.05) in NDF disappearance in cows supplemented with live yeast after 36h of incubation. Kikuyu and Eragrostis did not show any treatment effect while rye grass NDF disappearance showed a tendency (P<0.10) to be decreased by live yeast supplementation.

Table 4.17 Effect of live yeast supplementation on percentage neutral detergent fibre (NDFom) disappearance of different medium quality forages after being incubated *in sacco* for 12, 24 and 36h respectively for cows fed a TMR (n = 8)

Forage	Incubation Period	Treatments[1]		SEM[2]
		Live yeast	Control	
Lucerne	12	12.26	12.30	1.80
	24	23.74	23.36	2.05
	36	33.55[a]	25.47[b]	2.19
Kikuyu	12	14.03	12.29	1.80
	24	27.01	23.74	2.05
	36	41.18	45.88	2.19
Rye Grass	12	24.62	26.60	1.80
	24	39.73[d]	44.95[c]	2.05
	36	56.24	60.59	2.19
Eragrostis	12	14.08	12.17	1.80
	24	19.83	22.83	2.05
	36	27.95	29.51	2.19

[1]Live yeast: Cows received 0.5g of live yeast per cow per day directly through the cannula; Control: Cows did not receive any live yeast.

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd] Means the same row without a common superscript tends to differ (P<0.10)

Live yeast supplementation did not increase the NDFom disappearance in any of the low quality forages as presented in table 4.18. However the supplementation of live yeast caused a significant (P<0.05) reduction in NDFom disappearance after 36h for kikuyu and rye grass. To our knowledge there are no other studies that reported live yeast to have a negative impact on NDFom disappearance on any roughage. The reason for this remains unknown.

Table 4.18 Effect of live yeast supplementation on percentage neutral detergent fibre (NDFom) disappearance of different low quality forages after being incubated *in sacco* for 12, 24 and 36h respectively for cows fed a TMR (n = 8)

Forage	Incubation Period	Treatments[1]		SEM[2]
		Live yeast	Control	
Lucerne	12	23.73	23.37	1.80
	24	33.78	32.14	2.05
	36	44.73	41.09	2.19
Kikuyu	12	11.95	9.10	1.80
	24	21.97	22.08	2.05
	36	36.77[b]	44.61[a]	2.19
Rye Grass	12	24.13	26.11	1.80
	24	36.19	39.71	2.05
	36	51.23[b]	57.85[a]	2.19
Eragrostis	12	8.84	7.90	1.80
	24	14.99	16.46	2.05
	36	21.93	21.77	2.19

[1]Live yeast: Cows received 0.5g of live yeast per cow per day directly through the cannula; Control: Cows did not receive any live yeast.

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd] Means the same row without a common superscript tends to differ (P<0.10)

Evaluating the effect of live yeast supplementation on *in sacco* NDFom disappearance of the four forages, irrespective of forage quality within forage species, is presented in Table 4.19. A treatment effect can be seen after 36h for lucerne where the live yeast increased (P<0.05) overall NDFom disappearance by 10.9%. Kikuyu also showed a treatment effect after 36h but the live yeast reduced (P<0.05) the NDFom disappearance by 12.6%. NDFom disappearance for rye grass already showed a tendency (P<0.10) for NDFom disappearance to be decreased by live yeast treatment after 12h incubation and after 24h and 36h proved to be significant (P<0.05) with decreases of 9.1% and 7.3% respectively. Eragrostis NDFom disappearance seemed to be unaffected by live yeast supplementation in this study. There might be merit in the argument that there was no kikuyu or rye grass in the basal diet, hence the composition of the microbial population was not ideal for kikuyu or rye grass *in sacco* incubation studies. The reduction of NDFom disappearance could not be explained, especially if our results are compared to a recent

study that also evaluated the effect of live yeast on NDFom disappearance of feeds differing in quality (Sousa *et al*., 2018).

Sousa *et al* (2018) evaluated the *in sacco* NDFom digestibility of five different forages varying in aNDF, CP and lignin from 567g/kg to 661g/kg, 57g/kg to166g/kg and 71g/kg to 172g/kg respectively. The live yeast supplementation increased *in sacco* aNDF disappearance of all feedstuffs at 24h incubation (P<0.05) but not at 48h incubation. In our study when differences occurred, it was after 24h or 36h incubations but the major question still remains – why the negative effect of live yeast on *in sacco* NDFom disappearance?

Table 4.19 Effect of Live yeast on neutral detergent fibre (NDFom) disappearance of different forages, irrespective of quality, after being incubated *in sacco* for 12, 24 and 36h respectively for cows fed a TMR (n = 8)

		Treatments[1]		
Forage	**Incubation Period**	**Live yeast**	**Control**	**SEM[2]**
Lucerne	12	20.50	20.13	1.04
	24	30.84	29.41	1.18
	36	40.14[a]	36.19[b]	1.27
Kikuyu	12	12.45	10.97	1.04
	24	24.10	22.63	1.18
	36	37.67[b]	43.09[a]	1.27
Rye Grass	12	28.80[d]	31.51[c]	1.04
	24	44.33[b]	48.76[a]	1.18
	36	59.84[b]	64.50[a]	1.27
Eragrostis	12	10.88	10.52	1.04
	24	17.75	19.54	1.18
	36	25.78	26.63	1.27

[1]Live yeast: Cows received 0.5g of live yeast per cow per day directly through the cannula; Control: Cows did not receive any live yeast.

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd] Means the same row without a common superscript tends to differ (P<0.10)

Evaluating the effect of live yeast supplementation on all samples combined (Table 4.20), no treatment effect (P<0.05) was observed. This contradicts the findings of many authors (Wiedmeier *et al*., 1987, Erasmus *et al*., 1992, Plata *et al*., 1994; Wohlt *et al*., 1998;

Chaucheyras-Durand and Fonty, 2001; Guedes *et al*., 2008; Marden *et al*., 2008; Sousa *et al*., 2018) but support the findings of Angeles *et al*., (1998) and Corona *et al*., (1999). The majority of studies, however, have shown either a significant response or at least a trend of live yeast stimulating NDF digestibility, as pointed out by Sousa *et al* (2018).

Table 4.20 Overall effect of Live yeast on the percentage neutral detergent fibre (NDFom) disappearance, irrespective of forage type or quality, after being incubated *in sacco* for 12, 24 and 36h respectively for cows fed a TMR (n = 8)

	Treatments[1]		
Incubation Period	**Live yeast**	**Control**	**SEM[2]**
12	18.16	18.28	0.52
24	29.26	30.08	0.59
36	40.86	42.60	0.63

[1]Live yeast: Cows received 0.5g of live yeast per cow per day directly through the cannula; Control: Cows did not receive any live yeast.

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd] Means the same row without a common superscript tends to differ (P<0.10)

One of the main objectives of this study was to evaluate the effect of live yeast supplementation on different roughages but more importantly on different qualities within a forage species. The purpose was to investigate whether the lower quality forages respond better to live yeast treatment than higher quality roughages as reported by Guedes *et al*, (2008) who evaluated silage samples. Our roughages in each quality category for the forages varied considerably as seen in Table 3.6. High quality (40.7% to 75.67% NDFom), medium quality (43.2% to 78.52) and low quality (49.54 to 79.82%), however did not differ much in terms of spectrum of NDFom percentages. This means that the 3 sample sets did not differ much with regards to quality between the respective sample sets within each forage species. This could have affected the outcome of the one objective, namely the effect of the yeast on NDFom digestibility of different forage quality samples, within a forage, because of the narrow range. The differences in quality between the different forage species were substantial and the absence of any yeast effect cannot be explained. The mostly negative effect of yeast on NDF digestibility should be investigated further.

Table 4.21 Effect of live yeast on percentage neutral detergent fibre (NDFom) disappearance of different qualities of forages, irrespective of forage species, after being incubated *in sacco* for 12, 24 and 36h respectively for cows fed a TMR (n = 8)

Forage Quality	Incubation Period	Treatments[1]		SEM[2]
		Live yeast	Control	
High	12	21.06	22.39	0.90
	24	33.46	34.06	1.03
	36	44.17	46.11	1.10
Medium	12	16.25	15.84	0.90
	24	27.85	28.59	1.03
	36	39.73	40.36	1.10
Low	12	17.16	16.62	0.90
	24	26.73	27.60	1.03
	36	38.66[d]	41.33[c]	1.10

[1]Live yeast: Cows received 0.5g of live yeast per cow per day directly through the cannula; Control: Cows did not receive any live yeast.

[2]Standard error of the mean

[ab] Means the same row without a common superscript differs (P<0.05)

[cd] Means the same row without a common superscript tends to differ (P<0.10)

CHAPTER 5
CONCLUSION

It is clear that physio-chemical properties of forages play a role in the efficacy of live yeast to improve fibre digestion. What the exact criteria are for live yeast to be acting to its full potential needs to be researched in more depth due to the numerous biotic and abiotic factors influencing the efficacy of the additive. Our study did not find any treatment effect on the performance of the cows or on the fermentation patterns in the rumen, although those were secondary observations. Within forages the live yeast treatment did not affect NDFom disappearance in the high quality forage category. For lucerne hay the live yeast treatment appeared to increase NDFom disappearance in the medium quality category, and also the overall mean. For kikuyu and rye grass, the live yeast treatment decreased the NDFom disappearance in the lower quality category as well as the overall mean. For the Eragrostis curvula hay the live yeast treatment did not have any effect on NDFom disappearance. Eragrostis has the lowest initial NDF digestibility and if there was a response to yeast supplementation, one would have expected it to be with Eragrostis. The fact that yeast negatively affected the NDF digestibility of both pastures cannot be explained and further research is necessary. In addition, results are still inconclusive as to what role the basal diet plays in the effects of live yeast supplementation. Further research is needed to clarify and define the magnitude of every proposed mode of action as well as the role of the dietary components in the effect of live yeast supplementation in dairy cattle.

CHAPTER 6
CRITICAL EVALUATION

6.1 Forages evaluated

The four different forages did present us with an acceptable variety in roughage quality, but the extent of the difference between the lowest and highest quality within each forage type, were not sufficient. Better preparation with regards to sampling time and better screening of forages could lead to more accurate conclusions. Other factors such as ADF, lignin and CP content and invitro digestibility should be considered in the process of labelling a forage as high, medium or low quality.

6.2 Total Mixed Ration

The TMR that was fed was of lower quality than originally formulated. The animals received a diet with lower starch, lower CP and higher NDFom content than the formulated diet. A better approach would be to feed a maize silage based TMR with Lucerne hay and a dairy concentrate meal in stead of pellets to ensure a more consistent diet offered to the animals. The fact that a lower starch diet was fed, could have reduced the pH-challenge in the rumen thereby limiting the buffering effect of live yeast.

6.3 Yeast CFU count

Due to laboratory restrictions in South Africa no CFU counts were performed before the commencement of the trail and Lallemand supplied us with a fresh yeast product from their factory that has passed their quality control checks. Since we are dealing with live organisms, a recommendation would be to check the CFU counts of the product before and after the trial when similar projects are done in the future to ensure the viability of the product.

6.4 Statistical analysis

With the data from this trial, it was concluded that forage quality (within forage type) does not have a biologically significant effect on the response to yeast addition. But it is important to mention that this could be typical of a type (ii) error. This could be a failure of rejecting a false null hypothesis and warrants further research on this topic. By considering the aspects mentioned above it could impact the results of similar studies in the future.

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