Effect of live yeast supplementation on performance parameters of Jersey cows grazing ryegrass/kikuyu pasture

By

Carmen Coetzee

Submitted in fulfillment of the requirements for the degree

MSc (Agric) Animal Science: Nutrition Science

Department of Animal and Wildlife Sciences

Faculty of Natural and Agricultural Sciences

University of Pretoria

Dedicated to my Parents Koos and Sue Coetzee

INDEX

DECLARATION

I declare that this dissertation for the degree MSc (Agric) Animal Science: Nutrition Science at the University of Pretoria has not been submitted for a degree at any other University.

C Coetzee
Pretoria
July 2011

ACKNOWLEDGEMENTS

The success of this work was due to the contributions of the following institutions and people, who I'd like to recognise and extend my deep thanks and gratitude:

Firstly I would like to thank my Lord Jesus Christ, for the strength and abilities He has provided me throughout my life to fulfill his purpose.

My Mom and Dad, for providing the opportunity for me to study. my families loving support every step of the way has helped me reach my goals.

Doctor Ilse Trautmann, Director of Technology, Research and Development of the Department of Agriculture of the Western Cape for supplying the facilities and financial support to complete the study.

The Western Cape Dairy Research Trust for partly funding the research and my stay in George.

Lallemand for creating the opportunity for research on the yeast product supplied by them, and principally funding the project.

Professor Lourens Erasmus, my supervisor at the Department of Animal and Wildlife Sciences, University of Pretoria. I'm grateful for his leadership and knowledge he has taught me over the years of my studies. For providing the opportunity for me to further my postgraduate qualification on the Outeniqua Research Farm in George, for believing in me with his continual support and advice.

Professor Robin Meeske my co-supervisor at the Department of Agriculture Western Cape, Institute for Animal Production, Outeniqua Research Farm. His expertise and insight in animal production, continuous input, patience and support during my experimental study. The knowledge and experience I've gained since I've been in George due to his influence is greatly appreciated. It's an honor to have had the experience to be able to learn from him.

Doctor Philip Botha for his constant concern in my wellbeing, giving me the opportunity to attend study groups and congresses, publish popular articles and for the positive energy he emits to his students and colleagues, especially at tea times.

Hennie Gerber and Pieter du Plessis, the Outeniqua farm manager and farm chair-person for creating such a pleasant working environment, and the practical running of my trial on the farm.

Gerrit Van der Merwe the Outeniqua herd manager, in selecting and managing cows used and doing the body condition scoring and weighing.

Roelf Coertze, for his help in the statistical design and analysis of the project. His skill and patience throughout was greatly appreciated.

Janke Van der Colf, for her practical guidance and advice, her eagerness and willingness to help, and giving generously of her time. I have learned so much from you.

Werner August Lingnau, MSc student from the Department of Animal Production at Stellenbosch University. I worked alongside throughout my trial when times were stressful and tough. Your help with the study, constant support, friendship and time you sacrificed is priceless, I will be forever grateful.

Pieter Swanepoel, MSc student from the Department of Plant production, for his assistance in rumen and milk sampling in late evenings and early mornings, for always being willing to help and for his constant support and friendship.

Werner August von Zeuner for his unwavering support during the experimental study and dissertation write-up.

Pieter Cronje for his help and guidance with pasture measurements and allocation.

Titus Afrika, for his help with sorting and moving cows, milk sampling and concentrate weighing. Your diligence, dedication and eagerness to help where it was needed, ensured procedures ran smoothly.

Abraham Wildschut and Isak Wildskut for their assistance in managing and co-ordinating pasture, pasture allocation and cows.

Marius Herman for his help in ensuring that the water and electricity facilities were in working order and its maintenance thereafter.

Jerome Wildschut, Justin Smit and Themba Wisani for their daily assistance in moving, sorting and milking cows and always being eager to help where possible.

Hester De Klerk, Cina Smit and David Rondganger for their dedication and considerable input in milking cows.

Ilze Fourie and Kim Butler for their time and help when extra assistance was needed.

To the rest of the people on the Outeniqua Research Farm who have created such a positive and supportive environment to work in.

Wilma Hale at the Agricultural Research Council for conducting the yeast count analysis.

Elise Ferreira at the University of Pretoria Nutrilab, and the team of laboratory technicians; Karin Basson, Gertruida Smit, Gerda Kotzé, Tebogo Mogotsi and Ilze Smit , a very special thanks to all these ladies who gave kindly of their time and without their skill, patience and guidance, the lab analysis would not have been possible. It was a pleasure working with

such a fantastic team of ladies who have so much passion for their work. I'd like to extend a special thanks to the rest of the staff at Nutrilab and specifically the cleaning staff, and Moses who would so diligently have sorted the apparatus for use once again.

Nestle for calculating the milk prices for the two treatments.

To the lectures in the Department of Animal and Wildlife Sciences at the University of Pretoria.

SUMMARY

Supplementing live yeast to lactating Jersey cows grazing ryegrass/kikuyu pastures

By

C. Coetzee

Supervisor:	**Prof L. J. Erasmus**
Co-supervisor:	**Prof R. Meeske**
Department:	**Animal and Wildlife Sciences**
Faculty:	**Natural and Agricultural Sciences**
	University of Pretoria
	Pretoria
Degree:	**MSc (Agric) Animal Science: Nutrition Science**

Energy is the first-limiting nutrient for cows grazing pasture therefore, energy supplementation is necessary for high producing animals. In pasture-based systems, the concentrate is fed twice a day in the dairy parlour during the milking procedure. Consumption of large amounts of fermentable carbohydrates results in a drop in rumen pH and this may induce rumen acidosis. This may lead to reduced intake, lower fibre digestion and depressed milk yield. Supplemental yeast offer great possibilities in stabilising the rumen fermentation patterns and, therefore, improving dry matter intakes (DMI). This may increase milk production, milk composition parameters, rumen volatile fatty acid concentrations (VFA) which leads to higher profits.

Investigating these responses, a trial was conducted, where live yeast (Levucell SC 10 ME-Titan) was supplemented to Jersey cows grazing ryegrass/kikuyu pastures, supplemented with 6 kg (as is) dairy concentrate per day split over two milking periods.

Thirty multiparous high-producing Jersey cows between 30 and 120 days in milk (DIM) were selected, blocked and randomly allocated to control (no yeast) or live yeast treatment groups. Ten fistulated lactating cows were added in a cross-over design (two periods and two treatments) and all 40 cows strip grazed *Italian* ryegrass (*Lollium multiflorum*) and kikuyu pastures as one group. A new pasture strip was allocated after each milking and pasture was measured using a rising plate meter (RPM).

The yeast product Levucell SC 10 ME – Titan containing *Saccharomyces cerevisiae* CNCM I-1077 was supplied by Lallemand S.A.S (19 rue des Briquetiers, 31702 Blagnac cedex, France). The Levucell SC 10 ME – Titan is a micro-encapsulated formulation for premix and pelleted feeds. The yeast treatment group had the yeast pelleted in with the dairy concentrate at a concentration of 167 g of yeast per ton of concentrate, to obtain the required intake of 1 g yeast per cow per day as specified by Lallemand.

Milk yields were recorded daily and composite milk samples were taken every two weeks to determine, milk fat, protein, lactose, milk urea nitrogen (MUN) and somatic cell count (SCC) contents.

The fistulated cows were adapted to their respective diets and treatments for 21 days after which the pH measurment, rumen fluid sampling, and *In sacco* study were conducted.

Milk yield, 4% fat corrected milk (FCM) yield, milk protein and lactose percentages, SCC, body condition score (BCS), and live weight did not differ ($P > 0.05$) between treatments. The milk fat% however, was higher for the yeast supplemented cows at 4.24% compared to the control group of cows of 3.99% ($P < 0.05$).

The mean acetic and total VFA concentration (mmol/L) for the control treatment was higher compared to the yeast treatment ($P < 0.05$). There was no difference in the fermentation patterns of VFA, the pH and NH_3-N values measured between treatments ($P > 0.05$).

The mean *In sacco* neutral detergent fibre (NDF), organic matter (OM) and DM disappearance was higher for the yeast treatment group of cows after a 12 and 24 hour incubation compared to the control group ($P < 0.05$). The mean ruminal NDF disappearance of ryegrass in cows supplemented with yeast increased by 11.9% and 6.3% compared to the control at the 12 and 24 hour incubation periods, respectively.

With higher fibre digestibilities in the rumen and more acetate available at the mammary gland and subsequently higher milk fat percentages, the yeast effects on stimulating the cellulolytic bacteria in the rumen, may be a possible explanation for the results in the current study and it is well documented in previous studies. Live yeast supplementation resulted in higher milk fat percentages which improved milk price.

LIST OF ABBREVIATIONS

AA	Amino acids
AAFCO	Association of American Feed Control Officials
ADF	Acid detergent fibre
ADIN	Acid-detergent insoluble nitrogen
BCS	Body condition score
BHB	Beta-hydroxy butyrate
BR	Biting rate
BW	Body weight
C	Carbon
Ca	Calcium
CF	Crude fibre
CFU	Colony forming units
Cm	Centimeter
CP	Crude protein
CY	Yeast culture
°C	Degree Celsius
DIM	Days in milk
dL	Decilitre
DM	Dry matter
DMI	Dry matter intake
E_h	Redox potential
ECM	Energy corrected milk
EE	Ether extract
EU	European Union
FCM	Fat-corrected milk
FDA	Food and Drug Administration
G	Gram
GE	Gross energy
GIT	Gastrointestinal tract
GT	Grazing time
Ha	Hectare
H_2SO_4	Sulphuric acid

H_3PO_3	Ortho-phosphoric acid
HI	Herbage intake
IB	Quantity of herbage ingested per bite
Ile	Isoleucine
IVOMD	*In vitro* organic matter digestibility
Kg	Kilogram
KJ	Kilojoules
Leu	Leucine
LT	Long-term
Ly	Live yeast
Lys	Lysine
ME	Metabolisable energy
Met	Methionine
Mg	Milligram
MJ	Megajoules
Mmol	Millimole
mM	Millimole per litre
MUN	Milk urea nitrogen
mV	Millivolts
MY	Milk yield
N	Nitrogen
NE_L	Nett energy for lactation
NEFA	Non-esterified fatty acids
NaHCO3	Sodium bicarbonate
NAN	Non-ammonia nitrogen
NDF	Neutral detergent fibre
NDFIC	Neutral detergent fibre intake capacity
NDIN	Neutral detergent insoluble nitrogen
NH_3-N	Ammonia-nitrogen
NPN	Non-protein nitrogen
NSC	Non-starch carbohydrates
OM	Organic matter
P	Phosphorus
PA	Pasture allowance

pH	The negative logarithm to the base ten of the hydrogen ion activity in the solution
Phe	Phenylalanine
pK_a	Acid-dissociation constant
pTMR	Partial total mixed ration
r	Ruminal
R	South African rand
rH	Clarks exponent
ROI	Return on investment
RPM	Rising plate meter
RS	Standard energy requirements in reverse
SCC	Somatic cell count
SD	Standard deviation
SEM	Standard error of the mean
SI	Synchronization index
SR	Substitution rate
Temp	Temperature
Thr	Threonine
TMR	Total mixed ration
tt	Total-tract
USA	United States of America
Val	Valine
VFA	Volatile fatty acids
WOL	Weeks of lactation
Wt	Weight

LIST OF FIGURES

Chapter 2

Chapter 3

Chapter 4

LIST OF TABLES

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Appendix A

CHAPTER 1

INTRODUCTION

CHAPTER 1

Introduction

Commercial and emerging dairy farmers are under financial pressure as a result of low milk prices and increased input costs. Milk production from grazed forage systems is a low-cost, economical feeding system compared to that of a total mixed ration (Bargo *et al.*, 2002a; White *et al.*, 2002). White *et al.* (2002) found that a pasture-based system can be competitive, with a confinement system on the basis of having lower feed costs, culling costs and other economic factors (labour for animal care, manure handling and forage management), even when milk production is lower on pasture-based systems.

Profitable milk production in the Southern Cape region of SA are dependent on pasture-based systems consisting of predominately kikuyu (*Pennisetum clandestinum*) and ryegrass (*Lolium multiflorum*) (Meeske *et al.*, 2006). These grazing systems are intensive and require high input costs (Botha, 2003).

Energy is the first-limiting nutrient for cows grazing pasture necessitating energy supplementation for high producing animals (Kolver and Muller, 1998; Bargo *et al.*, 2002a; Bargo *et al.*, 2003). Nutritionists are continuously seeking new and innovative ways to manipulate the diet of the dairy cow to increase and optimize milk production.

In pasture-based systems, the concentrate is fed during the milking procedure, since the practical reality of a pasture situation requires this feeding pattern. The ideal system would, however, have concentrates fed together with roughages throughout the day. The amount of concentrate fed daily can vary between zero and eight kilograms (Pulido *et al.*, 2009). Pulido *et al.* (2009) fed grazing cows six kilograms of concentrate daily either once, twice, three or four times in equal amounts. No additional benefit was found feeding more than twice daily. Consumption of large amounts of fermentable carbohydrates may lead to rumen acidosis (Slyter, 1976), since such feeding practices can cause the rumen pH to decrease to a value of 5.6 or lower (Chiquette, 2009; Thrune *et al.*, 2009). The time period during which the rumen pH is below a value of six may be as long as nine hours per day (Bach *et al.*, 2007) possibly resulting in subclinical acidosis (Bach *et al.*, 2007; Desnoyers *et al.*, 2009b). Subclinical acidosis is characterized by a pH between 5.2 and 5.6 while a rumen pH below 5.2 is indicative of an extreme acute form of rumen acidosis (Chiquette, 2009; Thrune *et al.*, 2009). Acidosis effects may lead to reduced intake, lower fibre digestion (Owens *et al.*, 1998) and depressed milk yield. Subclinical acidosis, with its associated negative effects, such as rumenitis, reduced feed intake, abomasal ulcers and laminitis has

been suggested as one of the most important nutritional diseases burdening dairy cattle production (Enemark, 2008).

Direct-fed microbials have become increasingly popular since the ban of antibiotics from the animal feed industry in the European Union (EU) (1st January 2006). Thrune *et al.* (2009) states that these direct-fed microbials are recognised in the Food and Drug Administration (FDA) as being safe to implement. Direct-fed microbials include specific and non-specific yeasts, fungi and bacteria (Oetzel *et al.*, 2007). The most studied form of yeast is *Saccharomyces cerevisiae,* which is available in both viable and non-viable yeast cultures (Guedes *et al.*, 2008). Live yeast has the potential to stabilise rumen pH (Chaucheyras-Durand *et al.*, 2008; Desnoyers *et al.*, 2009a), as well as stimulate enzymatic and cellulolytic rumen activities (Harrison *et al.*, 1988; Guedes *et al.*, 2008). Yeast products offer great possibilities for stabilising the rumen fermentation patterns and, thereby, improving DM intakes (Desnoyers *et al.*, 2009a). This may increase milk production, improve milk compositional parameters and increase ruminal volatile fatty acid concentrations (VFA), all of which could result in higher profits (Desnoyers *et al.*, 2009a).

The dairy industry, as well as animal feed manufacturers, needs controlled studies to determine whether supplementation of yeast will be cost-effective. This study aims to contribute to the understanding of factors limiting milk production in pasture-based systems. The extensive research carried out with yeast supplementation and the results have been limited to total mixed ration system. There has been no such research done on pasture-based animal production systems, therefore future research should focus on supplementation of pasture-based systems.

The aim of this project was to determine the effect of supplementing live yeast to lactating Jersey cows grazing ryegrass/kikuyu pasture during spring.

The following hypotheses were tested in this study:

Ho = Supplementing live yeast to Jersey cows grazing ryegrass/kikuyu pasture will increase productivity.

H1 = Supplementing live yeast to Jersey cows grazing ryegrass/kikuyu pasture will have no effect on productivity.

CHAPTER 2

LITERATURE REVIEW

SUPPLEMENTING YEAST TO LACTATING JERSEY COWS GRAZING RYEGRASS/KIKUYU PASTURES

CHAPTER 2

Literature Review

2.1 Introduction

In many parts of the world feed additives such as yeast, also known as a direct-fed microbial or a probiotic, have been introduced and subsequently used extensively for ruminant animals. The definition of a feed additive is either substances, micro-organisms or preparations, that are not feed material or a premix, which are deliberately added to feed/water to achieve the following favourable outcomes; effecting the characteristics of the feed or animal products; to satisfy the nutritional requirements of animals; to effect the environmental aspects of animal production; favourably effecting animal production, performance or welfare, predominantly affecting the gastro- intestinal flora or digestibility of feedstuffs or having a coccidiostatic or histomonostatic effect (Regulation 1831/2003/EC and Regulation 183/2005/EC) (EU, 2009). The uses of such additives have become increasing popular since the ban of antibiotic in the European Union (EU) on 1st January 2006. The shift to more “natural” alternatives to manipulate production is mediated through the concern of built-up resistance by pathogens to antibiotics, to decrease the potential antibiotic load, increased consumer concerns about safety, quality of animal products and the impact on the environment.

The number of yeast products commercially available has increased significantly over the past ten years. The effect of yeast and various yeast products has been extensively researched, with conflicting results. It would therefore be naïve to assume that all yeast additives are synonymous in their effects on animal performance parameters. This may be due to the effect of various strains of yeast available in commercial yeast products.

There are many factors affecting the production responses of animals to supplemental yeast. Factors such as the diet, the strain of yeast, whether it’s a live yeast or a culture, the amount fed, the age of the animal, the metabolic status of the animal and all external environmental stimuli have an impact on the responses measured from a yeast supplement.

2.2 Nutritional imbalances and limitations on pasture-based systems

A major factor limiting production and milk yield of high producing cows grazing high quality pastures is low dry matter intakes (DMI) of pasture. Resulting in energy being the primary limiting nutrient (Bargo *et al.*, 2002b). The high moisture and low dry matter (DM) content of high quality pastures contributes to this effect which lowers the supply of

metabolisable energy (ME) (Kolver and Muller, 1998; Bargo *et al.*, 2003; Kolver, 2003). Energy content of the pasture is not posing the limitation, but rather the energy limitation rising from the cow unable to consume enough pasture to meet their energy requirements for production. Therefore high producing cows on pasture will meet their potential genetic merit for milk production if supplemental energy is provided (Kolver and Muller, 1998).

Cows consuming a grain supplement of more than 200 g/kg of the diet showed that milk production is further limited by specific amino acids (AA) such as methionine (Met) and lysine (Lys) (Kolver, 2003). Crude protein (CP) content of annual ryegrass varies from 13.6 to 31% on a DM basis, thus pasture analysis is necessary so adjustments to the concentrate CP content can be made and monitored (Meeske *et al.*, 2006). This is essential because the cows' protein requirements must be met to avoid limiting the potential production. The overfeeding of protein is costly and non-protein nitrogen (NPN) in pasture is highly rumen degradable. When a low level of non-starch carbohydrates (NSC) is fed the efficiency of nitrogen (N) utilisation is reduced (Kolver, 2003).

Kolver and Muller (1998) found that pasture fed cows consumed similar intakes of neutral detergent fibre (NDF) and CP as cows on a total mixed ration (TMR), but pasture offered less net energy for lactation, organic matter (OM) and 19% less DM. The supply of ME and protein with the profile of the supply of AA from pasture are affected by the degradation rate of fibre and protein, the lignin content and the effective fibre (Kolver, 2003).

Higher genetic merit cows partition more nutrients to milk production which therefore leads to higher responses due to supplementation (Bargo *et al.*, 2003). Kolver (2003) supports this, and declared that greater nutritional limitations are imposed by pasture diets, which highlights the importance of having cows that have the correct genetic merit which suites the type of farming system. To further explain this, animals on pasture partition more nutrients towards their energy depots which enable them to sustain themselves, and conception is not hindered, which would be ideal if lower genetic merit animals were selected. Alternatively, higher genetic merit cows, as what would be seen in a TMR system, partition less nutrients to their energy depots and conception rates are lower.

2.3 Pasture- based system versus a total mixed ration

Milk production of cows on pasture-only and pasture-based systems is lower than that of cows on TMR feeding systems. This could be owed to the fact that DM intakes are lower for pasture-only fed cows compared to cows consuming a TMR (Kolver and Muller, 1998). In a comparative study the DMI was 19 and 23.4 kg/day for grazing and TMR fed cows, respectively (Kolver and Muller, 1998). In a study reported by Bargo *et al.* (2002b) pasture-

based fed cows with a recorded total DMI of 21 kg DMI/day (12.9 kg DM was of pasture and 8.7 kg was concentrate) was lower compared to cows consuming a complete TMR (26.7 kg DMI/day) or a partial TMR (pTMR) (25.2 kg DMI/day) (Bargo *et al.*, 2002b). The pTMR consisted of cows consuming a combination of both pasture and a part TMR.

Milk production responses differ as a result of varying DM intakes between the systems, and generally follow a similar trend to DM intakes for which the milk production is lower for pasture-fed animals. This is supported by Kolver and Muller (1998) who reported milk yields of 44.1 kg/day for TMR fed cows compared to grazed cows producing 29.6 kg/day. Additionally, work done by Bargo *et al.* (2002b) reported milk production highest for TMR fed cows (38.1 kg/day) compared to the pasture and concentrate fed cows (28.5 kg/day), and intermediate between the two was the cows receiving a pTMR (32 kg/day). A lower milk production found in a pasture-based system is 61% accountable to the lower DM intakes simultaneously measured (Kolver, 2003).

Milk compositional differences exist between the feeding systems. Milk protein content was lower for grazing cows (2.61%) compared to the TMR fed cows (2.80%) (Kolver and Muller, 1998; Bargo *et al.*, 2002b). Bargo *et al.* (2002b) reported that cows receiving pasture and concentrate had lower milk fat percentages compared to TMR or pTMR fed cows. In general compared to a TMR, higher milk protein and fat percentages on pasture would be measured due to the lower milk yields, due to the presence of a dilution effect from higher milk yields found in TMR systems. However, contradictory to this, Bargo *et al.* (2002b) reported lower milk protein and milk fat percentages on pasture-based systems. This was due to the higher dietary energy intakes, highly digestible good quality pasture, and the concentrate fed twice a day separately to forages.

The energy requirements of grazing animals are not clearly defined (Caton and Dhuyvetter, 1997), this may be due to the fact that the additional energy required for grazing activities above maintenance vary widely, and the energy that would normally be used for production of milk is partitioned to these activities. The production differences for grazing versus TMR fed cows can be explained by the low energy intake of cows on pasture. The differences in intake of nett energy for lactation (NE_L) for a pasture and concentrate; a pTMR and a complete TMR were 147.7, 168.2 and 182.8 MJ/day, respectively (Bargo *et al.*, 2002b) and that the energy needed for maintenance, grazing and walking activities is higher (Bargo *et al.*, 2002b; Kolver, 2003). In a study by Robbins (1993) cited by Caton and Dhuyvetter (1997) it was found that energy costs for grazing animals (over and above that for just standing) is approximately 83 to 1255 KJ per animal per kg of DM ingested, this large range is contributed to time spent eating (which is longer for grazing animals and is dependent on

amount of forage consumed and its availability), forage maturity, pasture topography, locomotion of distances between the pasture and dairy parlour and rumination (National Research Council, 2001). The total daily energy expenditure for sheep on pasture compared to confinement is 6822.0 compared to 5349.2 KJ/day (Osuji. P. O., 1974). The difference between the energy on pasture and confinement consists of the energy required for eating, ruminating, standing, walking, muscular work, resting, metabolic and heat increment for animals on pasture (Caton and Dhuyvetter, 1997). The maintenance energy requirement for grazing animals increases when there is an increase in grazing time or when there is a decline in forage availability (Caton and Dhuyvetter, 1997). The maintenance requirement is approximately 2.3 MJ extra of NE_L for a total round trip of two kilometers to the milking parlour (National Research Council, 2001). The energy requirements for activity of a specific situation is too complex to try and quantify but a range varying from as little as 10 to 50% of an additional increase in the maintenance energy requirements is proposed (National Research Council, 2001).

Managing feed costs in conjunction with maintaining nutrient levels to be met for the animals is the crux of profitable dairy farming (Ishler *et al.*, 1996). A large percentage which is approximately 45 to 60% of the total costs of producing milk consists of the feed costs alone (Ishler *et al.*, 1996). Grazed forage is the most economical source of nutrients due to its low cost (Bargo *et al.*, 2003). Income over feed cost is a sufficient parameter to assess the economic impact between the pasture-based and confinement systems (White *et al.*, 2002). Pasture-based systems were shown to be competitive to confinement systems, as no significant difference between the two systems in terms of income over feed cost were measured, even though on pasture milk production was 11.1% lower, the lower feed costs, lower culling costs and other economic variables (herd health, longevity and labour) support this (White *et al.*, 2002).

2.4 Nutrient requirement and intake regulation of cows on pasture

Dry matter intake is essential for the health and production of the animal. The “exact” value of each nutrient which is required by the animal is practically impossible to know, however, accurate estimates and guidelines in published tables (National Research Council, 2001) are sufficient. These published tables will allow for the animals nutritional requirements for maintenance and production to be more closely met. If the efficiency of nutrient use and the milk production from dairy cows is to be maximised, with reduced nutrient loss to the environment, the animals must be fed to meet their requirements, with little excess of nutrients in the feed (National Research Council, 2001). This can be achieved when level of

production coincides with the level of intake (Forbes, 2007) and assessing the pasture is of utmost importance because one can more correctly match animal requirements and pasture production to reach precise supplementary feeding.

Intake and its regulation are affected by many factors. These factors are animal, feed or environmentally related which either impose physical or chemical constraints on the animal as stated by Mertens (1987) describing that intake regulation is influenced over the short and long term. Short-term regulation which refers to the events effecting the frequency, size and pattern of meals that occur within a day. These events are the endocrine and nervous stimuli. The integration of all these factors from their different sources contribute to the variable stimuli the animal receives, the physiological, and behavioural responses and feedback mechanisms that follow result in stimulating or depressing intake (Forbes, 1996). Forbes (1996) further reports that the various sources of the stimuli act together and effects can be additive. This is explained by Adams and Forbes (1981) cited by Forbes (1996), the infusion of sodium acetate in the rumen, and simultaneous infusion of sodium propionate in the hepatic portal vein of sheep additively depressed intake by 60%, where added separately it was depressed by 44 and 19%, respectively. When acetate was infused in the rumen and a balloon was inflated in the rumen, the separate effects caused a reduction in intake of 12 and 18%, respectively, but the simultaneous effect of both in the rumen reduced intake by 50%. Long-term intake regulation refers to a longer period of time for which daily intake is determined, when the animals requirements for maintenance and production are relatively constant (Mertens, 1987).

The methods used to predict voluntary feed intake, are either described under static models, dynamic models or estimates received from *In vivo* measurements of intake (Pienaar and Roux, 1989).

Static models are used when a dietary feed component such as ME or NDF are described or expressed as a function of intake. The concepts of long-term intake regulation forms the foundation on which static models are derived (Mertens, 1987). The advantage thereof is that diets are balanced with the use of linear programming and least cost computer techniques, where the desired intake is obtained from a specific composition of the diet (Pienaar and Roux, 1989).

Dynamic models depict the rumen digestion kinetics and flow (Pienaar and Roux, 1989). Dynamic models are either mathematical models, which involve algebraic formulas or simulation models, which may only be operated on a computer, due to its specific simulation language (Pienaar and Roux, 1989). The attributes of such models allows the inclusion of many more variables, and the outcome thereof is more reliable compared to static-models.

The complexity of dynamic models is its short-fall, as its application to the practical situation therefore limits its use (Pienaar and Roux, 1989).

In vivo measurements of voluntary intake is the last approach described by Pienaar and Roux (1989) and is the simplest method. Tabulated standard values are produced from reference grasses, which are then used to predict voluntary intake in the practical situation (Pienaar and Roux, 1989). The difficulty with such estimates is that, standard values between animal species need to be adjusted, as well as with animals in the same species, which are in different physiological stages (Pienaar and Roux, 1989).

Distension in the gastrointestinal tract (GIT) is a physical constraint that is responsible for a reduced voluntary intake of DM (Allen, 1996). However, the best predictor of voluntary DMI was proposed to be the NDF fraction, as this proportion of fibre is highly responsible for the filling effect and, the slow passage rate through the GIT (Allen, 1996). The rate of fibre digestion does not influence the intake of DM to the same degree, because the intake is influenced more by the quantity of indigestible fibre and rate of passage (Mertens and Ely, 1979). The static model of Mertens (1987) that uses the NDF value to predict intake is shown by the following equation:

$$NDFIC = 0.011\ (BW)$$

Neutral detergent fibre intake capacity (NDFIC) and body weight (BW) is used in above mentioned equation. The equation demonstrates the assumption that the NDF intake is constant and is approximately 1.1% of BW (kg). Pienaar and Roux (1989) states that the shortcomings of the model of Mertens (1987), is that intake is underestimated on legume-based diets and overestimated on grass-based diets. Particle size, chewing frequency and extent, particle fragility, indigestible NDF fraction, fermentation rate of NDF and reticular contractions are other factors which effect the fill of the GIT (Allen, 1996). Feed intake limited by gut fill is dependent on the forage type and its quality, the cows' energy demand, and the effect of age and stage of lactation on conditioning the GIT (Rayburn and Fox, 1993).

Behavioural factors when compared to the physiological factors, are more pronounced for grazing animals (Hodgson, 1985).

To quantify these behaviour factors Hodgson (1985) proposed a dynamic/mechanistic model, where:

$$HI = IB \times BR \times GT.$$

Herbage intake (HI), quantity of herbage ingested per bite (IB), the biting rate (BR) and grazing time (GT). The IB is largely influenced by the sward height, volume and density (Hodgson, 1985).

Pienaar and Roux (1987) concludes that the method or approach used to predict intake, should be one that describes the precise situation for which it is used, and in choosing the best model, a good insight in predicting intake with a variety of models is required.

2.5 Estimating Intake on pasture

Estimating intake for cows on pasture remains a challenge as it's difficult to quantify and its' accuracy is low. Various methods have been used to estimate intake, namely the sward cutting method, capacitance meter, alkanes, markers, equations and with the reverse energy requirement calculation.

The sward cutting method also known as the herbage disappearance method is the conventional method for estimating herbage intake. This method comprises measuring the herbage mass before and after grazing with a rising plate meter (RPM) and calculating the difference between the two measurements (Reeves *et al.*, 1996; Macoon *et al.*, 2003; Smit *et al.*, 2005). The RPM was calibrated for pre- and post-grazing estimates (Reeves *et al.*, 1996). Smit *et al.* (2005) showed that the variation of this method is large, but its outcome is more reliable when grazing periods are short. To achieve a satisfactory accuracy level, a large number of measurement readings are essential specifically in situations such as post-grazing when the pasture heights on an area are highly variable (Earle and McGowan, 1979). The co-efficient of variation averaged about 13% when the Ellinbank pasture meter was initially constructed and evaluated in measuring pasture heights (Earle and McGowan, 1979). The repeatability within operators was high but a high degree of variation exists between operators due to their differing pasture measurement techniques (Earle and McGowan, 1979). The RPM was therefore suitable for research purposes on the basis of its accuracy and a good pasture management aid for farmers in obtaining herd estimates for pasture intake (Earle and McGowan, 1979; Reeves *et al.*, 1996; Macoon *et al.*, 2003).

The RPM is superior to the capacitance meter in its robustness and ease of use (Earle and McGowan, 1979). The capacitance meter is difficult and slower to use, it suffers

calibration drift with changing environmental conditions and battery life (Earle and McGowan, 1979). On the contrary the RPM can take 100 readings in 5 minutes (Earle and McGowan, 1979). The indirect methods to determine pasture mass using an electronic capacitance meter, a RPM or a pasture ruler was studied and errors were found ranging from 26 to 33% (Sanderson *et al.*, 2001). These methods therefore are relatively inaccurate.

Alkanes as opposed to the RPM which measures group intake estimations, allows herbage intake estimations of individual animals (Dove and Mayes, 1991; Reeves *et al.*, 1996). To determine intake from the use of n-alkanes a representative pasture sample of that consumed by the animals must be collected, as well as the fecal recoveries of natural and synthetically dosed n-alkanes should be similar (Dove and Mayes, 1991). Pairs such as C_{28} and C_{29}, and C_{27} and C_{28} underestimate herbage intake by 3.5 and 7.6%, respectively (Mayes *et al.*, 1986). The actual herbage intake was identical to that estimated using C_{33} and C_{32} n-alkanes (Mayes *et al.*, 1986).

Using the standard energy requirements in reverse is an alternative method to determine the intake of cows on pasture. The ME requirements and the *In vitro* organic matter digestibility (IVOMD) of kikuyu which varied from around 63.9 to 65.2% was used to calculate the standard energy requirement in reverse (RS) (Reeves *et al.*, 1996). The use of *In vivo* digestibilities apposed to *In vitro* digestibility data for RS determination showed that calculated intakes were values closer to that estimated with the RPM and alkanes (Reeves *et al.*, 1996). When accurate animal production and feed quality parameters are available, the use of the RS technique is feasible (Reeves *et al.*, 1996).

Equations developed by the NRC (2001) to predict intake are as follows:

$$\text{DMI (\% BW)} = 4.048 - 0.00387 \times \text{BW (kg)} + 0.0584 \times \text{4\% FCM (kg)}$$

And

$$\text{DMI (kg/day)} = (0.372 \times \text{FCM} + 0.0968 \times \text{BW}^{0.75}) \times (1 - e^{(-0.192 \times (\text{WOL} + 3.67))})$$ (National Research Council, 2001)

Where WOL represents the weeks of lactation.

2.6 Pasture allowance

To allocate sufficient pasture to animals, the available pasture mass should first be correctly assessed and measured. Pasture allowance (PA) has an influence on DMI, concentrate supplementation of animals, substitution rate (SR) of pasture and milk responses.

To achieve maximum DM intakes on pasture, the quality and quantity of pasture available to the animals must be optimum (Bargo *et al.*, 2003). Pasture intake is effected by PA and is regulated by the structure of the herbage (Peyraud *et al.*, 1996). Herbage intake increased with sward surface height and daily herbage allowance, and further stated that defoliated sward height is a major factor affecting the intake of herbage (Tharmaraj *et al.*, 2003). Increasing the herbage allowance by 1 kg DM, the daily DMI increased by 0.13 kg DM (Wales *et al.*, 1999). Pasture allowance is therefore a tool to manipulate the DMI of cows on pasture, and a relationship therefore exists between the two parameters (Stockdale, 1985; Dalley *et al.*, 2001). Dalley *et al.* (2001) observed that the rate of intake increased (1.9 vs. 1.5 kg DM/hour) in response to increased PA (60 vs. 45 kg DM per cow per day), which may be responsible for the higher DMI observed. Increasing the frequency of pasture allocation had no effect on the DMI or milk production of early lactation cows, which would not validate the extra time and labour input needed to relocate cows (Dalley *et al.*, 2001). Dalley *et al.* (2001) concludes that the single method to increase the pasture DMI of early lactation cows is to increase the PA.

Dry matter intake without concentrate supplementation increased (from 17.7 to 20.5 kg/day) when PA increased (from 25 to 40 kg DM/cow per day) (Bargo *et al.*, 2002a). On the contrary a high PA (40 kg pasture/cow/day) with concentrate supplementation resulted in lower DMI on pasture when compared to the lower PA (25 kg pasture/cow/day) with supplementation (Bargo *et al.*, 2002a). This is because with concentrate supplementation, the SR increases, with a subsequent decrease in pasture DM intake. This illustrates that SR is a factor to consider. Bargo *et al.* (2003) reported from previous studies that the SR increases with increasing PA. This is concurrent with a study that measured a SR of 0.1 at the low PA level and 0.5 at a higher level of PA (Meijs and Hoekstra, 1984).

Milk responses due to supplementation decreased as the SR increased at higher PA (Bargo *et al.*, 2003). Wales *et al.* (1999) proposed that supplements are best used when pasture height is short as this will lead to lower substitution levels and it will optimise the returns in milk production. When feeding concentrates, the milk yields were higher at lower PA, than at higher PA (Grainger and Mathews, 1989). Manipulating the PA is a means to increase milk production in early lactation cows grazing irrigated pasture (Wales *et al.*, 2001).

2.7 Energy supplementation and substitution rate

Grazing cows are supplemented to achieve higher DM intakes which could increase the total energy intake compared to pasture-only diets (Reis and Combs, 2000; Bargo *et al.*, 2003). The significant mobilisation of body reserves for cows consuming pasture emphasises

the need for supplemental energy, specifically for high-producing cows (Kolver and Muller, 1998). Pulido *et al.* (2009) reported that milk production increased due to concentrate supplementation compared to cows consuming pasture-only diets. Energy supplementation may alter the grazing behaviour and increase the efficiency of nutrient utilisation which ultimately will affect the energy requirements of the grazing ruminant (Caton and Dhuyvetter, 1997). There is no benefit in providing animals with feed and energy in excess of what they require (Kellaway and Harrington, 2004).

Energy supplementation may lead to substitution of pasture which is measured in terms of a SR (Stockdale, 2000). This is defined as the decrease in pasture consumption per kilogram of feed supplemented (Kellaway and Porta, 1993; Stockdale, 2000; Bargo *et al.*, 2003; Kellaway and Harrington, 2004). Pulido *et al.* (2009) supports this, in that pasture DM intake decreased by 4.2 kg when 6 kg of concentrate was fed per day. The equation is as follows: SR (kg/kg) = (pasture DMI in unsupplemented treatment – pasture DMI in supplemented treatment)/ supplement DMI (Bargo *et al.*, 2003). Many factors affect the level of substitution such as the level of pasture and concentrate available to the animal, stage of lactation, forage digestibility, concentrates chemical and physical properties and duration of the change in feeding level (Stockdale, 2000; Kellaway and Harrington, 2004).

The SR increases as the quantity of concentrate in the diet increases (Wales *et al.*, 1999; Stockdale, 2000; Kellaway and Harrington, 2004) from a low to medium concentrate level, SR = 0.47, and from a medium to high concentrate level, SR = 0.67 (Faverdin *et al.*, 1991). This is similar to results of Fulkerson *et al.* (2006) who grazed cows on ryegrass and found that the SR increased from 0.58 to 1.18 when the initial concentrate level fed (1.57 kg concentrate/cow/day) was increased by an additional 1.28 kg concentrate/cow/day. Faverdin *et al.* (1991) further illustrates this using an equation:

$$SR = 0.093 * CI \text{ (CI, concentrate intake per cow per day) } (r = 0.96, n=16)$$

The mean SR found over many studies was a value of 0.69 Minsons (1990) cited by (Caton and Dhuyvetter, 1997). The SR increases with increasing the frequency of concentrate feeding from 0.65 to 0.8 and 0.95 for feeding 6 kg of concentrate fed over two, three and four meals, respectively (Pulido *et al.*, 2009). A substitution value below 1, implies that concentrate supplementation is continuing to increase the total DMI (Kellaway and Harrington, 2004). A concentrate feeding of 2.4 kg/cow/day resulted in the highest margin over feed cost and increasing the concentrate only diminished the returns obtained (Meeske *et al.*, 2006).

The SR is additionally effected by the type of roughage due to its digestibility, fill unit and its overall energy balance (Faverdin *et al.*, 1991). This is observed when maize silage was fed; the SR was 0.7, which is higher than that obtained with grass silage (0.53) and hay (0.44). Faverdin *et al.* (1991) concluded that SR values become more relevant the higher the energy balance. Caton and Dhuyvetter (1997) reported that the substitution coefficient increases as the forage CP increases. The total OM intake as well as the portion of OM digested in the intestines increased, while the forage OM decreased due to grain supplementation (Reis and Combs, 2000). The SR increases as a result of high pasture digestibilities (Kellaway and Harrington, 2004). Stockdale (2000b) found that substitution increased by 0.16 kg DM/kg DM of concentrate in less digestible (grass-dominant pastures) opposed to more digestible (white clover-dominant) pastures.

Season is an additional factor affecting substitution, where a substitution of 0.11 kg DM/kg DM of concentrate was lower in summer than in spring, and 0.11 kg DM/kg DM lower in autumn than in summer. The regression equation including season is described by Stockdale (2000b) is as follows:

Substitution = - 0.34+0.16 (± 0.035) PI + 0.16 (± 0.053) species + 0.11 (± 0.024) season + 0.03 (± 0.014) concentrate intake.

Species equates to a value of 0 and +1 for clover and grass, respectively, where the season equates to +1, 0 and -1 for spring, summer and autumn, respectively.

The relationship that exists between milk response and SR is inverse or negative; the higher the milk response from supplementation, the lower the SR (Bargo *et al.*, 2003).

The varying milk responses observed for concentrate supplementation on pasture, is mainly due to the rate of substitution (Kellaway and Porta, 1993; Kellaway and Harrington, 2004). A milk response of 1 kg milk/kg DM of cereal grain supplemented in the diet was acceptable for cows grazing perennial ryegrass pastures, therefore grain supplementation is a means to increase milk production in early lactation dairy cows (Wales *et al.*, 2001). Cereal grain based concentrates fed twice daily up to 3 kg DM/day resulted in a milk response of 1.1 kg FCM/kg DM cereal-based concentrate (Walker *et al.*, 2001). Responses diminished when the concentrate level increased, and cows were fed more than 3 kg DM/day (Walker *et al.*, 2001). In a pasture-based system, the milk production response tends to drop, when animals substitute pasture for concentrates (Stockdale, 2000), this may be that the highly fermentable concentrate effects pasture intake which impacts milk production. The frequency of concentrates fed affected their performance and was negative when given four times

opposed to twice a day. Frequent feeding of small amounts concentrate supplementation reduced time spent chewing and overall feed intake (Pulido *et al.*, 2009).

The reason for substitution is not clearly defined but a few possible theories are proposed. Kellaway and Harrington (2004), suggests that pasture intake decreased due to the concentrates effect of lowering the ruminal pH, once ingested, which decreased the activity of cellulolytic bacteria. Kellaway and Harrington (2004) further explains that organisms in the rumen, would preferably degrade starch instead of cellulose, which may be a reason for lower fibre digestion occurring, and lower pasture intakes thereafter. Grazing time and rumen capacity are two factors which contribute to the event of substitution (Kellaway and Harrington, 2004), and as the grazing time and rumen capacity increases the SR decreases. Bargo *et al.* (2003) found that 80% of the reduction in pasture DMI observed due to supplementation and the increased SR was due to the reduction in grazing time.

2.8 Yeast

2.8.1 Type of yeast products, live verse dead

Saccharomyces cerevisiae (*S. cerevisiae*) is the Latin name given to the single-cell organism more commonly known as “bakers yeast” (Stone, 2006). Yeast is a microscopic fungus which is five to ten microns in size. Yeasts are facultative anaerobes; this allows survival and growth with or without the presence of oxygen. Species of yeasts differ in terms of their location, shape, reproducing activities and the substrates they utilise (Stone, 2006).

A live “yeast culture” more correctly referred to as active dry yeast, consists of pure dried yeast cells with a high viable cell concentration without their culture medium (Lynch and Martin, 2002). The active dry yeast is formed from the drying of a yeast biomass to maintain metabolic activities and the cells viability vary from 15 to 25 billion colony forming units per gram (CFU/g) (Stone, 2006; Chaucheyras-Durand *et al.*, 2008). The definition according to the Association of American Feed Control Officials (AAFCO) is that an active dry yeast or live yeast is dried in such a way that a large portion of the fermenting capability is maintained, that contains no additional cereal or filler, with a CFU count of no less than 15 billion CFU/g. The active dry yeast is produced in three different forms which are dependent on the procedure used to dry the yeast. The granular powder form, is a product of tunnel-dried yeast; torpedo shaped yeast is a product of yeast dried with the fluid-bed drying process and yeast that is spherical in shape are dried using the rotolouver method (Stone, 2006). The active dry yeast (CNCM-1077, Lallemand Animal Nutrition) is a live yeast supplement (Thrune *et al.*, 2009).

In contrast to an active dry yeast, viable cells may be mixed with their fermentation mediums or may contain no viable cells (Stone, 2006; Chaucheyras-Durand *et al.*, 2008)

these are referred to as yeast cultures and can be fed as a sole source of nutrients when fed in large quantities. This is similar to the definition proposed by the AAFCO, that the yeast is dried to maintain some fermenting capabilities with fewer viable cells, and normally the fermentation medium on which it is grown is included. Brewer's yeast from riboflavin extraction is a yeast culture and is used as a source of energy and protein for animals (Besong *et al.*, 1996).

For the convenience of this review all yeast cultures and live yeast products will be referred to as supplemented yeast and no distinction will be made unless, the yeast products are specifically mentioned.

2.8.2 Yeast viability and processing

The viability of live yeast and its ability to remain viable was investigated and done in determining what the best incubation conditions would be to make this possible (Kung *et al.*, 1997). This study showed that yeast colonies began to decline only after a 24 hour exposure period of anaerobic conditions and are therefore able to remain viable for a long period of time, but their capacity to multiply under such conditions is limited (Kung *et al.*, 1997). Medina *et al.* (2002) showed that yeast cells are able to survive in the cecum and colon of the GIT of horses, but are unable to colonise it. This was concluded because viable yeast cells detected in the cecum of horses 4 hours post-feeding was similar to that initially dosed (± 10^6 CFU/g DM), but contents in the colon showed much lower levels of 4.5 ×10^4 CFU/g DM (Medina *et al.*, 2002).

The residual effect of yeast supplementation was maintained after which supplementing yeast during heat stress increased DMI ($P = 0.02$) and tended to increase milk production ($P = 0.08$) and, was maintained at 60 DIM even after withdrawal of yeast culture at 21 DIM. This suggests that the residual stimulatory effect that the yeast culture has on the animal remains prominent even after its withdrawal from the daily ration (Ward and McCormick, 2001). Vaneeta *et al.* (1998) had extracted the supernatant from yeast, and had autoclaved yeast, after which both had been added to the rumen of separate animals. Effects of the yeast up to 2 hours after addition was exerted on the animal, which indicates that stimulatory components are exhausted or destroyed shortly (after 2 hours) after being added to the rumen, which was present in the yeast cell filtrate and had not initially been destroyed by autoclaving (Vaneeta *et al.*, 1998).

Live yeast products remain viable during processing to allow the animal to fully benefit from post-consumption fermentation (Stone, 2006). Processing live yeast and the yeast maintaining its viability after pelleting (which adds heat) depends on the extremities of the

temperature and the time of such exposure. The approximate range at which feeds are pelleted is between 50 and 60 Degree Celsius (°C), this may vary as other studies used temperatures for steam pelleting as high as 85 °C (Hadjipanayiotou *et al.*, 1997). Yeast counts after pelleting decreased the viable yeast about tenfold (Aguirre-Guzmán *et al.*, 2002). Adding heat such as autoclaving (121 °C for 15 min) inactivates the yeast cells (Dawson *et al.*, 1990; Oeztuerk, 2009) and destroys all stimulatory activity (Vaneeta *et al.*, 1998). While y-irradiation of yeast kept 50% of the stimulatory activity because the yeast cells can no longer reproduce but remain metabolically active (Vaneeta *et al.*, 1998). The full effects of the yeast is only realized when the yeast is metabolically active (Vaneeta *et al.*, 1998).

In vitro studies carried out to investigate whether the yeasts viability (live yeast vs. autoclaved yeast) is a factor effecting responses in ruminal fermentation parameters, when 1.5 g/day of yeast was added with 9 g of feed (5 g meadow hay and 4 g pelleted concentrate) (Oeztuerk, 2009). The live yeast showed a resultant higher pH, ammonia nitrogen (NH_3-N) and propionate concentration compared with the autoclaved yeast (Oeztuerk, 2009). The live and autoclaved yeast both increased NH_3-N concentration by 15 and 8%, respectively. These results concluded that comparing the autoclaved and live yeast, the live yeast had a more prominent effect in stimulating fermentation in the rumen (Oeztuerk, 2009). Dawson *et al.* (1990) states that live yeast supplements stimulate cellulolytic micro-organism growth. *In vitro* fermentors have a high buffering capacity so studies used to measure yeast effects on pH may be inappropriate (Kung *et al.*, 1997).

Preparing the feed samples for viable yeast cell counts requires that the cells are dispersed well in the feed sample in the dilution medium, which ensures accurate yeast cell counts (Aguirre-Guzmán *et al.*, 2002).

2.8.3 Mode of action

The mode of action of *S. cerevisiae* has been investigated in many experiments. The results and responses across experiments of previous research have differing outcomes. The exact and true activity of yeasts in the rumen remains uncertain. Speculations' regarding the action of *S. cerevisiae* has been brought forward and the theories proposed will be discussed here.

The first and most widely supported theory is that the yeast stimulates the growth of certain microflora (Nisbet and Martin, 1991; Andrighetto *et al.*, 1993; Arakaki *et al.*, 2000; Abd El-Ghani, 2004). Cellulolytic, amylolytic, proteolytic bacteria and protozoa are among the microbes which have been reported to respond to the addition of yeast (Newbold *et al.*, 1996).

The theory proposed in an experiment to investigate the effects of yeast on lactate uptake and growth by *Selenomonas ruminantium* (*S. ruminantium*), is that the yeast stimulates this bacteria. *S. ruminantium* is a gram-negative bacterium which ferments lactate. Therefore, the yeast indirectly stabilised the rumen pH and its fermentation by improving the ability of the rumen bacteria to utilise lactic acid (Nisbet and Martin, 1991; Wallace, 1994). Quigley at al. (1992) elaborates and states that the lactate utilisers in the rumen are stimulated and respond to a specific substance known as L-malic acid which is contained in large amounts in a yeast culture that may be responsible for the reduced ruminal concentrations of lactate (Quigley *et al.*, 1992; Wallace, 1994).

Amylolytic bacteria are another group of bacteria which are affected by the presence of yeast in the rumen. Amylolytic bacteria proliferate due to yeast supplementation (Arakaki *et al.*, 2000). Enjalbert *et al.* (1999) explains that yeast prevents the decrease in amylolytic bacteria post-concentrate feeding, because the protozoal concentrations that proliferate and that are stimulated by yeast, are able to store starch and postpone bacterial fermentation.

The cellulolytic bacteria, which are the predominant fibre-digesters, promote higher intakes due to their increased activity in response to yeast supplementation, illustrated in Figure 2.1. Yeasts ability to improve the fibre digestion in stimulating cellulolytic bacterial numbers (Stella *et al.*, 2007) and their ruminal activities is well documented (Dawson *et al.*, 1990; Newbold *et al.*, 1996). A study conducted in horses where yeast was supplemented showed that the yeast increased most enzymes involved in plant cell-wall digestion, which would then lead to better fibre digestibilities (Jouany *et al.*, 2009). Another study involving yeast supplementation in horses suggested that the dietary acid detergent fibre (ADF) fraction was improved due to the positive effects of yeasts on the microflora to improve their activity in digesting nutrients (Jouany *et al.*, 2008). Supplementing lambs with yeast to investigate its effect on the colonization of the rumen of the newborn animals, found that yeast stimulated the growth of cellulolytic bacteria (Chaucheyras-Durand and Fonty, 2002)..

Protozoa populations form an integral part in the digestive process of fibre (Ishler *et al.*, 1996). The exact functions of protozoa micro-organisms remains uncertain, their numbers vary with the digestibility of the diet, and are numerous when the digestibility is high (Ishler *et al.*, 1996). Supplemented yeast increased the protozoa concentrations (Carro *et al.*, 1992; Plata *et al.*, 1994; Mathieu *et al.*, 1996); a possible reason for this may be that yeast increases the bacterial counts in the rumen. Higher bacterial numbers due to their growth is used as a source of protein and energy for protozoal growth (Ishler *et al.*, 1996; Arakaki *et al.*, 2000). The protozoa establishment for lambs receiving the yeast supplement, a product Levucell SC occurred earlier (Chaucheyras-Durand and Fonty, 2002).

Figure 2.1 further illustrates that increased feed intakes are driven by increased flow of absorbable N. This reactions stem simultaneously from the proliferation and higher viable cell counts of anaerobic bacteria in the ruminal fluid (Wallace, 1994). The higher NH_3-N concentration measured for the vessel in which live yeast was added compared to autoclaved yeast, suggest that the live yeast or some heat liable component of the yeast cells stimulates the proteolytic activity of rumen bacteria to influence ruminal fermentation (Oeztuerk, 2009). Crude protein digestibilities was largely increased by the combined fungal supplementation of a yeast culture and *Aspergillus oryzae* (*A. oryzae*), suggesting that while the yeast culture might promote proteolytic bacterial growth through supplying stimulatory factors, it might posses proteolytic activities (Wiedmeier *et al.*, 1987). More energy was available to the microbes for their growth when sheep fed berseem hay was supplemented with baker's yeast *S. cerevisiae* (on condition that N had unaffected release) (Kamel *et al.*, 2004). Ammonia nitrogen concentration was highest in the higher yeast supplementation level, which may suggest that the degradation of protein had been extensive and that yeast stimulates proteolytic bacterial activity (Kung *et al.*, 1997; Moallem *et al.*, 2009). Conversely a yeast additive *S. cerevisiae* CNCMI-1077 was investigated for its effects on protein degrading activities of bacteria *In vitro* and results from such a study suggest that the added live yeast limits the ability of bacteria to degrade proteins, because reduced bacterial proteolytic activities were measured (Chaucheyras-Durand *et al.*, 2005). The reason for this was assumed to be either that the live yeast and rumen bacteria compete for substrates or that the release of small peptides by the yeast may be the competitive structures responsible for the reduced protease activities measured in the bacteria (Chaucheyras-Durand *et al.*, 2005).

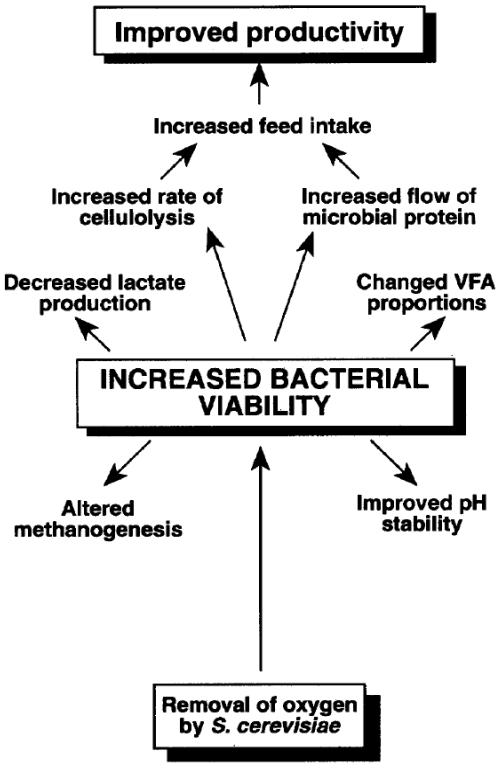

Figure 2.1 Representative scheme of the mode of action of *S. cerevisiae* (Wallace, 1994)

A firm understanding that yeast stimulates the microflora and fauna is evident but certain mechanisms are responsible for this. These mechanisms involve oxygen-scavenging, produced acids, growth and stimulatory factors which the yeast may be responsible for.

Respiratory activity of yeast in ruminal fluid drives its ability to stimulate the viable count of bacteria (Wallace, 1994). Oxygen molecules which are toxic to the ruminal anaerobes (Russell and Hespell, 1981; Wallace, 1994; Rode, 2000), are removed by the yeast at various times in the feeding cycle. To investigate this proposed idea that the yeast removes the oxygen in the rumen that may indirectly protect the anaerobic bacteria from the damage that oxygen molecules may have was carried out by Newbold *et al.* (1996). This theory was tested with different strains of yeast. The results showed that the rate of oxygen disappearance increased around 46 to 89% when yeast products were added to the rumen fluid *In vitro* at a rate of 1.3 mg/L (Newbold *et al.*, 1996). The investigated strains NCYC 240 and NCYC 1026 in rusitec stimulated the total and cellulolytic populations of bacteria, though when oxygen was excluded, the NCYC 240 increased cellulolytic bacterial numbers while ceasing to stimulate total bacterial numbers (Newbold *et al.*, 1996). Chaucheyras-Durand and Fonty (2002), Mathieu *et al.* (1996) and Marden *et al.* (2008) later found that oxygen-scavenging was due to the reduced redox potential of the ruminal fluid. This would create more

favourable environmental conditions in the rumen for anaerobic microflora (Mathieu *et al.*, 1996; Chaucheyras-Durand and Fonty, 2002). Marounek and Wallace (1984) investigated the influence of the E_h (redox potential) on the metabolism and growth of rumen bacteria (*S. ruminantium, Bacteroides amylophilus, Bacteroides succinogenes and Streptococcus bovis*) in batch culture. The study found that the growth yield and ratios of fermentation end-products produced by these bacteria, were unaffected by changing redox potentials induced, although the growth rate of these bacteria were effected when the redox potential values were above 0 mV (Marounek and Wallace, 1984). It was therefore found that the high E_h was not the toxic factor affecting the microbes, but the oxygen present (Marounek and Wallace, 1984).

The second proposed mechanism to be investigated was that the presence of malic and dicarboxilyic acids produced by the yeast may have a favourable effect in stimulating bacterial growth of some rumen micro-organisms (Newbold *et al.*, 1996) and was carried out in comparing the effects of malic acid and a yeast on fermentation responses. It was found that malic acid did not stimulate the total number of bacterial organisms, where yeast inclusion did (Newbold *et al.*, 1996). Therefore the mechanism by which the yeast stimulates the bacterial ruminal population is in itself respiratory mediated and not malic acid orientated (Newbold *et al.*, 1996). However, Martin and Nisbet (1992) suggested that the malate content in the extract of *A. oryzae* and *S. cerevisiae* may be involved in the stimulation of lactate utilisation by *S. ruminantium* (Martin and Nisbet, 1992).

Soluble growth factors (i.e. organic acids, branched-chain VFA, vitamins and AA) supplied by yeast may stimulate the growth of bacteria that utilise lactate, digest cellulose and protein (Wiedmeier *et al.*, 1987; Callaway and Martin, 1997). A rumen fungal strain of *Neocallimastix frontalis* was stimulated *In vitro* (by which its zoospores had germinated), by two strains of *S. cerevisiae* by Fonty and Chaucheyras-Durand (2006). *Sacharomyces cerevisiae* supplied thiamine which had been required by the rumen fungi for zoosporogenesis.

Yeast through selectively stimulating certain species of anaerobic bacteria, have been speculated to manipulate the AA profile of microbial protein (Harrison *et al.*, 1988; Dawson *et al.*, 1990; Erasmus *et al.*, 1992). Erasmus *et al.* (1992) further states that this may apply dominantly to the specific bacteria under investigation, such as *Selenomas ruminantium, Butyrivibrio fibrisolvens, Bacteroides amylophilus,* and *Bacteroides ruminicola* due to the large differences in AA concentrations (Erasmus *et al.*, 1992). The specific AA were Threonine (Thr), Valine (Val), Met, Isoleucine (Ile), Leucine (Leu), Lys and Phenylalanine (Phe) (Erasmus *et al.*, 1992). A population shift of rumen bacteria therefore occurs and is reflected

in the AA profile of the total bacterial mass that has been altered due to the stimulatory effect of the yeast on the growth of specific bacterial species (Erasmus *et al.*, 1992). The duodenal flow of Met was significantly higher for yeast supplemented cows, at 58 g/day compared to the control at 41 g/day (Erasmus *et al.*, 1992). The Lys was numerically higher for the yeast supplemented cows at 140 g/day compared to the control of 116 g/day (Erasmus *et al.*, 1992). Increased supply of these AA can increase milk protein content, yield, milk production and feed intake (National Research Council, 2001). This is of value to nutritionists, as it provides a means of possibly altering the duodenal AA profile, without inclusion of expensive rumen undegradable protein sources (Erasmus *et al.*, 1992). However, different results were found by Putnam *et al.* (1997) in which neither a shift of the AA profile, or the ratio of microbial protein to total protein passing to the duodenum occurred. Further experimentation to enrich the current research in which these theories and mechanisms can be accepted or ruled out is essential.

The mechanisms by which yeast affects the ruminal microorganisms has been discussed above. To conclude that supplemental yeast stabilises rumen pH, reduces ruminal lactate production, stimulates amylolytic, cellulolytic and proteolytic bacterial rumen activities.

2.9 The rumen environment

The rumen, also known as the fermentation chamber is inhabited by bacteria, fungi and protozoa which are specific to the type of diet being fed (Ishler *et al.*, 1996; Rode, 2000). The rumen contents is not a uniform composition not only as a result of varying feed types ingested at any given time, but the feed is distributed in such a way that it forms stratified layers (Ishler *et al.*, 1996). These layers range from the recently ingested material that are present as a floating mat and as the process of fermentation and digestion progresses, the particles hydrate, become more dense and move through the mat once reduced in size progressively to the bottom of the rumen (Ishler *et al.*, 1996). Food particles broken down through the process of rumination and microbial activities then leave the rumen and pass through the orifice to the lower GIT (Ishler *et al.*, 1996).

Bacterial numbers range from 10^{10} to 10^{11} cells/g of rumen contents (Ishler *et al.*, 1996). The bacteria may be classified into different groups with respect to their structure and shape (cocci, rods and spirilla), size (starting at 0.3 to 50µm) and the type of substrate they ferment and utilise (Ishler *et al.*, 1996). Plant material colonised by the bacteria, consist of cellulose, hemicellulose, starch, sugars, intermediate acids, protein and lipids (Ishler *et al.*, 1996). These structures are utilised by bacteria to produce energy, VFA and microbial protein (Ishler

et al., 1996). There are specific classes of bacteria, which perform different functions, and some may be classified in more than one type namely:

Cellulolytic species (*Bacteroides succinogens, Ruminococcus flavefaciens, Ruminococcus albus and Butyrivibrio fibrisolvens*); Pectinolytic species (*Butyrivibrio fibrisolvens, Bacteroides ruminicola, Lachnospira multiparus, Succinivibrio dextrinosolvens, Treponema bryantii, Streptococcus bovis*); Ureolytic species (*Succinivibrio dextrinosolvens, Selenomonas sp, Bacteroides ruminicola, Ruminococcus bromii, Butyrivibrio sp, Treponema sp)*; Sugar-utilising species (*Treponema bryantii, Lactobacillus vitulins, Lactobacillus ruminus)*; Proteolytic species (*Bacteroides amylophilus, Bacteroides ruminicola, Butyrivibrio fibrisolvens, Streptococcus bovis)*; Lipid-utilising species (*Anaerovibrio lipolytica, Butyrivibrio fibrisolvens, Treponema bryantii, Eubacterium sp., Fusocillus sp., Micrococcus sp.)*; Hemicellulolytic *species* (*Butyrivibrio fibrisolvens, Bacteroides ruminicola, Ruminococcus sp.)*; Amylolytic species (*Bacteroides amylophilus, Bacteroides ruminicola, Streptococcus bovis, Succinimonas amylolytica)*; Methan-producing species of bacteria (*Methanobrevibacter ruminantium, Methanobacterium formicicum, Methanomicrobium mobile*) the hydrogen gas together with the carbon dioxide to produce methane (Ishler *et al.*, 1996). These bacteria promote growth of other bacteria when hydrogen is removed and stimulate the hydrogen-producing bacteria which all results in higher yields of microbial cells, which is a protein source for the ruminant (Ishler *et al.*, 1996). Acid-utilising species (*Megasphaera elsdenii, Selenomonas ruminantium*); Ammonia-producing species (*Bacteroides ruminicola, Megasphera elsdenii, Selenomonas ruminantium*) source Church, D. C., Ed. The ruminant Animal: Digestive Physiology and Nutrition. Englewood Cliffs, N.J.: Prentice Hall, 1988. (Ishler *et al.*, 1996).

Bacteria function over a range of microbial conditions which are dictated by the diet which ultimately fluctuates pH levels (Ishler *et al.*, 1996). The pH of the rumen digesta of a cow on a well-balanced diet should range between 5.8 and 6.4, which should include the range of all microbial species (Ishler *et al.*, 1996). The two most commonly mentioned groups of bacteria that operate at different pH levels, are the fibre-digesters and starch-digesters (Ishler *et al.*, 1996). The fibre-digesting bacteria, function optimally at a pH that ranges from 6.2 to 6.8, and generally a drop below 6, sees the reduction in cellulolytic and methanogenic bacteria (Ishler *et al.*, 1996). The starch digesters function at a pH level ranging from 5.2 to 6, which indicates their acid tolerant nature (Ishler *et al.*, 1996).

The protozoa concentration in the rumen is approximately 10^5 to 10^6 cells per gram of rumen contents (Ishler *et al.*, 1996). Protozoa numbers under a pH of 5.5 are greatly reduced

(Ishler *et al.*, 1996). They grow and engulf bacteria, their exact role in the rumen is unclear though they play an important role in fibre digestion (Ishler *et al.*, 1996).

Fungi degrade cellulose and xylans (Ishler *et al.*, 1996)

Microbes present in the rumen exist in what could be simplified and divided into three phases. The first phase is the liquid-associated phase (which consists of 25% of the microbial biomass) in which rumen bacteria freely move and feed on the soluble constituents in the rumen such as soluble proteins and carbohydrates (Ishler *et al.*, 1996). The second phase is the solid-associated phase, in which 70% of the microbial biomass are closely associated with the less soluble constituents of starches, fibre and protein particles (Ishler *et al.*, 1996). The third phase (which consists of 5% of the microbial biomass) microbes may attach to the rumen walls epithelial cells or to protozoa (Ishler *et al.*, 1996). These phases being either the liquid or solid vary in terms of their retention time and passage rate through the rumen, these processes conduct the bacterial reproduction time and therefore their turnover rate (Ishler *et al.*, 1996). This further influences the type of bacteria one would expect to be associated in either phase, as to maintain a constant balance of bacteria being produced and removed from the rumen (Ishler *et al.*, 1996). The slower growing and producing bacteria are associated with the solid phase in which particles are retained longer and have a slow passage rate (Ishler *et al.*, 1996). The liquid- or fluid-phase have associated bacteria which are fast growing and producing and leave the rumen after a shorter retention and therefore have higher passage rates (Ishler *et al.*, 1996).

Gases produced as a result of fermentation are largely made up of carbon dioxide (65.5%) and methane (26.8%) in proportions dependant on the type of fermentation and ecology of the rumen (Ishler *et al.*, 1996). N, oxygen and hydrogen make up approximately, 7, 0.5 and 0.2% (Ishler *et al.*, 1996).

Ruminal papillae on the mucosal surface are highly vascularised which aid in maximizing absorption. The papilli characteristics are a function of the type of forage, the feeding pattern and digestibility (Ishler *et al.*, 1996). The changes in the diet of the ruminant constitute the ruminal papillae and ecosystem having to gradually adapt sufficiently, which is recommended over a period of two to three weeks (Ishler *et al.*, 1996).

Volatile fatty acids such as acetic acid (50-60%), propionic (18-20%), butyric (12-18%), iso-butyric, valeric and isovaleric are produced from fermentation of both structural and non-structural carbohydrates (Ishler *et al.*, 1996). The VFA suffice 80% of the animals energy requirements (Ishler *et al.*, 1996). The VFA proportions between diets with varying forage to concentrate ratios remains stable, but varies with the pH level (Ishler *et al.*, 1996). The main site of VFA absorption is the rumen, and maintaining a continuous supply of VFA by the

microbes and removal from absorption allows in stabilising the ruminal pH (Ishler *et al.*, 1996).

Acetic acid is the dominant acid produced in the rumen of animals consuming high fibre diets (Ishler *et al.*, 1996). The acetic acid produced is absorbed and is used to synthesis fatty acids which is deposited as fat in adipose tissue or to produce milk fat (Ishler *et al.*, 1996).

Propionic acid production in the rumen predominates when diets high in grain or energy, such as a concentrate are fed (Ishler *et al.*, 1996; Eastridge, 2006). The propionic acid is a precursor for glucose synthesis in the liver which is used for energy (Ishler *et al.*, 1996). This glucose is the source of milk sugars such as lactose (Ishler *et al.*, 1996).

Butyric acid is converted to beta-hydroxybutyrate by the rumen epithelium during absorption and provides energy to the rumen wall in this conversion (Ishler *et al.*, 1996). The beta-hydroxybutyrate is a ketone and used to produce fatty acids which are stored in the adipose tissue (Ishler *et al.*, 1996).

The first limiting nutrient for microbial growth is energy, and when large amounts of energy become available as when a dairy concentrate is fed, the microbes cannot limit the energy uptake and then deal with the carbohydrate overload in different ways (Rode, 2000). The bacteria may either store the excess carbohydrates as intra- or extracellular polysaccharides or shift their fermentation pathway. The shift will be towards microbes (*S. bovis*) producing lactate instead of acetate or propionate (Rode, 2000).

Dairy cows are prone to acute or chronic acidosis, when they ingest excessive amounts of readily fermentable carbohydrates. Accumulating acids in the rumen from VFA produced and lactic acid from lactic acid producing bacteria impose negative effects on the cows' health. The ruminal intestinal wall is damaged, the blood pH falls, laminitis and liver abscesses occur, the animal reduces its intake all of which effect overall animal performance (Owens *et al.*, 1998).

In a review covering efficiency of supplemental dietary neutralizing agents for lactating dairy cows, states that the ability of forage digesting organisms to grow and produce acetate is compromised by low pH levels more so than are the starch-digesting organisms producing propionate in the rumen (Staples and Lough, 1989). The absorption of propionate and butyrate are increased at low pH's, while acetate absorption is decreased (Staples and Lough, 1989).

Ruminal pH fluctuates before and after feeding, with higher values found before feeding and lower pH values measured at 2 and 4 hours after feeding (Guedes *et al.*, 2008). The rumen pH influences the ruminal end-products produced. The fibre-digesting bacteria produce acetate with hydrogen gas as a product. The accumulation of hydrogen gas blocks

further acetate produced. The removal of hydrogen gas is accomplished in two was, by either producing propionate, which is a usable energy source or methane which is not, and energy is lost. When the pH declines, it affects the fibrolytic and methane producing organisms. Therefore high concentrate diets result in propionate dominantly produced with lower acetate levels (Rode, 2000). High propionic acid produced from starch fermentation may overload the liver to metabolise these substrates and lead to lower milk and fat yields (Orskov, 1986). The cow spends less time eating and ruminating such highly fermentable diets and saliva production is therefore reduced, this all contributes to the drop in pH and reduced fibre digestion (Orskov, 1986). The rumination behaviour in the goats was observed and found that the rumination duration time decreased as the concentration of the diet increased (Desnoyers *et al.*, 2009b). Starch in the diet, increased the lag time for fibre digestion *In vitro*, which decreased the potential extent of digestion without effecting the rate of digestion (Mertens and Loften, 1980).

To improve the efficiency of feed used, it is important to increase the efficiency of ruminal fermentation and digestion (Eastridge, 2006).

2.10 Factors effecting yeast supplementation responses

Yeast supplementation will be discussed with respect to the following factors; diet and pasture; animal related factors such as carcass quality, body condition score (BCS), feed efficiency and BW; strain variation; stage of supplementation; yeasts interaction with other additives or substances; and, responses to supplementation. Piva *et al.* (1993) states that the cows response to supplementation of yeast depends on the lactational phase, the type of diet in terms of the forage to concentrate ratio and specifically roughage supplied and feeding technique.

2.10.1 Diet and forage : concentrate ratio

The diet has a substantial influence on the microbial species, numbers and their dominant activity in the rumen. Yeast and its influence is effected by the quality and composition of feed as reflected in *In vitro* (Zelenák *et al.*, 1994). Lack of response of yeast was found with yeast supplemented steers on low quality pasture such as tropical pastures consisting of 72.3 % star grass (*Cynodon plectostachyus*), 14.4 % *Paspalum conjugatum* , 8.9 % *Brachiaria mutica* and 4.4 % other (Cabrera *et al.*, 2000).

It is well known that concentrate supplementation reduces the pH, and Thrune *et al.* (2009) demonstrated that a diet fed consisting of 40% concentrate and 60% forage (corn silage 40.3%, alfalfa hay 17.3% and wheat straw 1.8%) with supplemented yeast resulted in

higher pH's measured, however, diets of similar ratios of concentrate (ground corn and barley, and rice bran) to forage (alfalfa) with yeast supplemented, showed little effects on ruminal fermentation (Malcolm and Kiesling, 1990).

Yeast supplemented goats were fed either a diet consisting of 20:80 or 50:50 concentrate (wheat, barley and maize) to forage (lucerne and grass hay, and sugarbeet pulp) ratios, which resulted in an adjustment in the feeding behaviour shown in a study where goats selected diets that were lower in fibre (Desnoyers *et al.*, 2009b). Additionally, steers grazing mixed-grass prairie supplemented with yeast resulted in the animals selecting a diet greater in soluble N and IVOMD ($P > 0.1$) (Olson *et al.*, 1994).

The addition of yeast to a moderate concentrate diet of 50% (wheat bran and molasses) and 50% forage (alfalfa hay and barley straw) increased the total tract percentage digestion of CP and hemicellulose compared with the control (Wiedmeier *et al.*, 1987). It was proposed that stimulatory factors were added by the yeast for rumen bacteria and for *A. Oryzae* when added together which additionally provided cellulase enzymes that resulted in an increase in CP, hemicellulose digestibility and percentage of cellulolytic organisms (Wiedmeier *et al.*, 1987). Abd El-Ghani (2004) fed Suffolk ewes a diet with the same ratios of 50% concentrate (sorghum grain, wheat bran and molasses) and 50% low quality forage (sugar cane tops) with yeast supplementation which had no effect on the total tract digestibility of DM, OM and NDF (Abd El-Ghani, 2004). This may be due to the fact that the quality of forage effects the response of yeast supplementation, and specifically influences NDF digestion, in which more favourable results are produced from the use of good quality forage (Roa V *et al.*, 1997). This is supported in a study where no significant interaction ($P > 0.1$) was found with yeast supplementation between the diets differing in oats straw level (40, 60 and 80%) which is a lower quality forage (Plata *et al.*, 1994) and for yeast supplemented steers on tropical pastures where no improvement of total tract digestibility of NDF or ADF was found (Cabrera *et al.*, 2000). Contradictory to this, in a study where the use of good quality forages such as corn silage or alfalfa hay, with corn meal concentrate, whole cotton seed, tallow and yeast, at a concentrate to forage ratio of 50:50, resulted in a lack of an interaction between yeast and the diet (Smith *et al.*, 1993).

There existed a significant interaction with yeast and forage at a concentrate to forage ratio of 55:45 specifically with the forage type alfalfa hay compared to yeast and corn silage (control) for milk yield ($P = 0.043$) and solid corrected milk ($P = 0.024$) (Adams *et al.*, 1995). Williams *et al.* (1991) concluded that the diets interaction with a yeast supplement at a higher concentrate to forage ratio (60:40) tended ($P < 0.061$) to increase FCM yields with significantly higher milk yields when the diet and yeast interaction was significant ($P < 0.05$) at

this level (60:40) for hay. The milk yield was not significantly affected, but the milk fat tended to increase when straw was fed with a yeast supplement.

Increasing the grain (concentrate) level at a rate of 2.5 kg per day for 4 days for Holstein heifers initially on an all forage diet created a dietary challenge reported by Moya *et al.* (2009), which increased the dietary upsets by 83.3%. The use of yeast reduced the strength of the foam formed in the rumen (Moya *et al.*, 2009). This suggests the possible advantage of reducing the incidence of bloat possible with diets having high concentrate to forage ratios (Moya *et al.*, 2009). *In vitro* studies investigating the effect of yeast supplementation of three different diets differing in their concentrate to forage ratio (high forage 70:30, a medium diet 50:50 and a high concentrate 30:70), revealed that yeast effects on fermentation is dependent on the diet, where high concentrate diets resulted in more dominant effects with yeast supplementation (Carro *et al.*, 1992). These effects stems from the increased microbial growth and activity of yeast when high concentrate diets were fed. The high concentrate diet and yeast resulted in increased degradabilities of DM and NDF, VFA and methane production and protozoa numbers, while ammonia production was reduced (Carro *et al.*, 1992). Lascano and Heinrichs (2009) fed corn silage based diets across a range of concentrate to forage ratios (20:80, 40:60 and 60:40) with yeast supplementation and recorded increased microbial activity and growth, in which the fermentation rate was improved (Lascano and Heinrichs, 2009). Guedes *et al.* (2008) fed maize silage based diets and agreed with this as higher fibre degradation occurred in the rumen, with reduced possibility of developing rumen acidosis. When animals are fed a high grain diet and a low pH in the rumen is produced, it was proven that despite the low pH the animals are able to adapt and avoid the subsequent drop in fibre digestibility and intake, and that added yeast will be of little value in adapted animals (Beauchemin *et al.*, 2003).

Moloney and Drennan (1994) have proved that yeasts (Yea-Sacc) positive attributes and effects on N metabolism were accountable to the N content of the basal diet. When the substrate starch and cellulose was incubated in both a yeast adapted and yeast unadapted rumen inoculums, results showed higher microbial N synthesis for both substrates in the yeast adapted inoculums (Kamalamma *et al.*, 1996). This is in agreement with a study where goats where goats were fed a low-N diet with yeast supplementation, which resulted in the more efficient use of NH_3-N incorporated into microbial protein (Giger-Reverdin *et al.*, 1996) and in Holstein cows a tendency towards a yeast and CP level interaction, that suggested a greater effect of yeast at the lower CP level (Putnam *et al.*, 1997). According to Giger-Reverdin *et al.* (1996), autolysis of yeast in the forestomach, the micro-organisms would use

the source of nutrients (nucleotides, AA and vitamins) thereafter for bacterial growth especially when N is limiting.

In vitro mixed ruminal micro-organism fermentations with yeast supplementation had little effect on final pH and fermentation end products for the substrates of ground corn, maltose, and lactate (Sullivan and Martin, 1999; O'Connor *et al.*, 2002). The rate (O'Connor *et al.*, 2002) and extent of digestion of either alfalfa hay or bemudagrass hay by mixed ruminal micro-organisms was minimally effected (Sullivan and Martin, 1999; O'Connor *et al.*, 2002). Neutral detergent fibre and ADF digestibility *In vitro* were not significantly affected by yeast adapted rumen inoculums' for diets consisting of either a commercial cattle feed or finger millet straw (Kamalamma *et al.*, 1996). An *In vitro* gas production technique was used to determine the effects of yeast supplementation on *In vitro* fermentation parameters of rice straw, wheat straw, maize stover and maize stover silage (Tang *et al.*, 2008). This study showed an increase in cumulative gas production, rate of gas production, *In vitro* DM digestibility and *In vitro* OM disappearance, with a decreased lag time for each type of straw when yeast was supplemented. The simultaneous supplementation of fibrolytic enzymes and yeast showed interactions between the two additives for all the *In vitro* gas parameters for each type of straw (Tang *et al.*, 2008).

2.10.2 Animal growth, physiological status and energy balance

The effect of yeast supplementation on the animals' physical growth characteristics, physiological status and energy balance is depicted by and measured as either carcass quality, BCS, feed efficiency and BW.

2.10.2.1 Carcass quality

The mean fat cover over the 12th rib, loin area, and dressing percentage of the yeast fed steers did not differ from that of the control group (Mir and Mir, 1994). Similarly no differences were measured between the carcass characteristics of the control and yeast treatment group of steers (Hinman *et al.*, 1998).

2.10.2.2 Body condition score

The BCS is a visual assessment of the fat depots and fat distribution over the body of the cow. This score is objective and varies widely between different individuals and is dependent on the assessors' previous experience.

The BCS was not affected by supplementation of *S. cerevisiae* in a study with Saanen dairy goats (Stella *et al.*, 2007). Similar results were found with cows supplemented with Yea-

Sacch[1026](Kamalamma *et al.*, 1996; Kalmus *et al.*, 2009) and Amaferm (*A. oryzae* extract) and Vitaferm (*A. oryzae* plus yeast plus mineral –vitamin supplement) (Kellems *et al.*, 1990). Wohlt *et al.* (1998) found that the inclusion, its level nor removal of a yeast supplement had an effect on the animals' BCS. The higher non-esterified fatty acids (NEFA) levels measured in early lactation goats supplemented with yeast, suggested that energy reserves were mobilised and induced by such supplementation causing a further negative energy balance which is typical for early lactation animals (Giger-Reverdin *et al.*, 1996). This would support the findings that resulted in higher FCM being produced (Giger-Reverdin *et al.*, 1996).

2.10.2.3 Feed efficiency

Feed efficiency is calculated as kilograms milk produced per kilogram DM consumed in a dairy system (Linn and Salfer, 2006) or in a feedlot system, a feed to gain ratio is evaluated. Feed efficiency is difficult to quantify due to other influencing factors such as milk production, BW, body condition change, genetics, feed digestibility, growth and production and nutrient imbalances (Linn and Salfer, 2006).

Yeast has the potential to increase the efficiency of production, of 4% FCM by 3.7% (Moallem *et al.*, 2009), of energy corrected milk by 7% (Schingoethe *et al.*, 2004) and higher tendencies for increased fat yield and energy corrected milk (ECM) of cows (Cooke *et al.*, 2007). Improvement of efficiency of production was achieved for heat stressed mid-lactation cows supplemented with yeast (Schingoethe *et al.*, 2004). A study with Holstein calves supplemented with either *S. cerevisiae* or *S. boulardii* showed no improvement or effect on feed efficiency (Pinos-Rodríguez *et al.*, 2008).

In a feedlot system, high energy concentrates/diets are fed for optimum gain from feed consumed. Energy from concentrates is used more efficiently than energy from forages, therefore efficiency of utilisation of dietary ME for maintenance and gain is influenced by the forage to concentrate ratio (National Research Council, 1984). The efficiency for feedlot lambs supplemented with yeast was higher (Tripathi and Karim, 2010), which was concurrent with the study for finishing Awassi lambs supplemented daily with either 0, 3 or 6 g of Diamond V yeast culture with feed to gain ratios averaging 5, 4.2 and 4.7, respectively (Haddad and Goussous, 2005). The *S. cerevisiae* of the three supplemented yeast treatments had the best potential to promote growth in feedlot lambs, which may be an alternative to the use of ionophores or antibiotics (Tripathi and Karim, 2010). Haddad and Goussous (2005) found that the dosage level of 3 g had the lowest feed to gain ratio which is superior to the higher ratios, at this point one is getting the optimum growth from as little feed as possible. Feed efficiencies improved by 4.5% have been measured for yeast

supplemented steers compared with the control with gain to feed ratios of 151.8 and 158.6 g/kg DMI, respectively ($P < 0.01$) (Hinman *et al.*, 1998).

However, contradictory findings in studies monitoring growth parameters in steers were found and the higher feed to gain ratios which contributes to decreased efficiencies were attributed to the yeast supplemented steers having significantly higher intakes and similar ADG compared with the control (Mir and Mir, 1994). The feed efficiency was not different between the treatments of supplemented yeast and control heifers (Lascano *et al.*, 2009b).

2.10.2.4 Body weight

Animals lost BW less rapidly post-calving for Jersey cows supplemented with diamond V XP yeast culture (Dann *et al.*, 2000) and for Holstein cows supplemented with a live yeast Biosaf Sc 47 (Grochowska *et al.*, 2009). Conversely goats fed a yeast culture (Yea-Sacc^{8217}) experienced greater BW losses compared to the control (Hadjipanayiotou *et al.*, 1997).

The lack of effect of yeast supplementation on BW has previously been recorded with differing yeast products on the market such as the use of diamond V XP yeast culture (Arambel and Kent, 1990; Robinson, 1997; Magalhaes *et al.*, 2008), Yea-Sacc^{1026} (Kamalamma *et al.*, 1996; Lascano *et al.*, 2009b), Amaferm or Vitaferm (Kellems *et al.*, 1990) and Biomate Yeast Plus (Kung *et al.*, 1997) fed group of cows or heifers were not significantly different. This was similar for the total BW gain in dairy calves which was unaffected by yeast treatment of *S. cerevisiae* (Quigley *et al.*, 1992; Pinos-Rodríguez *et al.*, 2008) or *S. boulardii* (Pinos-Rodríguez *et al.*, 2008). The large inconsistency in effects between different products on the BW may be explained by Wohlt *et al.* (1998) and Kung *et al.* (1997). Wohlt *et al.* (1998) showed that the BW was not affected by the level or the amount added/removed of a yeast supplement ,and Kung *et al.* (1997) showed that stage of lactation particularly early- to mid-lactation dairy cows had no significant difference in the BW change between yeast and control treatment groups (Kung *et al.*, 1997). The explanation thereof is that BW is largely influenced by many factors such as genetics and the diet, which may override the effects of a yeast additive.

2.10.3 Strain variation

Yeast products available in the animal feed industry are numerous. These products differ according to their strain of *S. cerevisiae*, the concentration of viable yeast cells, the growth medium, and the recommended dosages of the specific product. Products have been compared to each other in published research. Lascano and Heinrichs (2009) chose a dosage level that was constant across differing intakes to account for animals having higher

intakes in comparison to animals consuming less. Dosages of yeast specified and supplemented as yeast per kg feed instead of yeast per day would therefore have a constant and a subsequent superior effect, and the latter would dilute the effects of yeast and cause a possible loss in response (Lascano and Heinrichs, 2009).

2.10.3.1 Levucell SC

Levucell SC (Lallemand S.A.S., Blagnac Cedex, France) is a live yeast product and has been researched extensively in dairy cows (Doreau and Jouany, 1998; Bach *et al.*, 2007; Thrune *et al.*, 2009), non-lactating cows (Guedes *et al.*, 2008), dairy goats (Giger-Reverdin *et al.*, 1996; Stella *et al.*, 2007), calves (Pinos-Rodríguez *et al.*, 2008) and sheep (Angeles C *et al.*, 1998; García *et al.*, 2000). The dosage level across studies with Levucell yeast supplementation ranged from 0.2 (Giger-Reverdin *et al.*, 1996; Stella *et al.*, 2007), 0.3 (Guedes *et al.*, 2008), 0.5 (Doreau and Jouany, 1998; Thrune *et al.*, 2009), 1 (Angeles C *et al.*, 1998; Guedes *et al.*, 2008; Pinos-Rodríguez *et al.*, 2008) and 5 (Bach *et al.*, 2007) grams per animal per day. The CFU/g specified in previous research for Levucell SC 20 was 1.1 x 10^{10} (Giger-Reverdin *et al.*, 1996) and 2 x 10^{10} (Angeles *et al.*, 1998; García *et al.*, 2000; Stella *et al.*, 2007; Pinos-Rodríguez *et al.*, 2008; Thrune *et al.*, 2009), and Levucell SC 10 ME was 1 x 10^{10} (Guedes *et al.*, 2008). The significant ($P < 0.05$) effects on production parameters found with Levucell SC are higher DMI (Pinos-Rodríguez *et al.*, 2008) between 4 to 18 weeks post-partum (Stella *et al.*, 2007), higher milk yields (Stella *et al.*, 2007), lower milk fat percentages (Stella *et al.*, 2007). The significant effects found for ruminal parameters are higher ruminal pH's (Bach *et al.*, 2007; Guedes *et al.*, 2008; Thrune *et al.*, 2009) and higher VFA concentrations (Guedes *et al.*, 2008). The individual VFA and ruminal acids in previous studies found lower (García *et al.*, 2000) and higher (Guedes *et al.*, 2008; Pinos-Rodríguez *et al.*, 2008) ruminal acetate percentages, higher ruminal propionate percentages (García *et al.*, 2000; Guedes *et al.*, 2008), higher ruminal butyrate percentages (Guedes *et al.*, 2008; Thrune *et al.*, 2009) and lower ruminal lactic acid concentrations (Guedes *et al.*, 2008). Higher ruminal NDF digestibilities (Guedes *et al.*, 2008) were reported with supplementation.

2.10.3.2 Biosaf

Biosaf Sc 47 (Lesaffre Feed Additives, Marquette-Lez-Lille, France),is a live yeast and has been used in previous research with Holstein cows (Marden *et al.*, 2008; Grochowska *et al.*, 2009; Moallem *et al.*, 2009) at dosages ranging from 5 (Marden *et al.*, 2008), 6 (Moallem *et al.*, 2009) to 7 (Grochowska *et al.*, 2009) grams per cow per day. The CFU/g specified in

the study of Moallem *et al.* (2009) was 1 x 10^{11}. Significant ($P < 0.05$) changes in performance parameters found by Moallem *et al.* (2009) include higher DM intakes, milk yields, 4% FCM yields, milk lactose percentages and lower ruminal NH_3-N concentrations. Marden *et al.* (2008) reported that ruminal parameters were affected by supplementation such that higher ruminal pH's, total tract NDF digestion, ruminal VFA concentrations, ruminal acetate percentages, ruminal propionate percentages and lower lactate concentrations were found. Their study concluded that with yeast supplementation a better fibre digestion was achieved as lactate accumulation was prevented mediated through a lower ruminal E_h and rH (Clarks exponent) (Marden *et al.*, 2008). Grochowska *et al.* (2009) demonstrated that the BW loss of Holstein dairy cows had decreased post-calving.

2.10.3.3 Yea-Sacc^{1026}

Yea-Sacc® (Alltech Inc., Lexington, KY) is a yeast product, referred to in previous research as both a live yeast (Masek *et al.*, 2008) and yeast culture (Williams *et al.*, 1991), in this review, it will be referred to as a yeast culture. Yea-Sacc^{1026} has been extensively researched in Holstein cows (Williams *et al.*, 1991; Erasmus *et al.*, 1992; Kalmus *et al.*, 2009), steers (Mir and Mir, 1994; Moloney and Drennan, 1994; Plata *et al.*, 1994; Roa V *et al.*, 1997), Jersey cross (Kamalamma *et al.*, 1996), Holstein cross (Kamalamma *et al.*, 1996), heifers (Miranda *et al.*, 1996; Putnam *et al.*, 1997; Lascano and Heinrichs, 2009), Suffolk ewes or sheep (Newbold *et al.*, 1995; Angeles C *et al.*, 1998; Arcos-García *et al.*, 2000), lactating sheep (Masek *et al.*, 2008), horses (Medina *et al.*, 2002), buffalo calves (Vaneeta *et al.*, 1998) and *In vivo* (Newbold *et al.*, 1995). In the various studies the dosage rate has varied from 2 (Newbold *et al.*, 1995), 3 (Angeles C *et al.*, 1998; Masek *et al.*, 2008), 5 (Vaneeta *et al.*, 1998), 6 (Masek *et al.*, 2008), 8 (Lascano and Heinrichs, 2009) to 10 (Williams *et al.*, 1991; Erasmus *et al.*, 1992; Mir and Mir, 1994; Moloney and Drennan, 1994; Plata *et al.*, 1994; Kamalamma *et al.*, 1996; Miranda *et al.*, 1996; Putnam *et al.*, 1997; Roa V *et al.*, 1997; Medina *et al.*, 2002; Kalmus *et al.*, 2009) grams per animal per day. The CFU/g specified for Yea-Sacc^{1026} according to previous research was 1 x 10^8 (Angeles *et al.*, 1998; Arcos-García *et al.*, 2000), 6.5 x 10^8 (Moloney and Drennan, 1994), 10.1 x 10^8 (Moloney and Drennan, 1994), 6.57 x 10^4 (Newbold *et al.*, 1995) and 5 x 10^9 (Williams *et al.*, 1991; Mir and Mir, 1994). The significant ($P < 0.05$) production parameters found with Yea-Sacc^{1026} supplementation are higher milk and FCM yields (Masek *et al.*, 2008), milk protein yield (Kalmus *et al.*, 2009), milk lactose yield (Masek *et al.*, 2008) and milk fat yields (kg) (Masek *et al.*, 2008; Kalmus *et al.*, 2009). The significant ($P < 0.05$) ruminal parameters found with Yea-Sacc^{1026} supplementation were lower ruminal pH (Vaneeta *et al.*, 1998; Arcos-García *et al.*,

2000), higher (Roa V *et al.*, 1997) and lower (Moloney and Drennan, 1994; Vaneeta *et al.*, 1998; Lascano and Heinrichs, 2009) ruminal NH_3-N concentrations, higher ruminal VFA concentrations (Roa V *et al.*, 1997; Vaneeta *et al.*, 1998; Lascano and Heinrichs, 2009), higher ruminal butyric acid percentages (Roa V *et al.*, 1997) and lower peak (Erasmus *et al.*, 1992) and mean (Vaneeta *et al.*, 1998) ruminal lactic acid concentrations higher ruminal NDF and CP digestion (Roa V *et al.*, 1997), lower CP digestibilities in low quality diets (Moloney and Drennan, 1994),. It can be concluded from these studies that the strain Yea-Sacc^{1026} has the ability to modify the ruminal bacterial population and increase the total celulolytic bacterial numbers.

2.10.3.4 Diamond V XP

Diamond V XP (Diamond V Mills, Inc., Cedar Rapids, IA) yeast cultures which has been researched in studies with Jersey cows (Dann *et al.*, 2000), Holstein cows (Erdman and Sharma, 1989; Arambel and Kent, 1990; Harris *et al.*, 1992; Yoon and Stern, 1996; Robinson, 1997; Shaver and Garrett, 1997; Robinson and Garrett, 1999; Schingoethe *et al.*, 2004; Erasmus *et al.*, 2005; Cooke *et al.*, 2007; White *et al.*, 2008; Grochowska *et al.*, 2009; Longuski *et al.*, 2009), non-lactating cows (Wiedmeier *et al.*, 1987; Enjalbert *et al.*, 1999), heifers (Olson *et al.*, 1994; Moya *et al.*, 2009), Angus Hereford cross (Lehloenya *et al.*, 2008), Angus cross (Hinman *et al.*, 1998), Awassi lambs (Haddad and Goussous, 2005), steers (Olson *et al.*, 1994) and *In vitro* (Miller-Webster *et al.*, 2002). The dosage levels across previous research ranged from 3 (Haddad and Goussous, 2005), 6 (Haddad and Goussous, 2005), 14 (Moya *et al.*, 2009), 28.4 (Olson *et al.*, 1994), 50 (Enjalbert *et al.*, 1999), 56 (Cooke *et al.*, 2007; Lehloenya *et al.*, 2008; White *et al.*, 2008; Longuski *et al.*, 2009), 57 (Harris *et al.*, 1992; Yoon and Stern, 1996; Robinson, 1997; Shaver and Garrett, 1997; Robinson and Garrett, 1999; Miller-Webster *et al.*, 2002), 60 (Dann *et al.*, 2000; Schingoethe *et al.*, 2004; Grochowska *et al.*, 2009), 85 (Hinman *et al.*, 1998) to 90 (Wiedmeier *et al.*, 1987; Arambel and Kent, 1990) grams of yeast culture per day. The CFU/g specified in previous studies was 2×10^6 (Arambel and Kent, 1990) and 4×10^7 (Robinson and Garrett, 1999). The significant ($P < 0.05$) effects of Diamond V XP yeast cultures on the production parameters across previous research has found lower DMI (Harris *et al.*, 1992), higher milk yields (Shaver and Garrett, 1997), higher FCM yields (3.5%) (Longuski *et al.*, 2009), lower (Shaver and Garrett, 1997) and higher (White *et al.*, 2008) milk fat percentages, lower milk protein percentages (Shaver and Garrett, 1997; White *et al.*, 2008). The effects of supplementation on the digestibility of feed components have resulted in higher *In vitro* DM digestibility (Miller-Webster *et al.*, 2002), higher total tract DM, NDF, OM and CP digestibility (Haddad and

Goussous, 2005). The ruminal parameters due to supplementation resulted in higher VFA concentrations (Miller-Webster *et al.*, 2002), lower ruminal acetic acid percentages (Miller-Webster *et al.*, 2002), higher ruminal propionic acid percentages (Miller-Webster *et al.*, 2002), higher ruminal valerate percentages (Miller-Webster *et al.*, 2002) and decreased incidence of bloat (Moya *et al.*, 2009).

2.10.3.5 Biomate Yeast Plus

Biomate Yeast Plus® (Chr. Hansen BioSystems, Inc., Milwaukee, WI) contains a yeast culture, alpha-amylase, protease and lipase. Biomate Yeast Plus has been used in research with Holstein cows (Wohlt *et al.*, 1991; Kung *et al.*, 1997; Wohlt *et al.*, 1998; Soder and Holden, 1999) and *In vitro* (Kung *et al.*, 1997) with dosages ranging from 10 (Wohlt *et al.*, 1991; Kung *et al.*, 1997; Wohlt *et al.*, 1998) to 20 (Kung *et al.*, 1997; Wohlt *et al.*, 1998; Soder and Holden, 1999) grams per cow per day. The CFU/g specified in previous research for Biomate Yeast Plus was 3.5×10^9 (Kung *et al.*, 1997) and 5×10^9 (Wohlt *et al.*, 1998). The significant ($P < 0.05$) effects of Biomate Yeast Plus on production parameters are increases of DMI between week 5 and 18 of lactation (Wohlt *et al.*, 1998) and increased milk yield between week 5 to 18 (Wohlt *et al.*, 1998). The changes in ruminal parameters due to Biomate Yeast Plus supplementation such as higher CP and ADF digestibilities (Wohlt *et al.*, 1998) and lower ruminal valerate percentages (*In vitro*) have been reported.

2.10.3.6 A-Max

The yeast culture A-max XTRA (Varied Industry Co., Mason City, IA) is a product derived from *S. cerevisiae*. Bruno *et al.* (2009) had supplemented this product to Holstein dairy cows at a level of 30 grams per cow per day and found that significantly higher milk yields and milk fat percentages were measured. A-Max yeast concentrate (*Saccharomyces cerevisiae* yeast culture) an additional product from Varied Industry Co., Mason City, IA, that was added to continuous cultures *In vitro* which resulted in significantly higher DM digestions, VFA concentrations, valerate and propionate percentages, and lower acetate percentages (Miller-Webster *et al.*, 2002). The CFU/g of A-Max yeast culture was not specified in previous research.

2.10.3.7 Strain comparisons

Previous research was done with the use of two or more yeast products and different strains of yeast, and their effects were compared. Newbold *et al.* (1995) studied *In vivo* and *In vitro* the activity of different strains of *Saccharomyces* NCYC 240, NCYC 694, NCYC 1026,

NCYC 1088 and Yea-Sacc^{1026}, and concluded that there are strain differences in their ability to modify the ruminal bacteria population. The products NCYC 240, NCYC 1026, and Yea-Sacc were found to be superior to NCYC 694 and NCYC 1088 in their effect of stimulating total cellulolytic bacterial numbers (Newbold *et al.*, 1995). However, in other studies the effects of both a *S. cerevisiae* culture (1.16×10^4 CFU/g) and live *S. cerevisiae* cells (1.39×10^7 CFU/g) added to incubations at three concentrations (0, 0.35 and 0.73 g/L) on *In vitro* ruminal fermentation concluded that both the live yeast and the yeast culture supplement showed similar effects and neither was superior in terms of *In vitro* fermentation (Lynch and Martin, 2002). The effect of strain variation is demonstrated in feedlot lambs which were fed three individual yeast cultures and a mixed culture of *Kluyveromyces marximanus* NRRL3234, *S. cerevisiae* NCDC42 and *Saccharomyces uvarum* ATCC9080 (Tripathi and Karim, 2010). The *S. cerevisiea* of the three supplemented yeast cultures had the best potential to promote growth in feedlot lambs (Tripathi and Karim, 2010). Angeles *et al.* (1998) and Arcos-Garcia *et al.* (2000) compared the effects of supplementing the yeast culture Yea-Sacc^{1026} and the live yeast Levucell in Suffolk sheep. Angeles *et al.* (1998) found no difference between treatments and no beneficial improvement in fermentation using *Saccharomyces cerevisiae*. Arcos-Garcia *et al.* (2000) demonstrated that Levucell supplemented sheep had higher ruminal pH's and ruminal VFA concentrations, with lower ruminal NH_3-N measured compared with the Yea-Sacc^{1026}. Arcos-Garcia *et al.* (2000) concluded and agreed with Angeles *et al.* (1998), as no beneficial effect of either yeast was exerted on the fermentation or digestibility. Different modes of action are exhibited by different yeast cultures which are specific to that strain (Miller-Webster *et al.*, 2002). Diamond V XP and A-Max yeast cultures were compared in a study, where Diamond V XP had higher (*In vitro*) digestibilities, VFA concentrations, propionic acid and valerate concentrations, and lower acetate compared with A-Max (Miller-Webster *et al.*, 2002). Grochowska *et al.* (2009) compared effects of a live yeast (Biosaf Sc 47) and a yeast culture (Diamond V XP), where the live yeast had demonstrated reduced BW losses and higher blood glucose concentrations. The results suggest that better digestibilities by rumen microbes were achieved with live yeast supplementation, due to treatments having similar DMI but live yeast cows measuring higher blood glucose levels with potential for higher energy uptakes (Grochowska *et al.*, 2009).

The method of feeding yeast products depends on the form and dose of the yeast product that is to be supplemented. To give an example, Levucell SC10 ME is the micro-encapsulated formula of Levucell SC 20, the micro-encapsulated formulation is specific for addition to animal feeds that require pelleting and ensure that the yeast remains viable and

can withstand temperatures as high as 89/90 °C. Levucell SC 10 ME - Titan is the micro-encapsulated yeast product which is similar to Levucell SC 10 ME but can withstand more extreme temperatures (Personal communication, 2010, R. Venter, Vitam International, richardtv@vitam.co.za). Yeast products are therefore pelleted, top dressed (Wohlt *et al.*, 1991; Nocek *et al.*, 2003) or dosed intra-ruminally (Miranda *et al.*, 1996; Desnoyers *et al.*, 2009b).

2.10.4 Stage of supplementation

Yeast supplementation has been carried out with animals differing in age such as with young ruminants, and/or with lactating cows at differing stages of the lactational cycle. Piva *et al.* (1993) stated that the cows response to yeast supplementation is effected by the animals stage of lactation. Nutrient requirements of cows in differing stages of lactation vary and the utilisation and efficiency in partitioning nutrients differ. This enables researches to investigate whether yeast supplementation pre- or postpartum would have a beneficial effect on production. For simplicity the lactational cycle was split into the transitional, early-, mid- and late-lactational periods which correspond to the periods 3 weeks pre- to 3 weeks post-partum, 0 to 100 days, 100 to 200 days and 200 days and more in the lactational cycle, respectively. No significant interaction between yeast supplementation and stage of lactation or lactation number was evident (Swartz *et al.*, 1994).

2.10.4.1 Young ruminants

In the past, research on yeast supplementation has been conducted with young ruminants such as lambs and dairy calves. Tripathi and Karim (2010) supplemented yeast to feedlot lambs, and concluded that *S. cerevisiea,* of the three supplemented yeast cultures, had the best potential to promote growth, and was successful as an alternative to the use of growth promoters. Additional positive attributes is greater N, Zn and Fe balances which were found when lambs were supplemented with yeast (Cole *et al.*, 1992). The benefits of supplementing a live yeast (Levucell SC) was that in lambs, the functionality of the rumen was improved and accelerated to form a stable rumen ecosystem in the young animal (Chaucheyras-Durand and Fonty, 2002). Pinos-Rodriguez *et al.* (2008) supports this and elaborates in stating that the process of rumen development in calves is facilitated by the fact that rumen epithelial growth requires energy sources such as butyrate and to a lesser extent propionate. Pinos-Rodriguez *et al.* (2008) compared the yeast strains *S. boulardii* I-1079 and *S. cerevisiae*, to investigate when in the young ruminants life would yeast supplementation of these strains best support development and growth. They recommended the strain *S.*

boulardii I-1079 for non-ruminants and young ruminants early in life as it would promote and support better DM intakes, rumen fermentation processes and growth, while the effects of the strain *S. cerevisiae* would be beneficial to the non-ruminant as weaning approaches and when the rumen is nearly fully developed. Butyrate concentrations in the ruminal fluid were lower for *S. bouldarii* supplemented calves compared to *S. cerevisiae*, a possible explanation for this may be because of the increased uptake of ruminal butyrate by ruminal epithelium, which helps in developing the rumen, improving its functionality and enhancing its stability in a young ruminants gastro-intestinal tract (Pinos-Rodríguez *et al.*, 2008). Magalhaes *et al.* (2008) demonstrated that *S. cerevisiae* supplemented to Holstein calves improved fecal scores with reduced watery feces, fever, and diarrhoea and health problems. The mortality rate decreased past 13 days of age, which increased income per calf. Quigley *et al.* (1992) reported contradictory results in that there was no effect on the rate of gain or intake of supplemented yeast to dairy calves.

2.10.4.2 Prepartum, transition period and postpartum

Cows supplemented with yeast during the transitional period (-2 days, 0 day (calving), 1 day after calving) observed higher intakes and concluded that parturition stress could be lessened by yeast supplementation (Kim *et al.*, 2006). This was observed in other studies (Dann *et al.*, 2000). A *S. cerevisiae* supplement included from 14 days prepartum to 14 days postpartum resulted in no difference between treatments for pre- or postpartum DMI, milk production or milk composition (Robinson, 1997; Kim *et al.*, 2006). Higher fibre digestibilities were measured for the yeast supplemented cows both pre- and postpartum (Robinson, 1997). An experiment conducted to evaluate the effects of supplemental yeast, starting from 28 days prepartum and continuing through week 13 of lactation, found no effect of yeast supplementation on both pre and postpartum DMI or milk yield and composition (Soder and Holden, 1999).

2.10.4.3 Early lactation (0 to 100 days in milk)

Yeast supplementation for animals in early lactation had increased milk yield (Bruno *et al.*, 2009) and FCM yield (Longuski *et al.*, 2009). Bruno *et al.* (2009) reported that cows supplemented with yeast produced more milk with no effect on the DMI and, this may be due to the typical effect of temperature on DMI for heat stressed cows. In another study, yeast supplemented cows in early lactation (75 + 25 DIM) showed a tendency for higher milk production and FCM compared to the control (Kung *et al.*, 1997). In contrast, a number of studies did not show any effect of yeast supplementation on production parameters (Arambel

and Kent, 1990; Williams *et al.*, 1991; Wohlt *et al.*, 1991; Erasmus *et al.*, 1992; Swartz *et al.*, 1994; Putnam *et al.*, 1997; Doreau and Jouany, 1998; Marden *et al.*, 2008; Desnoyers *et al.*, 2009b; Kalmus *et al.*, 2009; Longuski *et al.*, 2009).

2.10.4.4 Mid-lactation (100 to 200 days in milk)

Mid-lactation Holstein were supplemented with yeast and positive effects in terms of higher FCM (Piva *et al.*, 1993; Moallem *et al.*, 2009) and milk yield (Piva *et al.*, 1993; Shaver and Garrett, 1997; Moallem *et al.*, 2009) were recorded. Conversely when mid-lactation Holstein cows were supplemented with yeast, DMI decreased according to Besong *et al.* (1996) and Harris *et al.* (1992) (early to mid-lactational cows) with tendencies towards lower DMI (Kung *et al.*, 1997). The lack of an effect of yeast on FCM (Erdman and Sharma, 1989; Kamalamma *et al.*, 1996; Shaver and Garrett, 1997; Schingoethe *et al.*, 2004; White *et al.*, 2008), milk production (Erdman and Sharma, 1989; Harris *et al.*, 1992; Kamalamma *et al.*, 1996; Kung *et al.*, 1997; Cooke *et al.*, 2007) or DMI (Erdman and Sharma, 1989; Piva *et al.*, 1993; Kamalamma *et al.*, 1996; Schingoethe *et al.*, 2004; White *et al.*, 2008) has been recorded in a number of studies.

2.10.4.5 Late-lactation (200 days and more in milk)

Late-lactational cows showed no effect of yeast on DMI (Bach *et al.*, 2007; Thrune *et al.*, 2009). Milk yields were not recorded in these studies, which may indirectly reflect the fact that yeast will exert a larger effect when animals are either earlier or just in mid-lactation, where the DMI could be manipulated. For many of the commercial products, the recommendation is early to mid lactation supplementation, anyway.

2.10.5 Interaction of yeast with other additives or substances

The simultaneous inclusion of yeast and additives such as selenium, sodium bicarbonate, fungal additives (*A. oryzae*), specific bacteria, enzymes or monensin are currently an active field of research in order to investigate if any antagonistic or synergistic relationships exists.

A selenium-yeast supplement (Sel-Plex produced from *S. cerevisiae* CNCM I-3060) was supplemented and the ruminal digestive micro-organisms and/or enzymes were stimulated by the selenium-yeast at a dose of 300 mg/kg DM of feed and suggested that with such products, the milk could be enriched with selenium, which has beneficial effects for the consumer. (Wang *et al.*, 2009).

Erdman and Sharma (1989) did a study where a yeast culture was combined with a buffer (sodium bicarbonate), which resulted in higher 4% FCM yields. Marden *et al.* (2008) compared the effects of a live yeast (Biosaf Sc 47) in combination with sodium bicarbonate and concluded that, while sodium bicarbonate acts only as a exogenous buffer, the live yeast permitted better fibre digestion and prevented lactate accumulation in the rumen resulting in a lower redox potential (Marden *et al.*, 2008).

Fungal additives containing yeast and *A. oryzae* had increased the CP digestibilities with both additives supplemented separately or in combination, with the combination resulting in the largest increase (Wiedmeier *et al.*, 1987). In a study reported by Yoon and Stern (1996) there were no effects of the two additive cultures supplemented separately (yeast and *A. oryzae)* but supplementing a mixture of the two cultures changed the ruminal VFA patterns of the iso-acids. Mathieu *et al.* (1996) observed that when a probiotic containing both yeast and *A. oryzae* were supplemented to refaunated or defaunated sheep, the ruminal pH had increased by 0.2 and 0.3 units in refaunated sheep, with no effect observed for the defaunated sheep. An interaction therefore exists between the added probiotics (yeast and *A. oryzae*) and protozoa, which was owed to the reduction of the ruminal redox potential (Mathieu *et al.*, 1996). Unfavourable responses with the addition of yeast and fungal organisms containing additives do exist and was reported by Kellems *et al.* (1990) who observed lower milk and 5.5% FCM yields with Vitaferm (*A. oryzae* plus yeast plus mineral–vitamin supplement) when compared to Amaferm (a registered *A. oryzae* extract).

In a study reported by Nocek *et al.* (2003) yeast was supplemented in conjunction with specific bacteria such as *Enterococcus faecium* bacteria to Holstein cows pre- and postpartum. The direct-fed microbials had no effect on DMI pre-partum, but postpartum DMI, milk yield and milk protein were higher for the first 21d in lactation when supplemented cows were compared to the control cows (Nocek *et al.*, 2003). The most consistent sign of clinical acidosis is reduced intake and in this study the function of the yeast and bacterial strain in the DFM was that of stimulating lactic acid utilisation after which a constant level of bacteria, which produces the lactic acid is cultivated. This creates a constant supply and utilisation of lactic acid, and forms a pH equilibrium balance and stabilization (Nocek *et al.*, 2003).

The inclusion of a yeast culture and enzymes (protease, lipase and alpha-amylase) has been demonstrated by Soder and Holden (1999) with no effects found for DMI, milk yields or composition. An *In vitro* gas production technique was used to determine the effects of yeast supplementation with simultaneous supplementation of fibrolytic enzymes (cellulase and xylanase) on *In vitro* fermentation parameters and interactions between the two additives for all the *In vitro* gas parameters such as *In vitro* OM digestibility and *In vitro* DM digestibility

improved for each type of straw (rice straw, wheat straw, maize stover and maize stover silage) was observed (Tang *et al.*, 2008).

A yeast supplement and monensin added simultaneously resulted in a significantly higher MUN compared to the control cows (Erasmus *et al.*, 2005) and no effect on the protozoa (Arakaki *et al.*, 2000).

The effects of supplementing a combination of yeast and various additives yielded inconsistent results, which demonstrate that multiple factors are present which is variable between studies and have a significant influence on responses. In addition, yeast may be in competition with other additives for substrate usage which will not reflect the full effect of either additive.

2.10.6 Responses to supplementation

The specific conditions under which supplemental yeast has a significant and favourable effect for production and ruminal parameters are difficult to quantify across published research as each study was carried out under different dietary and management conditions (Piva *et al.*, 1993; Moallem *et al.*, 2009). In the following discussions (2.10.6.1.1-2.10.6.5.2.6) significance were applied at levels $P < 0.05$ and tendencies at level $0.05 < P < 0.1$.

2.10.6.1 Milk yield and DMI production responses

2.10.6.1.1 Significantly higher dry matter intakes and milk yield responses

Milk yield responses tend to follow DMI and a possible interaction between the DMI potential and the milk production responses exists (Erasmus, 2000). Yeast supplementation during the hot season increased the daily DMI, which was on average 2.5% higher compared with the control (24.7 kg and 24.1 kg, respectively) (Moallem *et al.*, 2009). This lead to 4.1% higher mean milk yields for the yeast supplemented cows (37.8 vs. 36.3 kg/day) (Moallem *et al.*, 2009). The 4% FCM was 6.1% (2 kg) greater than the control group of cows, being 34.8 and 32.8 kg, respectively which was produced at a higher efficiency due to yeast supplementation (Moallem *et al.*, 2009). The increase in DMI and milk yield are consistent with observations by Stella *et al.* (2007), Desnoyers *et al.* (2009a), and Abd El-Ghani (2004) who conducted a study with Zaraibi goats. Abd El-Ghani (2004) states that this was due to the fact that yeast stimulated rumen microbes, which increased fibre digestion and subsequently increased intake, which drives milk production. Stella *et al.* (2007) suggested that the effects observed was as a result of creating an opportunity for which the yeast is able to stabilise the rumen pH and stimulate cellulolytic bacterial numbers, which was observed by

the inclusion of highly fermentable carbohydrates. This is in agreement with Chaucheyras-Durand and Fonty (2002).

A meta-analysis covering 110 papers, 157 experiments and 376 treatments for yeast supplementation effects carried out by Desnoyers *et al.* (2009a) reported that DMI increased on average by 0.44 g/kg BW and milk yield increased by 1.2 g/kg of BW. Erasmus *et al.*, (2000) states that the greater the cows' milking potential is, the stronger the positive effect of the yeast supplement; in addition the effect of yeast supplementation on the milk yield increases with DMI, concentrate level, NDF and CP proportion in the diet (Desnoyers *et al.*, 2009a). The dosage levels in the meta-analysis demonstrated that increasing such levels were linearly related to increasing DMI and milk yields. Nocek *et al.* (2003) owes the favourable response in DMI and milk yield to less fat mobilisation from the fat stores, due to more glucose being available.

2.10.6.1.2 Significantly higher and lower dry matter intakes

Feedlot lambs post-weaning supplemented with *S. cerevisiae* resulted in significantly higher ($P < 0.05$) DMI's compared with the *K. marximanus* and *S. uvarum* yeast cultures, which may suggest potential growth promoting characteristics exerted by the *S. cerevisiae* which are ideal for feedlot lambs (Tripathi and Karim, 2010). This was similar in calves supplemented with a live yeast, for which higher calf starter intakes were observed (Pinos-Rodríguez *et al.*, 2008). This is contradictory for dairy calves fed a yeast supplement, which resulted in significantly lower DMI (2.8 kg/day) compared to the control (3.2 kg/day) when intake was restricted, opposed to when calves were fed *ad libitum*, yeast supplementation had no effect on intake (Quigley *et al.*, 1992).

Yeast had effected the feeding behaviour in goats so that less fibrous feed was selected which increased the DMI of the afternoons main meal (Desnoyers *et al.*, 2009b).

Higher DM intakes were measured in steers (Mir and Mir, 1994) between 85 to 115 days of yeast supplementation (Hinman *et al.*, 1998). This might have been due to the higher gain to feed ratios (Hinman *et al.*, 1998), and the drive for higher intakes had precipitated from higher ADG (Hinman *et al.*, 1998) for steers supplemented with yeast.

In dairy cows, yeast supplementation had stimulated higher DMI's (Wohlt *et al.*, 1991; Dann *et al.*, 2000). Dann *et al.* (2000) measured higher DMI the last week before calving due to yeast supplementation which may be a means to lessen the stress post-calving where DMI in general would decline. Wohlt *et al.* (1991) had stated that higher DMI's was the reason peak milk yields were achieved sooner. This was concurrent with Dann *et al.* (2000) who found that yeast supplemented cows increased their intake more rapidly and thereafter peak

milk yields were reached at 43 DIM for yeast supplemented cows, as opposed to 57 DIM for the control. The effects of yeast on DMI have been unfavourable as significantly lower DMI were observed (Harris *et al.*, 1992; Besong *et al.*, 1996). Besong *et al.* (1996) increased the inclusion of the liquid yeast (control, 20% and 40%) in the diet which caused a linear decrease in DMI. Yeast supplemented to early to mid-lactation Holstein cows showed a significantly lower DMI compared to the control being 22.0 and 22.9 kg/day, respectively, which was unexpected as higher NDF digestibilities were measured (Harris *et al.*, 1992)

2.10.6.1.3 Significantly higher and lower milk yields

Higher milk (Piva *et al.*, 1993; Shaver and Garrett, 1997; Masek *et al.*, 2008; Bruno *et al.*, 2009) and FCM (Williams *et al.*, 1991; Piva *et al.*, 1993; Giger-Reverdin *et al.*, 1996; Longuski *et al.*, 2009) yields resulted from yeast supplementation. These studies included diets that had relatively low (30:70) (Shaver and Garrett, 1997) medium (50:50) (Piva *et al.*, 1993; Bruno *et al.*, 2009) to high (60:40) (Williams *et al.*, 1991) concentrate to forage ratios, with highly fermentable carbohydrates (Shaver and Garrett, 1997; Longuski *et al.*, 2009). Masek *et al.* (2008) does not specify the concentrate to forage ratio, just that 1 kg/day of a corn and barley concentrate was supplemented to ewes, compared to the 0.3 kg/day lucerne and available mixed pasture they consumed. This is a relatively high concentrate to forage ratio, which showed that with yeast supplementation of 6 g/ewe/day resulted in higher daily milk yields. Longuski *et al.* (2009) reported an interaction between yeast and a fermentable carbohydrate (high moisture corn) which was the concentrate used by Shaver and Garrett (1997), and was not significant for ground corn. The findings from Bruno *et al.* (2009b), Williams *et al.* (1991), Longuski *et al.* (2009), Piva *et al.* (1993) and Masek *et al.* (2008) could be owed to the kinetics of the digesta in the rumen, where yeast may be more effective in increasing the FCM or milk yield when a higher percentage or fermentable concentrate is included. This suggests that yeast increases mobilisation of the animals reserves and the use of the NEFA and beta-hydroxy butyrate (BHB) by the udder to produce in milk the long chain-fatty acids measured (Giger-Reverdin *et al.*, 1996). It may also be due to an increase of plasma BHB from pre-cursors of produced rumen acids, the plasma BHB are responsible for the production of the short chain fatty acids and corresponding higher FCM yields (Giger-Reverdin *et al.*, 1996).

Bruno *et al.* (2009b) had proved that a significant response for milk yields were achieved for heat stressed cows, which in general under such conditions without supplementation of yeast would compromise the milk production. Significantly lower milk

yields from yeast supplementation to heat stressed cows were not found in any of the literature research for this study.

2.10.6.1.4 No responses for dry matter intake and milk yield

Young ruminants such as dairy calves (Magalhaes *et al.*, 2008) and lambs (Haddad and Goussous, 2005; Tripathi and Karim, 2010) supplemented with yeast showed that no significant effect on the intake of N (Tripathi and Karim, 2010), ME (Haddad and Goussous, 2005) or DM (Haddad and Goussous, 2005; Magalhaes *et al.*, 2008) was evident. Haddad and Goussous (2005) further illustrated that DMI and ME intake was similar between treatments consisting of 3 levels of the supplemented yeast (0,3 and 6 g/day).

Smaller sized ruminants such as Damascus goats and Chios ewes were supplemented with yeast and thereafter due to the lack of effect of yeast on the DMI's, it was suggested that the concentrate inclusion was not high enough to elicit a response (Hadjipanayiotou *et al.*, 1997). This did not hold true in another study with goats, where yeast supplemented with either a high or low concentrate diet had no effect on the intake, but had changed their eating behaviour where animals chose a less fibrous diet (Desnoyers *et al.*, 2009b). Sheep supplemented with a yeast had no effect on the DMI measured when a live yeast was supplemented (Arcos-García *et al.*, 2000; García *et al.*, 2000) or yeast culture (Andrighetto *et al.*, 1993; Arcos-García *et al.*, 2000; Kamel *et al.*, 2004). Kamel *et al.* (2004) and Andrighetto *et al.* (1993) included different levels of yeast cultures with no difference between either dosage levels.

The DMI was not affected by *S. cerevisiae* supplementation in steers (Plata *et al.*, 1994; Hinman *et al.*, 1998) or when steers were fed high dry rolled barley grain or alfalfa silage (Mir and Mir, 1994).

Dry matter intake in dairy cows were not significantly different between yeast supplemented and control cows. This was observed in studies with primiparous and multiparous cows (Bach *et al.*, 2007; Moya *et al.*, 2009; Thrune *et al.*, 2009). Thrune *et al.* (2009) suggests that the lack in response for DMI is the relatively late stage in lactation 344 ± 60 DIM or 335 ± 42 DIM as with Bach *et al.* (2007), though a lack of response was observed in mid-lactation animals as well (Piva *et al.*, 1993; Shaver and Garrett, 1997). Bach *et al.* (2007) had observed that despite the lack of effect of yeast on the DMI that the meal frequency of the cows had increased.

Milk (Wohlt *et al.*, 1991; Harris *et al.*, 1992; Besong *et al.*, 1996; Dann *et al.*, 2000; Kalmus *et al.*, 2009) and FCM (Shaver and Garrett, 1997) yields were not different between control and yeast treatment groups. Although peak milk yields tended to be higher (P = 0.1)

and were reached earlier, when cows were supplemented with a yeast culture, with 29.5 kg/day reached at week seven compared to the control group of 28.7 kg/day reached at week 11 of the lactation cycle, this may be contributed by the fact that DMI was significantly higher for the first six weeks (Wohlt *et al.*, 1991).

Yeast supplement had no effect on DMI pre-partum and on the post-partum DMI and milk yield (Robinson, 1997; Wohlt *et al.*, 1998; Soder and Holden, 1999; Nocek *et al.*, 2003; Erasmus *et al.*, 2005) or the DMI and milk yield for studies with primiparous and multiparous cows (Erdman and Sharma, 1989; Putnam *et al.*, 1997; Robinson and Garrett, 1999; Cooke *et al.*, 2007; Longuski *et al.*, 2009). The DM intake tended to increase ($P < 0.062$) by an average of 1.2 kg/day in a study done by Williams *et al.* (1991) and 1.4 kg/day ($P < 0.1$) by Erasmus *et al.* (1992) for Friesian dairy cows with no significant difference between treatments for milk yield (Erasmus *et al.*, 1992). The latter study however, was conducted with only 4 fistulated cows.

Heat stressed cows were fed a yeast supplement with no difference for DMI or ECM observed between the treatments (Bruno *et al.*, 2009). A similar study in the summer months carried out by Schingoethe *et al.* (2004) showed that yeast had no effect on the DMI, milk yield and yields of FCM and ECM (Schingoethe *et al.*, 2004). The lack of response observed for ECM is because of the lower milk fat concentration measured in the yeast fed cows (Bruno *et al.*, 2009). The ECM tended to increase for yeast supplemented multiparous cows (Cooke *et al.*, 2007). The DMI, milk yield and FCM yield were similar between treatments when a yeast supplement was compared to the control (Arambel and Kent, 1990; Swartz *et al.*, 1994; Kamalamma *et al.*, 1996; Kung *et al.*, 1997; White *et al.*, 2008). Swartz *et al.* (1994) ran the study over seven herds with varying management and nutritional environments, investigating two yeast products and the lack of response could be due to the huge variation, although effects within herds were not significant.

2.10.6.2 Milk composition

2.10.6.2.1 Milk fat

Milk fat yield (kg) was significantly higher for a yeast supplemented group of cows in the hot season which may be due to the simultaneous higher milk production measured (Moallem *et al.*, 2009). The milk fat yield increased from 1.3 to 1.47 kg/day ($P < 0.05$) for the yeast supplemented group when high moisture corn grain was fed (Longuski *et al.*, 2009). Longuski *et al.* (2009), therefore concluded that the milk fat depression occurring due to a high fermentable starch inclusion can be lessened with yeast supplementation. Yeast supplementation increased milk fat yield significantly in studies by Kalmus *et al.* (2008) and

Piva *et al.* (1993) with yields 0.78 vs. 0.9 kg/day for the control and yeast supplemented groups of the latter study. Milk fat percentage was significantly higher in goats (Abd El-Ghani, 2004) and Holstein cows (White *et al.*, 2008) due to yeast supplementation. The goats milk fat percentage was significantly higher at the six grams per day dosage level compared to the three grams supplemented group and the control (Abd El-Ghani, 2004).

Milk fat percentage was significantly ($P = 0.001$) lower for yeast supplemented lactating animals in heat stressed (Bruno *et al.*, 2009) and mid-lactation (Shaver and Garrett, 1997) Holsteins. The significantly lower milk fat percentage found in the studies by Bruno *et al.* (2009) and Shaver and Garret (1997) were due to higher milk yield responses obtained for yeast supplemented cows which caused a dilution type effect, as milk fat yield was not different between treatments. This was the reason for lower milk fat percentages that were observed for dairy goats (Abd El-Ghani, 2004; Stella *et al.*, 2007). Stella *et al.* (2007) reported that the milk fat was 4.32% for yeast and 4.46% for the control. Abd El-Ghani (2004) reported that the dosage level of goats had effected the milk fat response with lower milk fat percentages measured when three grams per day was supplemented, compared to both the control and higher dosage level.

The meta-analysis carried out by (Desnoyers *et al.*, 2009a) showed that milk fat percentage tended ($P < 0.1$) to increase by 0.05 percentage units as a result of yeast supplementation

2.10.6.2.2 Milk protein

Yeast supplementation has the potential to increase the protein percentages (Nocek *et al.*, 2003; White *et al.*, 2008) and protein yields. Milk protein yields were significantly greater at 1.17 kg/day for the yeast fed cows compared with the control which produced 1.14 kg/day (Shaver and Garrett, 1997). This was similar to studies of Bruno *et al.* (2009) and Kalmus *et al.* (2009). The significant response may be due to the fact that yeast supplementation increases the digestive processes which occur in the rumen, and subsequently increase the nutrients available for absorption which is used for milk production (Bruno *et al.*, 2009). The higher microbial protein produced to be metabolised in the duodenum could possibly contribute to the higher protein output from the mammary gland (Bruno *et al.*, 2009; Kalmus *et al.*, 2009). The efficiency of protein utilisation is additionally increased as a result of yeast supplementation, as Bruno *et al.* (2009) had suggested as lower Blood urea N in the study was recorded.

Protein percentages as a result of yeast supplementation were significantly lower due to the dilution effect of higher milk yields, this was found in studies by Abd El-Ghani (2004) and Shaver and Garret (1997).

2.10.6.2.3 Milk lactose

The significantly higher lactose percentages and yields were measured in studies by Moallem *et al.* (2009) and Bruno *et al.* (2009b), respectively. These studies were conducted in the hot season (Moallem *et al.*, 2009) or when cows were experiencing heat stress (Bruno *et al.*, 2009). Despite the harsh environmental conditions these studies had additionally recorded higher milk yields due to yeast supplementation. It is well known that lactose is a driver for higher milk yields, which supports the findings. Therefore yeast supplementation has the potential to partition nutrients so that larger quantities of nutrients are available for milk synthesis (Bruno *et al.*, 2009).

2.10.6.2.4 Non-significant effects on the milk composition

A lack of the effect of yeast supplementation has been recorded for milk, lactose yield (Stella *et al.*, 2007; Moallem *et al.*, 2009), lactose % (Arambel and Kent, 1990; Erasmus *et al.*, 2005; Stella *et al.*, 2007; Bruno *et al.*, 2009; Longuski *et al.*, 2009), protein yield (Williams *et al.*, 1991; Swartz *et al.*, 1994; Soder and Holden, 1999; Cooke *et al.*, 2007; Stella *et al.*, 2007; White *et al.*, 2008); protein % (Erasmus *et al.*, 1992; Robinson and Garrett, 1999; Nocek *et al.*, 2003; Erasmus *et al.*, 2005; Stella *et al.*, 2007; Desnoyers *et al.*, 2009a; Moallem *et al.*, 2009), fat yield (Swartz *et al.*, 1994; Putnam *et al.*, 1997; Shaver and Garrett, 1997; Soder and Holden, 1999; Cooke *et al.*, 2007; Bruno *et al.*, 2009); fat % (Erasmus *et al.*, 1992; Harris *et al.*, 1992; Swartz *et al.*, 1994; Kung *et al.*, 1997; Robinson and Garrett, 1999; Erasmus *et al.*, 2005; Moallem *et al.*, 2009). Erasmus *et al.* (2005) states that the possible reason for the similar affects between treatments and the lack of a significant effect of the yeast on the fat % was that the NDF level of the diet was sufficient. Efficiency of milk production was found to be higher in the yeast supplemented group of cows compared to the control (Cooke *et al.*, 2007; Moallem *et al.*, 2009), due to an increase tendency of a higher milk fat yield and energy corrected milk yield (Cooke *et al.*, 2007).

Similar findings in that milk composition was unaffected by treatment is supported by Dann *et al.* (2000) and Kamalamma *et al.* (1996). Milk protein, lactose, ash, DM or urea concentrations were not affected by yeast supplemented goats (Giger-Reverdin *et al.*, 1996; Hadjipanayiotou *et al.*, 1997). The reason for a lack of response found in the study of Hadjipanayiotou *et al.* (1997) may be due to the yeasts inability to remain viable at 85 °C

when pelleted, but this had been ruled out since conditions had been met in which the viability due to the pelleting had not been compromised. Conditions such as prolonged storage at high temperatures and when moisture pick-up is large which is stipulated by Lewis (1990) cited by Hadjipanayiotou *et al.* (1997) is what will affect the yeasts viability.

2.10.6.3 Effects on rumen fermentation

2.10.6.3.1 Ruminal volatile fatty acids

Ruminal VFA concentration was significantly higher for yeast supplemented animals in both *In vivo* (Roa V *et al.*, 1997; Vaneeta *et al.*, 1998; Enjalbert *et al.*, 1999; Guedes *et al.*, 2008; Marden *et al.*, 2008; Desnoyers *et al.*, 2009a; Lascano and Heinrichs, 2009) and *In vitro* studies (Sullivan and Martin, 1999; Miller-Webster *et al.*, 2002).

VFA concentrations are influenced by the time after feeding. This is illustrated with the highest VFA concentration with yeast supplementation compared to the control measured one hour (Enjalbert *et al.*, 1999) and six hours post-feeding (Abd El-Ghani, 2004). Although the VFA concentrations measured at time zero, had shown that the VFA concentrations were significantly different which may have contributed to the effect (Enjalbert *et al.*, 1999; Abd El-Ghani, 2004). Andrighetto *et al.* (1993) stated that the greatest variation in VFA can be up to three hours post-feeding (even between animals consuming identical diets) when intense fermentation is taking place, and further emphasises that time of sampling is a factor to consider when comparing concentrations. This is demonstrated by Doreau and Jouany (1998) when three sampling times (09h00,11h00 and 15h00) had similar VFA concentrations in two of the samples and the 15h00 sample for the yeast fed cow had significantly higher ruminal VFA concentrations. The sudden increase in VFA concentration four hours post feeding for yeast fed cows could represent the *S. cerevisiae* short lived mode of action (Doreau and Jouany, 1998).

Studies conducted using two or more yeast supplements and comparing their effects on the ruminal VFA concentration will be discussed here. See section strain variation for more information on the commercial products mentioned here after. Significantly higher ruminal VFA concentrations were measured for Levucell compared to the Yea-Sacc^{1026} supplementation in a study with Suffolk ewes. These differences may be attributed to the lower CFU/g dosed of the Yea-Sacc^{1026} (1×10^{8}) compared to Levucell (20×10^{9}) (Arcos-García *et al.*, 2000). A live yeast such as Biosaf Sc 47 resulted in higher ruminal VFA concentrations compared to the control of 99.4 mM and 85.3 mM, respectively (Marden *et al.*, 2008) which was similar in the study by Guedes *et al.* (2008) with a live yeast. The *In vitro* study comparing the effect of the commercial yeast products, Diamond V-XP and A-Max, proved

that both yeast products significantly increased the ruminal VFA concentrations compared to the control, with Diamond V XP producing significantly higher concentrations compared to the A-Max (Miller-Webster *et al.*, 2002). Yeast supplementation in other studies with different strains of a live yeast or yeast culture has shown no effect on the ruminal VFA concentration. The VFA concentrations remained unaffected when a live yeast was supplemented to both (*In vitro*) continuous cultures and in *In vivo* studies with steers (Dawson *et al.*, 1990). Furthermore Dawson *et al*. (1990) showed that there was no significant difference between live yeast and dead yeast supplementation *In vitro* with respect to the VFA concentration. There was a tendency (P = 0.1) for a lower ruminal VFA concentration in Holstein cows supplemented with live yeast (Thrune *et al.*, 2009). No differences for ruminal VFA concentrations between treatments of two supplemented strains of yeast, *S. cerevisiae* and *S. bouldarii* in calves (Pinos-Rodríguez *et al.*, 2008) or yeast strains NCYC 240, 1026 and Yea-Sacc^{1026} in sheep (Newbold *et al.*, 1995).

The comparison of effects of yeast supplementation at differing dosages by Andrighetto *et al.* (1993) of 20 and 40 g had shown that there was no difference between the dosage levels, but both resulted in significantly higher ruminal VFA concentrations compared to the control. Desnoyers *et al.* (2009a) is in agreement and reported that increasing the yeast supplementation dosage levels, would linearly increase the ruminal VFA concentration. This was similar to the *In vitro* study of Sullivan and Martin (1999) with the addition of 0.35 g/L and 0.73 g/L of a yeast supplement fermented on coastal Bermuda grass hay, and thereafter measured that the total VFA concentration increased compared to the control, with no significant difference when comparing just the two levels. Andrighetto *et al.* (1993) had owed the higher ruminal VFA concentration to the effect of the yeast in increasing microbial activities. Guedes *et al.* (2008) had found differences in dosage levels, with the live yeast Levucell SC 10 ME resulted in significantly higher ruminal VFA concentrations for the dosage level of 1 g per cow per day.

Diet influences the effect of yeast addition on total VFA concentrations, such as in a study with differing concentrate to forage ratios (Lascano and Heinrichs, 2009). This is supported by Roa V *et al.* (1997) with higher VFA concentrations measured for alfalfa (88.2 vs. 74.3 mM) and coffee hull (69.6 vs. 51.7 mM) diets, compared to the control with no effect with yeast supplemented in a cornstalk fibre source diet. No difference were found between treatments for the concentration of total ruminal VFA (Piva *et al.*, 1993; Mir and Mir, 1994; Plata *et al.*, 1994; Besong *et al.*, 1996; Doreau and Jouany, 1998; García *et al.*, 2000; Erasmus *et al.*, 2005; Longuski *et al.*, 2009) even after a fermentable starch challenge (Longuski *et al.*, 2009), high starch diets (Medina *et al.*, 2002) or diets either high or low in CP

(Moloney and Drennan, 1994; Yoon and Stern, 1996; Putnam *et al.*, 1997). The *In vitro* addition of 0.35 g/L and 0.73 g/L of a yeast treatment fermented on ground corn, maltose or lactate had no effect on total VFA concentration (Sullivan and Martin, 1999). Total VFA were unaffected by yeast supplementation in calves (Quigley *et al.*, 1992), non-lactating Holstein cows (Wiedmeier *et al.*, 1987) or *In vitro* (Kung *et al.*, 1997).

2.10.6.3.2 Ruminal propionic acid

Ruminal propionic acid percentages and concentrations increased due to yeast supplementation in studies for heifers (Miranda *et al.*, 1996; Lascano and Heinrichs, 2009), early (Marden *et al.*, 2008) and mid (Besong *et al.*, 1996) lactation cows, sheep (García *et al.*, 2000), in non-lactating cows (Guedes *et al.*, 2008) and *In vitro* (Dawson *et al.*, 1990; Miller-Webster *et al.*, 2002). In contrast ruminal propionic concentrations were significantly lower for yeast supplemented calves (Quigley *et al.*, 1992) at 32 days of age (Pinos-Rodríguez *et al.*, 2008) and steers (Mir and Mir, 1994). Ruminal propionic concentrations and percentages are affected by sampling time as indicated by Guedes *et al.* (2008) with the highest propionate concentrations measured two to four hours after feeding (Guedes *et al.*, 2008). Calves fed a yeast supplement had significantly reduced ruminal propionate concentrations at four hours post-feeding compared to the control (Quigley *et al.*, 1992). Molar propionate percentage increased just before feeding as a result of yeast supplementation, with no significant difference observed after feeding (Enjalbert *et al.*, 1999).

Increasing the inclusion rate of a liquid yeast culture (20 vs. 40%) (Besong *et al.*, 1996) or live yeast (0.3 vs. 1 g) (Guedes *et al.*, 2008) had linearly increased the ruminal propionic acid concentration compared to the control.

The inclusion of a live yeast such as Biosaf Sc 37 (Marden *et al.*, 2008) and Levucell SC 10 ME (Guedes *et al.*, 2008) increased the propionic concentration compared with the control of 25.8 vs. 18 mM and 22.15 vs.31.7 mM, respectively. This was similar to the *In vitro* supplementation of a live yeast compared to a dead yeast culture, resulting in significantly higher propionic acid percentages (Dawson *et al.*, 1990). Although, the *In vitro* study comparing the effect of two commercial yeast cultures, Diamond V-XP and A-Max, has proved that both yeast products produced significantly higher propionate concentrations, with Diamond V XP producing significantly higher concentrations compared to the A-Max (Miller-Webster *et al.*, 2002). Marden *et al.* (2008) and Guedes *et al.* (2008) illustrated that the simultaneous decrease in lactic acid concentration and the increase in propionic concentrations may be a result of the greater conversion of lactic to propionic acid, which was as a result of the live yeast stimulating lactic acid-utilising bacteria. However, propionate

concentration was increased by *S. cerevisiae* (10 g) and *A. oryzae* (3 g) cultures (Miranda *et al.*, 1996), as yeast in previous research has found to stimulate lactic acid-utilising bacteria such as *Selenomonas ruminantium* (which increase propionate production) (Nisbet and Martin, 1991).

Yeast addition in diets irrespective of the ratio of forage to concentrate (20, 40 and 60% concentrate) resulted in significantly higher propionate concentrations compared to the control, which was attributed to the production and utilisation of fermentation end-products (Lascano and Heinrichs, 2009). This was inconsistent with results for steers receiving a yeast supplement and fed a high grain based diet, after which significantly lower ruminal propionic acid percentages were measured (Mir and Mir, 1994). Miranda *et al.* (1996) demonstrated that propionate concentration was increased by *S. cerevisiae* and *A. oryzae* cultures at a dietary NDF level of 37%, with an interaction between the NDF level and cultures.

The *In vitro* supplementation of *S. cerevisiae* at 0.73 g/L increased the propionate concentration when alfalfa hay was the substrate, and similarly the propionate concentration increased when coastal Bermuda grass hay was fermented at both (0.35 and 0.73 g/L) treatment levels (Sullivan and Martin, 1999).

The lack of effect of yeast supplementation on the ruminal propionic acid percentages and concentration has been recorded in studies for dairy cows (Piva *et al.*, 1993; Putnam *et al.*, 1997; Doreau and Jouany, 1998; Robinson and Garrett, 1999; Erasmus *et al.*, 2005; Thrune *et al.*, 2009), sheep (Andrighetto *et al.*, 1993; Newbold *et al.*, 1995; Arcos-García *et al.*, 2000; García *et al.*, 2000) and steers (Dawson *et al.*, 1990; Moloney and Drennan, 1994; Roa V *et al.*, 1997; Lehloenya *et al.*, 2008). A tendency (P = 0.06) towards higher propionate concentrations were measured for yeast supplemented Holstein steers (Plata *et al.*, 1994). The *In vitro* addition of 0.35 g/L and 0.73 g/L of a yeast treatment fermented on ground corn, maltose or lactate had no effect on the propionate concentration (Sullivan and Martin, 1999). This was found in other *In vitro* studies (Newbold *et al.*, 1995; Kung *et al.*, 1997).

2.10.6.3.3 Ruminal acetic acid

Yeast supplementation had significantly increased the ruminal acetic acid concentrations for calves (81.1 vs. 15.39 mol/L), sheep (Quigley *et al.*, 1992), cows (Marden *et al.*, 2008), non-lactating cows (Guedes *et al.*, 2008), horses (Medina *et al.*, 2002) and *In vitro* (Miller-Webster *et al.*, 2002). In contrast a lack of effect of supplemented yeast in dairy cows (Wiedmeier *et al.*, 1987; Erasmus *et al.*, 1992; Doreau and Jouany, 1998; Erasmus *et al.*, 2005; Thrune *et al.*, 2009), sheep (Andrighetto *et al.*, 1993; Newbold *et al.*, 1995; Arcos-García *et al.*, 2000; García *et al.*, 2000) and steers (Dawson *et al.*, 1990; Olson *et al.*, 1994;

Plata *et al.*, 1994; Hinman *et al.*, 1998; Lehloenya *et al.*, 2008) have been recorded. These findings could suggest that a significant difference would be difficult to acquire as yeast has been investigated to grow on acetate as a sole carbon source, and may be produced in amounts due to fibre digestion, that is then removed by yeast for growth (Chu *et al.*, 1981).

Acetate concentration was higher for live yeast supplemented cows compared to the control, 59.1 vs. 53.2 mM (Marden *et al.*, 2008) and 62.73 vs. 57.5 mM (Guedes *et al.*, 2008), respectively. However, this is does not hold true when comparing a control, live yeast and dead yeast in the *In vitro* study which reported that neither were different with regards to the acetate concentration measured (Dawson *et al.*, 1990). In other *In vitro* studies comparing the effect of the commercial yeast cultures, Diamond V-XP and A-Max, proved that both yeast products produced significantly lower acetate concentrations, 47.1 and 53.2 mmol/100 mmol, respectively compared to the control (57.3 mmol/100 mmol) (Miller-Webster *et al.*, 2002).

Increasing the dosage of a yeast product had increased acetic acid concentrations as recorded by Guedes *et al.* (2008), although Besong *et al.* (1996) found that increasing the inclusion rate of a liquid yeast, created a tendency for the acetate concentration to decrease ($P = 0.06$).

Yeast addition in all the diets irrespective of the ratio of forage to concentrate in the diet, resulted in higher ruminal acetic acid concentrations (Lascano and Heinrichs, 2009). This was observed in the cecum and colon of a high starch and high fibre based diets in yeast supplemented horses (Medina *et al.*, 2002). Medina *et al.* (2002) suggested that the addition of yeast promotes higher fibrolytic bacteria, though no subsequent increase in cellulolytic bacterial numbers was detected (Medina *et al.*, 2002). However, Andrighetto *et al.* (1993) states that in yeast supplemented sheep the ruminal acetic acid concentrations were maintained and stabilized despite the lower pH, which may be due to the fact that yeast stimulates cellulolytic processes in the rumen. This could be the reason why the *In vitro* concentrations of acetate increased, when 0.73 g/L of yeast was supplemented on coastal Bermuda grass hay (Sullivan and Martin, 1999). The effect of differing fibre sources with yeast supplementation had no effect on acetic acid concentration when yeast was supplemented on either alfalfa hay, cornstalk or coffee hulls compared to their controls (Roa V *et al.*, 1997) or ground corn, maltose or lactate (Sullivan and Martin, 1999). The lack of effect of yeast on acetic acid concentrations were observed in other *In vitro* studies (Newbold *et al.*, 1995; Kung *et al.*, 1997).

2.10.6.3.4 Ruminal butyric and valeric acid

Yeast supplementation has increased ruminal butyric (Roa V *et al.*, 1997; Guedes *et al.*, 2008; Pinos-Rodríguez *et al.*, 2008; Thrune *et al.*, 2009); valeric (Dawson *et al.*, 1990); and *In vitro* butyric (Sullivan and Martin, 1999) and valeric (Sullivan and Martin, 1999; Miller-Webster *et al.*, 2002) acid percentages. Iso-valerate and iso-butyrate were significantly higher for yeast supplemented dairy heifers (Lascano and Heinrichs, 2009).

Live yeast supplemented to calves and cows has increased the ruminal butyric acid concentrations. Calves supplemented with live yeast (*S. cerevisiae)* at 32 days of age had significantly ($P < 0.001$) higher butyric acid concentrations compared to the control and *S. bouldarii* supplemented calves (Pinos-Rodríguez *et al.*, 2008). This was similar for cows supplemented with a live yeast measuring 9.7 vs. 10.4% (Thrune *et al.*, 2009) and 10.95 vs. 11.73 and 12.3% (Guedes *et al.*, 2008). However, the yeast culture supplemented by Roa V *et al.* (1997) consuming coffee hulls as a fibre source and, Sullivan and Martin (1999) *In vitro* at the 0.73 g/L level on coastal Bermuda grass hay, measured significantly higher butyric acid concentrations.

Moreover, higher ruminal valeric concentrations were also measured in live yeast and yeast supplemented animals such as Jersey steers consuming a high forage diet (Dawson *et al.*, 1990) and *In vitro* on ground corn substrates for the 0.35 g/L treatment level and at the 0.73 g/L level on coastal Bermuda grass hay, respectively (Sullivan and Martin, 1999).

The *In vitro* study carried out by Miller-Webster *et al.* (2002) recorded significantly higher valerate concentrations when yeast cultures Diamond V-XP and A-Max were added compared to the control. Furthermore Biomate Yeast Plus addition (200 mg) into continuous cultures resulted in significantly lower valerate concentrations compared with the control (20 mg) (Kung *et al.*, 1997)

The lack of an effect of yeast supplementation on the butyrate concentrations and percentages in steers (Olson *et al.*, 1994; Plata *et al.*, 1994; Roa V *et al.*, 1997), sheep (Newbold *et al.*, 1995; Arcos-García *et al.*, 2000; García *et al.*, 2000) and dairy cows (Wiedmeier *et al.*, 1987; Piva *et al.*, 1993; Doreau and Jouany, 1998; Marden *et al.*, 2008; Lascano and Heinrichs, 2009) have been previously reported. Although a tendency towards lower ruminal butyrate concentrations in steers (Williams *et al.*, 1991) and in calves, the tendency towards higher butyrate concentrations existed (Quigley *et al.*, 1992).

Valeric acid concentration and percentages in dairy cows (Williams *et al.*, 1991) and dairy calves (Quigley *et al.*, 1992) were similar between yeast and control treatments. However, valerate concentrations tended ($P = 0.07$) to increase for yeast supplemented dairy heifers (Lascano and Heinrichs, 2009).

The occurrence of both butyric and valeric acid concentrations being unaffected by yeast supplementation in dairy cows (Erasmus *et al.*, 1992; Besong *et al.*, 1996; Yoon and Stern, 1996; Putnam *et al.*, 1997; Enjalbert *et al.*, 1999; Robinson and Garrett, 1999; Erasmus *et al.*, 2005; Longuski *et al.*, 2009), steers (Mir and Mir, 1994; Moloney and Drennan, 1994; Hinman *et al.*, 1998; Lehloenya *et al.*, 2008), sheep (Andrighetto *et al.*, 1993) and *In vitro* (Newbold *et al.*, 1995; Kung *et al.*, 1997; Miller-Webster *et al.*, 2002) has been recorded.

2.10.6.3.5 Acetate to propionate ratio

The acetate to propionate ratio in *S. cerevisiea* supplemented calves were significantly higher (1.7 compared to the control 1.4), and were not different to *S. boulardii* supplemented calves (Pinos-Rodríguez *et al.*, 2008). Conversely, yeast supplementation had significantly decreased the acetate to propionate ratio in steers (Williams *et al.*, 1991; Hinman *et al.*, 1998) and in cows with increasing yeast inclusion levels (Besong *et al.*, 1996; Guedes *et al.*, 2008). Guedes *et al.* (2008) states that the inclusion of yeast and its mode of action increases the glucogenic potential of the diet, which is represented by the higher ratio of propionic acid to acetic acid. This was shown *In vitro* with a live yeast (Dawson *et al.*, 1990) or yeast culture (Sullivan and Martin, 1999; Miller-Webster *et al.*, 2002), which illustrates the result of a lower ratio is due to higher propionic acid concentrations. The ratio was significantly ($P < 0.01$) reduced from 3.3:1 to 2.8:1 in steers supplemented with yeast, without the effect on the VFA (42 and 40.43 mM/L) $P > 0.05$ (Hinman *et al.* 1998) and (78 and 73 mM/L) ($P > 0.05$) (Williams *et al.* 1991) for control and yeast, respectively, which indicates the effect on the fermentation stochiometry that yeast may have (Williams *et al.*, 1991; Hinman *et al.*, 1998).

Yeast supplementation had failed to affect the acetate to propionate ratio in various studies (Erasmus *et al.*, 1992; Yoon and Stern, 1996; Erasmus *et al.*, 2005; Lehloenya *et al.*, 2008; Desnoyers *et al.*, 2009a; Longuski *et al.*, 2009; Moya *et al.*, 2009). Even though the acetate and propionate molar concentrations increased significantly, the acetic to propionic acid ratio were not different between treatments (Lascano and Heinrichs, 2009).

2.10.6.3.6 Ruminal lactic acid

Yeast supplementation has the potential to decrease the mean or peak concentration of lactic acid measured after feeding in cows (Williams *et al.*, 1991; Erasmus *et al.*, 1992; Guedes *et al.*, 2008; Marden *et al.*, 2008), buffalo calves (Vaneeta *et al.*, 1998) and horses (Medina *et al.*, 2002).

Marden *et al.* (2008) supplemented a live yeast (Biosaf Sc 47) and demonstrated a 67% decrease from the lactate compared to the control (Marden *et al.*, 2008). Similarly yeast supplementation has significantly lowered the mean ruminal lactic acid concentration or, the lactic acid concentrational peaks, evident after concentrate feeding. Williams *et al.* (1991) had shown that the mean lactic acid concentration had decreased from 3.55 to 1.43 mM due to yeast supplementation; as well the L-lactic acid peak found two hours after feeding a concentrate was inhibited. A possible explanation why the addition of yeast promotes stabilised lactate ruminal concentrations, may be that yeast competes with other micro-organisms for the uptake of oligosaccharides, which results in the decrease of total oligosaccharides in the rumen liquor. The oligosaccharide is then converted to glucose (which is the substrate the yeast utilises for growth) and utilised, resulting in less highly fermentable substrate present in the rumen liquor, which is coupled with less lactic acid production (Williams *et al.*, 1991). This is concurrent with studies by Erasmus *et al.* (1992) and Guedes *et al.* (2008). The peak lactic acid concentration found two to three hours after feeding had significantly decreased from 1.93 to 1.73 mM (Erasmus *et al.*, 1992) and reducing the extent of its increase, that would normally occurred after feeding for up to four hours (Guedes *et al.*, 2008) or in the case of horses (consuming a high starch diet) in the cecum and colon, were significantly lower with supplemented yeast compared to the control which was evident from two until ten hours post-feeding (Medina *et al.*, 2002).

The lack of the effect of yeast supplementation on the ruminal lactic acid concentrations is represented in cows (Erasmus *et al.*, 1992; Longuski *et al.*, 2009; Moya *et al.*, 2009) or steers (Mir and Mir, 1994; Lehloenya *et al.*, 2008), with a tendency to decreased on average by 0.9 mM units in a meta-analysis (Desnoyers *et al.*, 2009a).

The mean lactic acid measured as either the L- or D-lactate, for which yeast had no effect on either concentration (Quigley *et al.*, 1992; Putnam *et al.*, 1997). This coincides with Newbold *et al.* (1995) as the L-Lactate concentration was not different between strains NCYC 240, 1026 and Yea-Sacc compared to the control. The lactic acid concentration was not different between treatments, however, the incidence of acute ruminal acidosis was lower for steers receiving yeast supplementation possibly supporting the assumption that yeast promotes lactic acid utilisation in the rumen (Mir and Mir, 1994). The *In vitro* study supplementing yeast showed that the substrate maltose which was proposed to induce high lactate concentrations, although the added yeast had no effect on the lactate concentrations between the treatments (Sullivan and Martin, 1999) or strains of yeast (*In vitro*) NCYC 240, 694, 1026, 1088 and Yea-Sacc with no difference in L-Lactate concentration compared to the control (Newbold *et al.*, 1995).

2.10.6.3.7 Ruminal ammonia nitrogen

Yeast supplementation has increased the NH_3-N concentrations in calves (between 18 and 60 days of age) (Pinos-Rodríguez *et al.*, 2008), sheep (Arcos-García *et al.*, 2000; Kamel *et al.*, 2004), cows (Miranda *et al.*, 1996; Roa V *et al.*, 1997) and in *In vitro* studies (Kung *et al.*, 1997).

Higher NH_3-N concentrations were found in studies when live yeast (*S. cerevisiae*) CNCM I-1077 (Pinos-Rodríguez *et al.*, 2008) and a yeast culture Yea-Sacc^{1026} (Arcos-García *et al.*, 2000) were supplemented. However, the yeast culture (Yea-Sacc^{1026}) supplementation resulted in significantly higher ruminal NH_3-N concentrations than what was measured with the live yeast (Levucell) supplement. The higher NH_3-N concentrations should be absorbed due to the large concentration gradient created, but this was not the case as, the lower pH of the rumen and pK_a value resulted in similar absorptions. Furthermore, the difference in the NH_3-N concentrations may be a product of higher proteolytic bacterial populations (Arcos-García *et al.*, 2000). The higher proteolytic bacterial population caused by yeast supplementation is supported by other authors (Kung *et al.*, 1997; Moallem *et al.*, 2009). In addition, significantly lower NH_3-N concentrations were observed in buffalo calves (Vaneeta *et al.*, 1998)

Yeast may influence other bacterial populations which promote increased incorporation of NH_3-N into microbial protein as yeast may either effect the protein degradation by increasing or decreasing it (Moallem *et al.*, 2009). Moallem *at al.* (2009) had stated this after the study had resulted in significantly lower ruminal NH_3-N concentrations for the yeast supplemented animals (12.61 mg/dL compared to the control (15.19 mg/dL). This is in agreement with Erasmus *et al.* (1992), Enjalbert *et al.* (1999) and Lascano and Heinrichs (2009), who added that previous research for the addition of yeast causing a lower NH_3-N is due to either yeast stimulating NH_3-N uptake by bacteria, or alternatively stimulating cellulolytic bacterial growth.

The average effect of two yeast supplements (Yea-Sacc^{1026} and Levucell CNCM I-1077) tended to increase the NH_3-N concentration, compared to the control (Arcos-García *et al.*, 2000). This was similar to the *In vitro* study comparing live and dead yeast, which revealed that neither differed from the control for the NH_3-N concentrations (Dawson *et al.*, 1990) which were similar to other studies comparing different strains of yeast (Newbold *et al.*, 1995) and products, where the *In vitro* supplementation of a Diamond V-XP and Amax yeast culture tended ($P = 0.08$) towards higher NH_3-N concentrations (Miller-Webster *et al.*, 2002).

The flow, degradation and rate of AA, non-ammonia nitrogen (NAN) and total N through and in the GIT with yeast supplementation has been reviewed by previous authors with

conflicting results. It was suggested that yeast supplementation has the potential to alter the bacterial protein AA profile due to the fact that a significant effect was exerted on the duodenal AA profile and flow of Met in the GIT (Erasmus *et al.*, 1992). A higher bacterial N flow supported the fact that higher duodenal NAN flows had a tendency to be higher in yeast supplemented cows (Erasmus *et al.*, 1992). Similarly, Putnam *et al.* (1997) reported that NAN (non-microbial) flow to the duodenum tended to be higher for yeast supplemented cows, although the flow of essential AA and AA profiles of duodenal digesta and microbial proteins were not affected. Yoon and Stern (1996) found that the total N and NAN flow to the duodenum had decreased (due to tendencies towards higher CP degradabilities in the rumen) without a difference in bacterial N flow, but rather owing to a decrease in dietary N flow and endogenous N flow. Lehloenya *et al.* (2008) illustrated that feeding yeast did not affect the duodenal and microbial N flow. Microbial N flow, non-microbial N flow and NH_3-N were unaltered by yeast (CNCM I-1077) supplementation in the diet even though *In situ* N disappearance of soyabean meal was significantly higher at four hours and tended to be higher at eight hours (Doreau and Jouany, 1998). A bakers yeast supplementation showed no effect for the rate of N degradation (Kamel *et al.*, 2004).

Yeast supplementation and its effect or lack of effect on the diet has been reviewed (Glade and Biesik, 1986; Moloney and Drennan, 1994; Olson *et al.*, 1994; Roa V *et al.*, 1997; Medina *et al.*, 2002; Lascano and Heinrichs, 2009). This as represented by Roa V *et al.* (1997), with higher ruminal NH_3-N concentrations for alfalfa hay 36.3 vs. 27.7%, coffee hulls 36.4 vs. 26.9% and cornstalk 36.5 vs. 28.2% compared with the control, respectively (Roa V *et al.*, 1997). Yeast added to a urea-containing diet in horses, found that the yeast stimulated conversion of recycled urea to microbial protein and AA, the higher microbial activities enhanced ammonia and AA production from the fact that the fecal N absorbability increased by 47% (Glade and Biesik, 1986).

Moloney and Drennan (1994) reported that a high fibre-low protein diet was not affected by yeast inclusion in terms of ammonia concentrations in the rumen, but the NH_3-N concentration was significantly reduced when yeast was added to the low fibre-high protein diet. They furthermore revealed that yeasts effect on N metabolism is affected by the time after feeding and N content of the basal diet. This was similar to a study with horses consuming a high starch diet revealing significantly lower cecal NH_3-N concentrations for yeast supplemented animals five hours post-feeding compared to the control with no effect found for horses consuming a high-fibre diet (Medina *et al.*, 2002).

Lascano and Heinrichs (2009) on the other hand, found that the addition of yeast in all the diets irrespective of the ratio of forage to concentrate in the diet, resulted in significantly

lower NH_3-N concentration compared to the control, which simultaneously coincides with the significantly higher VFA concentration also recorded by Enjalbert *et al.* (1999). This indicates that the lower NH_3-N concentration measured was not limiting bacterial growth, but may be attributed to higher concentrations of cellulolytic and total bacteria (Lascano and Heinrichs, 2009).

Supplementing different levels of a yeast supplement has affected the NH_3-N concentrations. *In vitro* NH_3-N concentration was highest with the higher yeast supplementation level suggesting that the degradation of protein had been extensive and it was proposed that yeast stimulates proteolytic bacterial activity (Kung *et al.*, 1997). Furthermore, Kamel *et al.* (2004) concluded that the daily microbial N synthesis was significantly higher with the highest level of bakers yeast supplementation with a significant correlation between microbial N synthesis and SI (synchronization index) (Kamel *et al.*, 2004). The ratio between the hourly release of N and OM in the rumen is calculated and is known as the SI, the values for the control (berseem hay), control and low bakers yeast ,and control and high bakers yeast were 0.059, 0.61 and 0.72 ($P < 0.05$), respectively (Kamel *et al.*, 2004), therefore more energy was available to the microbes for their growth when HBSC was supplemented, on condition that N had unaffected release (Kamel *et al.*, 2004)

The sampling time and the subsequent analysis of the ruminal fluid for NH_3-N concentration, is a product of time of sampling after feeding. Generally, the NH_3-N concentration, increased two hours (Guedes *et al.*, 2008) and/or three hours post-feeding and decreased again at six hours post-feeding (Abd El-Ghani, 2004). However, with yeast supplementation lower ruminal NH_3-N values were found three hours post feeding for dairy cows (10.31 and 14.85 mg/dL) (Enjalbert *et al.*, 1999), four hours for steers (Moloney and Drennan, 1994) and for buck at three to six hours post-feeding (Abd El-Ghani, 2004). Moloney and Drennan (1994) confirmed that a time by yeast interaction had occurred.

Conversely, Miranda *et al.* (1996) measured significantly higher NH_3-N concentrations three hours post-feeding for Yea-Sacc^{1026} and *A. oryzae*, which was 19.15 and 18.44 mg/ dL, respectively compared to the control (15.0 mg/dL).

Ruminal fluid NH_3-N concentrations collected two or four hours after feeding, showed that there was no significant difference between yeast and control treatment with steers (Mir and Mir, 1994) or for dairy calves (Quigley *et al.*, 1992), respectively.

The lack of effect of a yeast supplement is supported by others (Wiedmeier *et al.*, 1987; Piva *et al.*, 1993; Olson *et al.*, 1994; Erasmus *et al.*, 2005; Guedes *et al.*, 2008; Longuski *et al.*, 2009).

2.10.6.3.8 Ruminal pH

Yeast supplementation and its effects on stabilising the rumen pH has been extensively researched. Yeast supplementation increased or stabilised rumen pH as found in studies with cows (Bach *et al.*, 2007; Guedes *et al.*, 2008; Marden *et al.*, 2008; Thrune *et al.*, 2009), steers (Roa V *et al.*, 1997), goats (Abd El-Ghani, 2004), buffalo calves (Vaneeta *et al.*, 1998) and in a meta-analysis study (Desnoyers *et al.*, 2009a). The mean ruminal pH was significantly higher for live yeast supplemented ruminants with ruminal pH values of 6.05 vs. 5.49 (Bach *et al.*, 2007), 6.14 vs. 5.94 (Marden *et al.*, 2008), 6.53 vs. 6.32 (Thrune *et al.*, 2009) and 6.55 or 6.51 vs. 6.41 for the two levels 1 and 0.3 g/day, respectively (Guedes *et al.*, 2008). Bach *et al.* (2007) suggested that active dry yeast may be more effective in stabilising the rumen pH, although the increased meal frequency recorded in their study may play a role in the consistent higher ruminal pH measured. Nevertheless, the lower ruminal lactic acid concentrations could be accountable for the lower ruminal pH (Guedes *et al.*, 2008; Marden *et al.*, 2008). The stabilising effect could be attributed to the pK_a value of lactate (National Research Council, 2001; Marden *et al.*, 2008) which is a quantitative measure of the dissociation constant of an acid, giving an indication of the strength of the acid (Kotz *et al.*, 2003). The higher the pK_a value, the smaller the extent of the dissociation of an acid. The ruminal VFA, are absorbed across the rumen wall, only when in the undissociative state (National Research Council, 2001). The VFA is propionate, acetate and butyrate have pK_a values of 4.87, 4.76 and 4.82, respectively (National Research Council, 2001). Lactate is a strong acid and has a lower pK_a value (3.86) (Marden *et al.*, 2008) compared to all the VFA's. The VFA in the undissociated state are absorbed rapidly compared to lactate, therefore lactic acid with a higher extent of dissociated acid, is absorbed more slowly across the rumen wall (National Research Council, 2001). In general higher ruminal lactic acid concentrations would be measured under those conditions, which is not the case for yeast supplemented cows in the studies of Marden *et al.* (2008) and Guedes *et al.* (2008). Marden *et al.* (2008) owes the effect on ruminal pH to the lower redox potential measured in ruminal fluid from yeast supplemented cows, as it indicates the strength and reducing power of yeast, the value of -149 mV proves this, having lower values than that of the control (-115 mV) group. The strength of the action of yeast is demonstrated by Guedes *et al.* (2008) where the diets fed posed no real risk of acidosis occurring due to a moderate NFC content, and yet yeast was able to elevate the pH levels in the rumen, alleviate the depression in pH that occurs after feeding, and significantly increased rumen pH compared to the control pH which was as high as 6.41. Furthermore, Desnoyers *et al.* (2009a) concluded from the quantitative findings of a meta-analysis, that the more concentrate and DM consumed, the more the positive effects on

the ruminal pH the yeast will express, but the extent of expression will be reduced by the NDF level (Desnoyers *et al.*, 2009a). In this study the effect of dose was prominent, in that increasing the dosage of yeast supplementation, contributed to increasing the pH linearly.

The drop in pH after large quantities of fermentable carbohydrates have been consumed is often difficult to avoid, due to the practical reality of a pasture-based system, where concentrates are fed at milking in the parlour, and not consumed at a steady rate throughout the day. As in the studies by Marden *et al.* (2000) and Guedes *et al.* (2008) which reported that the pH trend post-feeding caused lower pH values lasting for approximately four hours, was common between the control and yeast treatment group. Yeast altered the ruminal pH by reducing the drop in pH found typically after a large amount of concentrate was consumed, which may persist for 4 hours (Williams *et al.*, 1991) or three hours post-feeding (Abd El-Ghani, 2004) and in horses fed a high starch diet, revealed that the mean cecal pH's measured at four, six and eight hours post-feeding had increased compared to the control (Medina *et al.*, 2002). The *In vitro* study comparing the mean pH of Diamond V-XP and A-Max, showed that the mean pH was significantly higher when A-Max was supplemented and specifically at two and six hours post-feeding (Miller-Webster *et al.*, 2002). *In vitro* fermentors have high buffering capacities so studies used to measure yeast effects on pH may be inappropriate (Kung *et al.*, 1997). On the contrary yeast supplementation to steers (Plata *et al.*, 1994) or non-lactating dairy cows (Enjalbert *et al.*, 1999) had no effect on the pH measured at either three, six, nine or twelve hours after feeding, with no effect when the hours the pH is below six is compared to the control (Plata *et al.*, 1994) or one, three and five hours after feeding (Enjalbert *et al.*, 1999).

Yeast has the potential to increase the mean pH, additionally yeast increases the maximum and minimum pH levels experienced in the rumen and can decrease the time the ruminal pH is below a critical pH level. This is supported by Thrune *et al.* (2009) where significantly ($P < 0.05$) higher pH measurements with respect to the maximum (7.01 vs. 6.8) and minimum (5.97 vs. 5.69) values were recorded. Moreover, the pH was below 5.6 (subacute acidosis threshold), 5.8 and 6 for a shorter period of time (Thrune *et al.*, 2009). This is in line with Bach *et al.* (2007) who reported that the time the rumen pH was below 5.6 and 6 is significantly less for the yeast supplemented cows. This time variable may be represented by the area under the curve for the pH plotted on a graph of time vs. ruminal pH. The time the control cows ruminal pH was below 5.6 and 6, was 4 and 9.5 hours per day, respectively, which is significantly longer than 1.3 and 4.1 hours per day for the yeast supplemented cows (Bach *et al.*, 2007). This is in agreement with Roa V *et al.* (1997) with the time below a ruminal pH level of 6.2 was reduced for yeast supplemented steers. Bach *et al.*

(2007) concluded that yeast supplemented cows experienced a lesser intensity of subclinical acidosis when it arose (Bach *et al.*, 2007).

Contradictory studies exist where significantly lower ruminal pH's were reported due to yeast supplementation (Andrighetto *et al.*, 1993; Sullivan and Martin, 1999; Arcos-García *et al.*, 2000). This was demonstrated with different yeast products with their mean effect causing a lower pH compared to the control, and the Yea-Sacc (5.85) had a significantly ($P = 0.01$) lower pH compared to the Levucell (5.96) (Arcos-García *et al.*, 2000). Andrighetto *et al.* (1993) stated that the reason for the significantly lower ruminal pH may be due to the fact that higher VFA were measured for yeast supplemented animals (Andrighetto *et al.*, 1993).

The lack of an effect of a yeast supplement on the ruminal pH has been observed in studies where; high concentrate diets are fed (Longuski *et al.*, 2009); diets differ in their concentrate to forage ratios (Lascano and Heinrichs, 2009); high (low fibre-high protein) or low (high fibre-low protein) quality diets are fed (Moloney and Drennan, 1994); different strains of yeast in calves are compared (Pinos-Rodríguez *et al.*, 2008) and other strains comparing NCYC 240, NCYC1026 and Yea-Sacc (Newbold *et al.*, 1995); comparing both a live yeast and a killed yeast (Dawson *et al.*, 1990). Other studies in which the pH was unaffected by yeast supplementation were reported by (Erasmus *et al.*, 1992; Besong *et al.*, 1996; Robinson and Garrett, 1999; García *et al.*, 2000; Erasmus *et al.*, 2005; Lehloenya *et al.*, 2008) after four hours from concentrate consumption (Williams *et al.*, 1991) or four hours after feeding in dairy calves (Quigley *et al.*, 1992). A lack of response in these studies could be due to the stage of lactation, ration or source and type of supplemented yeast (Thrune *et al.*, 2009).

In a study with Holsteins pH tended ($P < 0.1$) to be higher for yeast supplemented cows (Moallem *et al.*, 2009). Conversely, Piva *et al.* (1993) found that the yeast supplemented mid-lactation cows tended to have a lower pH, and goats fed the high concentrate (50%) diet was not affected by the supplemented yeast, but tended to decrease the minimum rumen pH (Desnoyers *et al.*, 2009b). This was owed to the fact that they found the feeding behaviour of the yeast supplemented goats to select and ingest less fibrous portions of the diet and this could help such animals to cope better when consuming high concentrate diets (Desnoyers *et al.*, 2009b).

2.10.6.4 Micro-organisms

The most consistent mode of action of yeast is the fact the yeast stimulates ruminal micro-organisms (Nisbet and Martin, 1991; Andrighetto *et al.*, 1993; Arakaki *et al.*, 2000; Abd

El-Ghani, 2004). These micro-organisms that have been investigated in previous studies are protozoa and, cellulolytic, amylolytic and proteolytic bacteria.

The addition of yeast supplements to diets resulted in an increase number of rumen fluid associated bacteria or viable bacteria (Lascano *et al.*, 2009a), however, the total anaerobic bacteria counts were not different between treatments in other studies (Harrison *et al.*, 1988).

The counts or concentration of proteolytic bacteria which are involved in the breakdown of protein, have increased due to yeast supplementation and has been documented by Yoon and Stern (1996) (3.09 vs. 2.00 × 10^8/ml).

Cellulolytic bacteria concentrations were increased by yeast supplementation *In vitro* (Harrison *et al.*, 1988; Dawson *et al.*, 1990; Newbold *et al.*, 1995), in steers (Dawson *et al.*, 1990), in sheep (Newbold *et al.*, 1995), in cows (Harrison *et al.*, 1988) and buffalo calves (Vaneeta *et al.*, 1998). Cellulolytic bacterial populations are influenced by yeast supplementation thereby creating a better anaerobic environment for anaerobic bacteria such as cellulolytic bacteria which are oxygen sensitive (Chaucheyras-Durand and Fonty, 2002). The counts of ruminal micro-organisms, may differ when comparing *In vitro* to *In vivo* experimental conditions, although some researches have stated that the methods are highly comparable. Dawson *et al.* (1990) measured significantly higher cellulolytic and anaerobic bacteria (*In vitro* continuous culture) in live yeast compared to dead yeast cultures. When comparing a live yeast with the control, higher counts of 3.02 x 10^9/ml (log 9.48/ml) were measured *In vitro* compared to the control of 6.03 x 10^8/ml (log 8.78/ml) which was concurrent in steers (*In vivo*) fed a live yeast, compared to the control (Dawson *et al.*, 1990). Dawson *et al.* (1990) therefore concluded that similar responses were achieved with either *In vitro* or *In vivo* methods in measuring cellulolytic organisms, and rumen stimulating methods are as reliable. Newbold *et al.* (1995) further illustrates this with the use of different strains, as higher total viable and cellulolytic bacterial numbers were measured *In vitro* for strains NCYC 240, NCYC 1026 and Yea-Sacc compared to the control, however, when measured in sheep only higher cellulolytic bacterial concentrations were measured with strain NCYC 240 compared to the control. Harrison *et al.* (1988) measured significantly higher cellulolytic bacterial concentrations, coupled with a stable fermentation environment as measured in both *In vivo* and *In vitro* conditions due to the little variation between NH_3-N concentrations. The mechanism by which yeast supplements may influence certain populations of ruminal bacteria are elucid, nevertheless Wiedmeier *et al.* (1987) suggested the reason for increasing numbers of cellulolytic bacteria, may be either from the yeast providing stimulatory factors (B vitamins) or branched-chain VFA's, although the yeast itself is not cellulolytic. This is in

agreement with Callaway and Martin (1997) who suggested that yeast stimulated bacteria that utilise lactate and digest cellulose due to yeast addition of soluble factors such as B vitamins, AA and organic acids for their growth.

Failure to increase cellulolytic bacterial populations with supplemental yeast are reported by other authors (Dawson *et al.*, 1990; Erasmus *et al.*, 1992; Mir and Mir, 1994; Newbold *et al.*, 1995; Yoon and Stern, 1996). The *In vitro* supplementation of dead yeast cells (121 °C for 15 minutes) had a similar effect on the cellulolytic bacterial numbers compared to the control, this may which suggest the either some heat liable component or a component only present in live yeast cells stimulates the increase in cellulolytic numbers, and is absent in dead cells (Dawson *et al.*, 1990). This was demonstrated by Vaneeta *at al.* (1998) when autoclaved yeast had failed to affect microbial numbers, and that live yeast cell numbers or heat liable components present in the yeast supplements, are what is responsible for the influence on microbial bacterial numbers. Strains of yeast such as NCYC 694 and NCYC 1088 supplemented *In vitro* did not differ compared to the control with respect to the total number of viable and cellulolytic bacteria measured (Newbold *et al.*, 1995). Newbold *et al.* (1995) suggested that the difference between strains in their ability to stimulate microbial organisms may be attributed to the fermentation and assimilation of carbohydrate and N sources between strains. However, if the negative associated affect of concentrate supplementation becomes too large for the yeast to eliminate, it will fail to effectively stimulate the cellulolytic bacterial populations as in the study by Mir and Mir (1994).

Yeast affected a range of bacteria *In vitro* which are the major species found naturally in the rumen, such as *S. ruminantium* HD4 and H18, *Megasphaera elsdenii* B159 and T81, *Fibrobacter succinogenes* S85 and *Ruminococcus albus* B199 which were all stimulated by the addition of a yeast (Callaway and Martin, 1997). This resulted in *S. ruminantium* HD4 increasing the acetate and total VFA concentration, additionally the propionic and total VFA concentrations increased through the action of *S. ruminantium* H18 (Callaway and Martin, 1997).

Yeast supplementation has influenced the ruminal protozoal concentrations in lambs (Chaucheyras-Durand and Fonty, 2002) , steers (Plata *et al.*, 1994), sheep (García *et al.*, 2000) and heifers (Miranda *et al.*, 1996). Protozoa populations in the rumen of yeast supplemented young ruminants such as lambs were more diverse and had established themselves earlier (Chaucheyras-Durand and Fonty, 2002). Chaucheyras-Durand and Fonty (2002) highlights that the functionality of the rumen was improved and accelerated to form a stable rumen ecosystem, as yeast favours earlier microbial ecosystem maturation in the young animal. The lower redox potential in lambs supplemented with yeast confirms its

oxygen-scavenging activity which creates a better anaerobic environment (Chaucheyras-Durand and Fonty, 2002). Plata *et al.* (1994) showed that for yeast supplemented to older ruminants, the ruminal protozoal concentrations were significantly (P = 0.002) higher with counts of 341.8 × 10^3/ml vs. 254.1 × 10^3/ml. Additionally, Miranda *et al.* (1996) demonstrated that the Entodiniomorphid counts at a NDF level of 27% were higher for *S. cerevisiae* and *A. oryzae* cultures compared to the control, as there was an interaction between such cultures and NDF level for Entodiniomorphid counts, and as the NDF increased to 37% the counts decreased compared to the control. In contrast the ruminal protozoal concentrations significantly decreased as a result of live yeast supplementation measured in the study of Garcia *et al.* (2000), 39.7 × 10^4/ml compared to the control of 69.4 × 10^4/ml. However, in other studies yeast supplementation failed to effect the protozoa concentrations (Yoon and Stern, 1996; Doreau and Jouany, 1998; Arcos-García *et al.*, 2000) and for supplemented strains of NCYC 240, NCYC 1026 and Yea-Sacc to supplemented sheep (Newbold *et al.*, 1995; Arakaki *et al.*, 2000).

2.10.6.5 Digestion

The effects of supplemental yeast on digestion will be evaluated in this section, with the effects of yeast on both total tract digestibility as well as ruminal degradability/digestibility. Williams and Newbold (1990) cited by Erasmus *et al.* (1992) states that the total tract digestibility measurements carried out in experiments to show the effects of yeast supplementation may give an inaccurate depiction of yeast supplementation and its effects as the yeast may function in altering the site of digestion.

2.10.6.5.1 Total tract digestibility

Yeast supplementation has significantly influenced total tract digestibility of the following dietary nutrients, namely, DM, OM, CP, NDF, ADF and Hemicellulose.

The total tract DM digestibility has significantly increased with yeast supplementation for lambs supplemented with (3 g/day) Diamond V XP (Haddad and Goussous, 2005), heifers (Lascano *et al.*, 2009b) and steers (10 g/day) (Mir and Mir, 1994) and higher *In vitro* DM digestibilities were observed (Tang *et al.*, 2008). The lack of an effect of yeast on the total tract DM digestibility has been recorded in previous research (Harris *et al.*, 1992; Doreau and Jouany, 1998; García *et al.*, 2000; Abd El-Ghani, 2004; Cooke *et al.*, 2007; Moallem *et al.*, 2009; Tripathi and Karim, 2010).

The total tract OM digestibility increased due to yeast supplementation in the meta-analysis conducted by Desnoyers *et al.* (2009a), in heifers (Lascano *et al.*, 2009b) and in

lambs supplemented with (3 g/day) Diamond V XP (Haddad and Goussous, 2005) as well as *In vitro* OM disappearance (Tang *et al.*, 2008). Total-tract OM digestibility increased and this effect linearly increased with yeast dose, however, the positive effect on digestibility was negatively correlated with percentage of concentrate and positively correlated with DMI, NDF level and CP content in the diet (Desnoyers *et al.*, 2009a). The lack of an effect of yeast on total tract OM digestibility has been recorded in previous research (Moloney and Drennan, 1994; Doreau and Jouany, 1998; Arcos-García *et al.*, 2000; Abd El-Ghani, 2004; Lehloenya *et al.*, 2008; Moallem *et al.*, 2009) (Tripathi and Karim, 2010).

Total tract CP digestibility increased for cows supplemented with (10 g/day) Yea-Sacc (Erasmus *et al.*, 1992) and Biomate Yeast Plus (Wohlt *et al.*, 1998), lambs with (3 g/day) Diamond V XP (Haddad and Goussous, 2005) and steers with a yeast supplement (10 g/day) (Mir and Mir, 1994).

On the contrary the lack of an effect of yeast on the total tract CP (Wiedmeier *et al.*, 1987; Arambel and Kent, 1990; Harris *et al.*, 1992; Andrighetto *et al.*, 1993; Hinman *et al.*, 1998; Cooke *et al.*, 2007; Moallem *et al.*, 2009) or N digestibility (Lascano *et al.*, 2009b) has been recorded in previous research .

The cellulose, hemicelluloses and lignin (NDF) are structural carbohydrates, which are more resistant to digestion in the rumen. The total tract NDF digestibility increased in lambs supplemented with (3 g/day) Diamond V XP (Haddad and Goussous, 2005), cows with Biomate Yeast Plus (Wohlt *et al.*, 1998), (5 g/day) Biosaf Sc 47 (41.6 % vs. 29.6 %) (Marden *et al.*, 2008), heifers supplemented with Yea-Sacc (Lascano *et al.*, 2009b). The total tract ADF digestibility increased for cows supplemented with (10 g/day) Yea-Sacc (Erasmus *et al.*, 1992) or (5 g/day) Biosaf Sc 47 (32.3 % vs. 18.1 %) (Marden *et al.*, 2008) and lambs supplemented with (6 g/day) Diamond V XP (Haddad and Goussous, 2005). Marden *et al.* (2008) concluded that with live yeast supplementation a better fibre digestion was achieved as lactate accumulation was prevented mediated through a lower ruminal E_h and rH (Clarks exponent).

Hemicellulose digestibility (Glade and Biesik, 1986; Wiedmeier *et al.*, 1987; Jouany *et al.*, 2008) was significantly higher for yeast supplementated animals (horses and cows) compared to the control, this is supported by the significantly higher cellulolytic bacterial population measured for yeast supplemented cows (Wiedmeier *et al.*, 1987).

The lack of an effect of yeast on the total tract NDF (Wiedmeier *et al.*, 1987; Wohlt *et al.*, 1991; Harris *et al.*, 1992; Mir and Mir, 1994; Doreau and Jouany, 1998; Cabrera *et al.*, 2000; Moallem *et al.*, 2009), ADF (Arambel and Kent, 1990; Moloney and Drennan, 1994; Arcos-García *et al.*, 2000; Cooke *et al.*, 2007; Lehloenya *et al.*, 2008; Lascano *et al.*, 2009b;

Moallem *et al.*, 2009), cellulose (Wohlt *et al.*, 1991; Wohlt *et al.*, 1998; Cooke *et al.*, 2007) and hemicelluloses (Wohlt *et al.*, 1991; Wohlt *et al.*, 1998; Cooke *et al.*, 2007; Tripathi and Karim, 2010) digestibility has been recorded in previous research.

The above effects are a function of diet which has an influence on yeast failing to effect the nutrient digestibility, and is common when high concentrate diets (Arambel and Kent, 1990; Abd El-Ghani, 2004) or when corn gelatinized diets (Cooke *et al.*, 2007) are fed. Similarly for steers fed 88% barley and potato-based concentrate and alfalfa and corn silage based roughage, yeast had no effect on the total tract digestibilities (Hinman *et al.*, 1998). This was evident in other studies such as that reported by Mir and Mir (1994) with steers consuming either an alfalfa or corn silage diets and for cows fed a corn silage based diet (Wohlt *et al.*, 1991). Tang *et al.* (2008) revealed that the total tract *In vitro* DM and OM digestibility of forages was higher for all types of cereal straws namely, rice straw, wheat straw, maize stover and maize stover silage. A study conducted with live yeast supplementation to horses concluded that regardless of the diet, the yeast had the potential to improve the cellulose digestibility (Jouany *et al.*, 2008).

The inclusion of a yeast supplement interacted significantly with diet type in terms of CP digestibility, which was not affected in low fibre-high protein diets but CP digestibilities decreased significantly when the high fibre-low protein diet was fed (Moloney and Drennan, 1994). Yeast supplemented steers on tropical pastures, had no improvement in total tract digestibility of NDF or ADF (Cabrera *et al.*, 2000). An *In vitro* gas production technique was used to determine the effects of yeast on *In vitro* fermentation parameters of rice straw, wheat straw, maize stover and maize stover silage with a decreased lag time for each type of straw (Tang *et al.*, 2008).

The ADF and CP digestibilities were higher for the group of cows supplemented with yeast and proved that comparing a 20 or a 10 g/day level of yeast did not result in higher digestibilities (Wohlt *et al.*, 1998).

Yeast supplementation in both the commercial product of Levucell and Yea-Sacc^{1026} failed to effect the diet digestibility of the above stated nutrients (Arcos-García *et al.*, 2000). DM, OM, NDF and ADF digestibilities were all unaltered by yeast supplementation CNCM I-1077 in the diet for early lactation Holstein cows (Doreau and Jouany, 1998). Neutral detergent fibre and DM digestibility in Levucell supplemented sheep were not different compared to the control or to sheep supplemented with monensin and Levucell simultaneously (García *et al.*, 2000). Crude protein digestibilities tended to increase with both the separate and the combined fungal supplementation of yeast (90 g/day) and *A. oryzae*, with the combination resulting in the largest increase (Wiedmeier *et al.*, 1987).

2.10.6.5.2 Ruminal digestion

The ruminal digestion is a function of the ruminal retention time and the rate of ruminal OM digestion, of the OM that is potentially digestible (Milligan *et al.*, 1986). The ruminal retention time is inversely proportional to the rate of passage, and the rate of digestion is dependent on the chemical characteristics of the substrate and the attachment of bacteria to such substrates (Milligan *et al.*, 1986).

The ruminal digestion of nutrients as affected by yeast supplementation has been investigated in previous research, nutrients such as DM, OM, CP/N, NDF and ADF.

2.10.6.5.2.1 Dry matter

Dry matter digestibility is dependent on the type of feed and time of exposure or incubation in the rumen fluid, such as the digestibility of hay that has increased in response to supplemental yeast after 12 hours (Williams *et al.*, 1991) thereafter no effect of yeast was found (24 hours). Hovell *et al.*, 1986 cited by Williams *et al.*, (1991) described the possible reason for the yeast failing to affect the DM digestibility of the diet after 12 hours as bacterial numbers may shift to increase the rate of digestion of the fibre fraction in the diet, the diets digestibility is more associated with the ruminal retention time and the physiochemical characteristics of the feed. The responses to yeast supplementation seem to be optimum, when the environment as well as the feeding regime the cow is exposed to, compromises the cellulolytic activity in the rumen (Williams *et al.*, 1991) and, this type of behaviour supports the fact that the effect of the yeast may be alleviating the possible negative effects on the digestion of cellulose that the cows' environment would normally impose. The supplementation of yeast has failed to improve the ruminal DM digestion (Lascano and Heinrichs, 2009) of corn stalk (Doreau and Jouany, 1998), hay (Enjalbert *et al.*, 1999), specifically alfalfa and bermuda grass hay (Sullivan and Martin, 1999), various raw materials (barley grain, soybean meal, barley straw, barley hay, and lucerne hay) (Hadjipanayiotou *et al.*, 1997), wheat straw at 12 and 24 hours (Erasmus *et al.*, 1992), and furthermore the nature of the basal diet had no effect (Moloney and Drennan, 1994).

The degradation of DM with yeast supplementation is effected by the type of yeast product and strain. The degradation of DM of straw was significantly higher for the specific strain, NCYC 1026 at 72 and 96 and Yea-Sacc at 72 hours compared to the control (Newbold *et al.*, 1995). This was similar to an *In vitro* study where the *In vitro* DM digestibility was higher for a TMR in a continuous culture with added yeasts of Diamond V XP and A max, compared to the control (Miller-Webster *et al.*, 2002). However, contradictory findings by Arcos-Garcia *et al.* (2000) demonstrated that yeast supplement or type of yeast supplement had no effect on

the ruminal DM disappearance at the 12, 24, 72 and 96 hour incubation periods, though at 48 hours the yeast supplemented animals tended to be superior compared to the control, with Levucell being significantly higher at 48 hours compared to the Yea-Sacc in terms of DM digestibility (Arcos-García *et al.*, 2000). Similarly, *In vitro* DM digestibility after 24 and 48 hours were not different between various strains of yeast (NCYC 240, NCYC 694, NCYC 1026, NCYC 1088 and Yea-Sacc) (Newbold *et al.*, 1995).

2.10.6.5.2.2 Organic matter

The OM ruminal digestion increased for steers grazing mixed prairie grass with yeast supplementation (Olson *et al.*, 1994) and when higher levels of bakers yeast was supplemented (Kamel *et al.*, 2004).

Lehloenya *et al.* (2008), Yoon and Stern (1996), Doreau and Jouany (1998), Kamel *et al.* (2004) and Miller-Webster *et al.* (2002), demonstrated that supplementing yeast had no effect on OM digestibility in the rumen.

Desnoyers *et al.* (2009a) reported that the OM digestibility increased due to yeast supplementation, and the positive effect of yeast was increased with the NDF level of the diet and decreased with the level of concentrate include.

2.10.6.5.2.3 Crude protein or nitrogen

The ruminal N degradation of soybean meal was significantly higher at four hours and tended to be higher at eight hours (Doreau and Jouany, 1998). The higher ruminal N degradation suggests that the proteolytic bacteria were stimulated by the supplemental yeast, which implies that the proteolytic bacteria, had increased their activity and numbers due to the presence of yeast in the rumen, which is known to stimulate such activities (Doreau and Jouany, 1998). Similarly Roa V *et al.* (1997) showed that yeast supplementation increased the potentially digestible CP from 85.7 to 90.2% when total mixed diets with alfalfa hay were fed to steers (Roa V *et al.*, 1997). However, CP (Hadjipanayiotou *et al.*, 1997) and N (Kamel *et al.*, 2004) digestion in other studies were not affected by yeast supplementation.

2.10.6.5.2.4 Neutral detergent fibre

Yeast supplementation had increased ruminal NDF digestion at 24 hours (43.4 vs. 36.9) (P = 0.01) due to the fact that a significant protozoa concentration was measured in steers (Plata *et al.*, 1994). Roa V *et al.* (1997) suggests that the effect of direct-fed microbials is dependent on the fibre source, as in the case of Roa V *et al.* (1997) for total mixed diets with alfalfa hay (55.0 vs. 46.6%).

However, yeast failed to affect the NDF digestibility in other studies with steers (56 g) (Lehloenya *et al.*, 2008), dairy cows (50 g) (Enjalbert *et al.*, 1999), (0.5 g) (Doreau and Jouany, 1998), (10 g) (Putnam *et al.*, 1997), (57 g) (Yoon and Stern, 1996), *In vitro* (Miller-Webster *et al.*, 2002) or *In vivo* after 6, 12 (28.6 vs. 20.2) (P = 0.06), 48 (52.5 vs. 45.3) (P = 0.07) and 72 (60.4 vs. 48.6) (P = 0.08) (Plata *et al.*, 1994) and 48 and 72 hours of rumen incubation (Arcos-García *et al.*, 2000).

Ruminal NDF disappearances differed between strains, being significantly (P = 0.002) higher for the Yea-Sacc^{1026} than for the Levucell strain at 48 hours (Arcos-García *et al.*, 2000).

Increasing the level of live yeast supplementation (from 0.3 g to 1 g/day) significantly increased the degradation of NDF of the low fibre degradation group of maize silages compared to the control and the 0.3 g supplemented cows (Guedes *et al.*, 2008). Yeast response is effected by not only its inclusion level but additionally by the initial digestibilities of the maize silages as a response occurred only in the low NDF silages (Guedes *et al.*, 2008). However, a lower NDF level reduced the *In situ* alfalfa NDF disappearances in another study (Miranda *et al.*, 1996). *In vitro* NDF digestibility was not affected by yeast adapted rumen inoculums' for diets consisting of either a commercial cattle feed and/or finger millet straw (Kamalamma *et al.*, 1996).

2.10.6.5.2.5 Acid detergent fibre

The ruminal degradation of ADF in corn stalk was significantly higher at 6 hours incubation for yeast CNCM I-1077 supplemented cows, with no difference found at other times (Doreau and Jouany, 1998). Doreau and Jouany (1998) reported that the first 6 hours is coupled with increased concentration of live yeast cells, which in turn promotes carbohydrate digestion.

Although, in other studies it was found that yeast supplementation had failed to effect ruminal ADF digestion (Enjalbert *et al.*, 1999), in steers (56 g) (Lehloenya *et al.*, 2008), dairy cows (57 g) (Yoon and Stern, 1996) and *In vitro* (Kamalamma *et al.*, 1996).

2.10.6.5.2.6 Rate of Digestion

The rate of digestion ultimately influences digestion, and retention time, which in turn affects the passage rate of digesta through the rumen or GIT (Milligan *et al.*, 1986). Yeast supplementation has the potential to increase the rate of digestion (Newbold *et al.*, 1995; Callaway and Martin, 1997; Kamel *et al.*, 2004). The rate of degradation was higher for Yea-Sacc, NCYC 240 and NCYC 1026 compared to the control with Yea-Sacc significantly higher than NCYC 1026 (Newbold *et al.*, 1995). The increase in rate of straw degradation was as a

result of the increase in total bacterial numbers (Newbold *et al.*, 1995) or, as a result of specific bacteria such as *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* (Callaway and Martin, 1997). In a study reported by Kamel *et al.* (2004) the increased inclusion of bakers yeast supplementation increased the rate of OM degradation (0.065, 0.071 and 0.075 per hour) for the control (Berseems hay), lower and higher yeast inclusion, respectively.

The increase in rate of digestion can be viewed alternatively as a reduction in lag time. The lag time is the time required for the attachment of bacteria to their substrates for efficient inoculation (Milligan *et al.*, 1986). Yeast tended to decrease the lag time for fibre digestion in steers (Williams *et al.*, 1991) and *In vitro* for different types of straw (Tang *et al.*, 2008).

The lack of an effect of yeast supplementation on the rate of digestion has been reported in previous studies (Wiedmeier *et al.*, 1987; Miranda *et al.*, 1996; Roa V *et al.*, 1997). The degradation rates of potentially digestible DM (PDDM), NDF (PDNDF), and CP (PDCP) were not affected by either the inclusion of a yeast culture or the type of fibre source (alfalfa hay, coffee hull and cornstalks) (Roa V *et al.*, 1997). This was further supported by Miranda *et al.* (1996) who found that ruminal starch digestion was unaffected by either fungal culture (Yea-Sacc^{1026} or A. oryzae) or NDF level which suggest the rate of starch digestion does not depend on a forage-associated rumen environment (Miranda *et al.*, 1996). The increased tendency towards higher DM intakes ($P < 0.1$) due to yeast supplementation observed by Erasmus *et al.* (1992), was most probably the cause of higher rates of digestion although it was not directly measured.

Ruminal and total-tract digestion as a result of yeast supplementation has yielded contradictory results. This is due to the varying digestibility and quality of test feedstuff, the nature of the basal diet, the retention time and hence the incubation periods. The CFU/g of yeast varies between strains coupled with the specified dosage size and rate of the yeast supplement is an additional factor to consider when quantifying results.

2.10.6.6 Health

Yeast supplementation and its positive, negative or lack of effect on production and ruminal parameters is well documented. The longevity health and well-being of the animal may be compromised due to the constant drive for high productivities, where eventually a physiological limit or threshold is reached. Yeast supplementation may assist in, increasing the health and immune function of the animal.

The gut has acquired a flushing effect, in that any harmful substances such as toxins and pathogens are bound and absorbed to the yeast cell wall fractions in the gut, which

protect the animal from potential harm (Stone, 2006). Moreover, the yeast cell wall consists mostly of beta-glucans and mannans. Beta-glucans is a structure consisting of glucose molecules linked together with Beta 1,3 and 1,6 bonds. Beta-glucan activates the macrophages by stimulating the immune system (Stone, 2006). The probiotic effect is contributed by the mannans which are a chain of mannose sugars consumed by the beneficial bacteria in the gut thereafter promoting growth of the beneficial bacteria which naturally suppresses and inhibits the growth of the harmful bacteria in the gut (Stone, 2006).

Greater stability of the intestinal flora was found in a study conducted with yeast supplementation in Saanen goats, in that *E. coli* fecal counts were reduced and lactobacilli counts increased (Stella *et al.*, 2007). This precedes the subsequent digestive disorders which may be created, yet as observed in calves receiving *yeast* CNCM I-1077 supplementation, the occurrence of diarrhoea and pneumonia was narrowed (Pinos-Rodríguez *et al.*, 2008), this is similar to the study by Magalhaes *et al.* (2008). Conversely, Cole *et al.* (1992) measured no significant effect supplemental yeast on calve performance or health, though morbid calves required reduced days of antibiotic therapy, and calves compromised with disease had maintained higher DMI and weights. Magalhaes *et al.* (2008) showed demonstrated lower mortality rates, which might have been due to improved N, Zn and Fe metabolism (Cole *et al.*, 1992). This is echoed in the study by Piva *et al.* (1993) who had measured improved blood plasma Zn levels ($p = 0.14$) suggested that yeast may supply the Zn that plays a role in reproductive performance (Piva *et al.*, 1993).

Reproductive performance was unaffected by either the Amaferm or Vitaferm supplementation (Kellems *et al.*, 1990). Similar findings of yeast having no significant effect on reproductive performance have been reported by Kalmus *et al.* (2009), and explains that in order to successfully measure the effect on reproductive performance, large cow numbers are required. However, Harris *et al.* (1992) proposed that the lower blood urea N value for cows supplemented with yeast may contribute to increased conception rates (Harris *et al.*, 1992).

Yeast supplementation to heat stressed cows (Shwartz *et al.*, 2009) or cows with digestive upsets (Moya *et al.*, 2009), have shown that body temperatures in the former and foam strength of rumen fluid in the latter study were reduced.

2.10.6.7 Milk sensory and manufacturing characteristics

Milk flavour was enhanced by the inclusion of a liquid yeast supplement to a TMR probably due to the antioxidant properties of riboflavin (Besong *et al.*, 1996). This was

evaluated with lower off-flavours assessments for yeast supplemented milk, and would therefore lead to a increased shelf-life when yeast was fed to cows (Besong *et al.*, 1996).

Milk manufacturing is able to benefit from cows supplemented with yeast, as in a study done by Piva *at al.* (1993), measured the dairy processing characteristics, found that yeast showed a tendency to produce a higher creaming capacity with a higher curd firming rate.

2.11 Conclusion of Literature review

From this extensive review, it is clear that many factors such as dietary ingredients, forage to concentrate levels, levels of starch, strain of yeast, CFU per gram yeast product, levels of yeast supplementation, animal species, stage of lactation, frequency and amount of concentrate fed influences the response obtained from yeast supplementation. Responses can either be effects on production parameters or rumen fermentation. The variable and inconsistent responses, complicates the process of making steadfast conclusions and recommendations for yeast supplementation. Results from research therefore suggests that positive responses could be expected when feeding high levels of starch during early lactation, and during adaptation periods, when dietary changes takes place, when high levels of concentrate are fed (slug feeding) and when cows are expected to feed selectively, such as when cows are under heat stress. No comparative study could be found where yeast products were supplemented to grazing cows receiving concentrate post milking.

The use of Meta-analyses to interpret yeast effects on productivity would be more reliable, and such results as done by Desnoyers *et al.* (2009a) covered 110 papers, 157 experiments and 376 treatments for yeast supplementation. Such analysis, removes the inconsistency between experimental studies, and may be the key to forming clear cut recommendations and more such analysis would supplement and concrete the information available on the effects of yeast supplementation at present.

In Appendix A, the information and results from reviewed studies involving yeast supplementation are summarized in Tables A8 to A13. The aim of this study was to investigate the effects of live yeast on the supplementation on the production of lactating Jersey cows grazing ryegrass/kikuyu pastures. The materials and methods conducted during the study will be discussed in the Chapter 3.

CHAPTER 3

MATERIALS AND METHODS

CHAPTER 3

Materials and Methods

3.1 Experimental Design

3.1.1 Location

The study was conducted at the Outeniqua Research Farm (Altitude 201 m, 33 ° 58' 38'' S and 22 ° 25' 16''E) (Botha, 2003) in George, Western Cape, South Africa. The study was executed out over a period of approximately three months during the spring of 2009 (September, October and November).

3.1.2 Animals

Thirty multiparous high-producing Jersey cows between 30 and 120 DIM were selected for the trial. A block design was used and the cows were blocked according to milk production (4% FCM), DIM and lactation number (See blocking of cows in Appendix A; Table A1). The blocking was done first according to the lactation number, DIM and then FCM yield. The cows within blocks were randomly assigned to one of the two treatment groups. The control treatment groups DIM was on average 84.3 ± 31.57 days (standard deviation; SD) and the yeast treatments DIM averaged 83.0 ± 31.43 days (SD) as at the 31st of August 2009. Additionally a rumen study was performed using ten rumen-fistulated animals which were randomly allocated to one of the two treatment groups in a cross-over design.

3.1.3 Treatment

The two treatment groups were a no-yeast (control), and a yeast supplement treatment group, each consisting of 20 cows (15 cows and 5 rumen fistulated cows). The allocation was done at random using the random function in Microsoft® Excel (Microsoft Office, 2007). Once assigned the cows in each o the groups were recorded and tagged. The control treatment group was identified with a pink coloured tag and the yeast treatment group was identified with a yellow coloured tag (Figure 3.1). The coloured tags were all numbered according to the corresponding block number starting from number 1 to number 20. A lightweight neck chain was used to suspend the coloured tags. The tags allowed for easy identification and sorting of the two treatments groups.

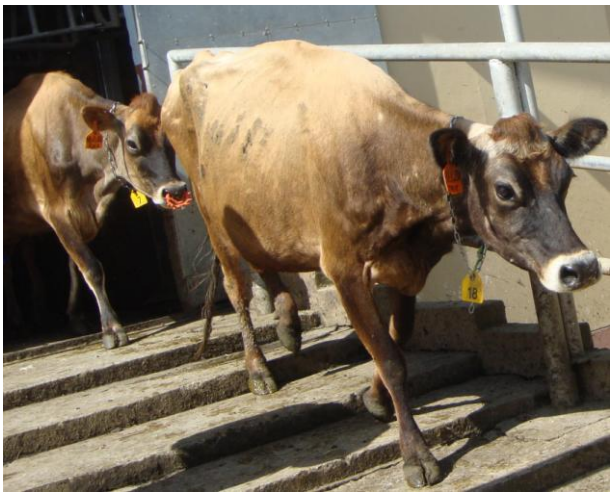

Figure 3.1 Jersey cows with a yellow tag represented the cows in the yeast treatment group which facilitated in sorting cows from the group herd into their treatment groups.

3.1.4 Feeds

The concentrate was formulated, mixed and pelleted at Nova Feeds (Nova Feeds George, Industrial Area, George Western Cape, South Africa). The ingredient and chemical composition of the concentrate is shown in Table 3.1 and 3.2, respectively. The control and yeast treatment concentrate had the same nutrient composition with the yeast added to the yeast treatment before pelleting. The cows were sorted and separated at each milking into their two respective groups. Cows received 6 kg concentrate (as is) daily, fed in equal portions in the milking parlour during milking. The 20 troughs on either side of the 20 point swing over parlour accommodated all 40 cows. The troughs were cleaned before feed was administered. Three kilograms of the control and yeast treatment concentrates were manually weighed with a Bizerba scale (model FC.15 with 0.001 g accuracy), and placed in separate plastic bags. The yeast treatment concentrate was fed on the left side of the milking parlour, and the control treatment was fed on the right side of the milking parlour. This was kept consistent throughout the trial period to avoid cross contamination of concentrates.

Table 3.1 Ingredient composition of experimental dairy concentrates fed to the Jersey cows.

Ingredient	DM %
Maize meal	82.37
Soyabean oilcake	10
Molasses syrup	4.0
Salt	0.5
MgO	0.3
Feed lime	2.0
MonoCaP	0.5
Premix[1]	0.33

DM – Dry matter; MgO – Magnesium oxide, MonoCaP – Monocalcium phosphate

[1]Premix contained ; Vitamin A, D3 and E; Zn; Mn; Cu; Se; Co and I.

Table 3.2 Chemical composition (g/kg DM) of experimental dairy concentrates fed to Jersey cows.

Nutrient	Dairy concentrate
DM %	87.10
ME MJ/kg DM[1]	12.41
CP (g/kg)	124.3
Fat (g/kg)	39.1
NDF (g/kg)	98.0
ADF (g/kg)	40.8
Ca (g/kg)	9.4
P (g/kg)	4.7
Mg (g/kg)	3.0

DM – Dry matter; ME – metabolisable energy; CP – Crude protein; NDF – Neutral detergent fibre; ADF – Acid detergent fibre

[1] Calculated ME MJ/kg DM = 0.82 × GE × IVOMD (Robinson *et al.*, 2004)

3.1.5 Yeast characteristics

The yeast product to be supplemented was supplied by Lallemand S.A.S (19 rue des Briquetiers, 31702 Blagnac cedex, France). The yeast from the strain (*Saccharomyces cerevisiae* CNCM I-1077) registered at the Pasteur Institute collection (CNCM), Paris, under the number I-1077, is a product manufactured as Levucell SC 10 ME–Titan which was produced from batch number 22aIN17UVS008. The package product and yeast is illustrated

in Figure 3.2. The Levucell SC 10 ME is a micro-encapsulated formulation for premix and pelleted feeds. The yeast treatment group had the yeast pelleted together with the dairy concentrate at a concentration of 167 g of the yeast per ton of concentrate i.e. 0.167 g/kg. This supports the requirement of an intake of 1 g yeast per cow per day as specified by Lallemand. The yeast has a concentration of 1×10^{10} CFU/g. The cows in the yeast treatment group therefore ingested 1×10^{10} CFU of yeast per day and the concentrate contained 1.67×10^{6} CFU yeast per gram of dairy feed concentrate.

Figure 3.2 The yeast product before pelleted together with the dairy concentrate ingredients

3.1.6 Pasture

Approximately seven hectares (ha) of an Estcourt type soil (Soil Classification Working Group, 1991) under permanent irrigation with kikuyu as a pasture base was used in the study. Italian ryegrass (*Lollium multiflorum*) of the cultivar Jeane was oversown (20 kg seed/ha) into kikuyu pasture using an Aitchinson planter. The pasture was divided into 41 pasture strips with the dimensions of ± 15 ×123 m. The divided strip would facilitate specific pasture allocation. Fertiliser was applied post-grazing at 56 kg N (limestone ammonium nitrate) per ha. Fresh clean water was available *ad libitum* to the cows on pasture. The cows were allocated a new pasture strip twice a day after milking. Water toughs were placed at a range of approximately 100 m walking distance.

3.1.7 Milking procedure

The milking machine in the milking parlour was a twenty point Dairy Master (Total Pipeline Industries, 33 Van Riebeeck St., P. O. Box 252, Heidelberg 6665, Republic of South Africa) swing over machine with weigh-all electronic milk meters. The cows covered an average distance of two kilometers for each milking procedure. The cows were sorted and separated at each milking period according to their treatment groups. The cows were milked twice daily at 06:00 and 14:00. The cows supplemented the yeast were milked daily on the left side of the milking parlour and control treatment group were milked on the right side of the milking parlour.

3.2 Measurements

The study consisted of a production and a rumen study.

3.2.1 Production Study

3.2.1.1 Cows

The 40 cows were weighed and their BCS determined at the beginning (31 August 2009 & 1 September 2009) and end (23 & 24 November 2009) of the experimental period. This was done after the afternoon milking (15:00) over two consecutive days to account for variation between days for each cows' water consumption, urination and defecation activities (See Appendix A; Table A2 and A3). A BCS of one to five (Wildman *et al.*, 1982) was allotted by one person, Gerrit Van der Merwe, the Jersey herd manager at the Outeniqua Research Farm.

3.2.1.2 Pasture management

The Jersey cows were allowed to strip graze the *Italian* ryegrass/kikuyu pastures (Figure 3.3) with clean water available *ad libitium* at all times. Pasture allocation was managed by conducting pasture height measurements before and after grazing. This was done for each new strip of ryegrass given to the herd. Pasture heights before and after grazing were measured with an Ellinbank RPM (Stockdale, 1984; Fulkerson, 1997) with an area of 0.098 m^2. The pasture RPM integrates sward height and density into a single value. To calibrate the RPM a linear regression was calculated between the meter reading and herbage DM mass. The calibration equation of $Y = aX + b$, was used to predict pasture mass where y = yield (kg DM ha^{-1}), a = factor as in gradient, (X) is the height measured on the RPM and b is a constant. The DM determination follows by utilising a reliable regression equation and reading the estimated DM availability at the specific height measured on the RPM.

Pasture regressions were performed every seven days on the camp to be grazed the next day. The pasture regression procedures were carried out by visually dividing the pasture strip into three sections. In each section the pasture heights were visually examined and three points of pasture that were at either a low, medium or high representative height of the rest of the pasture in that sectional strip were identified. The pasture disk meter reading was done on each of the three points selected in each of the three sections. The disks ring was placed over that area where the height had been recorded, and the pasture was cut at the level of the ring which is approximately three centimeters above the ground level. This level was chosen according to previous pasture management experience, as cows do not graze below this level.

Figure 3.3 Jersey cows grazing the *Italian* ryegrass/kikuyu pastures in allocated strips

3.2.1.3 Pasture sampling

In addition to the nine circles cut for the regression, a pasture sample was also collected on the same day which was sent to the laboratory for analysis. This pasture sample was taken at random on the strip to be grazed. The disks' ring was randomly tossed into the air over the pasture strip and where ever it landed on the pasture, and the grass was cut at a height of three centimeters above ground level. All the cut pastures above the disk ring were collected and placed in plastic sample bags and labelled; low one, two and three; medium one, two and three and high one, two and three. In addition the pasture sample collected was

numbered and placed in a plastic bag. All the sample bags were transferred to corresponding labelled paper bags and the wet material was weighed (Precisa 3100C scale, with accuracy to 0.01 g). All wet pasture samples were weighed and placed in an oven at 60 °C for 72 hours (Botha, 2003). The bags were removed from the oven after drying and were weighed (Sartorius L420P scale, with accuracy to 0.001 g). Pasture samples were taken on average at 12:00 weekly, starting on the 10th September 2009, and pooled for every two weeks, to result in six pasture samples.

3.2.1.4 Concentrate sampling

The sampling started on Monday the 7th of September 2009, where samples were continually taken every Wednesday, Friday and Monday thereafter. The sampling of both the control concentrate and the yeast treatment concentrates were done, by taking 350 g grab samples of each from the open pelleted concentrate bags to be used on that day. The samples were labelled and pooled for two weeks. This resulted in six sub-samples of concentrate sample taken over two weeks. There were, therefore, six concentrate samples of both the control and yeast supplemented concentrates at the end of the trial.

3.2.1.5 Milk sampling

Composite milk samples were taken every two weeks starting on Monday the 21st of September 2009 (after an adaption period of 20 days) and ended on the 23rd of November 2009. The milk samples of 30 cows were taken at each sampling period. A composite milk sample (24 ml) of morning and afternoon milkings were sampled. This equated to a volume of 16 ml and 8 ml of milk sampled at the morning and afternoon milkings, respectively. These volumes represent the interval of 16 and 8 hours between milkings (a millilitre sampled every hour). The same procedure of milk collection was carried out for each cow in the experimental group. The milk collecting procedure was as follows: the milk collecting bottles were removed once the cow was done being milked; the milk collecting bottle was swirled round twice and turned over twice before transferred into milk sample bottles to allow for even distribution of milk fat and milk contents. A total of five milk samples were collected over the experimental period for each cow.

3.2.2 Rumen study

3.2.2.1 Cows

The rumen-fistulated cows which had a mean 14 day milk yield of 18.5 ± 1.95 kg (SD) (September to the 14 September 2009) and were 105 ± 67.6 (SD) DIM on the 1st September

2009, were selected from the Outeniqua Research Farm in George. The cannula had rolled inner flanges of a diameter of ten centimeters (Bar Diamond, Inc., P. O. Box 60, Parma, Idaho, USA). The fistulated cows were adapted to their respective diets and treatments for 15 days. Thereafter, the first period of sampling was done, which consisted of both the pH and the rumen fluid sampling, and the *In sacco* study. A cross-over of treatments followed the first period, with a 21 day adaptation period. The second period of rumen study sampling then commenced where the same procedures were repeated.

3.2.2.2 pH

Automatic pH/temperature loggers (TruTrack Data Logger, Model pH-HR mark 4, the pH probe with Pt100 temperature sensor (www.intech.co.nz) were inserted through the plug of the cannulas into the rumens of the cows. The automatic pH loggers were started on the program omnilog, and were calibrated using pH buffer solutions of pH seven and four. Prior to insertion and between each calibration with the two buffer solutions, the loggers' electrode was rinsed with distilled water to remove the former buffer solution. This is illustrated in Figure 3.4a.This calibration procedure was done for all the automatic pH loggers. The loggers were labeled from one to ten to represent the number of the fistulated cows that were monitored during that period as illustrated in Figure 3.4b. The automatic pH loggers measure the pH throughout the day, over a period of four days at ten minute intervals.

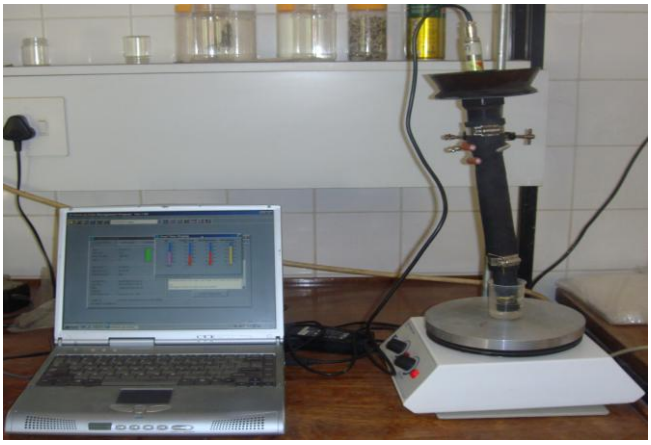

Figure 3.4a Illustrate the calibration of the logger on the computer

Figure 3.4b The final product to be inserted through the cannula of the cow

3.2.2.3 Rumen fluid sampling

Rumen fluid samples were extracted at 08:00, 14:00 (before milking), 20:00 and 02:00 on the 28th of September 2009 during the first period and on the 27th of November 2009 during the second period. Fistulated cows, were safely restrained, their cannula plug removed and a handheld suction pump was inserted into the contents in the rumen. The pump is manipulated to remove the liquid portion of the contents into a sample bottle. The fluid was drawn with a large pump to create a vacuum in the jar so that the fluid could easily be obtained. This is illustrated in Figures 3.5a and b. The rumen samples from all ten fistulated cows were collected in separate containers labelled one to ten to represent the ten fistulated cows. Once the sample was obtained the pH was measured immediately with a portable pH meter (WTW pH340i pH data meter/ data logger connected to a WTW SenTix 41 pH electrode). The electrode of the portable pH meter was placed in the container immediately after collection to measure pH. The pH might change when rumen digesta is exposed to oxygen, so it was important to take the reading just after collection. The rumen samples in the ten collected bottles were filtered through two layers of cheese cloth to remove solid particles as illustrated in Figure 3.6. Two laboratory sample bottles per cow were labelled corresponding to the fistulated cow number, the time of sampling, the date and whether the sample will be analysed for VFA or NH_3-N determination. Of the filtered liquor, 15 ml was drawn with a syringe and placed in one of the sample containers used per cow to be preserved with 2.5 ml of a 50% sulphuric acid (H_2SO_4) solution for NH_3-N determination. Then 18 ml of the filtered rumen liquor was placed in the second sample container, which was then preserved with 2 ml of a 25% ortho-phosphoric acid (H_3PO_3). The 20 sample bottles were frozen and sent for laboratory analysis. The sample bottles used are illustrated in Figure 3.7.

Figure 3.5a Insertion of the suction pump into the rumen

Figure 3.5b Manipulating the suction pump, used in retrieving rumen fluid samples for collection

Figure 3.6 The rumen fluid filtered through cheese cloth to remove larger particles

Figure 3.7 Illustrates the empty rumen fluid sample bottles for NH_3-N and VFA for cow 1 at the various sampling times

3.2.2.4 In sacco

Approximately 10 kg wet mass of ryegrass/kikuyu pasture was cut with pasture clippers at a height of 30 mm (when 1.2 ton DM/ha of ryegrass was available above 30 mm). The freshly cut ryegrass was placed in several brown paper bags (12.6 cm x 20.7 cm x 40 cm). The brown paper bags were placed in an oven and dried at 60 °C for 72 hours (Botha, 2003). The dried ryegrass was removed from the bags and cut into average lengths of five millimeters using a sharp scissors. The averaged length of a sample of 100 ryegrass pieces was 6 and 7 mm for period 1 and period 2, respectively. The ryegrass was then accurately weighed to three decimals on a Sartorius L420P scale in sample sizes of approximately five grams, which were placed in Dacron bags and recorded. The empty Dacron bags with dimensions of 10 cm by 20 cm and a pore size of 53 microns (Vanzant *et al.*, 1998) were labelled, and weighed (Sartorius L420P scale, with accuracy to 0.001 g) prior to ryegrass insertion. A cable tie was used to seal the bag and the weight of the whole unit (bag, grass and cable tie) was measured and recorded. The *In sacco* method used stockings to insert the bags into the rumen (Cruywagen, 2006). This method facilitates bag retrieval and prevents unnecessary exposure to oxygen for bags intended for later removal. Since oxygen exposure affects microbial degradation. Six Dacron bags were placed in stockings (three bags per stocking) and the two stockings inserted in each of the ten fistulated cows. The entire process is illustrated in Figures 3.8 (a, b, c and d) and Figure 3.9. Three bags were removed after a 12 and 24 hours of rumen incubation. The removed bags were then washed in clean water for 15 minutes until the water ran clear. The Dacron bags were dried at 60 °C for 72 hours (Botha, 2003). The bags were weighed back on a three decimal Sartorius L420P scale and recorded.

Figure 3.8a Dried and cut ryegrass/kikuyu grass inserted into Dacron bag

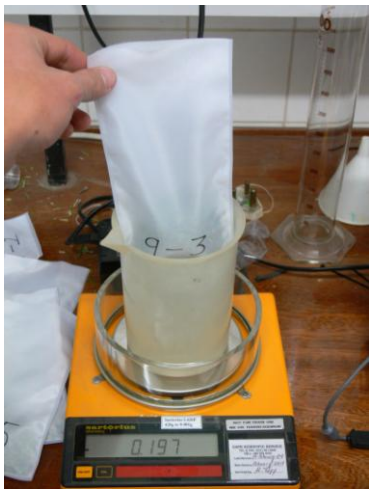

Figure 3.8b Dacron bag weighed

Figure 3.8c Dacron bag sealed with a cable tie

Figure 3.8d Dacron bags placed into a stocking

Figure 3.9 Insertion of the six *In sacco* Dacron bags in their stockings into the rumen through the cannula of each cow

3.3 Laboratory analysis

3.3.1 Production study

3.3.1.1 Pasture analysis

The six pasture samples collected over the experimental period were milled (SWC Hammer mill, 1mm sieve) and analysed at UP-Nutrilab (Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria) for DM (AOAC, 2000, procedure 934.01), IVOMD (Tilley and Terry, 1963), Ash (AOAC, 2000, procedure 942.05), CP (N was determined using a Leco N analyser, model FP-428, Leco Corporation, St Joseph, MI, USA and CP (calculated as N × 6.25 (AOAC, 2000, procedure 968.06)), NDF (filter bag technique with the Ankom[2000]

fibre analyser) (Robertson and Van Soest, 1981), neutral detergent insoluble nitrogen (NDIN) (determined from having done both the NDF (Robertson and Van Soest, 1981)) followed by the N analysis (AOAC, 2000, procedure 968.06), ADF (Ankom^{2000} fibre analyser), acid detergent insoluble nitrogen (ADIN) (determined from having done both the ADF (Goering and Soest, 1970) followed by the N analysis (AOAC, 2000, procedure 968.06)), ether extract (EE) (AOAC, 2000, procedure 920.39), gross energy (GE) (MC – 1000 Modular Calorimeter, Operators Manual), Ca (AOAC, 2000, procedure 965.09) and P (AOAC, 2000, procedure 965.17) (See Appendix A; Table A6).

3.3.1.2 Concentrate analysis

The control concentrate and the treatment concentrate samples, both groups numbered one to six, were milled (SWC Hammer mill, 1mm sieve) and analysed at UP-Nutrilab (Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria) for the analysis as stated in section 3.3.1.1 (Pasture analysis) (See Appendix A; Table A4 and A5).

3.3.1.3 Milk production and sample analysis

Milk production was measured daily from 16 September 2009 to 24 November 2009 after an adaptation period of 15 days. Milk samples collected were stored in containers preserved with potassium dichromate, and sent to Lactolab Pty (Ltd) (ARC, Main Rd., Irene, 0062) for analysis. The milk samples were analysed for milk fat, protein, lactose and MUN and SCC, according to the IDF standard 141B (IDF, 1996) with the Milkoscan FT 6000 analyser (Foss Electric, Denmark), a midrange infrared spectrophotometer (See Appendix A; Table A7).

3.3.2 Rumen study

3.3.2.1 Rumen-fluid analysis

The 160 rumen sample bottles (80 VFA, and 80 NH_3N) were sent in to UP-Nutrilab (Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria). The rumen VFA with modifications (for the following; acetic, propionic, butyric, valeric and iso-butyric acids) and rumen NH_3-N was determined according to (Broderick and Kang, 1980).

3.3.2.2 In sacco analysis

The Dacron bag residues after incubation, washing and drying, were emptied. The residues from three bags incubated at 0, 12 and 24 hours were pooled for that specific hour. This was done for each of the ten fistulated cows. The residues were milled (SWC Hammer

mill, 1mm sieve) and stored in labelled plastic containers, stating the cows' number, the treatment and the hour of incubation. The samples were analysed at the UP-Nutrilab (Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria) for DM, Ash, OM (from (DM – ASH = OM)) and NDF according to preceding analysis described in section 3.3.1.1.

3.4 Yeast analysis

A representative sample of approximately 200 g (half a handful) of each of the six concentrate samples taken per treatment over the experimental period, were collected. The samples were labelled Y1-Y12, representing the six yeast concentrate samples (Y1-Y6) and the six control concentrate samples (Y7-Y12) taken over the trial. The 12 samples were sent for analysis on the 19 of February 2010. The yeast analysis consisted of a yeast counting technique at 25 °C standard 94B (IDF, 1990) that was conducted at the ARC-API (Old Olifantsfontein rd, Irene, 0062).

3.5 Statistical analysis

The production study was analysed using a randomised complete block design with two treatments allocated to 20 blocks. The data was analysed by two-way ANOVA. using the general linear model of SAS (Statistical Analysis Systems Institute, 2009). The model used in the production study was:

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

Where Y_{ij}, is the dependent variable which is the observation of the ith treatment of the jth cow, μ is the overall mean, T_i is the treatment effect from the ith treatment group i.e. contol or yeast, B_j is the effect of the jth cow (blocking effect), and e_{ij} is the experimental or residual error. A P-value $P \leq 0.05$ is considered significant (Samuels, 1989), where a $P \leq 0.1$ represents a tendency.

The rumen study was analysed using a general linear model in a cross-over design, which ensures that both treatments are present in both periods. This allows the removal of period effects when comparing treatments.

The model used in the rumen study was:

$$Y_{ik} = \mu + T_i + P_k + e_{ik}$$

Where Y_{ik}, is the dependent variable which is the observation of the ith treatment and for the kth period, μ is the overall mean, T_i is the treatment effect from the ith treatment group i.e. contol or yeast, P_k is the effect of the kth period (k = 1, 2), and e_{ik} is the random error. Repeated measures analysis of variance with the GLM model was used for repeated time effects.

CHAPTER 4

RESULTS

CHAPTER 4

Results

4.1 Production study

4.1.1 Climatic conditions

The mean minimum, maximum and overall daily temperatures for the experimental period for September to November 2009, and the 44 year long-term means as of the 1st of January 1967 to the 12 December 2010 (Council, 2009) are illustrated in Table 4.1. The mean monthly values for maximum, minimum and mean overall temperature for the study are within ranges of the mean long-term temperatures. The mean total rainfall for October and November during the study were slightly lower compared to the long-term mean rainfall, due to the drought experienced in George. This however, did not impact the study negatively, as water was available to irrigate pastures throughout the study.

Table 4.1 Mean maximum, minimum and daily temperatures (°C), and mean total rainfall (mm) for the months of September, October and November 2009 during the study and the 44 year long term means in George.

Month	Mean maximum Temp (°C)		Mean minimum Temp (°C)		Mean Temp (°C)		Mean total rainfall (mm)	
	Study	LT	Study	LT	Study	LT	Study	LT
September	18.8	19.1	8.5	8.5	13.6	13.8	72.9	53.7
October	20.8	20.2	10.7	10.4	15.7	15.3	69.6	79.3
November	22.6	21.6	11.8	12.0	17.0	16.8	43.0	74.8

Temp = temperature

LT = Long term

4.1.2 Pasture management

Pasture management necessitates accurate pasture height measurements and subsequently correct pasture allocation for the grazing herd of cows. The regression calibration equation used during the trial was the following; Y= (H) 65 - 360. The mean pre-grazing RPM reading was 28.9 ± 7.55 (SD) (n = 108) and the mean post-grazing RPM reading was 11.8 ± 2.16 (SD) (n = 108). The mean pasture available before grazing was on average measured to be 1521 ± 490.0 kg DM/ha (SD) (n = 108). This equates to a mean PA of 8.9 ± 1.84 kg DM/cow/day (SD) (n = 108) with 357 ± 73.76 kg DM/day (SD) (n = 108) available to graze. The residual pasture mass was on average 404 ± 140.29 kg/DM/ha (SD)

(n = 108). Therefore the mean pasture removed was 1117 kg DM/ha (n = 108). The PA was adjusted according to pasture height before and after, ensuring post-grazing RPM heights were kept between 10 and 15. This ensures that ryegrass quality and growth is not compromised for the next grazing cycle, simultaneously ensuring enough pasture was available for cows to consume. The pasture intake was measured as 5.15 ± 1.89 kg DM/cow/day (SD) (n = 108).

The calculated regression equations were pooled and averaged to obtain the following regression throughout the trial; Y= (H) 86.187 - 296.35 (n = 11). The mean pasture available for grazing was on average measured to be 2197.99 ± 649.77 kg DM/ha (SD) (n = 108). This equates to a mean PA of 12.89 ± 2.50 kg DM/cow/day (SD) (n = 108), with 515.67 ± 100 kg DM/day (SD) (n = 108) available to graze. The residual pasture mass was on average 716 ± 186.02 kg/DM/ha (SD) (n = 108). The mean pasture therefore removed was 1481 kg DM/ha (n = 108). The estimated pasture intake is 6.83 ± 2.50 kg DM/cow/day (SD) (n = 108).

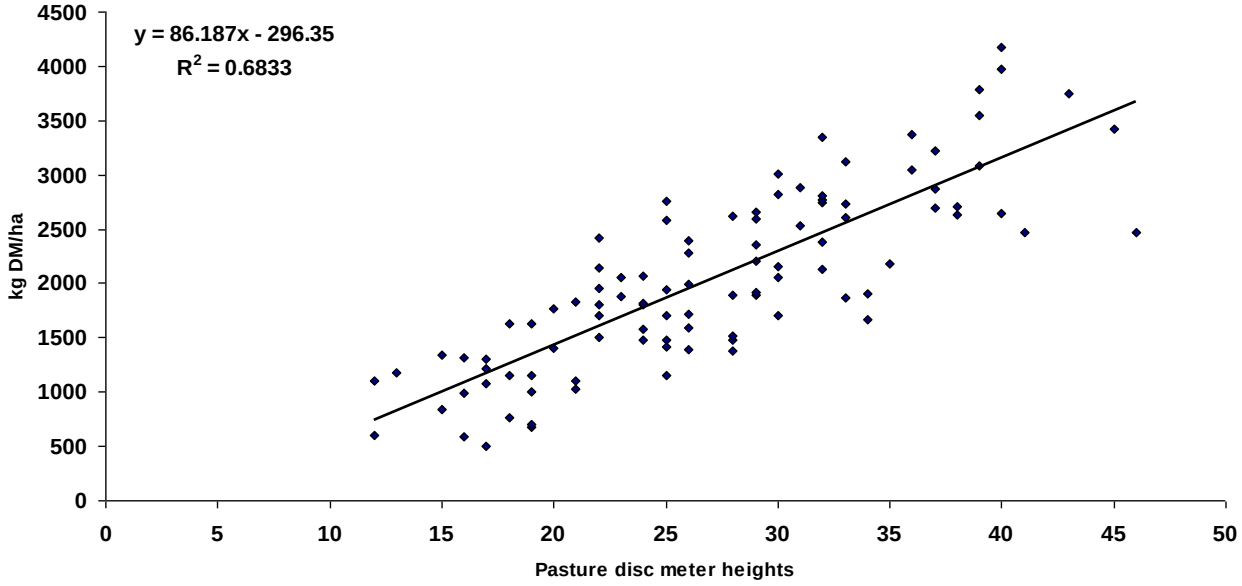

Figure 4.1 Regression equation illustrating the relationship between the rising plate meter (RPM) reading and the pasture yield (kg DM/ha) and the equation constructed for the experimental study for September, October and November: Y = 86.187X - 296.35, (R^2 = 0.68; n = 99)

4.1.3 Pasture and concentrate analysis

The chemical composition of dairy concentrates and ryegrass/kikuyu pasture are shown in Table 4.2. The control and yeast concentrate consisted of identical ingredients and the

chemical analysis represents the similarities between the concentrates. The pasture compositional components of CP, NDF and IVOMD over the experimental study for the pasture samples, taken weekly and pooled for every two weeks is illustrated in Figure 4.2.

Table 4.2 Chemical composition of dairy concentrates fed (6 kg as is) and ryegrass/kikuyu pasture grazed by Jersey cows during the trial (n = 6)

Nutrient[1]	Concentrate[2]		Pasture
	Control	Yeast	
DM (g/kg as is)	884	884	155[3]
CP (g/kg DM)	104	105	233
NDF (g/kg DM)	88.0	81.7	512
NDIN (g/kg DM)	0.47	0.44	12.0
ADF (g/kg DM))	34.3	32.4	305
ADIN (g/kg DM)	0.41	0.40	9.4
EE (g/kg DM)	34.2	32.7	34.3
GE (MJ/kg)	17.2	17.1	17.4
IVOMD (%DM)	93.1	92.0	76.1
ME MJ/kg DM[4]	13.1	12.9	10.8
Ca (g/kg DM)	9.2	9.1	4.0
P (g/kg DM)	9.2	8.7	3.7
Ca: P	1:1	1.05:1	1.08:1

[1]DM - Dry matter; CP - Crude protein; NDF - Neutral detergent fibre; NDIN - Neutral detergent insoluble nitrogen; ADF - Acid detergent fibre; ADIN – Acid detergent insoluble nitrogen; EE - Ether extract; GE - Gross energy; IVOMD - *In vitro* organic matter digestibility; ME - Metabolisable energy; Ca - Calcium; P - Phosphorus

[2]Control: dairy concentrate containing no yeast; Yeast: dairy concentrate containing yeast at 167g/ ton

[3]n = 11 pasture samples taken at each regression to determine, actually pasture DM%, which were later pooled to create 6 pasture samples used for other analysis

[4] Calculated ME MJ/kg DM ME = 0.82 × GE × IVOMD (Robinson *et al.*, 2004)

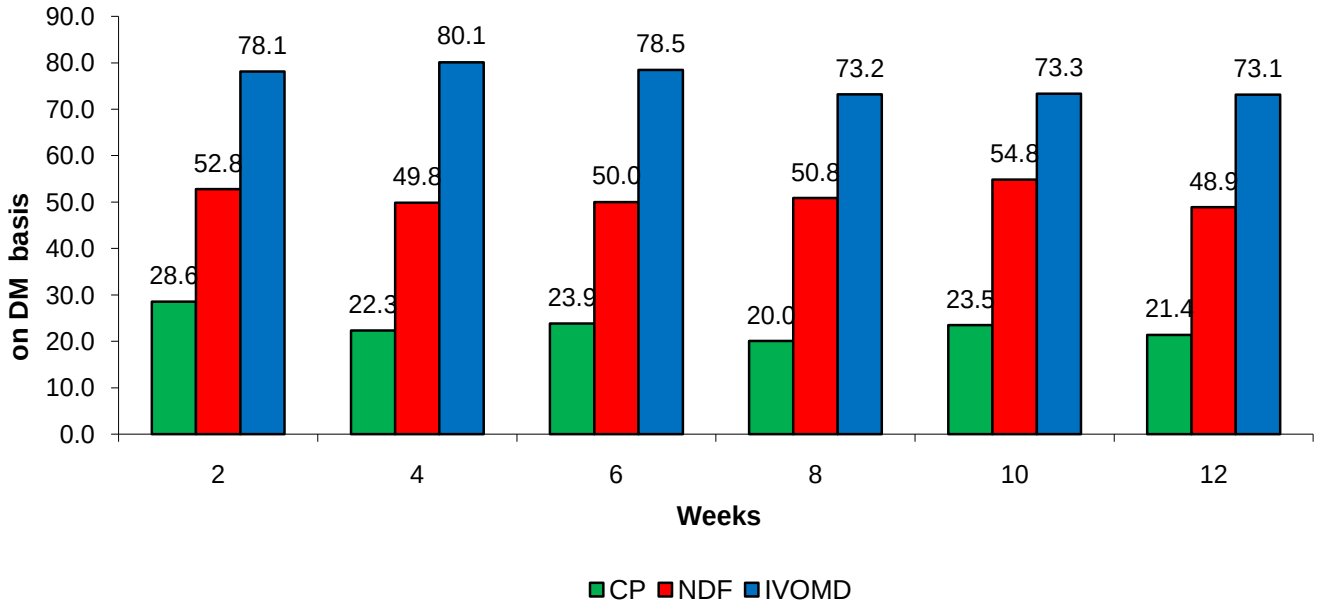

Figure 4.2 The crude protein (CP), neutral detergent fibre (NDF) and *In vitro* organic matter digestibility (IVOMD) on a DM basis of the grazed ryegrass/kikuyu pasture sampled weekly and pooled for every two weeks during the experimental study. Week 2 = sampled on the 10 and 17th of September 2009, week 4 = sampled on the 24 of September and 10 October 2009, week 6 = sampled on the 8th and 15 of October 2009, week 8 = sampled on the 22nd and 29th of October 2009, week 10 = sampled on the 5 and 12th of November 2009 and week 12 = sampled on the 19 November 2009.

4.1.4 Milk yield and analysis

The milk yield and analysis from composite sampling of milk is presented in Table 4.3. Milk yield and 4% FCM did not differ significantly between treatments ($P > 0.05$). Milk protein and lactose percentages, as well as the SCC was unaffected by yeast supplementation. Milk fat% was significantly higher ($P < 0.05$) for the yeast supplemented cows at 4.24% compared to the control group of cows at a fat% of 3.99.

4.1.5 Body condition score and body weights

Body condition score, BW and the resulting changes is represented in Table 4.3. The BW and BCS at the beginning, the end and the change of the study was not different between the treatment groups ($P > 0.05$).

Table 4.3 Effect of live yeast supplementation on milk yield, milk composition, somatic cell count, body weight and body condition score of cows grazing ryegrass/kikuyu pasture supplemented with 6 kg of dairy concentrate (as is) per day (n = 15)

Parameter	Experimental treatment[1]		SEM[2]	P value
	Control	Yeast		
Milk yield (kg/day)	20.1	19.7	0.534	0.59
4 % FCM[3] (kg/day)	20.1	20.3	0.513	0.72
Milk fat (%)	3.99[a]	4.24[b]	0.080	0.04
Milk protein (%)	3.51	3.58	0.049	0.31
Milk Lactose (%)	4.68	4.73	0.033	0.28
MUN (mg/dL)	10.7	11.0	0.390	0.58
SCC x 1000	254	155	76.466	0.38
BW beginning (kg)	335	331	6.232	0.65
BW end (kg)	371	369	2.320	0.59
BW change (kg)	+37.8	+36.4	2.259	0.67
BCS beginning	2.08	2.09	0.032	0.77
BCS end	2.27	2.23	0.052	0.65
BCS change	+0.185	+0.149	0.050	0.62

[1]Control: dairy concentrate containing no yeast; Yeast: dairy concentrate containing yeast at 167g/ ton fed a 1g/cow/day.

[2]Standard error of the mean, FCM - 4 % Fat-corrected milk; MUN - Milk urea N; SCC - Somatic cell count; BW - Body weight; BCS - Body condition score (scale 1-5).

[3]4% FCM (kg) = (0.4 × kg of milk) + (15 × kg of milk fat) (National Research Council, 2001)

[a, b] Means in the same row with different superscripts differ (P<0.05)

4.2 Rumen Study

The ruminal parameters such as the total and individual VFA concentration (mmol/L), individual VFA molar percentages, NH_3-N (mg/dL) and pH measurements for the control and yeast treatments are represented in Table 4.4 which are the means over both periods combined. The mean acetic and total VFAs concentrations (mmol/L) for the control treatment was higher ($P < 0.05$) compared to the yeast treatment. The pH measurements were taken both with the logger pH and portable pH meter.

Table 4.4 Effect of live yeast supplementation on the mean daily ruminal volatile fatty acids production, ammonia nitrogen concentration and pH of cows grazing ryegrass/kikuyu pasture supplemented with 6 kg of dairy concentrate (as is) per day (n = 10)

Parameter	Experimental diets[1]		SEM[2]	P value
	Control	Yeast		
Total VFA (mmol/L)	106.3[a]	99.3[b]	2.030	0.04
Acetic acid (mmol/L)	65.8[a]	61.3[b]	1.283	0.04
Propionic acid (mmol/L)	24.7	23.3	0.513	0.09
Butyric acid (mmol/L)	13.4	12.5	0.442	0.23
Valeric acid (mmol/L)	1.60	1.36	0.097	0.11
Iso butyric acid (mmol/L)	0.835	0.779	0.037	0.32
Total VFA molar %				
Acetic acid %	62.0	61.8	0.348	0.69
Propionic acid %	23.3	23.4	0.433	0.91
Butyric acid %	12.4	12.7	0.286	0.53
Valeric acid %	1.49	1.35	0.066	0.17
Iso butyric acid %	0.788	0.791	0.030	0.94
NH_3-N (mg/dL)	10.1	9.54	0.642	0.58
pH				
Portable mean	6.01	6.06	0.044	0.52
Logger mean	6.09	6.11	0.069	0.84

[1]Control: dairy concentrate containing no yeast; Yeast: dairy concentrate containing yeast at 167g/ ton

[2]Standard error of the mean

[a, b] Means in the same row with different superscripts differ (P<0.05)

The total ruminal VFA concentration (mmol/L) measured at 02:00, 08:00, 14:00 and 20:00 are averaged over both periods combined and are presented in Table 4.5. The total ruminal VFA concentration (mmol/L) measured at 02:00, 08:00, 14:00 and 20:00 is illustrated in Figure 4.3. At 08:00 and 14:00 the control treatment groups VFA concentration was higher, and at 20:00 the yeasts VFA concentration was higher ($P < 0.05$).

Table 4.5 Effects of live yeast supplementation on the total mean ruminal volatile fatty acid concentration (mmol/L) measured at 02:00, 08:00, 14:00 and 20:00 for cows grazing ryegrass/kikuyu pasture supplemented with 6 kg dairy concentrate (as is) per day (n = 10)

Time	Experimental diets[1]		SEM[2]	P value
	Control	Yeast		
02:00	99.0	99.6	2.091	0.83
08:00	102[a]	83.4[b]	3.783	0.01
14:00	99.6	90.0	4.164	0.14
20:00	125	124	1.964	0.88

[1]Control: dairy concentrate containing no yeast; Yeast: dairy concentrate containing yeast at 167g/ ton

[2]Standard error of the mean

[a, b] Means in the same row with different superscripts differ (P < 0.05)

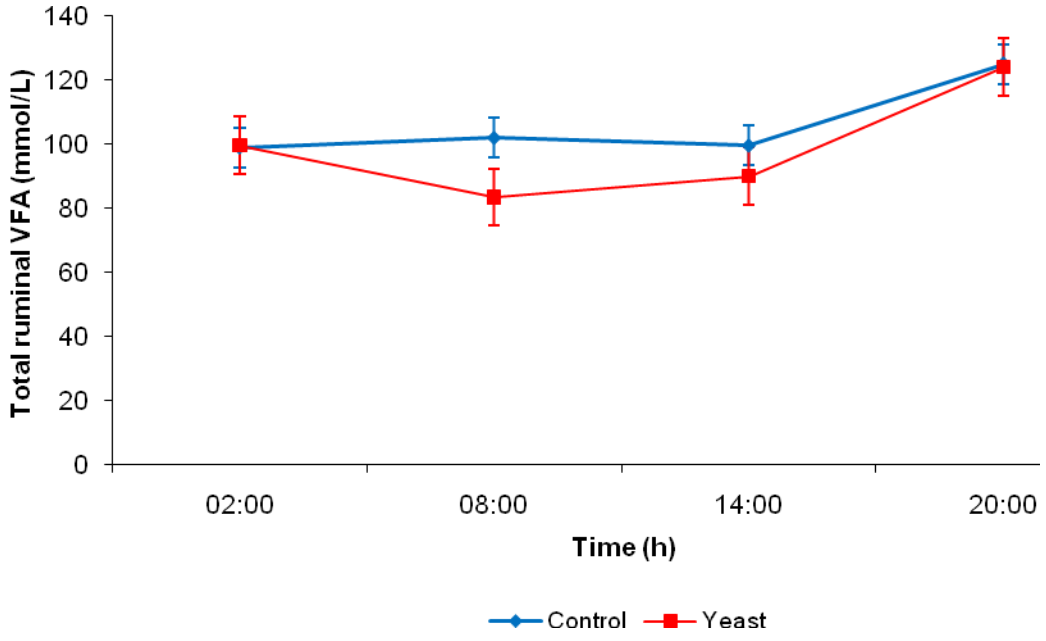

Figure 4.3 Ruminal concentration of total VFA (mmol/L) of cows supplemented with live yeast and grazing ryegrass/kikuyu pasture supplemented with 6 kg dairy concentrate (as is) per day (n = 10)

The ruminal acetic acid concentration (mmol/L) measured at 02:00, 08:00, 14:00 and 20:00 are averaged over both periods combined, and are represented in Table 4.6. The mean

ruminal acetic acid concentration (mmol/L) measured at 02:00, 08:00, 14:00 and 20:00 is illustrated in Figure 4.4. The mean ruminal acetic acid concentration differed between the control and yeast treatment group at 08:00, with the controls acetic acid value higher ($P < 0.05$).

Table 4.6 Effects of live yeast supplementation on the mean ruminal acetic acid concentration (mmol/L) measured at 02:00, 08:00, 14:00 and 20:00 for cows grazing ryegrass/kikuyu pasture supplemented with 6 kg dairy concentrate (as is) per day (n = 10)

Time	Experimental diets[1]		SEM[2]	P value
	Control	Yeast		
02:00	62.8	63.3	1.489	0.80
08:00	63.1[a]	51.3[b]	2.356	0.01
14:00	63.0	56.6	2.607	0.12
20:00	74.5	74.1	1.284	0.84

[1]Control: dairy concentrate containing no yeast; Yeast: dairy concentrate containing yeast at 167g/ ton

[2]Standard error of the mean

[a, b] Means in the same row with different superscripts differ ($P < 0.05$)

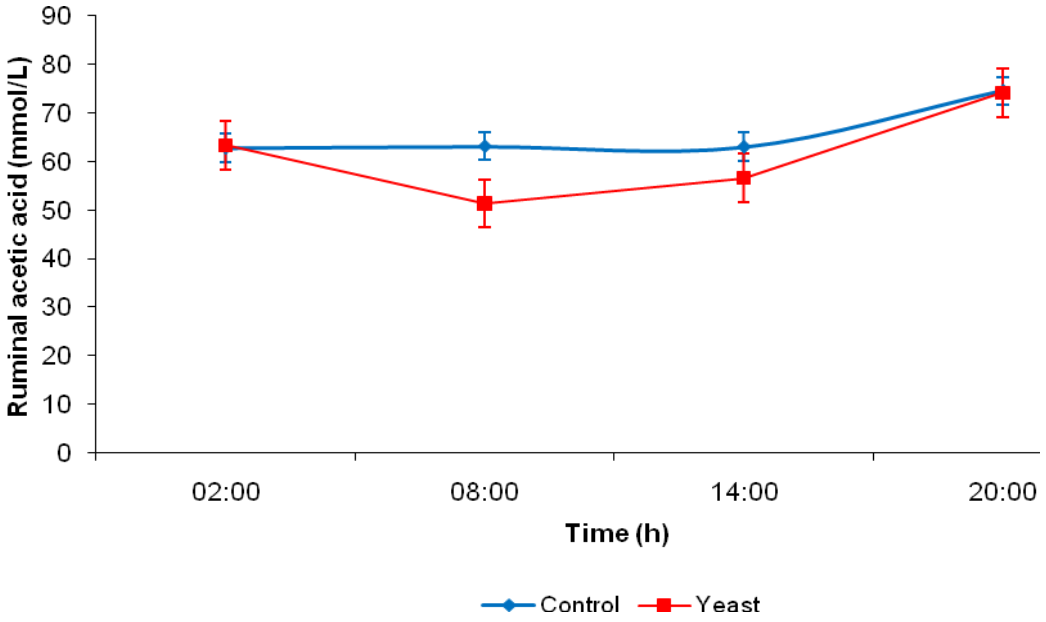

Figure 4.4 Ruminal concentration of acetic acid (mmol/L) for cows supplemented with live yeast and grazing ryegrass/kikuyu pasture supplemented with 6 kg dairy concentrate (as is) per day (n = 10)

The ruminal propionic acid concentration (mmol/L) measured at 02:00, 08:00, 14:00 and 20:00 are averaged over both periods combined, and are represented in Table 4.7. The mean ruminal propionic acid concentration (mmol/L) measured at 02:00, 08:00, 14:00 and 20:00 is illustrated in Figure 4.5. The control had a higher ($P < 0.05$) ruminal propionic acid value at 08:00 for the mean over both periods combined.

Table 4.7 Effects of live yeast supplementation on the mean ruminal propionic acid concentration (mmol/L) measured at 02:00, 08:00, 14:00 and 20:00 for cows grazing ryegrass/kikuyu pasture supplemented with 6 kg dairy concentrate (as is) per day (n = 10)

Time	Experimental diets[1]		SEM[2]	P value
	Control	Yeast		
02:00	21.8	22.2	0.722	0.72
08:00	24.3[a]	19.1[b]	1.028	0.01
14:00	21.8	20.5	0.797	0.28
20:00	30.8	31.2	1.131	0.80

[1]Control: dairy concentrate containing no yeast; Yeast: dairy concentrate containing yeast at 167g/ ton

[2]Standard error of the mean

[a, b] Means in the same row with different superscripts differ (P < 0.05)

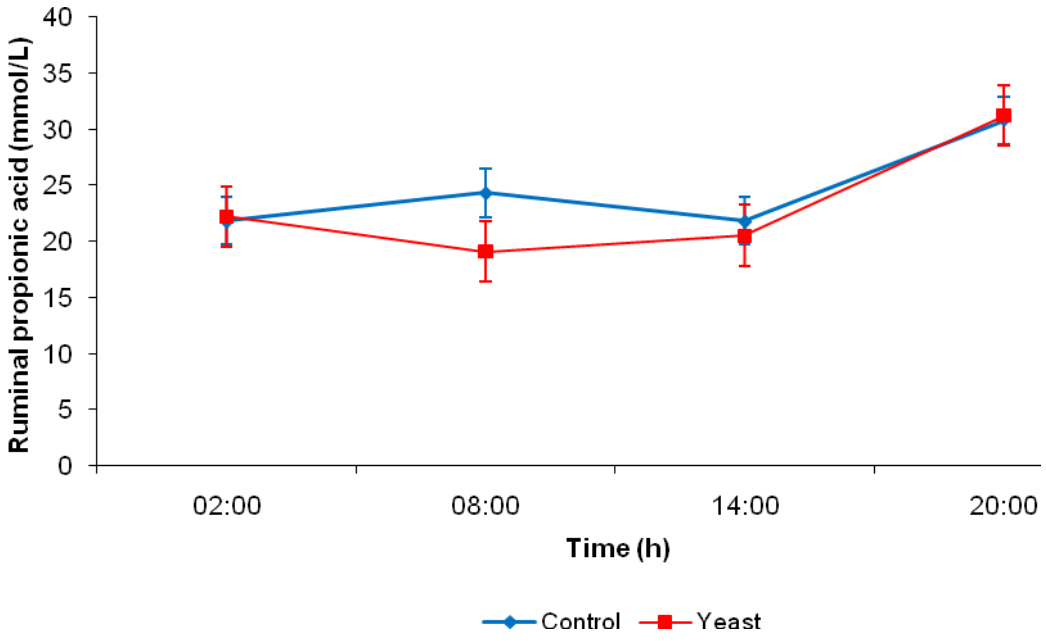

Figure 4.5 Ruminal concentration of propionic acid (mmol/L) for cows supplemented with live yeast and grazing ryegrass/kikuyu pasture supplemented with 6 kg dairy concentrate (as is) per day (n = 10)

The ruminal butyric acid concentration (mmol/L) measured at 02:00, 08:00, 14:00 and 20:00 are averaged over both periods combined, and are represented in Table 4.8. The mean

ruminal butyric acid concentration (mmol/L) measured at 02:00, 08:00, 14:00 and 20:00 is illustrated in Figure 4.6. No differences between the control and yeast treatment group were found.

Table 4.8 Effects of live yeast supplementation on the mean ruminal butyric acid concentration (mmol/L) measured at 02:00, 08:00, 14:00 and 20:00 for cows grazing ryegrass/kikuyu pasture supplemented with 6 kg dairy concentrate (as is) per day (n = 10)

Time	Experimental diets[1]		SEM[2]	P value
	Control	Yeast		
02:00	12.1	11.9	0.276	0.62
08:00	12.4	11.1	0.671	0.22
14:00	12.8	11.1	0.792	0.18
20:00	16.2	16.0	0.318	0.75

[1]Control: dairy concentrate containing no yeast; Yeast: dairy concentrate containing yeast at 167g/ ton

[2]Standard error of the mean

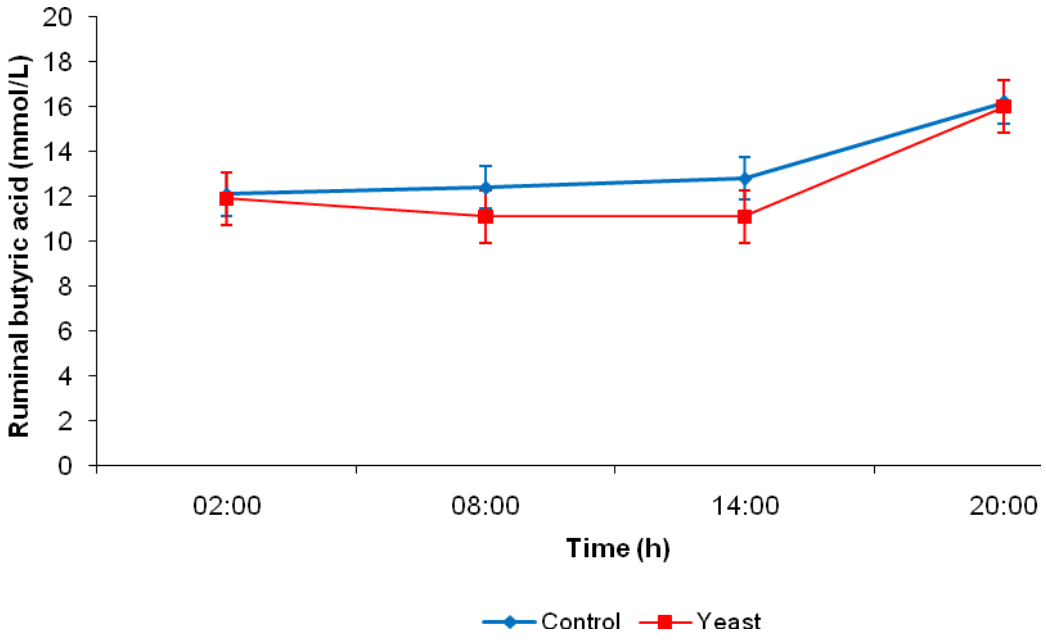

Figure 4.6 Ruminal concentration of butyric acid (mmol/L) for cows supplemented with live yeast and grazing ryegrass/kikuyu pasture supplemented with 6 kg dairy concentrate (as is) per day (n = 10)

The ruminal valeric acid concentrations (mmol/L) measured at 02:00, 08:00, 14:00 and 20:00 are averaged over both periods combined, and are represented in Table 4.9. No differences between the control and yeast treatment group were found.

Table 4.9 Effects of live yeast supplementation on the mean ruminal valeric acid concentration (mmol/L) measured at 02:00, 08:00, 14:00 and 20:00 for cows grazing ryegrass/kikuyu pasture supplemented with 6 kg dairy concentrate (as is) per day (n = 10)

Time	Experimental diets[1]		SEM[2]	P value
	Control	Yeast		
02:00	1.49	1.42	0.158	0.75
08:00	1.42[a]	1.08[b]	0.085	0.025
14:00	1.30	1.04	0.110	0.13
20:00	2.22	1.90	0.220	0.34

[1]Control: dairy concentrate containing no yeast; Yeast: dairy concentrate containing yeast at 167g/ ton

[2]Standard error of the mean

[a, b] Means in the same row with different superscripts differ (P < 0.05)

The ruminal iso-butyric acid concentrations (mmol/L) measured at 02:00, 08:00, 14:00 and 20:00 are averaged over both periods combined, and are represented in Table 4.10. No differences between the control and yeast treatment group were found.

Table 4.10 Effects of live yeast supplementation on the mean ruminal iso-butyric acid concentration (mmol/L) measured at 02:00, 08:00, 14:00 and 20:00 for cows grazing ryegrass/kikuyu pasture supplemented with 6 kg dairy concentrate (as is) per day (n = 10)

Time	Experimental diets[1]		SEM[2]	P value
	Control	Yeast		
02:00	0.816	0.791	0.0346	0.62
08:00	0.902	0.765	0.0586	0.14
14:00	0.769	0.700	0.0606	0.44
20:00	0.854	0.858	0.0479	0.96

[1]Control: dairy concentrate containing no yeast; Yeast: dairy concentrate containing yeast at 167g/ ton

[2]Standard error of the mean

The ruminal NH_3-N concentration (mg/dL) measured at 02:00, 08:00, 14:00 and 20:00, are averaged over both periods combined, and are represented in Table 4.11. The mean

ruminal NH_3-N concentration (mg/dL) measured at 02:00, 08:00, 14:00 and 20:00 is illustrated in Figure 4.7. No differences between the control and yeast treatment group were found.

Table 4.11 Effects of live yeast supplementation on the mean ruminal ammonia nitrogen concentration (mg/dL) measured at 02:00, 08:00, 14:00 and 20:00 for cows grazing ryegrass/kikuyu pasture supplemented with 6 kg dairy concentrate (as is) per day (n = 10)

Time	Experimental diets[1]		SEM[2]	P value
	Control	Yeast		
02:00	7.06	6.29	0.4744	0.29
08:00	13.2	11.4	1.203	0.31
14:00	7.32	5.93	0.907	0.31
20:00	12.7	14.6	1.446	0.38

[1]Control: dairy concentrate containing no yeast; Yeast: dairy concentrate containing yeast at 167g/ ton

[2]Standard error of the mean

[a, b] Means in the same row with different superscripts differ (P<0.05)

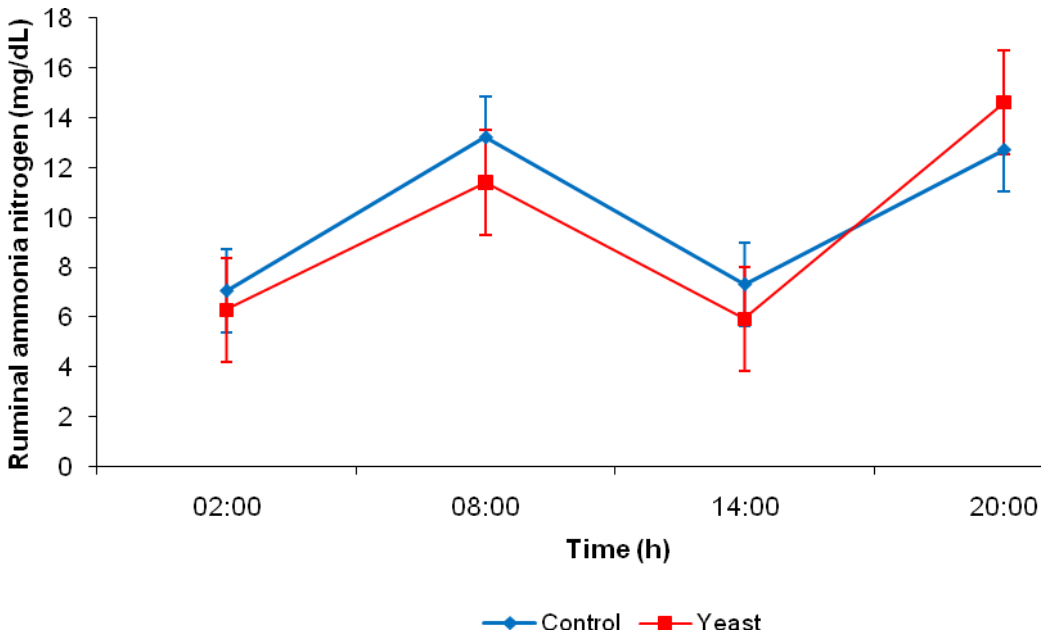

Figure 4.7 The ruminal ammonia nitrogen concentration (mg/dL) for cows supplemented with live yeast and grazing ryegrass/kikuyu pasture supplemented with 6 kg dairy concentrate (as is) per day (n = 10)

The ruminal pH's measured at 02:00, 08:00, 14:00 and 20:00 are averaged over both periods combined, and are represented in Table 4.12. The mean ruminal pH's measured at 02:00, 08:00, 14:00 and 20:00 is illustrated in Figure 4.8. The mean ruminal pH at 20:00 for the yeast treatment group was higher ($P < 0.05$) compared to the control. Figure 4.9 illustrates the mean pH fluctuations over a period of 24 hours which was averaged from the pH logger over a period of 4 days for every half an hour.

Table 4.12 Effects of live yeast supplementation on the mean ruminal pH measured at 02:00, 08:00, 14:00 and 20:00 for cows grazing ryegrass/kikuyu pasture supplemented with 6 kg dairy concentrate (as is) per day (n = 10)

Time	Experimental diets[1]		SEM[2]	P value
	Control	Yeast		
02:00	6.20	6.17	0.0284	0.55
08:00	6.11	6.20	0.0544	0.26
14:00	6.04	5.90	0.1038	0.38
20:00	5.70[a]	5.94[b]	0.0550	0.02

[1]Control: dairy concentrate containing no yeast; Yeast: dairy concentrate containing yeast at 167g/ ton

[2]Standard error of the mean

[a, b] Means in the same row with different superscripts differ (P < 0.05)

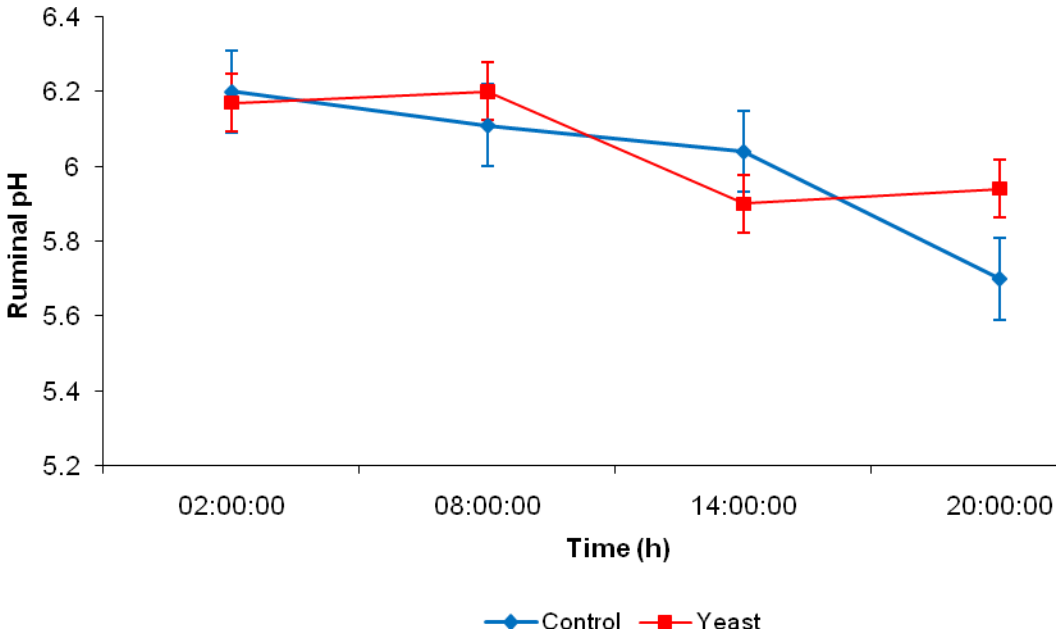

Figure 4.8 The ruminal pH for cows supplemented with live yeast and grazing ryegrass/kikuyu pasture supplemented with 6 kg dairy concentrate (as is) per day (n = 10)

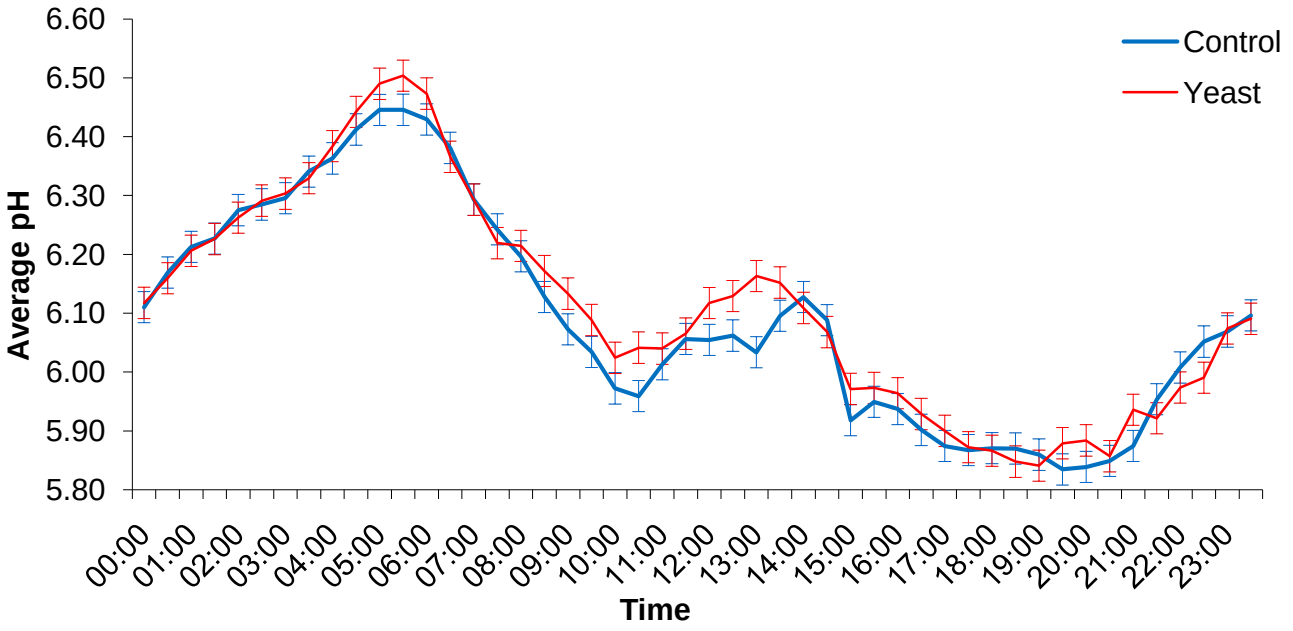

Figure 4.9 The mean ruminal pH of the control and yeast treatment group over a 4 day period for Jersey cows grazing ryegrass/kikuyu pasture supplemented with 6 kg dairy concentrate (as is) per day (n = 10)

The mean percentage *In sacco* disappearance of NDF, OM and DM of ryegrass, at 12 and 24 hours of ruminal incubation are averaged over both periods combined, and are represented in Table 4.13. This is also illustrated in Figure 4.10. The mean NDF, OM and DM disappearance was higher ($P<0.05$) for the yeast treatment group of cows after a 12 and 24 hour incubation compared to the control group. The mean NDF disappearance of ryegrass in cows supplemented with yeast increased by 12.0% and 6.3% compared to the control at the 12 and 24 hour incubation periods, respectively.

Table 4.13 Effects of live yeast supplementation on the mean percentage disappearance *In sacco* of neutral detergent fibre (NDF), organic matter (OM) and dry matter (DM) of ryegrass/kikuyu, at 12 and 24 hours of ruminal incubation for cows grazing ryegrass/kikuyu pasture supplemented with 6 kg dairy concentrate (as is) per day (n = 10)

Parameter	Experimental treatments[1]		SEM[2]	P value
	Control	Yeast		
NDF 12h	46.6[a]	52.2[b]	0.963	0.004
NDF 24h	65.1[a]	69.2[b]	1.142	0.04
OM 12h	60.5[a]	64.3[b]	0.697	0.01
OM 24h	76.1[a]	78.4[b]	0.723	0.05
DM 12h	63.8[a]	67.2[b]	0.632	0.01
DM 24h	77.9[a]	80.0[b]	0.660	0.05

[1]Control: dairy concentrate containing no yeast; Yeast: dairy concentrate containing yeast at 167g/ ton

[2]Standard error of the mean

[a, b] Means in the same row with different superscripts differ (P < 0.05)

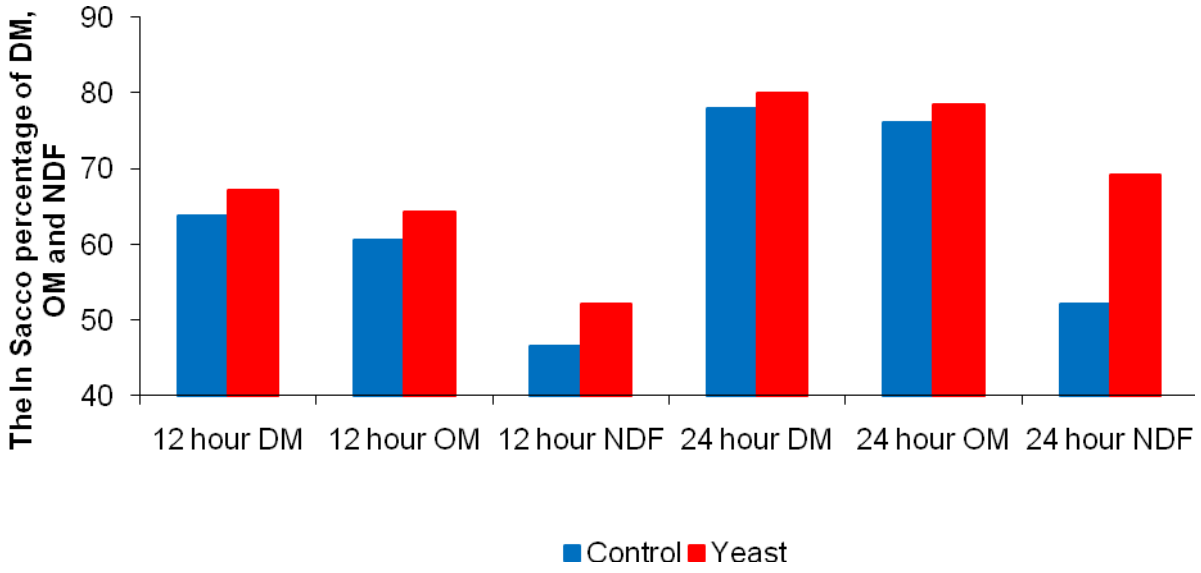

Figure 4.10 The effect of yeast supplementation on the mean DM, OM and NDF In sacco percentage disappearance of ryegrass/kikuyu pastures at 12 and 12 hours of rumen incubation, for Jersey cows grazing ryegrass/kikuyu pasture supplemented with 6 kg dairy concentrate (as is) per day (n = 10)

4.3 Yeast Analysis

The yeast was added at 167 g/ton and pelleted. One gram of the yeast contains 1×10^{10} CFU. Therefore a gram of dairy concentrate should contain 1.67×10^{6} CFU. The analysis for yeast count on the dairy concentrate samples is represented in Table 4.14.

Table 4.14 Yeast analysis of the dairy concentrate of the control (Y7-Y12) and yeast (Y1-Y6) treatment

Dairy concentrate	Yeast (CFU/g of dairy concentrate)
Y1	300000
Y2	85000
Y3	1500
Y4	25000
Y5	70000
Y6	75000
Y7	<10
Y8	<10
Y9	<10
Y10	<10
Y11	<10
Y12	<10

The results confirm that the control treatment did not receive any added yeast product. A discussion of the results, as presented in this chapter will follow in Chapter 5.

CHAPTER 5

DISCUSSION

CHAPTER 5

Discussion

5.1 Production Study

5.1.1 Pasture management and quality

5.1.1.1 Pasture allowance and intake

Dry matter intakes are dominantly affected by PA. This relationship is positively correlated, which supports the statement that when increasing the PA, the DMI of the dairy cows will also increase (Wales *et al.*, 1999; Dalley *et al.*, 2001). The following regression equation Y= (H) 86.187-296.35 (R^2 = 0.68) was calculated during the duration of the trial. The mean pasture available for grazing was 2198 ± 650 kg DM/ha (SD), which is represented by an estimated mean RPM reading of 28.94 ± 7.54 (SD). This equates to a mean PA of 12.89 ± 2.50 kg DM/cow/day (SD), with 515.67 ± 100 kg DM/day (SD) available to graze. The PA in the current study is lower than the data reported by Bargo *et al.* (2002) where cows grazed at two PA either low or high representing 26.7 and 48.9 ± 1.4 kg DM/cow/day (SD). The residual pasture mass was on average 716 ± 186.02 kg/DM/ha (SD), which represents a mean estimated RPM of 11.76 ± 2.16 (SD). A single regression equation was used for pre- and post-grazing, where previous studies have found higher accuracies with separate equations (Stockdale, 1984). To ensure optimum pasture growth and quality, the pasture is grazed to a residual height of no less than five centimeters which is set as a target height (Stockdale, 2000). The estimated pasture intake in our study was 6.83 ± 2.50 kg DM/cow/day (SD). The grazing cycle over the 41 strips of available pasture was approximately 30 days. This allowed for sufficient re-growth and rest after application of N fertilizer (Fulkerson and Donaghy, 2001).

The pasture intake determination using the RPM is inaccurate as is also indicated by the high standard error value, and the method is insufficient whether used for a single group or for determining intake differences between treatment groups (Reeves *et al.*, 1996; Malleson, 2008). Determining intake with the use of the RPM was not the purpose in this study. The purpose thereof was the management of pasture allocation to ensure sufficient pasture availability for daily grazing. Allocating pasture correctly is of utmost importance as over-utilisation of the pasture compromises pasture re-growth and under-utilisation will affect the pasture composition and nutritive value (Stockdale, 2000).

Alternatively the intake estimation was determined according to the NRC (2001). This requires inputs from the animals' description, production parameters and the

management/environment factors to calculate the ME intake requirements for maintenance, lactation and live weight gain. A back calculation (National Research Council, 2001) was carried out to determine intake of pasture, since amount of concentrate and the ME content of the concentrate and pasture is known. The back calculation, involves calculating the total daily energy requirements in terms of maintenance, live weight gain and lactation. The daily energy intake from concentrate is then subtracted from the total daily energy requirement. The balance of the energy requirement would then be obtained from pasture. Since the energy content of pasture is known, the pasture DM intake to meet the balance of energy requirement, can then be calculated by dividing the energy provided from pasture by the pasture energy content. This is illustrated in Table 5.1.

Table 5.1 The NRC model predictions for energy requirements and intake for cows grazing ryegrass/kikuyu pasture and supplemented with 5.3 kg DM of a dairy concentrate per day

Parameter[2]	**Experimental treatments**[1]	
	Control	**Yeast**
Energy requirements for maintenance ME MJ/day	58.45	57.03
Energy requirements for lactation ME MJ/day	104.81	106.91
Energy requirements for live weight gain[3] ME MJ/day	17.81	20.52
Total energy intake needed ME MJ/day	181.07	184.46
Energy intake from concentrates[5] ME MJ/day	69.5	68.4
Energy provided from the pasture ME MJ/day	111.58	116.04
Pasture intake[6] kg DM/day	10.33	10.74
Total intake kg DM/day	15.63	16.04
Total intake as a % BW	4.43	4.59

[1]Control: dairy concentrate containing no yeast; Yeast: dairy concentrate containing yeast at 167g/ ton

[2]ME - Metabolisable energy; DM - Dry matter; BW – Body weight

[3]40 MJ/kg live weight gain required (National Research Council, 2001)

[4]309 Mcal/condition score gain (National Research Council, 2001)

[5]Concentrate intake on a DM basis is 5.3 kg

[6] The pasture energy content is 11 MJ ME

The pasture intake of the yeast supplemented cows as explained in Table 5.1 is higher than the control cows. It is well known that an increase in energy requirements drives DM intake (Faverdin *et al.*, 1995). Williams *et al.* (1991) reported that yeast fed animals, had an additional energy requirement, to support the additional milk produced and the live weight

gained. Pasture intake according to the NRC (2001) is higher than that estimated using the RPM, additionally production values achieved in the current study could not have been supported by the pasture intake values estimated using the RPM. It can therefore be concluded that the RPM underestimated pasture intake. The total DM intake as a percentage of BW was similar to that reported by Malleson (2008) of 4.0%. However, the value measured in the current study is higher than that reported by Fulkerson *et al.* (2005) who measured a DMI of 3.6% BW. The variation between studies can be attributed to the forage to concentrate ratio, as it's noted that for grazing cows, supplementation is used to achieve higher DMI and increase the total energy intake compared to pasture-only diets (Reis and Combs, 2000; Bargo *et al.*, 2003).

The daily pasture intake can be calculated according to the assumption that total NDF intake is 1.5% of BW according to Kolver and Muller (1998) and knowing that the NDF content of the pasture in our study is 51.2%. The daily pasture DM intakes for the control and yeast treatments were calculated to be 10.33 and 10.24 kg per day, respectively. Concentrate supplementation leads to substitution, which is defined as the reduction in pasture in kilograms DM per day for every kilogram concentrate DM consumed per day (Stockdale, 2000). Considering a SR of 0.49% (5.3 kg DM of concentrate/cow/day * 0.093) (Faverdin *et al.*, 1991) the amount of pasture substituted is 2.61 kg. A substitution value below 1, implies that concentrate supplementation is continuing to increase the total DMI (Kellaway and Harrington, 2004). Pasture intake after considering pasture substitution is reduced to 7.72 and 7.63 kg and the NDF intake from pasture has reduced to 3.95 and 3.91 kg for the control and yeast treatment, respectively. The total NDF intake for the control and yeast treatment, after the concentrate NDF content is included, is 4.42 and 4.34 kg, respectively. This equates to a total NDF% of BW as 1.25 and 1.24% for the control and yeast treatment, respectively. This is in accordance with that described by Bargo *et al*. (2002) of 1.3% of BW as NDF, and lower than Kolver and Muller (1998) who found values as high as 1.5% ,and Fulkerson *et al.* (2006) varying from 1.6% to 2.2% for kikuyu and 1.5% to 1.6% for ryegrass of BW as NDF.

The energy calculations based on the pasture DM intakes described according to the NDF% of BW, results in a deficit of ME between 26 and 31 MJ ME per day. This cannot support the milk produced and it could therefore be concluded that the pasture intakes calculated according to the total NDF intake as a percentage of BW underestimated pasture intake.

The above mentioned methods to accurately determine pasture intake remains a challenge and such methods should be used as a guideline and not as absolute values. The methods described above either over estimate or under estimate pasture intake

measurements, and more accurate methods for pasture DMI are needed to accurately determine total daily DMI for cows in pasture-based systems. These methods however, should not be excluded, but used as pasture and herd management tools.

5.1.2 Pasture chemical composition

The nutritive value of the pasture is a function of the simultaneous digestibility and the efficiency of end-product use, which is dictated by the stage of growth of the pasture, the botanical and morphological composition and the environmental conditions during the pasture growth (Lambert and Litherland, 2000). The ryegrass/kikuyu pasture produces a consistent availability of forage, which results in a consistent grazing capacity and production of milk with little variation compared to that of kikuyu pastures (Botha *et al.*, 2008b).

The pasture DM of 15.5 ± 2.84 % (SD) (n = 11) is higher than that reported by Botha *et al.* (2003) of 12, 12.1 and 12.8% in year 1, 2 and 3, respectively. In our study, the first pasture sample collected on the 10th of September 2009 had a DM% of 12.9% and DM content increased progressively thereafter to 21.5% on the 19th of November 2009. This illustrates the beginning transformation of a ryegrass-dominant pasture in spring, towards summer in which the kikuyu-dominant pasture prevails (Botha *et al.*, 2008a).

The ME content of 10.8 ± 0.51 MJ/kg DM (SD) (n = 6) is lower than that reported by Botha *et al.* (2003) in year 2 (11.47) and 3 (11.27) of his study. The ME ranged from 9.7 – 10.4 in spring (Fulkerson *et al.*, 2007), and in other studies a value of 9.4 (Lowe *et al.*, 1999), 10.9 (Meeske *et al.*, 2006) and 11.3 (Fulkerson *et al.*, 2006) was measured. Our results is in agreement with the value reported by Meeske *et al.* (2006) in a study conducted at Outeniqua Research Farm.

The CP of 23.3 ± 2.95 % DM (SD) (n = 6) is higher than that reported by Botha *et al.* (2003) of 21.8 and 20.8% DM in year 2 and 3, respectively. The CP content is similar to studies where values as high as 24.7 – 25.6% DM were found in studies done by Fulkerson *et al.* (2007) and 25.5% DM in Lowe *et al.* (1999). Meeske *et al.* (2006) and Fulkerson *et al.* (2006) measured values as low as 18% DM and 22% DM, respectively. The CP content decreased as the study progressed, this was due to the pasture compositional change towards kikuyu-dominant pastures in which the lowest CP content was found (Botha *et al.*, 2008a).

The NDF comprises the cellulose, hemi-cellulose and lignin fractions (National Research Council, 2001). The NDF of 51.2 ± 2.22 % DM (SD) (n = 6) is higher than that reported by Fulkerson *et al.* (2006) of 44.4% DM and Botha *et al.* (2003), where values of 50.1 and 46% DM were measured in year 2 and 3, respectively. The NDF content was similar

to the 51% reported by Meeske *et al.* (2006) and 49.5 - 53.1% NDF reported by Fulkerson *et al.* (2007). The NDF% of the pasture increases from spring to summer and autumn, as kikuyu becomes more prominent following the trend from ryegrass dominant-pasture towards kikuyu-dominant pasture and finally towards kikuyu-only pasture, respectively (Botha *et al.*, 2008a).

The NDIN, is the protein and N components that are bound to the cell walls (National Research Council, 2001). The NDIN was measured as 1.20 ± 0.14 % DM (SD) (n = 6).

The ADF includes the cellulose and lignin fraction of the feedstuffs (National Research Council, 2001). The ADF content of 30.5 ± 1.40 % DM (SD) (n = 6) is higher than that reported by other authors such as 22.1% DM (Fulkerson *et al.*, 2006), 27.6% DM (Lowe *et al.*, 1999), 28.0% DM (Meeske *et al.*, 2006), 25.6 and 28.2% DM reported by Botha et. al (2003) in year 2 and 3, respectively. The ADF content is somewhat above the ranges 26.2 – 27.7% DM reported by Fulkerson *et al.* (2007) for annual ryegrass. The reason for the higher ADF% of pasture, may be due to the compositional variability of the pasture, which consisted of both ryegrass and kikuyu. The kikuyu component increases from spring to summer, and is dominant in summer and autumn pastures, while the ryegrass component decreased. The kikuyu component of pastures is therefore responsible for the higher ADF%.

In vitro organic matter digestibility for the pasture was 76.1 ± 3.19% DM (SD) (n = 6) which is similar to 80.2 ± 3.34 (SD) (Malleson, 2008) on ryegrass pasture.

The EE was measured to be 3.4 ± 0.33% DM (SD) (n = 6) which is relatively similar to 3.19 (Erasmus, 2009) and values ranging between 2.3 – 2.5 measured in the study done by Fulkerson *et al.* (2007).

The Ca percentage reported by Botha *et al.* (2003) of 0.47 and 0.59% in year 2 and 3 is higher than that measured in the current study of 0.4 ± 0.02% DM (SD) (n = 6).

The P% of 0.48 and 0.53 reported by Botha *et al.* (2003) measured in year 2 and 3 was higher than that measured of 0.37 ± 0.08% DM (SD) (n = 6) (Botha, 2003)

5.1.3 Concentrate Sampling

The chemical analysis on the concentrates were similar to that of the theoretical formulation and also confirmed that the concentrate was correctly mixed according to the formulation.

5.1.4 Milk yield, Dry matter intake, Milk composition, Milk urea nitrogen and Somatic cell count

5.1.4.1 Milk yield and dry matter intake

Milk production, 4% FCM yields and DMI were similar between the control and yeast treatment cows. This is in agreement with published research (Erdman and Sharma, 1989; Shaver and Garrett, 1997; Robinson and Garrett, 1999; Soder and Holden, 1999; Bach *et al.*, 2007; Kalmus *et al.*, 2009).

Higher milk yields have been reported after yeast supplementation; which may be due to the fact that higher DMI responses were recorded (Abd El-Ghani, 2004; Stella *et al.*, 2007; Desnoyers *et al.*, 2009a; Moallem *et al.*, 2009). Furthermore, results from the meta-analysis carried out by Desnoyers *et al.* (2009a) revealed that the effect of yeast supplementation on milk yield increases with DMI, concentrate level, NDF and CP proportion in the diet. Stella *et al.* (2007) found in Saanen dairy goats, that the inclusion of a 47% concentrate, which is highly fermentable may give added yeast the opportunity to stabilise rumen pH and stimulate cellulolytic bacterial numbers.

Dry matter intake was significantly higher for yeast supplemented dairy cows (Wohlt *et al.*, 1991; Dann *et al.*, 2000; Desnoyers *et al.*, 2009b) and steers (Mir and Mir, 1994; Hinman *et al.*, 1998). Dann *et al.* (2000) and Wohlt *et al.* (1991) found that peak milk yield was achieved sooner with yeast supplementation, peak milk yield tended to be higher (P = 0.1) and was reached earlier, which can be attributed by the fact that DMI was significantly higher for the first 6 weeks of lactation (Wohlt *et al.*, 1991).

Higher milk yields without a simultaneous increase in DMI have been previously reported due to yeast supplementation (Williams *et al.*, 1991; Piva *et al.*, 1993; Shaver and Garrett, 1997; Bruno *et al.*, 2009; Longuski *et al.*, 2009). The findings from Williams *et al.* (1991), Longuski *et al.* (2009) and Piva *et al.* (1993) could be owed to the kinetics of the digesta in the rumen, with varying forage to concentrate ratios as well as the fermentability of the concentrate, where yeast may be more effective in increasing the FCM when a higher percentage of fermentable concentrate is included.

Although results were variable it would seem that an increase in milk production is normally accompanied by an increase in DMI and when cows were fed high concentrate diets. In our study it was not possible to accurately measure DMI but was most probably not sufficient to cause an unfavourable rumen environment that would have enabled the live yeast supplement to elicit a response. Unfortunately no published pasture data could be found to compare our results.

5.1.4.2 Milk fat

The yeast supplemented cows had higher milk fat percentages (P = 0.04) compared with the control. Higher milk fat yields achieved with yeast supplementation were reported by Moallem *et al.* (2009), Longuski *et al.* (2009), Piva *et al.* (1993) and Kalmus *et al.* (2009). Longuski *et al.* (2009) had fed high moisture corn grain which is highly fermentable, and concluded that yeast may aid in preventing milk fat depression which would normally occur due to the drop in acetate production at low pH's. This was similar to a study involving yeast supplementation to goats, where higher fat percentages were measured when 6 g of a yeast culture was fed per day.

A lower milk fat percentage, however, was measured due to yeast supplementation (Shaver and Garrett, 1997; Bruno *et al.*, 2009). The significant lower milk fat found was possibly due to higher milk yield responses for yeast supplemented cows (Shaver and Garrett, 1997; Bruno *et al.*, 2009). The fat percentage measured for lactating goats was significantly lower (Abd El-Ghani, 2004; Stella *et al.*, 2007) and Abd El-Ghani (2004) experienced this when the 3 g per day dosage level was fed compared to the control.

Yeast supplementation failed to alter the fat composition of milk according to previous studies (Robinson, 1997; Robinson and Garrett, 1999; Nocek *et al.*, 2003; Schingoethe *et al.*, 2004; Cooke *et al.*, 2007; Stella *et al.*, 2007; Bruno *et al.*, 2009). Erasmus *et al.* (2005) states that the similar effect between treatments and the possible reason for the lack of effect of the yeast was due to the sufficient NDF (31.2% DM) in the diet. Although the NDF content of the pasture was above 30%, the effect of less effective fibre, and lower saliva production from pastures should be taken into account.

The milk fat yield tended to increase for yeast supplemented cows (Putnam *et al.*, 1997; Cooke *et al.*, 2007; White *et al.*, 2008). There was a tendency for an interaction with the CP level in the diet, where a higher (tendency) effect in the low CP diet was found (Putnam *et al.*, 1997). The meta-analysis carried out by Desnoyers *et al.* (2009a) showed that milk fat percentage tended (P < 0.1) to increase by 0.05% units as a result of yeast supplementation (Desnoyers *et al.*, 2009a). This is lower than the 0.25% increase in milk fat found in the present study.

As is the case with milk production, responses in fat were variable in our study, the low effective fibre content and lower buffering in the rumen due to less salivation probably created a rumen environment favouring yeast action.

5.1.4.3 Milk protein

The milk protein percentage was similar between treatments. A lack of response for the milk protein composition is in agreement with other authors (Robinson and Garrett, 1999; Dann *et al.*, 2000; Nocek *et al.*, 2003; Erasmus *et al.*, 2005; Bruno *et al.*, 2009; Desnoyers *et al.*, 2009a).

Milk protein content is largely influenced by the forage to concentrate ratio, and the amount and source of both dietary fat and protein (Sutton, 1989; Jenkins and McGuire, 2006). Decreasing the forage to concentrate ratio to 10% or less forage in the diet DM, would increase the milk protein content by 0.4 percentage units (Jenkins and McGuire, 2006). This is not practical as forage should be included in at least 40% of the diet to avoid the risk of metabolic disorders. However, Jenkins and McGuire (2006) concluded that energy intake and not the percentage of forage in the diet was the reason the protein content in the milk was influenced. Diets differing in protein levels, with either a high or low protein level did not influence the effect of supplemented yeast on milk protein percentage (Putnam *et al.*, 1997). Sutton (1989) supports this by stating that dietary protein has little effect on the milk protein concentration compared to the milk fat concentration, and effects are largely inconsistent. Jenkins and McGuire (2006) elaborates on this and states that the inability of dietary protein content and fractions to manipulate the milk protein is due to the lower transfer efficiency of dietary protein to milk which is around 25 to 30%. Therefore to quantify the lack of effect of milk protein content between treatments, the energy intake and its subsequent conversion to ruminal propionate is assessed. Jenkins and McGuire (2006) reported that cows will produce more milk protein due to the greater production of propionate and microbial protein when rapidly fermentable carbohydrates are fed. The reason may be that microbes require energy to capture NH_3-N and AA, and convert it into microbial protein, and/or that higher propionate is produced in the rumen, which increases the incorporation of protein in milk. The similar milk protein content measured in the present study is due to the similar ruminal propionate concentrations between treatment groups. This was observed in other studies by Robinson and Garret (1999), Erasmus *et al.* (1992), Longuski *et al.* (2009), Piva *et al.* (1997), Putnam *et al.* (1997) and Erasmus *et al.* (2005). Besong *et al.* (1996) however, showed that although higher propionate concentrations were observed for yeast supplemented cows, the milk protein percentage was similar.

Cows supplemented with yeast tended to produce milk with higher protein percentages (Erdman and Sharma, 1989; Cooke *et al.*, 2007) or higher protein yields for yeast supplemented cows consuming a high concentrate diet (Williams *et al.*, 1991).

Contrary to studies when there was no significant responses, Nocek *et al.* (2003) found that a direct-fed microbial containing yeast fed starting at pre-partum and continuing post-partum, significantly increased the protein percentage from 8 days post-partum to 70 days in lactation. This is in agreement with studies reported by White *et al.* (2008). Similarly in studies where milk protein yield was measured, it was found that yields were higher due to yeast supplementation (Shaver and Garrett, 1997; Bruno *et al.*, 2009; Kalmus *et al.*, 2009). Yeast supplementation increases digestion in the rumen, and subsequently increases the nutrients available for absorption, which are used for milk production (Bruno *et al.*, 2009). The higher protein output from the mammary gland could be a result of higher microbial protein produced and available to be metabolized in the duodenum (Bruno *et al.*, 2009; Kalmus *et al.*, 2009). Additionally, the efficiency of protein utilization is increased as a result of yeast supplementation, as Bruno *et al.* (2009) has suggested because lower blood urea N in the study was recorded.

Yeast supplementation in studies of Abd El-Ghani (2004) and Shaver and Garrett (1997) resulted in significantly lower milk protein percentages. The supplemented goats produced milk with a significantly lower protein percentage with a dosage level of 6 g of yeast per day (Abd El-Ghani, 2004) and Shaver and Garret (1997) owe the decreased milk protein percentages to the dilution effect of a simultaneous higher milk yield recorded.

5.1.4.4 Milk lactose

The milk lactose percentages did not differ between the control and yeast supplemented animals. This was expected, as stated by Sutton (1989) that lactose concentrations are generally not effected by dietary changes and if changes occur due to dietary changes, they are small and inconsistent and are of no value. Jenkins and McGuire (2006) supports Sutton (1989) and additionally reported that changes in lactose concentrations are only brought about by severe feeding situations.

5.1.4.5 Milk urea nitrogen

Animals in agriculture contribute to N pollution, via urea excreted due to the over feeding of protein and the subsequent decrease in efficiency of N utilization (Jonker *et al.*, 1998). Urea excreted in the urine is directly proportional to both the amount of urea in the blood and the milk of the cow (Jonker *et al.*, 1998). Therefore urinary N excretion can be predicted from the MUN level.

The MUN concentration was not different between the control and the yeast treated cows in our study, values were a mean 10.72 and 11.04 mg/dL, respectively. Bargo *et al.*

(2002a) concluded that MUN levels decrease as concentrate supplementation increased irrespective of the PA. This was due to the fact that supplementation increased the proportion of true protein in total protein, which would proportionally decrease the NPN content of milk and subsequently MUN (Bargo *et al.*, 2002a). The values obtained with supplementation were 11.1 and 11.6 mg/dL in the Bargo *et al.* (2002a) study, which are slightly higher compared to the values recorded in the current study. The target MUN values of 10 to 16 mg/dL when cows were fed according to NRC (2001) recommendation, indicate that MUN levels in the present study are within this range (Jonker *et al.*, 1999). The MUN levels in the present study therefore indicated that dietary protein was not limiting results and was not fed in excess.

5.1.4.6 Somatic cell count

The SCC was similar between treatments (P = 0.62). It was however, not expected to be affected by live yeast supplementation.

5.1.4.7 Body weight

The BW at the beginning, the end, and the BW change of the yeast supplemented cows during the study did not differ from the control, similar to previous studies (Erdman and Sharma, 1989; Kamalamma *et al.*, 1996; Kung *et al.*, 1997; Erasmus *et al.*, 2005; Lascano *et al.*, 2009b). Yeast supplementation had no effect on the mean BW or its change (Robinson, 1997), not only post-partum, but Wohlt *et al.* (1998) also found no effect of level of yeast supplementation on BW. This was confirmed by Kung *et al.* (1997) who fed Biomate Yeast Plus at both a level of 10 and 20 g/day, with no effect on BW. Jersey cows, showed beneficial effects in that supplemented cows lost BW less rapidly (42 DIM) post-calving (Dann *et al.*, 2000). From this study it was furthermore concluded that the different rates of weight loss between treatments were owed to the different DM intakes observed during the first 42 DIM. The NRC (2001) states that changes in tissue weight are not reflected by the changes in BW, this is because in early lactation the increase in DMI and hence gut fill masks the decrease in tissue mass due to tissue mobilisation. Therefore BW is difficult to quantify, and is not a true reflection of tissue mass, as gut fill makes up 15% of the BW. This contributed to the lack of a response on BW in our study.

Alternatively, there are studies which reported that yeast had positively influenced growth parameters; for example yeast culture improved ADG of steers by 6.9% (Hinman *et al.*, 1998). Yeast culture improved the total ADG of finishing Awassi lambs (266 vs. 212 g/day) with only 3 g/day (Haddad and Goussous, 2005) but ADG in other feedlot lambs by

21% (Tripathi and Karim, 2010). Tripathi and Karim (2010) suggested that yeast cultures had the best potential to promote growth in feedlot lambs, which may be an alternative to the use of ionophores or antibiotics.

5.1.4.8 Body condition score

The BCS is a visual assessment of the fat depots and fat distribution over the body of the cow. This score is subjective and varies widely between different individuals and is dependent on the assessors' previous experience. The body compositional changes in the lactational cycle are a function of diets which either provide insufficient or excess amounts of energy which either cause a depletion or excess replenish of body tissue (National Research Council, 2001). The body condition is an important factor to consider as cows that are too thin or too fat risk complications such as metabolic diseases, low conception rates, decreased milk yield and dystocia (National Research Council, 2001). The normal physiological process during early lactation negates that energy reserves are mobilised, to support the energy requirements for milk production. Therefore in early lactation, cows are in a negative energy balance. As mid- and late-lactation approach, the cows body condition improves, and the cow replenishes the lost body reserves, and the cows energy balance approaches 0 and eventually becomes positive (National Research Council, 2001).

The BCS at the beginning, the end, and the BCS change of the yeast supplemented cows during the study did not differ from the control ($P > 0.05$). Wohlt *et al.* (1998) found that neither the inclusion level nor removal of a yeast supplement had an effect on BCS in Holstein cows. Although no difference between treatments were observed in the present study, the NRC (2001) supports that the BCS in early lactation as with the cows in the beginning of the study was lower than the BCS that was observed at the end of the study and with cows later in lactation, within treatment groups.

5.2 Rumen Study

5.2.1 Rumen pH

The rumen pH of a cow on a well-balanced diet should range between 5.8 and 6.4 which were true for both treatment groups in the present study. This pH range accommodates all relevant microbial species (Ishler *et al.*, 1996). The two most commonly mentioned groups of bacteria that operate at different pH levels, are the fibre-digesters and starch-digesters (Ishler *et al.*, 1996). The fibre-digesting bacteria also known as the cellulolytic bacteria, function optimally at a pH that ranges from 6.2 to 6.8, and generally a drop below 6, sees the reduction in cellulolytic and methanogenic bacteria. The starch digesters function at a pH

level ranging from 5.2 to 6.0, which indicates their acid tolerant nature (Ishler *et al.*, 1996). The first-limiting nutrient for microbial growth is energy, and when large amounts of energy become available the microbes deal with the carbohydrate overload in different ways (Rode, 2000). The bacteria may either store the excess carbohydrates as intra- or extracellular polysaccharides or shift their fermentation pathway. The shift will be towards microbes producing lactate instead of acetate or propionate (Rode, 2000).

The extent of the pH decrease in the presence of produced acids is dependent on the rate at which the acids are produced, the total amount produced, the rate of absorption of acids across the rumen wall and the buffering capacity of the salivary secretions to neutralize the acid (National Research Council, 2001).

The mean pH was not different between the control and yeast treatment groups. No differences in ruminal pH that were reported studies could be an effect of the stage of lactation, ration or source and type of supplemented yeast (Thrune *et al.*, 2009). Feeding system has no effect on the mean ruminal pH, which was similar for the pasture and concentrate, pTMR and full TMR, therefore studies across feeding systems are comparable (Bargo *et al.*, 2002c). The ruminal pH was not different between yeast and control treatments in *In vitro* studies (Newbold *et al.*, 1995; Kung *et al.*, 1997), in cow studies (Besong *et al.*, 1996; Miranda *et al.*, 1996; Putnam *et al.*, 1997; Doreau and Jouany, 1998; Robinson and Garrett, 1999), in sheep studies (Newbold *et al.*, 1995; García *et al.*, 2000) and also in studies with steers (Dawson *et al.*, 1990; Olson *et al.*, 1994; Lehloenya *et al.*, 2008). Ruminal pH did not differ between yeast supplemented and control cows during a fermentable starch dietary challenge (Longuski *et al.*, 2009) or when different quality diets, differing in their forage to concentrate ratio were consumed (Moloney and Drennan, 1994; Lascano and Heinrichs, 2009). The mean rumen pH in a group of goats fed a high concentrate (50%) diet was not affected by the supplemented yeast, but tended to decrease the minimum rumen pH (Desnoyers *et al.*, 2009b). This was owed to the fact that the feeding behaviour of the yeast supplemented goats to select and ingest less fibrous portions of the diet and thus animals coped better with the high concentrate diets they were consuming (Desnoyers *et al.*, 2009b). The concentrate to forage ratio in the present study, was approximately 34:64, this level of concentrate may not have been sufficient to elicit a significant response in ruminal pH between treatment groups. The concentrate percentage of the total diet in previous studies, for which significant responses (higher and lower) were observed, ranged between 15 and 52% although 15% was the one extreme and excluding the latter the range averaged approximately 47% which is higher than the percentage measured in our study. Bargo *et al.* (2003) concluded that the relationship between pH and the amount of concentrate fed is

complex due to the large inconsistencies observed for grazing cows supplemented with various amounts of concentrate. Enjalberta *et al.* (1999) supplemented yeast to non-lactating dairy cows, and found no difference between treatment groups for the ruminal pH, which were both above 6; this is similar to that found in the present study. Enjalberta *et al.* (1999) claimed that the possibility of a significant difference was masked by the high pH of the control.

Different strains of yeast or other fungal supplements in studies by Pinos-Rodríguez *et al.* (2008), Wiedmeier *et al.* (1987), Miranda *et al.* (1996), Yoon and Stern (1996) and Newbold *et al.* (1995) were similar in their effects on ruminal pH.

In continuous culture studies, when comparing both a live yeast and a killed yeast with each other and/or to the control, no differences in ruminal pH were found (Dawson *et al.*, 1990). The same was found in other *In vitro* studies on different substrates (Sullivan and Martin, 1999) and strains (Newbold *et al.*, 1995). *In vitro* fermentors have high buffering capacity so studies used to measure yeast effects on pH may be inappropriate (Kung *et al.*, 1997).

Ruminal pH measured is time dependent, logger pH values measured over four days have revealed that the lowest and highest pH measured for control cows was 5.83 at 19:30 and 6.45 at 05:30, respectively (Guedes *et al.*, 2008). The yeast supplemented cows revealed that the lowest and highest pH measured was 5.84 at 19:00 and 6.50 at 05:30, respectively. This is in agreement with Guedes *et al.* (2008) who found that for the two treatment groups that generally just before feeding the ruminal pH is the highest. The lower ruminal pH values are measured two and four hours after feeding (Williams *et al.*, 1991; Guedes *et al.*, 2008; Marden *et al.*, 2008), in the case of this study it was approximately four to five hours after the afternoon feeding.

The 20:00 sampling time yielded a significantly higher ruminal pH for the yeast supplemented cows compared to the control. Contradictorily *S. cerevisiae* supplemented to steers had no effect on pH measured at either 3, 6, 9 or 12 hours after feeding, with no effect for the hours the pH is below six compared to the control (Plata *et al.*, 1994) or on non-lactating dairy cows when measured one, three and five hours after feeding (Enjalbert *et al.*, 1999). Bargo *et al.* (2002c) compared the effect of three different feeding systems on ruminal pH patterns and concluded that a TMR feeding system measured provided the most constant pH measurements compared to both a pTMR or pasture and concentrate, which should be considered when comparing daily ruminal pH variations. This was expected because with a pasture and concentrate feeding system, as in this study, the daily concentrate intake remains constant, and the pasture intake varies. Therefore the concentrate to forage ratio fluctuates daily and depends on the pasture DMI (Bargo *et al.*, 2002c).

A significantly higher ruminal pH has been measured when a live yeast had been supplemented in other studies (Bach *et al.*, 2007; Guedes *et al.*, 2008; Marden *et al.*, 2008). There is a possibility that a live yeast may be more effective in stabilising the rumen pH, although increased meal frequency may play a role in the consistently higher ruminal pH measured in yeast supplemented cows (Bach *et al.*, 2007). The higher pH according to Guedes *et al.* (2008) and Marden *et al.* (2008) could be a result of the lower lactate concentrations measured in the yeast supplemented cows. The pK_a value, which is a quantitative measure of the dissociation constant of an acid, gives an indication of the strength of the acid (Kotz *et al.*, 2003). The higher the pK_a value, the smaller the extent of the dissociation of an acid. Ruminal VFA, are absorbed across the rumen wall, only when in the undissociative state (National Research Council, 2001). The VFA is namely propionate, acetate and butyrate have pK_a values of 4.87, 4.76 and 4.82, respectively (National Research Council, 2001). Lactate is a strong acid and has a lower pK_a value (3.86) (Marden *et al.*, 2008) compared with all the VFA's. The VFA in an undissociated state are absorbed rapidly compared with lactate (National Research Council, 2001). Therefore lactic acid with a higher extent of dissociated acid, is absorbed more slowly across the rumen wall (National Research Council, 2001). Yeast may therefore function by either removing lactate, by reducing the bacteria initially producing the acid, and by increasing the lactic-acid utilizing bacteria. The redox potential of the rumen fluid with supplemented yeast indicates the strength and reducing power of the yeast, the value of -149 mV proves this, having lower values than that of the control (-115 mV) group (Marden *et al.*, 2008). Yeasts ability in reducing the redox potential has been observed in other studies (Mathieu *et al.*, 1996; Chaucheyras-Durand and Fonty, 2002).

Additionally, yeasts strength in this regard is illustrated in the study of Guedes *et al.* (2008), where the diet posed no risk of acidosis, and pH differences would be difficult to detect between treatments. Despite this the yeast was able to elevate the pH significantly and alleviate the depression in pH that occurs after feeding (Guedes *et al.*, 2008). This was not found in the present study, because in the study of Guedes *et al.* (2008), the use of three cows increases the cow effect, and the yeast supplement was dosed directly into the rumen. In the present study, five replicates were performed in a cross-over design. Increasing the replicates allows the study to be reproducible, it increases the precision and less replicates will have a higher error variance. In the present study the yeast supplement was pelleted in the concentrate and fed, which assumes that the yeast supplement was fully viable and present at 1 g per cow per day. The study of Guedes *et al.* 2008 has no assumptions whether

the yeast was ingested or not, as it was dosed directly through the cannula, as well as the yeast supplement was not processed (such as pelleted).

In a study reported by Bach *et al.* (2007), the mean minimal and maximum ruminal pH values were higher for yeast supplemented cows. This is supported by another study investigating a live yeast supplement, and results revealed higher ($P < 0.05$) pH measurements with respect to the maximum (7.01 vs. 6.8), minimum (5.97 vs. 5.69) and mean (6.53 vs. 6.32) measurements of the rumen fluid pH (Thrune *et al.*, 2009). The pH was below 5.6 (sub-acute acidosis threshold), 5.8 and 6 for a shorter period of time (Thrune *et al.*, 2009). This is in agreement with Bach *et al.* (2007) who reported that the time the rumen pH value is below 5.6 and 6 is significantly less in yeast supplemented cows. This time, represented by the area under the curve, for the control is 308 (hours/day pH < 5.6) and 232% (hours/day pH < 6) higher than the yeast supplemented cows, which suggests that yeast supplemented cows experience a lesser intensity of subclinical acidosis when it arises (Bach *et al.*, 2007). The time below a pH of 6.2 was significantly reduced for yeast (Yea-Sacc) supplemented steers compared to the control for steers consuming alfalfa hay and cornstalk (Roa V *et al.*, 1997). The pH of the control remaining below 6, while the pH of the yeast supplemented cows gradually increased (Marden *et al.*, 2008). These studies highlight the pH stabilising ability of a live yeast (Bach *et al.*, 2007; Guedes *et al.*, 2008; Marden *et al.*, 2008; Thrune *et al.*, 2009).

A live yeast (Levucell SC 10 ME) supplemented at two different levels (0.3 and 1g/cow/day), revealed no significant difference between the two levels for the measured pH, but significantly higher pH values were found compared to that of the control (Guedes *et al.*, 2008).

Yeast cultures opposed to live yeast supplementation has equally proved to increase the ruminal pH in goats (three hours after feeding) (Abd El-Ghani, 2004) and cecal pH in horses (Medina *et al.*, 2002). The quantitative findings of a meta-analysis showed that the rumen pH increased on average by 0.03 units and that the more concentrate and DM consumed, the more the positive effects on the ruminal pH the yeast will express (Desnoyers *et al.*, 2009a). In the present study the yeast supplemented group of cows had higher ruminal pH's for the portable and logger measurements, by 0.05 and 0.02 units, respectively however, these findings were not significant due to the variation between cows. Williams *et al.* (1991) showed that added yeast to a growth medium altered the ruminal pH by reducing the drop in pH typically found after a large amount of concentrate was consumed.

In a study reported by Desnoyers *et al.* (2009a) the positive effect on ruminal pH was increased by the DMI and concentrate level of the diet and reduced by the NDF level.

Enjalberta *et al.* (1999) although found no effect on the ruminal pH between control and yeast supplemented cows, proposing that the ruminal protozoal concentrations that are increased due to yeast supplementation, store starch that is a readily fermentable carbohydrate, which renders it unavailable to the amylolytic bacteria. This mechanism may be responsible for the pH stabilising effect, which could reduce the drastic falls in pH observed after concentrate feeding.

An *In vitro* study comparing the mean pH of Diamond V-XP and A-Max, showed that the mean pH was significantly higher two and six hours post-feeding when A-Max was supplemented (Miller-Webster *et al.*, 2002).

In contrast yeast supplementation may in effect lower the ruminal pH significantly as shown by Arcos-García *et al.* (2000), Andrighetto *et al.* (1993) and Sullivan and Martin (1999). Arcos-García *et al.* (2000) investigated the effects of the differences between two commercial supplements (Yea-Sacc and Levucell) with the Yea-Sacc (5.85) fed sheep having a lower pH ($P = 0.01$) compared with the Levucell (5.96) fed sheep, with both yeast products having a lower pH compared to the control (6.05). Andrighetto *et al.* (1993) showed that even though two dosage levels of yeast (20 and 40 g) resulted in significantly lower ruminal pH compared to the control, there was no difference between the two dosage levels. Lower ruminal pH's measured in animals supplemented with a yeast culture may be owed to higher VFA's measured which reflects the increased microbial activity in the rumen (Andrighetto *et al.*, 1993). However, a higher ruminal VFA coupled with a higher ruminal pH with yeast supplementation was observed by Desnoyers *et al.* (2009a), Marden *et al.* (2008) and Guedes *et al. (*2008), which may be the effect of the use of a live yeast supplement.

Supplementing yeast and its effects on rumen pH as discussed above in previous research reveals that results across studies are contradictory, however, what can be concluded that yeast has the ability to stabilize the rumen pH, specifically with high concentrate diets, and therefore reduce the risk of metabolic disorders.

5.2.2 Ruminal ammonia nitrogen and volatile fatty acid concentration

5.2.2.1 Ruminal ammonia nitrogen

Ruminal ammonia concentrations are affected by fermentability of the diet, and the endogenous and recycled N to the rumen (Olson *et al.*, 1994). The inclusion of non-structural carbohydrates in the diet results in reduced loss of N during digestion of high quality pasture, due to the dilution effect of N intake (Kolver *et al.*, 1998). The minimum ruminal NH_3-N which is required in the ruminal fluid to not compromise microbial production is a concentration of 5 mg/dL rumen fluid (Satter and Roffler, 1974; Satter and Slyter, 1974). In our study higher

concentrations were, namely 10.10 and 9.54 mg/dL for control and yeast treatment, respectively.

The ruminal NH_3-N concentration was significantly higher on a pasture and concentrate feeding system (19.96 mg/dL) compared to the pTMR (10.75 mg/dL) and TMR (9.74 mg/dL) (Bargo *et al.*, 2002c). The ruminal NH_3-N in the present study for the control and yeast treatment is lower than that for the same feeding system reported by Bargo *et al.* (2002c) and the values are closer to the pTMR and TMR feeding system. The explanation could stem from the differences between the studies such as the larger Holstein cows (BW, 653 ± 79 kg) (Bargo *et al.*, 2002c) compared to smaller Jerseys in the current study. The Holstein cows consumed more DM per day (22.6 ± 1.4 kg) and the pastures CP percentage was higher at 26.3% (Bargo *et al.*, 2002c) compared to an estimated total DMI of 15.8 kg/day and pasture CP percentage of 23.3% in the present study. The high fibre-low protein diet was not affected by yeast inclusion in terms of NH_3-N concentrations in the rumen, but the NH_3-N concentration was significantly reduced when yeast was added to the low fibre-high protein diet (Moloney and Drennan, 1994). This reveals that yeasts effect on N metabolism is related to the time after feeding as well as the N content of the basal diet (Moloney and Drennan, 1994).

Ruminal NH_3-N concentration is dependent on sampling time (Bargo *et al.*, 2002c; Marden *et al.*, 2008). The ruminal NH_3-N concentration (mg/dL) measured in the current study at 02:00, 08:00, 14:00 and 20:00, averaged over both periods combined did not differ between treatment groups. It is generally found that peak NH_3-N concentrations are found around two to four hours after feeding (Guedes *et al.*, 2008; Marden *et al.*, 2008). In the study reported by Abd El-Ghani (2004) the lower concentrations were found around six hours-post feeding. In the current study, peak values reached for the control (13.2 mg/dL) and yeast (14.6 mg/dL) cows were at two hours post morning feeding and milking (08:00) and five hours post afternoon feeding (20:00), respectively. In the study reported by Bargo *et al.* (2002c) the peak NH_3-N concentrations were measured after concentrate ingestion and when cows were placed back on the paddock which was at 13:00 (20.7 mg/dL) and 21:00 (25.8 mg/dL). Those values are much higher compared to the values measured in the present study. However, samples of NH_3-N in the present study were taken at set time intervals and periods, and the concentrations of NH3-N outside those specific samples are unknown and could represent higher or lower values. Yeast supplemented goats yielded significantly lower ammonia concentrations at three to six hours post-feeding (Abd El-Ghani, 2004); in cows significantly lower ruminal NH_3-N concentrations were measured three hours post-feeding (Enjalbert *et al.*, 1999); and steers yielded lower NH_3-N concentration at four hours post-feeding (Moloney and

Drennan, 1994). However, Mir and Mir (1994) found that there was no significant difference for samples collected two hours after feeding in steers, similar to Quigley *et al.* (1992) who found no difference four hours after feeding in dairy calves.

Yeast culture supplementation increased ruminal NH_3-N concentration in sheep (Arcos-García *et al.*, 2000), calves (Pinos-Rodríguez *et al.*, 2008) and cows (Roa V *et al.*, 1997). Yeast supplementation may therefore increase the microbial protein production, which was illustrated when the daily microbial N synthesis was significantly higher, with the highest level of bakers yeast supplementation (Kamel *et al.*, 2004). The microbial N synthesis and Synchronisation index (SI) are significantly correlated and therefore it was concluded that more energy was available to the microbes for their growth when the highest level bakers yeast was supplemented, on condition that N had not affected release (Kamel *et al.*, 2004).

This suggests that yeasts effects has the potential to alter the AA profile of microbial protein due to the fact that a significant effect was exerted on the duodenal AA profile and flow of Met in the GIT (Erasmus *et al.*, 1992). The increase in NH_3-N has been suggested to be due to the fact that yeast stimulates the proteolytic activity of certain bacteria (Kung *et al.*, 1997). In horses fed diets containing urea, it was shown that yeast supplementation stimulated conversion of recycled urea to microbial protein and AA (Glade and Biesik, 1986).

In contrast it was also recorded that yeast supplementation may significantly decrease ruminal NH_3-N concentration. This has been observed in studies with cows where Moallem *et al.* (2009) concludes that yeast influences either the protein degradation by increasing or decreasing it, and/or larger quantities of NH_3-N is incorporated into microbial protein. Enjalbert *et al.* (1999) and Erasmus *et al.* (1992) confirm the theory that increased microbial activity and hence incorporation of NH_3-N into microbial protein was the reason for lower NH_3-N measured. Similarly in another study, the yeast addition, irrespective of the ratio of forage to concentrate in the diet, resulted in significantly lower NH_3-N concentration compared to the control, which simultaneously coincides with the significantly higher VFA concentration (Lascano and Heinrichs, 2009). This indicates that the lower NH_3-N concentration measured was not limiting bacterial growth, but may be attributed to higher concentrations of cellulolytic and total bacteria (Lascano and Heinrichs, 2009). Lascano and Heinrichs (2009) states that previous research for the addition of yeast causing a lower NH_3-N is due to either yeast stimulating NH_3-N uptake by bacteria, or alternatively stimulating cellulolytic bacterial growth. Cellulolytic bacteria, primarily use NH_3-N as their N source, where amylolytic bacteria possess proteolytic activity and therefore prefer the use of AA (Bach *et al.*, 2005).

When starch and cellulose substrates were incubated in both a yeast adapted and yeast unadapted rumen inoculums (*In vitro*), results showed higher productions of microbial N

synthesis for both substrates in the yeast adapted inoculum (Kamalamma *et al.*, 1996). This higher bacterial N flow supported the tendency of higher duodenal NAN that was observed in yeast supplemented cows which then affected the duodenal AA profile and flow of Met in the GIT (Erasmus *et al.*, 1992). Conversely, Putnam *et al.* (1997) found that NAN (non-microbial) flow to the duodenum tended to be higher for yeast supplemented cows, while the flow of essential AA and AA profiles of duodenal digesta and microbial proteins were not affected. Yoon and Stern (1996) found that the total N and NAN flow to the duodenum had decreased (due to tendencies towards higher CP degradabilities in the rumen) without a difference in bacterial N flow, but rather owing to a decrease in dietary N flow and endogenous N flow. Lehloenya *et al.* (2008) and Doreau and Jouany (1998) showed that feeding yeast did not affect the duodenal N and microbial N flow even though *In situ* N degradation of soyabean meal was significantly higher at four hours and tended to be higher at eight hours (Doreau and Jouany, 1998). Supplementing yeast had no effect on bacterial N flow in the study by Moloney and Drennen (1994) although a tendency was reported towards higher duodenal bacterial N flows in late July (Olson *et al.*, 1994). The *In vitro* supplementation of a Diamond V-XP and Amax yeast culture products tended ($P = 0.08$) towards higher NH_3-N concentrations compared to the control (Miller-Webster *et al.*, 2002).

Concurrent with the results of no difference found between treatments in the present study, it is supported by other authors (Wiedmeier *et al.*, 1987; Yoon and Stern, 1996; Putnam *et al.*, 1997; Robinson and Garrett, 1999; Erasmus *et al.*, 2005; Guedes *et al.*, 2008). Olson *et al.* (1994) found numerically lower ($P > 0.05$) NH_3-N concentrations in yeast fed steers and suggested a more rapid fermentation rate could explain why increasing the dietary soluble N concentrations with yeast culture supplemented steers did not result in higher ruminal ammonia concentrations. The *In vitro* study comparing live and killed yeast revealed that neither differed from the control for the NH_3-N concentrations (Dawson *et al.*, 1990) which applied to another study comparing different strains of yeast (Newbold *et al.*, 1995).

5.2.2.2 Ruminal volatile fatty acids

The ruminal VFA were similar between cows in our study and a study reported by Bargo *et al.* (2002c) comparing different feeding systems (pasture and concentrate, pTMR and TMR), which therefore indicates that results across studies can be compared if similar amounts of concentrates are fed. The ruminal VFA measured on average over both periods combined in our study were significantly lower for the yeast supplemented cows (99.27 mmol/L) compared to the control (106.31 mmol/L). This may be attributed to the significantly higher mean quantity of acetic acid produced by the control cows, with no difference in the

acetic acid molar percentage. This is contradictory to other studies where a significantly higher production of total VFA was found due to yeast supplementation (Roa V *et al.*, 1997; Guedes *et al.*, 2008; Marden *et al.*, 2008; Desnoyers *et al.*, 2009a). The higher VFA production may be indicative of higher microbial activities (Andrighetto *et al.*, 1993).

The inconsistency of the significant differences between the treatments for the mean VFA concentration may be because on any given diet the concentration of VFA at a specific time, is dependent on the rate of fermentation and absorption, as well as the volume of rumen liquor (Balch and Rowland, 1957). These factors can vary largely between animals.

In our study the mean ruminal VFA was a product of rumen samplings at four time periods (02:00, 08:00, 14:00 and 20:00) over two periods. Abd EI-Ghani (2004) found that time of sampling after feeding had a significant effect on the VFA concentration and is in agreement with other studies (Andrighetto *et al.*, 1993; Doreau and Jouany, 1998). In our study a significantly higher mean ruminal VFA concentration was measured for the control treatment at 08:00 which is approximately two hours post-concentrate (3 kg as is) consumption. The greatest variation in VFA concentrations can be up to three hours post meal ingestion when intense fermentation is taking place (Andrighetto *et al.*, 1993). This is further evident from the VFA concentrations measured in this study at the various sampling times split into both periods. The fermentation pattern of cows on pasture is different to cows consuming TMR which have forages of uniform composition. This is due to the fact that as cows graze a specific paddock, the ratio of leaf to stem is reduced, which will alter the fermentation pattern and hence VFA production (Holden *et al.*, 1994).

5.2.2.3 Ruminal acetate

Acetic acid is the dominant acid produced in the rumen of animals consuming high fibre diets (Ishler *et al.*, 1996). The molar percentage of acetate was significantly higher for dairy heifers consuming a low concentrate diet (80 forage: 20 concentrate) compared to high concentrate (40 forage: 60 concentrate) diets, 63.2 and 59.3%, respectively (Lascano and Heinrichs, 2009). The acetic acid produced is absorbed and used to synthesise fatty acids, which are deposited as fat in adipose tissue or to produce milk fat (Ishler *et al.*, 1996). The ruminal acetic acid produced for the two treatment groups in the current study did not differ, with molar proportions of 62.0 and 61.8%, for the control and yeast treatment, respectively. The acetic acid percentages in the present study, is similar to that measured by Bargo *et al.* (2002c) namely 63.1% (results between the three different feeding systems were similar and therefore averaged) and is close to the expected range described by Ishler *et al.* (1996) of 50 to 60%. The similar ruminal acetic acid percentages between treatments in the present study

were not expected, because significantly higher NDF disappearances and milk fat percentages were observed for the yeast supplemented cows. Ishler *et al.* (1996) states that the fatty acid deposited in the milk is as a result of the absorbed ruminal acetic acid produced from the fermentation of fibre in the diet. A lack of response in acetate concentration due to yeast supplementation was recorded for dairy cows (Erasmus *et al.*, 1992; Piva *et al.*, 1993; Putnam *et al.*, 1997; Robinson and Garrett, 1999; Longuski *et al.*, 2009; Thrune *et al.*, 2009), calves (Pinos-Rodríguez *et al.*, 2008), sheep (Andrighetto *et al.*, 1993; Newbold *et al.*, 1995; Arcos-García *et al.*, 2000; García *et al.*, 2000) and steers (Mir and Mir, 1994; Olson *et al.*, 1994; Hinman *et al.*, 1998; Lehloenya *et al.*, 2008). These findings, which are similar to the current study suggest that a significant difference would be difficult to acquire as yeast has been investigated to grow on acetate as a sole carbon source (Chu *et al.*, 1981). This implies that acetate produced over and above the control by specific rumen microflora due to fibre digestion (assuming yeast influence on improving fibre digestion increases acetate concentration), may be removed by yeast for growth, and therefore differences in concentration between treatment groups are difficult to detect (Chu *et al.*, 1981). At lower pH values, cellulolytic bacteria cease to function optimally and hence fibre digestion and acetic acid production is compromised (Ishler *et al.*, 1996). However, Andrighetto *et al.* (1993) found that despite the significantly lower pH in yeast supplemented sheep, acetate concentrations were maintained and stabilised, and did not differ compared with the control this may be due to the fact that yeast stimulates cellulolytic processes in the rumen. The *In vitro* addition of a yeast supplement to different substrates such as ground corn, maltose or lactate had no effect on the acetate concentration (Sullivan and Martin, 1999). An *In vitro* study comparing a control, live yeast and dead yeast, reported that neither were different with regards to the acetate concentration measured (Dawson *et al.*, 1990). This was also found in other *In vitro* studies (Newbold *et al.*, 1995; Kung *et al.*, 1997).

The highest acetic acid concentration for the control and yeast supplemented cows were measured at 20:00, which is approximately five hours post-feeding. The highest acetate concentrations were affected by sampling time, and where found two to four hours after feeding (Guedes *et al.*, 2008). The mean ruminal acetic acid concentration measured at 08:00 were significantly lower ($P < 0.05$) for the yeast supplemented group.

Higher ruminal acetic acid concentrations for yeast supplemented calves (Quigley *et al.*, 1992), cows (Guedes *et al.*, 2008; Marden *et al.*, 2008) and *In vitro* studies (Sullivan and Martin, 1999) have been recorded .

Higher acetate concentrations were measured for yeast supplemented heifers irrespective of the forage to concentrate ratio, with no effect on the acetate percentage

(Lascano and Heinrichs, 2009). Similarly diets high in starch or fibre had increased the molar acetate percentage in the cecum and colon of horses (Medina *et al.*, 2002). This suggests that the addition of yeast promotes higher fibrolytic bacterial activity, though no subsequent increase in cellulolytic bacterial numbers was detected (Medina *et al.*, 2002).

Significantly lower acetate concentration (*In vitro)* (for the commercial yeast products, Diamond V-XP and A-Max) proved that both yeast products produced significantly lower acetate concentrations (Miller-Webster *et al.*, 2002). Besong *et al.* (1996) found that increasing the inclusion rate of a liquid yeast, created a tendency for the acetate concentration to decrease (P = 0.06).

5.2.2.4 Ruminal propionate

Propionic acid production in the rumen predominates when diets high in grain or when concentrate mixtures are fed (Ishler *et al.*, 1996; Eastridge, 2006; Lascano and Heinrichs, 2009). Propionic acid is a precursor for glucose synthesis in the liver, which is used for energy and is the source of milk sugars such as lactose (Ishler *et al.*, 1996).

Ruminal propionic acid measured in the control and yeast treatment groups in the current study did not differ. This is in agreement with reported studies in dairy cows (Erasmus *et al.*, 1992; Yoon and Stern, 1996; Robinson and Garrett, 1999; Longuski *et al.*, 2009), sheep (Andrighetto *et al.*, 1993; Newbold *et al.*, 1995; Arcos-García *et al.*, 2000; García *et al.*, 2000), steers (Dawson *et al.*, 1990; Moloney and Drennan, 1994; Olson *et al.*, 1994; Roa V *et al.*, 1997; Hinman *et al.*, 1998; Lehloenya *et al.*, 2008) as well as in *In vitro* (Newbold *et al.*, 1995; Kung *et al.*, 1997; Sullivan and Martin, 1999). A tendency (P = 0.06) towards higher propionate concentrations, however, was measured for yeast supplemented Holstein steers in study reported by Plata *et al.* (1994).

The lack of differences found between treatment groups in the present study, could be owed to the low concentrate to forage ratios, the possible high “inherent” variation between cows or the physiology of the cow in early lactation. The concentrate to forage ratio in the present study, was estimated to be 34:64 which is relatively low, in comparison to the studies where significant differences (for propionic acid percentages) were observed. Studies that had yeast supplemented animals with higher (P < 0.05) ruminal propionic acid concentrations, consumed diets with concentrate to forage ratios, such as 43:57 (Marden *et al.*, 2008), 45:55 (Besong *et al.*, 1996), 47:53 (Miller-Webster *et al.*, 2002), 50:50 (García *et al.*, 2000) and 52:58 (Guedes *et al.*, 2008). However, Bargo *et al.* (2002c) recorded that the mean propionic acid percentage was 20.6% when averaged between three feeding systems which differed in concentrate to forage ratios. The propionic acid percentages measured in

the present study were slightly higher for the control and yeast (23.3 and 23.4%) treatments when compared to results reported by Bargo *et al.* (2002c) (19.42%) but are still within the normal range to be expected (Ishler *et al.*, 1996).

Bargo *et al.* (2002c) had fed cows (according to Bargo *et al.* (2002b) who fed 8.7 kg DM/cow/day concentrate) a higher level of concentrate compared to that in the present study of approximately 5.3 kg DM/cow/day, and the lower propionic acid percentage measured by Bargo *et al.* (2002c) compared to that measured in the present study, was not expected. This could support the fact that the concentrate to forage ratio may have not been a factor which had affected the propionic acid percentages. The high variation between the fistulated cows in the present study could contribute to the inability to detect significant differences between treatment groups. The cow in early lactation is in a negative energy balance, and propionate is the glucose precursor, which is used for energy. Therefore ruminal propionate is readily absorbed through the rumen wall which could explain why differences between treatments may not be easily detectable. This is supported by Balch and Rowland (1957) who suggested that rapid fermentation and absorption is supported by high concentrate diets.

Yeast supplementation had resulted in higher propionate concentrations when a live yeast supplement was fed to cows (Guedes *et al.*, 2008; Marden *et al.*, 2008). This was evident with the *In vitro* supplementation of a live yeast culture compared to a killed yeast culture, which resulted in significantly higher propionic acid detection (Dawson *et al.*, 1990). Guedes *et al.* (2008) and Marden *et al.* (2008) illustrated that with live yeast supplementation the simultaneous decrease in lactate and increase in propionate concentrations may be a result of the greater conversion of lactate to propionate. Commercial yeast cultures, Diamond V-XP and A-Max, proved that both yeast products produced significantly higher propionate concentrations *In vitro*, with Diamond V XP producing significantly higher concentrations compared to A-Max (Miller-Webster *et al.*, 2002).

Yeast addition in diets irrespective of the ratio of forage to concentrate, resulted in significantly higher propionate concentrations compared with the control (Lascano and Heinrichs, 2009). *In vitro* supplementation of *S. cerevisiae* at 0.73 g/L increased the propionate concentration when alfalfa hay was the substrate, and similarly the propionate concentration increased when coastal Bermuda grass hay was fermented at both treatment levels (Sullivan and Martin, 1999).

Guedes *et al.* (2008) reported that the highest propionate concentrations were affected by sampling time, and were found two to four hours after feeding for a control (0h, 15.6; vs. 2h, 28.3; and 4h, 26.5 mmol/L), a 0.3 g (0h, 15.4; vs. 2h, 29.3 ; and 4h, 28.5 mmol/L) and a 1 g (0h, 24.3; vs. 2h, 38.5 ; and 4h, 36.6 mmol/L) yeast treatment level. Similarly the highest

propionic concentrations were found approximately five hours post-afternoon feeding for both treatment groups in the current study (control, 36.6 and yeast, 32.6 mmol/L). Though two hours post-morning feeding, showed that the control (28.2 mmol/L) had significantly higher ruminal propionic concentrations compared with the yeast (19.6 mmol/L) supplemented cows.

Similar to yeast effects on acetate production, many factors affect propionic acid production and no clear conclusion could be made on the effect of yeast on propionate production.

5.2.2.5 Ruminal butyrate and valerate

Butyric acid is converted to beta-hydroxybutyrate by the rumen epithelium during absorption and provides energy to the rumen wall during this conversion (Ishler *et al.*, 1996). Beta-hydroxy butyrate is a ketone used to produce fatty acids, which are stored in the adipose tissue (Ishler *et al.*, 1996).

Ruminal butyrate and valerate concentrations were not different between the control and yeast supplemented cows in the present study. This is supported by Bargo *et al.* (2002c) who reported that ruminal butyrate and valerate percentages were similar for cows on three different feeding systems, and averaged 12 and 2.03%, respectively. This is in range with what had been found in the current study for ruminal butyrate (control, 11.17% and yeast, 12.35%) and valerate (control, 1.87 and yeast, 1.6%). The lack of an effect on ruminal butyrate and valerate concentrations in yeast supplemented animals was found in previous studies with dairy cows (Yoon and Stern, 1996; Putnam *et al.*, 1997; Enjalbert *et al.*, 1999; Robinson and Garrett, 1999; Erasmus *et al.*, 2005; Longuski *et al.*, 2009), steers (Mir and Mir, 1994; Moloney and Drennan, 1994; Hinman *et al.*, 1998; Lehloenya *et al.*, 2008) and sheep (Andrighetto *et al.*, 1993).

Significantly higher butyrate concentrations were measured in yeast supplemented calves at 18 (estimation from the figure of 11 vs. 5%) and 32 (estimation from the figure of 18 vs. 8%) days of age (Pinos-Rodríguez *et al.*, 2008). The same was found in other studies with cows as reported by Guedes *et al.* (2008) (11.73 and 12.3 vs. 10.95), Roa V *et al.* (1997) (15.23 vs. 13.15) and Thrune *et al.* (2009) (10.4 vs. 9.7). No effect of yeast supplementation on butyrate levels were measured in studies with steers (Olson *et al.*, 1994; Plata *et al.*, 1994), sheep (Newbold *et al.*, 1995; Arcos-García *et al.*, 2000; García *et al.*, 2000) or dairy cows (Wiedmeier *et al.*, 1987; Piva *et al.*, 1993; Doreau and Jouany, 1998; Marden *et al.*, 2008; Lascano and Heinrichs, 2009). The butyrate concentration was not different for steers consuming either alfalfa hay or corn stalk as a fibre source and supplemented with 10 g/day of a yeast culture (Roa V *et al.*, 1997). I*n vitro* addition of 0.35 g/L and 0.73 g/L of a yeast

treatment fermented on ground corn, maltose or lactate had no effect on butyrate concentration (Sullivan and Martin, 1999). This was evident in other *In vitro* studies (Newbold *et al.*, 1995; Kung *et al.*, 1997; Miller-Webster *et al.*, 2002). Yeast supplemented steers had a tendency for lower ruminal butyrate levels (Williams *et al.*, 1991) and for calves the tendency towards higher butyrate concentrations existed (Quigley *et al.*, 1992).

Higher valerate concentrations were measured in Jersey cows on a high fibre diet (Dawson *et al.*, 1990) and *In vitro* (Sullivan and Martin, 1999; Miller-Webster *et al.*, 2002). With no effect of yeast supplementation on the ruminal valerate concentration in other studies (Williams *et al.*, 1991; Quigley *et al.*, 1992). The valerate concentration tended ($P = 0.07$) to increase for yeast supplemented dairy heifers (Lascano and Heinrichs, 2009).

Highest butyrate concentrations were measured two to four hours after feeding (Guedes *et al.*, 2008). The highest concentration of butyrate and valerate measured in both treatment groups in the current study was measured approximately five hours post-afternoon feeding. There existed a significantly lower ruminal valeric acid concentration two hours post-morning feeding for the yeast supplemented cows compared with the control. Similarly *In vitro* yeast supplementation into a continuous culture, measured significantly lower valerate concentration with 200 mg Biomate Yeast Plus, compared to the 20 mg and control treatment (Kung *et al.*, 1997).

The percentage of butyrate and valerate of the total VFA is small, and often significant differences from changes in butyrate and valerate levels due to yeast supplementation, compared to the control, do not occur.

5.2.3 *In sacco* disappearance

The *In sacco* disappearance of DM, NDF and OM in ryegrass/kikuyu pastures at 12 and 24 hours incubation was higher ($P < 0.05$) for the yeast supplemented cows, compared with the control.

Yeast supplementation increased the DM degradability of hay at 12 hours of rumen incubation, and there after no effect of yeast was found (Williams *et al.*, 1991). DM disappearance was not affected by yeast supplementation according to Erasmus *et al.* (1992) for wheat straw, and Lascano and Heinrichs (2009) for a low (20%), medium (40%) or high (60%) concentrate based on ground corn and corn silage. The variable effect on the DM digestibilities of supplemented yeast cultures lies in the type of feed being digested. DM degradability of different feed resources such as barley grain, soybean meal, barley straw, barley hay, and lucerne hay in the rumen was not affected by yeast supplementation (Hadjipanayiotou *et al.*, 1997). Similarly Moloney and Drennan (1994) found that the nature of

the basal diet or the inclusion of yeast had no effect on the DM disappearance of straw. However, DM disappearance of cellulose (cotton) after 48 hours tended to decrease in the high fibre-low protein diet and tended to increase in the low fibre-high protein diet of yeast supplemented steers (Moloney and Drennan, 1994). The *In situ* degradation of DM of hay was not affected by yeast supplementation (Enjalbert *et al.*, 1999). *In vitro* DM disappearance for both substrates of alfalfa hay and Bermuda grass hay was not affected when a yeast culture was supplemented (Sullivan and Martin, 1999).

Degradabilities are affected by incubation time. Hay incubated in the rumen of steers fed a hay and barley diet, found that with yeast supplementation there was a significant increase in DM degradation at 12 hours of incubation, but no difference between the treatments for DM degradation at 24 hours of incubation (Williams *et al.*, 1991). The yeast culture or type of yeast culture supplemented had no effect on the *In situ* DM disappearance at the 12, 24, 72 and 96 hour incubation periods, though at 48 hours the yeast tended to be superior compared to the control (Arcos-García *et al.*, 2000).

I*n vitro* DM digestibility was higher for a TMR in a continuous culture, with added yeasts of Diamond V XP and A max, compared to the control (Miller-Webster *et al.*, 2002). The *In situ* degradation of DM of straw was significantly higher for NCYC 1026 at 72 and 96 ,and Yea-Sacc at 72 hours compared to the control (Newbold *et al.*, 1995). The *In situ* DM disappearance of sugar cane tops in sheep supplemented with Levucell being significantly higher at 48 hours compared to Yea-Sacc^{1026} (Arcos-García *et al.*, 2000). However, the *In situ* DM degradation was not altered by live yeast CNCM I-1077 supplementation (Doreau and Jouany, 1998) or with other various strains of a yeast culture measured *In vitro* (Newbold *et al.*, 1995).

Hovell *et al.* (1986) cited by Williams *et al.* (1991) described the possible reason for the yeast failing to affect the DM digestibility of the diet after 12 hours as bacterial numbers may shift to increase the rate of digestion of the fibre fraction in the diet. Therefore the diets digestibility is more associated with the ruminal retention time and the physiochemical characteristics of the feed, than the increased bacterial numbers and rate. The responses to yeast supplementation seem to be optimum when the environment as well as the feeding regime the cow is exposed to, compromises the cellulolytic activity in the rumen (Williams *et al.*, 1991). This type of behaviour supports the fact that the effect of the yeast may be alleviating the possible negative effects on the digestion of cellulose that the cows' environment would normally impose. The depression of cellulolytic activity is not only due to the absolute lowered level of the pH, but also to the accumulated time the pH remains at depressed levels (Williams *et al.*, 1991).

True ruminal OM digestion had increased in late June and July (early grazing season) for yeast supplemented steers grazing mixed prairie grass (Olson *et al.*, 1994). From 3 to 48 hours of rumen incubation of Berseem hay, the OM disappearance increased for the higher level of bakers yeast (22.5 g per day) supplementation compared with the control, with no effect between the levels (11.25 and 22.5 g per day) after 72 hours (Kamel *et al.*, 2004). Organic matter disappearance was not affected by yeast supplementation in steers fed a sorghum silage-based TMR (Lehloenya *et al.*, 2008) and dairy cows fed a maize-silage (60%) based diet (Doreau and Jouany, 1998). *In vitro* OM digestion was not affected by yeast treatment (Diamond V-XP & A-Max) compared with the control (Miller-Webster *et al.*, 2002). Organic matter flow to the duodenum was lower, which further supports the fact that the OM digestion tended ($P < 0.1$) to be higher in the rumen with yeast supplementation (Yoon and Stern, 1996).

Yea-Sacc^{1026} supplemented to Holstein steers found that the *In situ* NDF percentage disappearance of oat straw based diets tended to be higher after 6 hours of rumen incubation and persisted to be higher at 12 (28.6 vs. 20.2) ($P = 0.06$), 24 (43.4 vs. 36.9) ($P = 0.01$), 48 (52.5 vs. 45.3) ($P = 0.07$) and 72 (60.4 vs. 48.6) ($P = 0.08$) hours compared to the control, respectively (Plata *et al.*, 1994). These researches furthermore suggested that the increase in percentage NDF disappearance was due to a significantly higher protozoal concentration. Increasing the level of live *S. cerevisiae* supplementation from 0.3 g to 1 g/day significantly increased the degradation of NDF for the low fibre degradation group of maize silages compared to the control and the 0.3 g supplemented cows (Guedes *et al.*, 2008). It was furthermore concluded that the yeasts response is affected by not only its inclusion level, but additionally by the initial digestibilities of the maize silages as yeast had no effect on the higher NDF fibre level. Roa V *et al.* (1997) suggests that the effect of direct-fed microbials is dependent on the fibre source. The potentially digestible (PD) NDF increased from 46.6 to 55% when yeast (Yea-Sacc^{1026}) was added to complete diets with alfalfa hay fed to steers (Roa V *et al.*, 1997). *In situ* NDF disappearances being significantly ($P = 0.002$) higher for the Yea-Sacc^{1026} than for the Levucell at 48 hours (Arcos-García *et al.*, 2000). The beneficial effect of live yeast supplementation in the present study may be similar to that suggested by Guedes *et al.* (2008) and Roa V *et al*. (1997) which are due to the relatively good quality ryegrass pasture and acceptable NDF levels associated. Yeast supplementation had not improved the NDF digestibilities in other studies (Miranda *et al.*, 1996; Yoon and Stern, 1996; Doreau and Jouany, 1998; Enjalbert *et al.*, 1999; Miller-Webster *et al.*, 2002; Lehloenya *et al.*, 2008).

The *In situ* degradation of ADF in corn stalk was significantly higher at six hours incubation for the yeast CNCM I-1077 supplemented cows, with no significant difference found at other times (Doreau and Jouany, 1998). Doreau and Jouany (1998) reported that the first six hours are coupled with an increased concentration of live yeast cells, which in turn promotes carbohydrate digestion. Acid detergent fibre disappearance was not affected by yeast supplementation according to the following authors (Kamalamma *et al.*, 1996; Yoon and Stern, 1996; Enjalbert *et al.*, 1999; Lehloenya *et al.*, 2008).

In situ N degradation of soybean meal was significantly higher at four hours and tended to be higher at eight hours which suggests that the proteolytic bacteria were stimulated to degraded the protein (Doreau and Jouany, 1998). The potentially digestible CP, increased from 85.7 to 90.2 % when yeast (Yea-Sacc^{1026}) was added to complete diets with alfalfa hay fed to steers (Roa V *et al.*, 1997). N disappearance was not affected by yeast supplementation according to the following authors (Hadjipanayiotou *et al.*, 1997; Kamel *et al.*, 2004).

5.3 Yeast Count

Heat stability and processing are factors to consider when including a live yeast product as the stability and viability of the product may be compromised. Levucell SC 10 ME - Titan is a micro-encapsulated formulation where the live yeast cells are protected and remain stable to heat extremes of 112 °C (20 – 30 seconds) where yeast cells had survived (personal communication, 2010, R. Venter, richardtv@vitam.co.za). Dairy meal pelleting temperatures in South Africa range between 50 and 60 ° C (personal communication, 2010, R. Venter, richardtv@vitam.co.za). The viability of the product used in this study, therefore was not compromised at all.

CHAPTER 6

ECONOMIC EVALUATION

CHAPTER 6

Economic Evaluation

The economic implication of supplementing yeast to cows grazing ryegrass/kikuyu pasture is illustrated with application of the results from the current study to a hypothetical situation. This situation closely resembles the farming herd structure and mean milk price in the Southern Cape at the time that the study was conducted. An average herd size was assumed to consist of 280 lactating cows. The milk price was obtained by Nestle to determine the milk price within each experimental treatment represented in Table 6.1. According to the results obtained with regards to milk fat, it is clear that a milk payment scheme that favours higher milk fat content or yield, would increase the milk price obtained. In such situations yeast supplementation would be more favourable. Milk payments schemes are considered confidential by the milk buyers, therefore stating additional hypothetical situations was not considered necessary.

Table 6.1 The milk price calculated according to the fat and protein percentages for cows grazing ryegrass/kikuyu pasture supplemented with 6 kg/day (as is) of a dairy concentrate and live yeast (1 g/cow/day)

Parameter	Experimental treatments[1]	
	Control	Yeast
Milk yield (kg/cow/day)	20.08	19.67
Milk yield (Kg/day) for 280 cows	5622	5508
4% FCM[3] (kg/cow/day)	20.05	20.32
4% FCM (kg/day) for 280 cows	5614	5690
Milk fat %	3.99	4.24
Milk protein %	3.51	3.58
Milk lactose %	4.68	4.73
MUN (mg/dL)	10.72	11.04
Milk price (R^2/L/day)	3.04	3.13

[1]Control: dairy concentrate containing no yeast; Yeast: dairy concentrate containing yeast at 167g/ ton or 1 g/cow/day

[2]R- South African rand

[3]FCM: fat corrected milk yield

The performance of supplementing yeast for an average herd over a month can be calculated and described using the financial ratio (Phillips, 2002); return on investment:

(ROI) % = [(benefits – costs)/costs]*100

And is represented in Table 6.2.

Table 6.2 The return on investment for a herd consisting of 280 cows grazing ryegrass/kikuyu pasture and supplemented with 6 kg/day (as is) of a dairy concentrate and live yeast (1 g/cow/day)

Item	**Experimental treatments[1]**	
	Control	**Yeast**
Herd 280 milk yield (kg/month[3])	168672	165228
Milk income (R[2]/month[3])	512762.88	517163.64
Benefit[6]	-	4400.76
Cost	-	4200
Yeast[4] (R[2]/month[3])	-	4200
ROI %[5] per month[3]	-	4.78

[1]Control: dairy concentrate containing no yeast; Yeast: dairy concentrate containing yeast at 167g/ton

[2]R- South African rand

[3]30 days equivalent to one month

[4]Supplemented at 1 g per cow per day; the cost R0.50 per gram (Lallemand specifications)

[5]Return on investment; ROI % = [(benefits – costs)/costs] x 100

[6] Milk price (R[2]/month[3]) for the yeast – Milk price (R[2]/month[3]) for the control

As stated by Hutjens (2003) the anticipated response, economic return, available research and field responses are variables to asses in considering the use of a specific feed additive. The benefit to cost ratio of yeast and yeast cultures in previous research with yeast supplementation has been reported to be 4:1 and its status thereafter is recommended (Hutjens, 2003). The benefit to cost ratio in the current study is 1.05:1. According to Hutjens (2003) a guideline to follow for considering the use of an additive is if two or more rand is benefited for every one rand invested. To achieve the 4:1 benefit to cost ratio as stated by Hutjens (2003), the yeast supplemented cows should have produced 0.5 litres more than the control cows, but had only produced 0.2 litres more. The yeast supplement in the current

study according to Hutjens (2003) would therefore not be feasible when a 5% return on investment is achieved.

CHAPTER 7

CONCLUSION

CHAPTER 7

Conclusion

Cows grazing high quality pasture are often supplemented with high levels of concentrate after milking resulting in reduced performance due to lower rumen pH, reduced fibre digestibility and depressed dry matter intake. Supplementation with yeast has improved performance of TMR fed cows but limited data is available for grazing cows.

Supplementation with live yeast (1 g of Levucell SC 10 ME-Titan) did not affect milk yield, 4% FCM, milk protein % or BW change ($P > 0.05$). Results for control and yeast supplemented cows were as follows; milk yield (20.1 and 19.7 kg/d), 4% FCM (20.1 and 20.3 kg/d), milk protein % (3.51 and 3.56) and BW change (+37.8 and +36.4 kg/cow). Milk fat %, however, was increased ($P < 0.05$) by live yeast supplementation (3.99 and 4.24%), respectively. Using a generalized milk payment formula that takes milk solids into account, it could increase milk price from R 3.04 to R3.13/litre. The cost of the supplement, therefore, would largely determine the economic viability.

In addition, an *In sacco* study revealed that NDF disappearance was 46.6 and 52.2% after 12 hours and 65.1 and 69.2% after 24 hours of rumen incubation for control and live yeast supplementated cows. Live yeast supplementation increased NDF disappearance at 12 hours by 11.9% ($P < 0.05$). None of the other rumen fermentation parameters, however, were affected by live yeast supplementation.

Results suggest that grazing dairy cows may benefit from live yeast supplementation through increased milk fat% and increased ruminal fibre digestibility.

Future research could further investigate the effects of interfering factors on yeast supplementation through the use of meta-analyses, that would clearly define responses to yeast supplementation.

CHAPTER 8

CRITICAL EVALUATION

CHAPTER 8

Critical Evaluation

8.1 Production Study

8.1.1 Cow numbers

In the current study 30 cows were used in the production study (15 cows per treatments representing 15 blocks), and 10 cows were used in the rumen study (5 cows per treatment representing 5 blocks). If such a study was conducted in which the number of cows, blocks and therefore replicates is not limited to available facilities and cows, it would then increase the precision for treatment means that could possibly contribute to finding significant differences between treatments. Universities and research institutions are urged to maintain and increase the size of their dairy herds in order to conduct meaningful dairy cattle research.

8.1.2 Pasture intake estimation

The pasture height estimation was calculated from the use of the RPM. This method as stated previously is inaccurate. The practical reality of accommodating grazing cows on pasture makes it impossible to determine individual cow intake within a herd. Therefore a herd average would have to suffice from estimations using the RPM. Large variations which create large errors when using a RPM originate from the operator, technique or pattern of sampling, correct cutting of regressions which are representative to pasture grazed, climatic conditions etc. Intake drives production, and in research it remains a challenge to accurately estimate intake of individual cows on pasture. Additional research in this field is encouraged.

8.1.3 Yeast dosage-supplementation

The dosage level of supplemented Levucell was 1 g of yeast per cow per day, comparable to previous research where levels are as high as 10, 56 or 90 g, this level is low. Though levels are recommended on number of CFU which vary between products, which gives the differing dosage levels, it would have been interesting to investigate a higher inclusion level above the recommended 1 g per cow per day.

8.1.4 Milk sampling

The milking machine is set to test, which is a setting that governs the milk sampling process. At this setting the milking machine diverts a specific volume of milk per litre milk recorded by each animal. The diverted milk is collected in a milk sample bottle connected at

each milk-meter which may then be manually removed after which the composite milk sample is then taken. The flow rate of milk being collected at each milk-meter varied, which was evident by the extreme difference in certain milk volumes collected in the sampling bottles. This would create large errors in that a representative sample was not collected. Furthermore, simply pouring the milk into sample bottles creates variation in results as milk solids separate. To avoid this, once removing the each sampling bottle, the bottle was swirled and the mouth sealed (with the hand) and physically rotated and turned over back and forth, to distribute the milk solids before collecting the composite sample.

8.2 Rumen Study

8.2.1 *In sacco* disappearance incubation periods

The incubation periods of 12 and 24 hours had resulted in creating a linear regression from two points. The results from the current study reveal the significant difference in the disappearance of DM, OM and NDF at both incubation periods differed significantly ($P < 0.05$). The picture therefore described over the incubation period would be more informative if additional incubation periods were established. The additional periods of incubation (0, 2, 4, 8, 12, 18, 24, 36, and 72 hours) would have been of value as degradation rate could then have been determined for different treatments. Including the additional time periods, the degradation rate could be more correctly estimated, established and compared between treatment groups. Alternatively a six hour time point would have enabled the use of the Van Amburgh rate calculator to estimate rate of NDF disappearance. The use of two time points was due to cost implications.

8.2.2 Rumen sampling times

There were four set times at which rumen samples was collected and this was repeated after the cross-over of treatments. The question then arises how representative is the rumen sampling? bearing in mind that the sample is collected from a rumen with an approximate volume of 150 litres for each Jersey cow. Are four samplings adequate to describe the fermentation patterns? More sampling times at least shortly after feeding, would better describe the effect of concentrate supplementation. This however, would increase labour and a corresponding increase in the budget.

8.2.3 Rumen sampling acids

The rumen samples were analyzed for VFA and not for lactic acid. The lactic acid which has a pK_a value lower than the major VFA (propionate, butyrate and acetate) may have a

greater influence on the overall ruminal pH.Therefore the question that arises is whether lactic acid sampling should have been done? With this in mind, the nadir pH for the current study was 5.8, and lactic acid accumulation would be unlikely and therefore lactic acid sampling was not needed.

8.2.4 Ruminal micro flora

The ruminal microbes consisting of the major cellulolytic and amylolytic bacteria, the protozoa and the fungi should have been sampled and counted within each treatment group. In previous studies the bacterial numbers of specific species have assisted in explaining the fermentation patterns produced as a result of yeast supplementation and subsequent findings. In South Africa, however, there is a lack of laboratories capable of performing high level rumen microbiological work.

8.2.5 Ruminal yeast count

It has been shown in previous research that different strains of yeast differ in their modes of action. This being said, the viability of yeast cells in the rumen could possibly differ between strains. The specific strain of yeast used in this study was (*Saccharomyces cerevisiae* CNCM I-1077) registered at the Pasteur Institute collection (CNCM), Paris, under the number I-1077, is a product manufactured as Levucell SC 10 ME – Titan which was produced from batch number 22aIN17UVS008. The viability of this specific strain could have been tested against time in the rumen, to more effectively explain the life cycle and its effect in the rumen.

8.2.6 Concluding remark

The need for more samples, analysis and animals in order to achieve more accurate results on the one hand, and financial constraints on the other hand, will always remain a balancing act.

REFERENCES

Abd El-Ghani, A.A., 2004. Influence of diet supplementation with yeast culture (*Saccharomyces cerevisiae*) on performance of Zaraibi goats. Small Ruminant Research 52, 223-229.

Adams, A.L., Harris, B., Jr., Van Horn, H.H., Wilcox, C.J., 1995. Effects of Varying Forage Types on Milk Production Responses to Whole Cottonseed, Tallow, and Yeast. J. Dairy Sci. 78, 573-581.

Aguirre-Guzmán, G., Ricque-Marie, D., Cruz-Suárez, L.E., 2002. Survival of agglomerated *Saccharomyces cerevisiae* in pelleted shrimp feeds. Aquaculture 208, 125-135.

Allen, M.S., 1996. Physical constraints on voluntary intake of forages by ruminants. J. Anim Sci. 74, 3063-3075.

Andrighetto, I., Bailoni, L., Cozzi, G., Berzaghi, P., 1993. Effects of yeast culture addition on digestion in sheep fed a high concentrate diet. Small Ruminant Research 12, 27-34.

Angeles C, S.C., Mendoza M, G.D., Cobos P, M.A., Crosby G, M.M., Castrejón P, F.A., 1998. Comparison of two commercial yeast cultures (*Saccharomyces cerevisiae*) on ruminal fermentation and digestion in sheep fed on corn-stover diet. Small Ruminant Research 31, 45-50.

Angeles , C.S.C., Mendoza, M.G.D., Cobos, P.M.A., Crosby, G.M.M., Castrejón, P.F.A., 1998. Comparison of two commercial yeast cultures (*Saccharomyces cerevisiae*) on ruminal fermentation and digestion in sheep fed on corn-stover diet. Small Ruminant Research 31, 45-50.

AOAC, 2000, procedure 920.39. Official method of analysis 920.39 (17th Edition) Volume I. Association of Official Analytical Chemists, Inc. Maryland, USA.

AOAC, 2000, procedure 934.01. Official method of analysis 934.01 (17th Edition) Volume I. Association of Official Analytical Chemists, Inc.Maryland, USA. .

AOAC, 2000, procedure 942.05. Official method of analysis 942.05 (17th Edition) Volume I. Association of Official Analytical Chemists, Inc.Maryland, USA.

AOAC, 2000, procedure 965.09. Official method of analysis 965.09 (17th Edition) Volume I. Association of Official Analytical Chemists, Inc.Maryland, USA.

AOAC, 2000, procedure 965.17. Official method of analysis 965.17 (17th Edition) Volume I. Association of Official Analytical Chemists, Inc. Maryland, USA.

AOAC, 2000, procedure 968.06. Official method of analysis 968.06 (17th Edition) Volume I. Association of Official Analytical Chemists, Inc. Maryland, USA. .

Arakaki, L.C., Stahringer, R.C., Garrett, J.E., Dehority, B.A., 2000. The effects of feeding monensin and yeast culture, alone or in combination, on the concentration and generic composition of

rumen protozoa in steers fed on low-quality pasture supplemented with increasing levels of concentrate. Animal Feed Science and Technology 84, 121-127.

Arambel, M.J., Kent, B.A., 1990. Effect of Yeast Culture on Nutrient Digestibility and Milk Yield Response in Early- to Midlactation Dairy Cows. J. Dairy Sci. 73, 1560-1563.

Arcos-García, J.L., Castrejón, F.A., Mendoza, G.D., Pérez-Gavilán, E.P., 2000. Effect of two commercial yeast cultures with Saccharomyces cerevisiae on ruminal fermentation and digestion in sheep fed sugar cane tops. Livestock Production Science 63, 153-157.

Bach, A., Calsamiglia, S., Stern, M.D., 2005. Nitrogen Metabolism in the Rumen. Journal of Dairy Science 88, E9-E21.

Bach, A., Iglesias, C., Devant, M., 2007. Daily rumen pH pattern of loose-housed dairy cattle as affected by feeding pattern and live yeast supplementation. Animal Feed Science and Technology 136, 146-153.

Balch, D.A., Rowland, S.J., 1957. Volatile fatty acids and lactic acid in the rumen of dairy cows receiving a variety of diets. British Journal of Nutrition 11, 288-298.

Bargo, F., Muller, L.D., Delahoy, J.E., Cassidy, T.W., 2002a. Milk Response to Concentrate Supplementation of High Producing Dairy Cows Grazing at Two Pasture Allowances. J. Dairy Sci. 85, 1777-1792.

Bargo, F., Muller, L.D., Delahoy, J.E., Cassidy, T.W., 2002b. Performance of High Producing Dairy Cows with Three Different Feeding Systems Combining Pasture and Total Mixed Rations. J Dairy Sci 85, 2948-2963.

Bargo, F., Muller, L.D., Kolver, E.S., Delahoy, J.E., 2003. Invited Review: Production and Digestion of Supplemented Dairy Cows on Pasture. J. Dairy Sci. 86, 1-42.

Bargo, F., Muller, L.D., Varga, G.A., Delahoy, J.E., Cassidy, T.W., 2002c. Ruminal digestion and fermentation of high-producing dairy cows with three different feeding systems combining pasture and total mixed rations. J Dairy Sci 85, 2964-2973.

Beauchemin, K.A., Yang, W.Z., Morgavi, D.P., Ghorbani, G.R., Kautz, W., Leedle, J.A.Z., 2003. Effects of bacterial direct-fed microbials and yeast on site and extent of digestion, blood chemistry, and subclinical ruminal acidosis in feedlot cattle. J. Anim Sci. 81, 1628-1640.

Besong, S., Jackson, J.A., Hicks, C.L., Hemken, R.W., 1996. Effects of a Supplemental Liquid Yeast Product on Feed Intake, Ruminal Profiles, and Yield, Composition, and Organoleptic Characteristics of Milk from Lactating Holstein Cows. J. Dairy Sci. 79, 1654-1658.

Botha, P.R., 2003. Die Produksiepotensiaal van Oorgesaaide Kikoejoeweiding in die Gematigde Kusgebied van die Suid-Kaap. University of the Free-State. PhD.

Botha, P.R., Meeske, R., Snyman, H.A., 2008a. Kikuyu over-sown with ryegrass and clover: dry matter production, botanical composition and nutritional value. African journal of Range and Forage science 25(3), 93-101.

Botha, P.R., Meeske, R., Snyman, H.A., 2008b. Kikuyu over-sown with ryegrass and clover:grazing capacity, milk production and milk composition. African journal of Range and Forage science 25(3), 103-110.

Broderick, G.A., Kang, J.H., 1980. Automated Simultaneous Determination of Ammonia and Total Amino Acids in Ruminal Fluid and In Vitro Media1. J Dairy Sci 63, 64-75.

Bruno, R.G.S., Rutigliano, H.M., Cerri, R.L., Robinson, P.H., Santos, J.E.P., 2009. Effect of feeding *Saccharomyces cerevisiae* on performance of dairy cows during summer heat stress. Animal Feed Science and Technology 150, 175-186.

Cabrera, E.J.I., Mendoza, M.G.D., Aranda, I.E., Garcia-Bojalil, C., Bárcena, G.R., Ramos, J.J.A., 2000. *Saccharomyces cerevisiae* and nitrogenous supplementation in growing steers grazing tropical pastures. Animal Feed Science and Technology 83, 49-55.

Callaway, E.S., Martin, S.A., 1997. Effects of a *Saccharomyces cerevisiae* Culture on Ruminal Bacteria that Utilize Lactate and Digest Cellulose. J. Dairy Sci. 80, 2035-2044.

Carro, M.D., Lebzien, P., Rohr, K., 1992. Influence of yeast culture on the *in vitro* fermentation (Rusitec) of diets containing variable portions of concentrates. Animal Feed Science and Technology 37, 209-220.

Caton, J.S., Dhuyvetter, D.V., 1997. Influence of energy supplementation on grazing ruminants:requirements and responses. J Animal Sci 75, 533-542.

Chaucheyras-Durand, F., Masséglia, S., Fonty, G., 2005. Effect of the Microbial Feed Additive *Saccharomyces cerevisiae* CNCM I-1077 on Protein and Peptide Degrading Activities of Rumen Bacteria Grown In Vitro. Current Microbiology 50, 96-101.

Chaucheyras-Durand, F., Walker, N.D., Bach, A., 2008. Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. Animal Feed Science and Technology 145, 5-26.

Chaucheyras-Durand, F.d.r., Fonty, G.r., 2002. Influence of a Probiotic Yeast (*Saccharomyces cerevisiae* CNCM I-1077) on Microbial Colonization and Fermentations in the Rumen of Newborn Lambs. Microbial Ecology in Health and Disease 14, 30 - 36.

Chiquette, J., 2009. Evaluation of the protective effect of probiotics fed to dairy cows during a subacute ruminal acidosis challenge. Animal Feed Science and Technology 153, 278-291.

Chu, M.I., Hartig, A., Freese, E.B., Freese, E., 1981. Adaptation of Glucose-grown *Saccharomyces cerevisiae* to Gluconeogenic Growth and Sporulation. J Gen Microbiol 125, 421-430.

Cole, N.A., Purdy, C.W., Hutcheson, D.P., 1992. Influence of yeast culture on feeder calves and lambs. J. Anim Sci. 70, 1682-1690.

Cooke, K.M., Bernard, J.K., West, J.W., 2007. Performance of Lactating Dairy Cows Fed Whole Cottonseed Coated with Gelatinized Starch plus Urea or Yeast Culture. J. Dairy Sci. 90, 360-364.

Council, A.R., 2009. Agro-Climatology database. The Agricultural Research Council's Institute for Soil Climate and Water, Department of Agro-Climatology. R. Wentzel, wentzelr@arc.agric.za. Stellenbosch.

Cruywagen, C.W., 2006. Technical Note: A Method to Facilitate Retrieval of Polyester Bags Used in *in sacco* Trials in Ruminants. Journal of Dairy Science 89, 1028-1030.

Dalley, D.E., Roche, J.R., Moate, P.J., Grainger, C., 2001. More frequent allocation of herbage does not improve the milk production of dairy cows in early lactation. Aust. J. Exp. Agric 39, 923-931.

Dann, H.M., Drackley, J.K., McCoy, G.C., Hutjens, M.F., Garrett, J.E., 2000. Effects of Yeast Culture (*Saccharomyces cerevisiae*) on Prepartum Intake and Postpartum Intake and Milk Production of Jersey Cows. J. Dairy Sci. 83, 123-127.

Dawson, K.A., Newman, K.E., Boling, J.A., 1990. Effects of microbial supplements containing yeast and lactobacilli on roughage-fed ruminal microbial activities. J. Anim Sci. 68, 3392-3398.

Desnoyers, M., Giger-Reverdin, S., Bertin, G., Duvaux-Ponter, C., Sauvant, D., 2009a. Meta-analysis of the influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminants. J. Dairy Sci. 92, 1620-1632.

Desnoyers, M., Giger-Reverdin, S., Sauvant, D., Bertin, G., Duvaux-Ponter, C., 2009b. The influence of acidosis and live yeast (*Saccharomyces cerevisiae*) supplementation on time-budget and feeding behaviour of dairy goats receiving two diets of differing concentrate proportion. Applied Animal Behaviour Science 121, 108-119.

Doreau, M., Jouany, J.P., 1998. Effect of a *Saccharomyces cerevisiae* Culture on Nutrient Digestion in Lactating Dairy Cows. J. Dairy Sci. 81, 3214-3221.

Dove, H., Mayes, R.W., 1991. The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: a review. Australian Journal of Agricultural Research 42, 913-952.

Earle, D.F., McGowan, A.A., 1979. Evaluation and calibration of an automated rising plate meter for estimating dry matter yield of pasture. Australian Journal of Experimental Agriculture 19, 337-343.

Eastridge, M.L., 2006. Major advances in applied dairy cattle nutrition. J Dairy Sci 89, 1311-1323.

Enemark, J.M.D., 2008. The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. The Veterinary Journal 176, 32-43.

Enjalbert, F., Garrett, J.E., Moncoulon, R., Bayourthe, C., Chicoteau, P., 1999. Effects of yeast culture (*Saccharomyces cerevisiae*) on ruminal digestion in non-lactating dairy cows. Animal Feed Science and Technology 76, 195-206.

Erasmus, L., 2009. Milk production from cows grazing kikuyu-ryegrass pasture systems. University of Pretoria. MSc thesis.

Erasmus, L.J., 2000. Associative effects in dairy feeds. Afma Matrix (South Africa) 9(1), 5-9.

Erasmus, L.J., Botha, P.M., Kistner, A., 1992. Effect of Yeast Culture Supplement on Production, Rumen Fermentation, and Duodenal Nitrogen Flow in Dairy Cows. J. Dairy Sci. 75, 3056-3065.

Erasmus, L.J., Robinson, P.H., Ahmadi, A., Hinders, R., Garrett, J.E., 2005. Influence of prepartum and postpartum supplementation of a yeast culture and monensin, or both, on ruminal fermentation and performance of multiparous dairy cows. Animal Feed Science and Technology 122, 219-239.

Erdman, R.A., Sharma, B.K., 1989. Effect of Yeast Culture and Sodium Bicarbonate on Milk Yield and Composition in Dairy Cows. J. Dairy Sci. 72, 1929-1932.

EU, 2009. European Feed Additives and PreMixtures Quality System. European Code of Practice for Feed Additive and Premixture Operators, page 6.

Faverdin, P., Baumont, R., Ingvartsen, K.L., 1995. Control and prediction of feed intake in ruminants. In: Journet, M., Grenet, E., Farce, M.-H., Theriez, M., Demarquilly, C. (Eds.), Recent developments in the nutrition of herbivores. Proceedings of the IVth International Symposium on the Nutrition of Herbivores. INRA Editions, Paris, pp. 95-120.

Faverdin, P., Dulphy, J.P., Coulon, J.B., Vérité, R., Garel, J.P., Rouel, J., Marquis, B., 1991. Substitution of roughage by concentrates for dairy cows. Livestock Production Science 27, 137-156.

Forbes, J.M., 2007. Voluntary food intake and diet selection in farm animals. CABI.

Fulkerson, W.J., 1997. Use of the rising plate meter to allocate pasture. Research to Farm. NSW Agriculture Wollungbar Agricultural Institute, May 1997.

Fulkerson, W.J., Donaghy, D.J., 2001. Plant soluble carbohydrate reserves and senescence- key criteria for developing an effective grazing management system for ryegrass-based pastures: a review. Aust. J. Exp. Agric 41, 261-275.

Fulkerson, W.J., Nandra, K.S., Clark, C.F., Barchia, I., 2006. Effect of cereal-based concentrates on productivity of Holstein-Friesan cows grazing short-rotation ryegrass (*Lolium multiflorum*) or kikuyu (*Pennisetum cladestinum*) pastures. Journal of Livestock Science 103, 85-94.

Fulkerson, W.J., Neal, J.S., Clark, C.F., Horadagoda, A., Nandra, K.S., Barchia, I., 2007. Nutritive value of forage species grown in the warm temperate climate of Australia for dairy cows: Grasses and legumes Journal of Livestock Science 107, 253-264.

García, C.C.G., Mendoza, M.G.D., González, M.S., Cobos, P.M., Ortega, C.M.E., Ramirez, L.R., 2000. Effect of a yeast culture (*Saccharomyces cerevisiae*) and monensin on ruminal fermentation and digestion in sheep. Animal Feed Science and Technology 83, 165-170.

Giger-Reverdin, S., Bezault, N., Sauvant, D., Bertin, G., 1996. Effects of a probiotic yeast in lactating ruminants: interaction with dietary nitrogen level. Animal Feed Science and Technology 63, 149-162.

Glade, M.J., Biesik, L.M., 1986. Enhanced Nitrogen Retention in Yearling Horses Supplemented with Yeast Culture. J. Anim Sci. 62, 1635-1640.

Goering, H.K., Soest, P.J., 1970. Forage Fiber Analyses (Apparatus, reagents, procedures and some applications). Agricultural Handbook No.379.A.R.S, U.S. Dept. of Agric. ADSRI - JAN 1988.

Grainger, C., Mathews, G.L., 1989. Positive relation between substitution rate and pasture allowance for cows receiving concentrates. Australian Journal of Experimental Agriculture 29, 355-360.

Grochowska, S., Nowak, W., Mikula, R., 2009. Effect of *Saccharomyces cerevisiae* live cells and *Saccharomyces cerevisiea* culture on the performance and blood biochemical indices in dairy cows. Bull Vet Inst Pulawy 53, 747-751.

Guedes, C.M., Gonçalves, D., Rodrigues, M.A.M., Dias-da-Silva, A., 2008. Effects of a *Saccharomyces cerevisiae* yeast on ruminal fermentation and fibre degradation of maize silages in cows. Animal Feed Science and Technology 145, 27-40.

Haddad, S.G., Goussous, S.N., 2005. Effect of yeast culture supplementation on nutrient intake, digestibility and growth performance of Awassi lambs. Animal Feed Science and Technology 118, 343-348.

Hadjipanayiotou, M., Antoniou, I., Photiou, A., 1997. Effects of the inclusion of yeast culture on the performance of dairy ewes and goats and the degradation of feedstuffs. Livestock Production Science 48, 129-134.

Harris, B., Jr., Dorminey, D.E., Smith, W.A., Van Horn, H.H., Wilcox, C.J., 1992. Effects of Feather Meal at Two Protein Concentrations and Yeast Culture on Production Parameters in Lactating Dairy Cows. J. Dairy Sci. 75, 3524-3530.

Harrison, G.A., Hemken, R.W., Dawson, K.A., Harmon, R.J., Barker, K.B., 1988. Influence of Addition of Yeast Culture Supplement to Diets of Lactating Cows on Ruminal Fermentation and Microbial Populations. J. Dairy Sci. 71, 2967-2975.

Hinman, D.D., Sorensen, S.J., Momont, P.A., 1998. Effect of Yeast Culture on Steer Performance, Apparent Diet Digestibility, and Carcass Measurements When Used in a Barley and Potato Finishing Diet. The Professional Animal Scientist 14, 173-177.

Hodgson, J., 1985. The control of herbage intake in the grazing ruminant. Proceedings of the Nutrition Society 44, 339-346.

Holden, L.A., Muller, D.L., Varga, G.A., Hillard, P.J., 1994. Ruminal digestion and duodenal nutrient flows in dairy cows consuming grass as pasture, Hay or silage. J Dairy Sci 77, 3034-3042.

Hutjens, M., 2003. Economics of feed additives. Penn State Dairy Cattle Nutrition Workshop. University of Illinois, Urbana, pp. 1-4.

IDF, 1990. International Dairy Federation, Standard 94B. Milk and milk products/ enumeration of yeasts and moulds. .

IDF, 1996. International Dairy Federation, Standard 141B, IDF General Secretariat, DiAmmannt Building, Boulevard Auguste Reyers 80, 1030, Brussels, Belguim, E-mail: info@fil-idf.org.

Ishler, V., Heinrichs, J., Varga, G.A., 1996. From feed to milk:understanding rumen function. Extension circular 422. Pennsylvania state university, university park, PA, pp. 1-27.

Jenkins, T.C., McGuire, M.A., 2006. Major advances in nutrition: Impact on milk composition. J Dairy Sci 89, 1302-1310.

Jonker, J.S., Kohn, R.A., Erdman, R.A., 1998. Using milk urea nitrogen excretion and utilization efficiency in lactating dairy cows. J Dairy Sci 81, 2681-2692.

Jonker, J.S., Kohn, R.A., Erdman, R.A., 1999. Milk urea nitrogen target concentrations for lactating dairy cows fed according to national research council recommendations. J Dairy Sci 82, 1261-1273.

Jouany, J.P., Gobert, J., Medina, B., Bertin, G., Julliand, V., 2008. Effect of live yeast culture supplementation on apparent digestibility and rate of passage in horses fed a high-fiber or high-starch diet. J. Anim Sci. 86, 339-347.

Jouany, J.P., Medina, B., Bertin, G., Julliand, V., 2009. Effect of live yeast culture supplementation on hindgut microbial communities and their polysaccharidase and glycoside hydrolase activities in horses fed a high-fiber or high-starch diet. J. Anim Sci. 87, 2844-2852.

Kalmus, P., Orro, T., Waldmann, A., Lindjarv, R., Kask, K., 2009. Effect of yeast culture on milk production and metabolic and reproductive performance of early lactation dairy cows. Acta Veterinaria Scandinavica 51, 32.

Kamalamma, Krishnamoorthy, U., Krishnappa, P., 1996. Effect of feeding yeast culture (Yea-sacc1026) on rumen fermentation *in vitro* and production performance in crossbred dairy cows. Animal Feed Science and Technology 57, 247-256.

Kamel, H.E.M., Sekine, J., El-Waziry, A.M., Yacout, M.H.M., 2004. Effect of *Saccharomyces cerevisiae* on the synchronization of organic matter and nitrogen degradation kinetics and microbial nitrogen synthesis in sheep fed Berseem hay (*Trifolium alexandrinum*). Small Ruminant Research 52, 211-216.

Kellaway, R., Harrington, T., 2004. Feeding concentrates supplements for dairy cows. Landlinks Press, 150 Oxford Street, Collingwood VIC 3066, Australia.

Kellaway, R., Porta, S., 1993. Feeding concentrates supplements for dairy cows. Dairy Research and Development Corporation, Melbourne, Australia.

Kellems, R.O., Lagerstedt, A., Wallentine, M.V., 1990. Effect of Feeding *Aspergillus oryzae* Fermentation Extract or *Aspergillus oryzae* plus Yeast Culture plus Mineral and Vitamin Supplement on Performance of Holstein Cows During a Complete Lactation. J. Dairy Sci. 73, 2922-2928.

Kim, H.S., Ahn, B.S., Chung, S.G., Moon, Y.H., Ha, J.K., Seo, I.J., Ahn, B.H., Lee, S.S., 2006. Effect of yeast culture, fungal fermentation extract and non-ionic surfactant on performance of Holstein cows during transition period. Animal Feed Science and Technology 126, 23-29.

Kolver, E.S., 2003. Nutritional limitations to increased production on pasture-based systems. Proceedings of the Nutrition Society 62, 291-300.

Kolver, E.S., Muller, L.D., 1998. Performance and Nutrient Intake of High Producing Holstein Cows Consuming Pasture or a Total Mixed Ration. J. Dairy Sci. 81, 1403-1411.

Kolver, E.S., Muller, L.D., Varga, G.A., 1998. Ruminal utilisation of pasture nitrogen in response to inclusion of dietary starch. Proceedings of the New Zealand Society of Animal Production, pp. 106-109.

Kotz, J.C., Treichel, P.M., Harman, P.A., 2003. Chemisty & Chemical Reactivity. Thomson Brooks/Cole, USA.

Kung, L., Jr., Kreck, E.M., Tung, R.S., Hession, A.O., Sheperd, A.C., Cohen, M.A., Swain, H.E., Leedle, J.A.Z., 1997. Effects of a Live Yeast Culture and Enzymes on *in vitro* Ruminal Fermentation and Milk Production of Dairy Cows. J. Dairy Sci. 80, 2045-2051.

Lambert, M.G., Litherland, A.J., 2000. A practioner's guide to pasture quality. Proceedings of the New Zealand Grassland Association, pp. 111-115.

Lascano, G.J., Heinrichs, A.J., 2009. Rumen fermentation pattern of dairy heifers fed restricted amounts of low, medium, and high concentrate diets without and with yeast culture. Livestock Science 124, 48-57.

Lascano, G.J., Zanton, G.I., Heinrichs, A.J., 2009a. Concentrate levels and *Saccharomyces cerevisiae* affect rumen fluid-associated bacteria numbers in dairy heifers. Livestock Science 126, 189-194.

Lascano, G.J., Zanton, G.I., Suarez-Mena, F.X., Heinrichs, A.J., 2009b. Effect of limit feeding high- and low-concentrate diets with *Saccharomyces cerevisiae* on digestibility and on dairy heifer growth and first-lactation performance. J. Dairy Sci. 92, 5100-5110.

Lehloenya, K.V., Krehbiel, C.R., Mertz, K.J., Rehberger, T.G., Spicer, L.J., 2008. Effects of Propionibacteria and Yeast Culture Fed to Steers on Nutrient Intake and Site and Extent of Digestion. J. Dairy Sci. 91, 653-662.

Linn, J., Salfer, J., 2006. Feed Efficiency. University of Minnesota Extension Service www.extension.umn.edu/dairy.

Longuski, R.A., Ying, Y., Allen, M.S., 2009. Yeast culture supplementation prevented milk fat depression by a short-term dietary challenge with fermentable starch. J. Dairy Sci. 92, 160-167.

Lowe, K.F., Bowdler, T.M., Casey, N.D., Moss, R.J., 1999. Performance of temperate perennial pastures in the Australian subtropics 1. Yeild, persistence and pasture quality. Aust. J. Exp. Agric 39, 663-676.

Lynch, H.A., Martin, S.A., 2002. Effects of *Saccharomyces cerevisiae* Culture and *Saccharomyces cerevisiae* Live Cells on *in vitro* Mixed Ruminal Microorganism Fermentation. J. Dairy Sci. 85, 2603-2608.

Macoon, B., Sollenberger, L.E., Moore, J.E., Staples, C.R., Fike, J.H., Portier, K.M., 2003. Comparison of three techniques for estimating the forage intake of lactating dairy cows on pasture. J. Anim Sci. 81, 2357-2366.

Magalhaes, V.J.A., Susca, F., Lima, F.S., Branco, A.F., Yoon, I., Santos, J.E.P., 2008. Effect of Feeding Yeast Culture on Performance, Health, and Immunocompetence of Dairy Calves. J. Dairy Sci. 91, 1497-1509.

Malcolm, K.J., Kiesling, H.E., 1990. Effects of whole cottonseed and live yeast culture on ruminal fermentation and fluid passage rate in steers. J. Anim Sci. 68, 1965-1970.

Malleson, E.R., 2008. Fishmeal supplementation to high producing Jersey cows grazing ryegrass or kikuyu pasture. Department of Animal and Wildfife Sciences. University of Pretoria. MSc thesis, Pretoria, p. 242.

Marden, J.P., Julien, C., Monteils, V., Auclair, E., Moncoulon, R., Bayourthe, C., 2008. How Does Live Yeast Differ from Sodium Bicarbonate to Stabilize Ruminal pH in High-Yielding Dairy Cows? J. Dairy Sci. 91, 3528-3535.

Marounek, M., Wallace, R.J., 1984. Influence of Culture Eh on the Growth and Metabolism of the Rumen Bacteria *Selenomonas ruminantium, Bacteroides amylophilus, Bacteroides succinogenes* and *Streptococcus bovis* in Batch Culture. J Gen Microbiol 130, 223-229.

Martin, S.A., Nisbet, D.J., 1992. Effect of Direct-Fed Microbials on Rumen Microbial Fermentation. J. Dairy Sci. 75, 1736-1744.

Masek, T., Mikulec, Z., Valpotic, H., Antunac, N., Mikulec, N., Stojevic, Z., Filipovic, N., Pahovic, S., 2008. Influence of Live Yeast Culture (*Saccharomyces cerevisiae*) on Milk Production and Composition, and Blood Biochemistry of Grazing Dairy Ewes during the Milking Period. Acta Veterinaria Scandinavica 77, 547-554.

Mathieu, F., Jouany, J.P., Senaud, J., Bohatier, J., Bertin, G., Mercier, M., 1996. The effect of *Saccharomyces cerevisiae* and *Aspergillus oryzae* on fermentations in the rumen of faunated and defaunated sheep; protozoal and probiotic interactions. Reprod. Nutr. Dcv. 36, 27, 1-287.

Mayes, R.W., Lamb, C.S., Colgrove, P.M., 1986. The use of dosed and herbage n-alkanes as markers for the determination of herbage intake. The Journal of Agricultural Science 107, 161-170.

Medina, B., Girard, I.D., Jacotot, E., Julliand, V., 2002. Effect of a preparation of *Saccharomyces cerevisiae* on microbial profiles and fermentation patterns in the large intestine of horses fed a high fiber or a high starch diet. J. Anim Sci. 80, 2600-2609.

Meeske, R., Rothuge, A., van der Merwe, G.D., Greyling, J.F., 2006. The effect of concentrate supplementation on the productivity of grazing Jersey cows on a pasture based system. SA J Animal Sci 36, 105-110.

Meijs, J.A.C., Hoekstra, J.A., 1984. Concentrate supplementation of grazing dairy cows. Grass & Forage Science 39, 59-66.

Mertens, D.R., 1987. Predicting Intake and Digestibility Using Mathematical Models of Ruminal Function. J. Anim Sci. 64, 1548-1558.

Mertens, D.R., Ely, L.O., 1979. A Dynamic Model of Fiber Digestion and Passage in the Ruminant for Evaluating Forage Quality. J. Anim Sci. 49, 1085-1095.

Mertens, D.R., Loften, J.R., 1980. The effect of starch on forage fiber digestion kinetics in vitro. J Dairy Sci 63, 1437-1446.

Microsoft Office, 2007. Microsoft Excel ®. Microsoft. Redmond, Washington, United States.

Miller-Webster, T., Hoover, W.H., Holt, M., Nocek, J.E., 2002. Influence of Yeast Culture on Ruminal Microbial Metabolism in Continuous Culture. J. Dairy Sci. 85, 2009-2014.

Milligan, L.P., Grovum, W.L., Dobson, A., 1986. Control of digestion and metabolism in ruminants: proceedings of the Sixth International Symposium on Ruminant Physiology, held at Banff, Canada, September 10th-14th, 1984. Prentice-Hall.

Mir, Z., Mir, P.S., 1994. Effect of the addition of live yeast (*Saccharomyces cerevisiae*) on growth and carcass quality of steers fed high-forage or high-grain diets and on feed digestibility and in situ degradability. J. Anim Sci. 72, 537-545.

Miranda, R.L.A., Mendoza, M.G.D., Bárcena-Gama, J.R., González, M.S.S., Ferrara, R., Ortega, C.M.E., Cobos, P.M.A., 1996. Effect of *Saccharomyces cerevisiae* or *Aspergillus oryzae* cultures and NDF level on parameters of ruminal fermentation. Animal Feed Science and Technology 63, 289-296.

Moallem, U., Lehrer, H., Livshitz, L., Zachut, M., Yakoby, S., 2009. The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. J. Dairy Sci. 92, 343-351.

Moloney, A.P., Drennan, M.J., 1994. The influence of the basal diet on the effects of yeast culture on ruminal fermentation and digestibility in steers. Animal Feed Science and Technology 50, 55-73.

Moya, D., Calsamiglia, S., Ferret, A., Blanch, M., Fandino, J.I., Castillejos, L., Yoon, I., 2009. Effects of dietary changes and yeast culture (*Saccharomyces cerevisiae*) on rumen microbial fermentation of Holstein heifers. J. Anim Sci. 87, 2874-2881.

National Research Council, 1984. Nutrient requirements of beef cattle National Academy Press, Washington, DC.

National Research Council, 2001. Nutrient requirements of dairy cattle. National academy press, Washington, DC.

Newbold, C.J., Wallace, R.J., Chen, X.B., McIntosh, F.M., 1995. Different strains of *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers *in vitro* and in sheep. J. Anim Sci. 73, 1811-1818.

Newbold, C.J., Wallace, R.J., McIntosh, F.M., 1996. Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. British Journal of Nutrition 76, 249-261.

Nisbet, D.J., Martin, S.A., 1991. Effect of a *Saccharomyces cerevisiae* culture on lactate utilization by the ruminal bacterium *Selenomonas ruminantium*. J. Anim Sci. 69, 4628-4633.

Nocek, J.E., Kautz, W.P., Leedle, J.A.Z., Block, E., 2003. Direct-Fed Microbial Supplementation on the Performance of Dairy Cattle During the Transition Period. J. Dairy Sci. 86, 331-335.

O'Connor, M.H., Martin, S.A., Hill, G.M., 2002. Effects of *Saccharomyces cerevisiae* on *in vitro* Mixed Ruminal Microorganism Fermentation. The Professional Animal Scientist 18, 358-362.

Oetzel, G.R., Emery, K.M., Kautz, W.P., Nocek, J.E., 2007. Direct-Fed Microbial Supplementation and Health and Performance of Pre- and Postpartum Dairy Cattle: A Field Trial. J. Dairy Sci. 90, 2058-2068.

Oeztuerk, H., 2009. Effects of live and autoclaved yeast cultures on ruminal fermentation *in vitro*. Journal of Animal and Feed Sciences 18, 142-150.

Olson, K.C., Caton, J.S., Kirby, D.R., Norton, P.L., 1994. Influence of yeast culture supplementation and advancing season on steers grazing mixed-grass prairie in the northern Great Plains: II. Ruminal fermentation, site of digestion, and microbial efficiency. J. Anim Sci. 72, 2158-2170.

Orskov, E.R., 1986. Starch digestion and utilization in ruminants. J Animal Sci 63, 1624-1633.

Osuji. P. O., 1974. The physiology of eating and the energy expenditure of the ruminants at pasture. J. Range Manag. 27, 437-442.

Owens, F.N., Secrist, D.S., Hill, W.J., Gill, D.R., 1998. Acidosis in cattle: a review. J. Anim Sci. 76, 275-286.

Peyraud, J.L., Comeron, E.A., Wade, M.H., Lemaire, G., 1996. The effect of daily herbage allowance, herbage mass and animal factors upon herbage intake by grazing dairy cows. Ann Zootech 45, 201-217.

Phillips, P.P., 2002. The bottomline on ROI: Basics, benefits & barriers to measuring. CEP Press, 2300 Peachford Rd, Suite 2000, Atlanta, GA 30338, USA.

Pienaar, J.P., Roux, C.Z., 1989. A perspective on the prediction of voluntary feed intake for ruminants. Research developments in ruminant nutrition, Proceedings of an Information day held at the animal and dairy research institute, Irene, p. 53.

Pinos-Rodríguez, J.M., Robinson, P.H., Ortega, M.E., Berry, S.L., Mendoza, G., Bárcena, R., 2008. Performance and rumen fermentation of dairy calves supplemented with *Saccharomyces cerevisiae1077* or *Saccharomyces boulardii1079*. Animal Feed Science and Technology 140, 223-232.

Piva, G., Belladonna, S., Fusconi, G., Sicbaldi, F., 1993. Effects of Yeast on Dairy Cow Performance, Ruminal Fermentation, Blood Components, and Milk Manufacturing Properties. J. Dairy Sci. 76, 2717-2722.

Plata, F.P., M, G.D.M., Bárcena-Gama, J.R., M, S.G., 1994. Effect of a yeast culture (*Saccharomyces cerevisiae*) on neutral detergent fiber digestion in steers fed oat straw based diets. Animal Feed Science and Technology 49, 203-210.

Pulido, R.G., Muñoz, R., Lemarie, P., Wittwer, F., Orellana, P., Waghorn, G.C., 2009. Impact of increasing grain feeding frequency on production of dairy cows grazing pasture. Livestock Science 125, 109-114.

Putnam, D.E., Schwab, C.G., Socha, M.T., Whitehouse, N.L., Kierstead, N.A., Garthwaite, B.D., 1997. Effect of Yeast Culture in the Diets of Early Lactation Dairy Cows on Ruminal Fermentation and Passage of Nitrogen Fractions and Amino Acids to the Small Intestine. J. Dairy Sci. 80, 374-384.

Quigley, J.D., III, Wallis, L.B., Dowlen, H.H., Heitmann, R.N., 1992. Sodium Bicarbonate and Yeast Culture Effects on Ruminal Fermentation, Growth, and Intake in Dairy Calves. J. Dairy Sci. 75, 3531-3538.

Rayburn, E.B., Fox, D.G., 1993. Variation in Neutral Detergent Fiber Intake of Holstein Cows. Journal of Dairy Science 76, 544-554.

Reeves, M., Fulkerson, W.J., Kellaway, R.C., Dove, H., 1996. A comparison of three techniques to determine the herbage intake of dairy cows grazing kikuyu (*Pennisetum clandestinum*) pasture. Australian Journal of Experimental Agriculture 36, 23-30.

Reis, R.B., Combs, D.K., 2000. Effects of Increasing Levels of Grain Supplementation on Rumen Environment and Lactation Performance of Dairy Cows Grazing Grass-Legume Pasture. J. Dairy Sci. 83, 2888-2898.

Roa V, M.L., Bárcena-Gama, J.R., González M, S., Mendoza M, G., Ortega C, M.E., García B, C., 1997. Effect of fiber source and a yeast culture (*Saccharomyces cerevisiae1026*) on digestion and the environment in the rumen of cattle. Animal Feed Science and Technology 64, 327-336.

Robertson, J.B., Van Soest, P.J., 1981. The Analysis of Dietary Fibre in Food. Dekker, New York. ADSRI-JAN 1988.

Robinson, P.H., 1997. Effect of Yeast Culture (*Saccharomyces cerevisiae*) on Adaptation of Cows to Diets Postpartum. J. Dairy Sci. 80, 1119-1125.

Robinson, P.H., Garrett, J.E., 1999. Effect of yeast culture (*Saccharomyces cerevisiae*) on adaptation of cows to postpartum diets and on lactational performance. J. Anim Sci. 77, 988-999.

Robinson, P.H., Givens, D.I., Getachew, G., 2004. Evaluation of NRC, UC Davis and ADAS approaches to estimate metabolisable energy values of feeds at maintenance energy intake from equations utilizing chemical assays and *in vitro* determinations. Animal Feed Science and Technology 114, 75-90.

Rode, L.M., 2000. Maintaining a healthy rumen-An overview. Advances in Dairy Technology 12, 101.

Russell, J.B., Hespell, R.B., 1981. Microbial rumen fermentation. J Dairy Sci 64, 1153-1169.

Samuels, M.L., 1989. Statistics for the life sciences. Collier MacMillan Publishers, London.

Sanderson, M.A., Rotz, C.A., Fultz , S.W., Rayburn , E.B., 2001. Estimating forage mass with a commercial capacitance meter, rising plate meter, and pasture ruler. Agron. J. 93, 1281-1286.

Satter, L.D., Roffler, R.E., 1974. Nitrogen requirement and utilization in Dairy Cattle. J Dairy Sci 58, 1219-1237.

Satter, L.D., Slyter, L.L., 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. British Journal of Nutrition 32, 199-208.

Schingoethe, D.J., Linke, K.N., Kalscheur, K.F., Hippen, A.R., Rennich, D.R., Yoon, I., 2004. Feed Efficiency of Mid-Lactation Dairy Cows Fed Yeast Culture During Summer. J. Dairy Sci. 87, 4178-4181.

Shaver, R.D., Garrett, J.E., 1997. Effect of Dietary Yeast Culture on Milk Yield, Composition, and Component Yields at Commercial Dairies. The Professional Animal Scientist 13, 204-207.

Shwartz, G., Rhoads, M.L., VanBaale, M.J., Rhoads, R.P., Baumgard, L.H., 2009. Effects of a supplemental yeast culture on heat-stressed lactating Holstein cows. J. Dairy Sci. 92, 935-942.

Slyter, L.L., 1976. Influence of Acidosis on Rumen function. J. Anim Sci. 43, 910-929.

Smit, H.J., Taweel, H.Z., Tas, B.M., Tamminga, S., Elgersma, A., 2005. Comparison of Techniques for Estimating Herbage Intake of Grazing Dairy Cows. J. Dairy Sci. 88, 1827-1836.

Smith, W.A., Harris, B., Jr., Van Horn, H.H., Wilcox, C.J., 1993. Effects of Forage Type on Production of Dairy Cows Supplemented with Whole Cottonseed, Tallow, and Yeast. J. Dairy Sci. 76, 205-215.

Soder, K.J., Holden, L.A., 1999. Dry Matter Intake and Milk Yield and Composition of Cows Fed Yeast Prepartum and Postpartum. J. Dairy Sci. 82, 605-610.

Soil Classification Working Group, 1991. Soil Classification: A Taxonomic System for South Africa. Memoirs on the Natural Agricultural Resources of South Africa. Nr 15. Pretoria: Department of Agricultural Development.

Staples, C.R., Lough, D.S., 1989. Efficacy of supplemental dietary neutralizing agents for lactating dairy cows. A review. Animal Feed Science and Technology 23, 277-303.

Statistical Analysis Systems Institute, 2009. SAS User's Guide: Statistics Version 12. SAS Institue Inc,, Cary, North Carolina, USA.

Stella, A.V., Paratte, R., Valnegri, L., Cigalino, G., Soncini, G., Chevaux, E., Dell'Orto, V., Savoini, G., 2007. Effect of administration of live *Saccharomyces cerevisiae* on milk production, milk composition, blood metabolites, and faecal flora in early lactating dairy goats. Small Ruminant Research 67, 7-13.

Stockdale, C.R., 1984. Evaluation of techniques for estimating the yeild of irrigated pastures intensively grazed by dairy cows 2. The rising plate meter. Aust. J. Exp. Agric 24, 305-311.

Stockdale, C.R., 1985. Influence of some sward characteristics on the consumption of irrigated pastures grazed by lactating dairy cows. Grass & Forage Science 40, 31-39.

Stockdale, C.R., 2000. Levels of pasture substitution when concentrates are fed to grazing dairy cows in northern Victoria. Aust. J. Exp. Agric 40, 913-921.

Stone, C.W., 2006. Yeast products in the feed industry:A practical guide for feed professionals. Diamond V. Mills,Inc. Bulletin, Cedar Rapids, IA, 52407, USA, pp. 1-16.

Sullivan, H.M., Martin, S.A., 1999. Effects of a *Saccharomyces cerevisiae* Culture on In Vitro Mixed Ruminal Microorganism Fermentation. J. Dairy Sci. 82, 2011-2016.

Sutton, J.D., 1989. Altering milk composition by feeding. J Dairy Sci 72, 2801-2814.

Swartz, D.L., Muller, L.D., Rogers, G.W., Varga, G.A., 1994. Effect of Yeast Cultures on Performance of Lactating Dairy Cows: A Field Study. J. Dairy Sci. 77, 3073-3080.

Tang, S.X., Tayo, G.O., Tan, Z.L., Sun, Z.H., Shen, L.X., Zhou, C.S., Xiao, W.J., Ren, G.P., Han, X.F., Shen, S.B., 2008. Effects of yeast culture and fibrolytic enzyme supplementation on in vitro fermentation characteristics of low-quality cereal straws. J. Anim Sci. 86, 1164-1172.

Tharmaraj, J., Wales, W.J., Champman, D.F., Egan, A.R., 2003. Defoliation pattern, foraging behaviour and diet selection by lactating dairy cows in response to sward height and herbage allowance of a ryegrass-dominated pasture. Grass For. Sci. 58, 225-238.

Thrune, M., Bach, A., Ruiz-Moreno, M., Stern, M.D., Linn, J.G., 2009. Effects of *Saccharomyces cerevisiae* on ruminal pH and microbial fermentation in dairy cows: Yeast supplementation on rumen fermentation. Livestock Science 124, 261-265.

Tilley, J.M.A., Terry, R.A., 1963. A two stage technique for the *in vitro* digestion of forage crops. J Brit. Grassld Soc. 18, 104.

Tripathi, M.K., Karim, S.A., 2010. Effect of individual and mixed live yeast culture feeding on growth performance, nutrient utilization and microbial crude protein synthesis in lambs. Animal Feed Science and Technology 155, 163-171.

Vaneeta, K., Umesh, K., Vijay, K.S., Sudarshan, S., 1998. Mode of action of yeast culture (YEA-SACC 1026) for stimulation of rumen fermentation in buffalo calves. Journal of the Science of Food and Agriculture 77, 407-413.

Vanzant, E.S., Cochran, R.C., Titgemeyer, E.C., 1998. Standardization of *in situ* techniques for ruminant feedstuff evaluation. J. Anim Sci. 76, 2717-2729.

Wales, W.J., Doyle, P.T., Stockdale, C.R., Dellow, D.W., 1999. Effects of variations in herbage mass, allowance, and level of supplement on nutrient intake and milk production of dairy cows in spring and summer. Aust. J. Exp. Agric 39, 119-130.

Wales, W.J., Williams, Y.J., Doyle, P.T., 2001. Effect of grain supplementation and the provision of chemical or physical fibre on marginal milk production responses of cows grazing perennial ryegrass pastures. Aust. J. Exp. Agric 41, 465-471.

Walker, G.P., Stockdale, C.R., Wales, W.J., Doyle, P.T., Dellow, D.W., 2001. Effect of level of grain supplementation on milk production responses of dairy cows in mid-late lactation when

grazing irrigated pastures high in paspalum (*Paspalum dilatatum* Pior.). Aust. J. Exp. Agric 2001, 1-11.

Wallace, R.J., 1994. Ruminal microbiology, biotechnology, and ruminant nutrition: progress and problems. J. Anim Sci. 72, 2992-3003.

Wang, C., Liu, Q., Yang, W.Z., Dong, Q., Yang, X.M., He, D.C., Zhang, P., Dong, K.H., Huang, Y.X., 2009. Effects of selenium yeast on rumen fermentation, lactation performance and feed digestibilities in lactating dairy cows. Livestock Science 126, 239-244.

Ward, J.D., McCormick, M.E., 2001. Effect of Diamond V XP Yeast Culture fed during the transition period on production, health and reproduction of Holstein cows during hot weather. Louisiana State University AgCenter. Southeast Research Station, Franklinton, pp. DA017-s.

White, R.A., Harrison, J.H., Yoon, I., Sanchez, W.K., Nicholson, N., 2008. Effect of Yeast Culture on Efficiency of Nutrient Utilization for Milk Production and Impact on Fiber Digestibility and Fecal Particle Size. The Professional Animal Scientist 24, 114-119.

White, S.L., Benson, G.A., Washburn, S.P., Green, J.T., Jr., 2002. Milk Production and Economic Measures in Confinement or Pasture Systems Using Seasonally Calved Holstein and Jersey Cows. J. Dairy Sci. 85, 95-104.

Wiedmeier, R.D., Arambel, M.J., Walters, J.L., 1987. Effect of Yeast Culture and *Aspergillus oryzae* Fermentation Extract on Ruminal Characteristics and Nutrient Digestibility. J. Dairy Sci. 70, 2063-2068.

Wildman, E.E., Jones, G.M., Wagner, P.E., Boman, R.L., Troutt, H.F., Jr., Lesch, T.N., 1982. A Dairy Cow Body Condition Scoring System and Its Relationship to Selected Production Characteristics. J. Dairy Sci. 65, 495-501.

Williams, P.E., Tait, C.A., Innes, G.M., Newbold, C.J., 1991. Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. J. Anim Sci. 69, 3016-3026.

Wohlt, J.E., Corcione, T.T., Zajac, P.K., 1998. Effect of Yeast on Feed Intake and Performance of Cows Fed Diets Based on Corn Silage During Early Lactation. J. Dairy Sci. 81, 1345-1352.

Wohlt, J.E., Finkelstein, A.D., Chung, C.H., 1991. Yeast Culture to Improve Intake, Nutrient Digestibility, and Performance by Dairy Cattle During Early Lactation. J. Dairy Sci. 74, 1395-1400.

Yoon, I.K., Stern, M.D., 1996. Effects of *Saccharomyces cerevisiae* and *Aspergillus oryzae* Cultures on Ruminal Fermentation in Dairy Cows. J. Dairy Sci. 79, 411-417.

Zelenák, I., Jalc, D., Kmet, V., Siroka, P., 1994. Influence of diet and yeast supplement on *in vitro* ruminal characteristics. Animal Feed Science and Technology 49, 211-221.

APPENDIX A

APPENDIX A

Production Study

Blocking of cows

The 30 cows selected from the Outeniqua Research Farm were used in the production study. The cows considered for selection were kept between 60 and 120 DIM, the mean DIM of the herd was 83. The lactation numbers of the cows used were kept between two and six. Cows in their first lactation or cows too far in milk were not included, due to the less likelihood of a milk response. The cows were blocked according to lactation number, DIM and fat-corrected milk yield. The treatment groups were randomly allocated within each block. This is represented in Table A1.

Table A1 The parameters for which the individual cows were blocked, with their treatments allocated

Name	Lactation no.	Calving date	DIM[1]	Milk yield[2]	FCM[3]	Block	Treatment[4]
BELL 145	3	2009/05/29	93	24.05	24.74	1	Y
SYMB 67	4	2009/07/04	57	23.39	22.9	1	P
FIRE 46	6	2009/05/19	103	23.19	26.71	2	P
JAPN 64	5	2009/06/26	65	22.71	28.03	2	Y
ALET 105	3	2009/07/17	44	22.76	23.65	3	Y
GRET 31	6	2009/07/27	34	22.7	23.68	3	P
MARL 70	5	2009/07/22	39	27.27	26.7	4	Y
MART 155	4	2009/07/23	38	22.53	26.75	4	P
GERL 24	3	2009/06/10	81	22.57	27.64	5	Y
SYMB 65	4	2009/06/18	104	22.71	27.47	5	P
GRET 52	2	2009/07/20	41	22.35	22.42	6	P
MARL 87	2	2009/05/08	114	22.41	22.34	6	Y
JAPN 82	3	2009/06/14	77	22.15	24.47	7	P
MAGDA86	2	2009/07/21	40	21.84	24.04	7	Y
LAUR 27	8	2009/06/08	84	21.46	25.03	8	Y
MARL 47	8	2009/08/02	54	21.93	25.02	8	P
GRET 49	2	2009/06/15	76	22.69	24.87	9	P
MART 160	4	2009/05/16	106	21.26	24.61	9	Y
LAUR 34	5	2009/04/23	129	20.83	19.26	10	P
MARL 68	5	2009/05/03	119	20.81	19.94	10	Y
BELL 135	4	2009/05/31	91	20.96	22.94	11	Y
DORA 102	6	2009/05/14	108	20.68	23.56	11	P
DONN 9	2	2009/06/11	80	20.61	22.28	12	Y
DORA 137	2	2009/07/19	41	20.69	22.34	12	P
ALET 113	2	2009/06/03	88	19.43	20.86	13	Y
MART 175	2	2009/05/19	103	20.41	20.87	13	P
ALTA 34	3	2009/05/08	114	20.18	19.48	14	P
MAGD 81	4	2009/05/12	110	20.39	20.48	14	Y
DORA 109	5	2009/05/02	120	18.97	22.56	15	Y
JAPN 76	3	2009/04/14	138	19.89	23.23	15	P

[1]DIM- Days in milk as of the 31 of August 2009. [2]Milk yield daily means from the 18 to the 24 August. [3]FCM- Fat corrected milk from the following calculation (0.4* kg Milk Yield) + (15* kg Milk fat)(National Research Council, 2001). [4]Treatments; P- Control; and Y- Yeast.

Table A2 The Body weight and BCS measured and averaged for the control cows at the beginning and at the end of the experimental trial period

Name	Block	Beginning				End			
		Wt 1	Wt 2	Ave Wt 1&2	BCS	Wt 3	Wt 4	Ave Wt 3&4	BCS
ALTA 34	14	316	324	320	2.00	360	356	358	2.5
DORA 102	11	349	364	356.5	2.00	390	422	406	2.25
DORA 137	12	294	296	295	2.00	330	325	328	2
FIRE 46	2	365	387	376	2.25	400	423	412	2.5
GRET 31	3	366	373	369.5	2.25	409	442	426	2.25
GRET 49	9	315	334	324.5	2.00	362	365	364	2.25
GRET 52	6	278	297	287.5	2.00	328	329	329	2.25
JAPN 76	15	308	322	315	2.00	334	343	339	2.5
JAPN 82	7	291	300	295.5	2.00	341	345	343	2.25
LAUR 34	10	431	447	439	2.25	458	491	475	2.5
MARL 47	8	369	385	377	2.00	396	417	407	2.25
MART 155	4	297	297	297	2.00	316	325	321	2
MART 175	13	300	306	303	2.25	326	340	333	2
SYMB 65	5	342	340	341	2.25	376	393	385	2.5
SYMB 67	1	339	354	346.5	2.00	377	407	392	2
AVE		**330.67**	**341.73**	**336.20**	**2.08**	**366.87**	**381.53**	**374.20**	**2.27**

AVE – Average; BCS – Body Condition Score

Wt1 = Weight 1 - 31 August 2009; Wt2 = Weight 2 -1 September 2009; Wt3 = Weight 3 - 23 November 2009; Wt4 = Weight 4 - 24 November 2009

Table A3 The Body weight and BCS measured and averaged for the yeast cows at the beginning and at the end of the experimental trial period

Name	Block	Beginning				End			
		Wt 1	Wt 2	Ave Wt 1&2	BCS	Wt 3	Wt 4	Ave Wt 3&4	BCS
ALET 105	3	331	334	332.5	2.00	388	382	385	2
ALET 113	13	264	267	265.5	2.00	320	333	326.5	2.25
BELL 135	11	303	308	305.5	2.00	333	346	339.5	2.25
BELL 145	1	300	311	305.5	2.00	327	346	336.5	2.25
DONN 9	12	299	303	301	2.25	329	335	332	2.5
DORA 109	15	311	314	312.5	2.00	347	362	354.5	2
GERL 24	5	304	311	307.5	2.00	332	340	336	2
JAPN 64	2	350	362	356	2.25	369	392	380.5	2.25
LAUR 27	8	410	418	414	2.25	439	448	443.5	2.25
MAGD 81	14	352	359	355.5	2.25	376	391	383.5	2.75
MAGDA 86	7	302	303	302.5	2.00	318	349	333.5	2
MARL 68	10	348	369	358.5	2.00	375	403	389	2.25
MARL 70	4	348	342	345	2.00	376	386	381	2.25
MARL 87	6	319	329	324	2.00	347	377	362	2
MART 160	9	347	351	349	2.00	379	397	388	2.5
AVE		**325.87**	**332.07**	**328.97**	**2.07**	**357.00**	**372.47**	**364.73**	**2.23**

AVE – Average; BCS – Body Condition Score

Wt1= Weight 1 - 31 August 2009;Wt2 = Weight 2 -1 September 2009; Wt3 = Weight 3 - 23 November 2009; Wt4 = Weight 4 - 24 November 2009

Table A4 Chemical compositions on a 2 weekly basis of the supplemental control concentrate experimental diet

Parameter	Control 1	Control 2	Control 3	Control 4	Control 5	Control 6
DM (g/kg as is)	883.90	884.38	883.31	882.46	882.55	885.23
CP (g/kg DM)	103.63	103.56	103.66	105.69	102.47	103.88
NDF (g/kg DM)	86.61	87.91	88.44	89.07	86.72	89.38
ADF (g/kg DM)	33.24	35.52	32.12	32.25	35.51	36.93
EE (g/kg DM)	33.03	34.19	33.46	38.48	35.65	30.19
GE (MJ/kg)	17.17	17.14	17.16	17.20	17.25	17.14
IVOMD (%DM)	93.3	94.2	91.9	91.1	93.9	94.2
ME (MJ/kg)	13.1	13.2	12.9	12.8	13.3	13.2
Ca (g/kg DM)	9.34	9.14	9.08	9.34	9.03	9.41
P (g/kg DM)	9.24	9.10	9.21	9.19	9.24	9.24
Ca:P	1.01:1	1.00:1	0.99:1	1.02:1	0.98:1	1.02:1

DM - Dry matter; CP - Crude protein; NDF - Neutral detergent fibre; NDIN - Neutral detergent insoluble nitrogen; ADF - Acid detergent fibre; EE - Ether extract; GE - Gross energy; IVOMD - *In Vitro* organic matter digestibility; ME - Metabolisable energy; Ca - Calcium; P - Phosphorus

Table A5 Chemical compositions on a 2 weekly basis of the supplemental yeast concentrate experimental diet

Parameter	Yeast 1	Yeast 2	Yeast 3	Yeast 4	Yeast 5	Yeast 6
DM (g/kg as is)	882.60	885.79	882.82	887.11	884.61	881.40
CP (g/kg DM)	104.25	104.06	103.59	105.59	105.19	106.94
NDF (g/kg DM)	81.99	82.02	81.62	81.24	80.50	82.92
ADF (g/kg DM)	30.99	31.11	32.69	31.34	35.13	33.27
EE (g/kg DM)	33.19	31.64	34.20	35.29	30.80	30.92
GE (MJ/kg)	17.18	17.10	17.00	17.26	17.06	17.21
IVOMD (%DM)	94.1	92.8	92.4	91.2	90.5	90.8
ME (MJ/kg)	13.3	13.0	12.9	12.9	12.7	12.8
Ca (g/kg DM)	9.10	9.12	9.35	8.84	8.75	9.25
P (g/kg DM)	9.12	9.22	9.12	8.78	8.98	6.83
Ca:P	1.00:1	0.99:1	1.03:1	1.01:1	0.97:1	1.36:1

DM - Dry matter; CP - Crude protein; NDF - Neutral detergent fibre; NDIN - Neutral detergent insoluble nitrogen; ADF - Acid detergent fibre; EE - Ether extract; GE - Gross energy; IVOMD - *In Vitro* organic matter digestibility; ME - Metabolisable energy; Ca - Calcium; P - Phosphorus

Table A6 Chemical compositions on a 2 weekly basis of the ryegrass/kikuyu pasture

Parameter	Pasture 1	Pasture 2	Pasture 3	Pasture 4	Pasture 5	Pasture 6
DM (g/kg as is)	127.98	144.37	136.42	164.95	172.60	215.12
CP (g/kg DM)	285.69	223.13	238.56	200.47	234.97	213.72
NDF (g/kg DM)	527.83	498.19	499.88	508.46	548.35	488.92
ADF (g/kg DM)	330.26	296.34	292.89	308.97	305.84	295.06
EE (g/kg DM)	37.14	35.90	37.24	32.17	34.16	28.88
GE (MJ/kg)	17.68	17.26	17.43	17.09	17.32	17.38
IVOMD (%DM)	78.1	80.1	78.5	73.2	73.3	73.1
ME (MJ/kg)	11.3	11.3	11.2	10.3	10.4	10.4
Ca (g/kg DM)	4.09	3.61	4.04	4.06	4.20	4.10
P (g/kg DM)	3.85	3.82	4.06	4.12	4.13	2.05
Ca:P	1.06:1	0.94:1	1.00:1	0.99:1	1.02:1	2.00:1

DM - Dry matter; CP - Crude protein; NDF - Neutral detergent fibre; NDIN - Neutral detergent insoluble nitrogen; ADF - Acid detergent fibre; EE - Ether extract; GE - Gross energy; IVOMD - *In vitro* organic matter digestibility; ME - Metabolisable energy; Ca - Calcium; P - Phosphorus

Table A7 Milk compositional analysis of five milk samples collected over the experimental period for live yeast supplemented and control Jersey cows grazing ryegrass/kikuyu pasture and supplemented with (6 kg as is) concentrate (n = 15)

Compositional parameter	Treatment	Milk sample number[1]				
		1	2	3	4	5
Milk fat (%)	Control	3.97	4.47	3.95	3.96	3.60
	SE	0.418	0.778	0.490	0.575	0.334
	Yeast	3.66	4.06	4.28	4.83	4.38
	SE	0.472	0.515	0.635	0.688	0.616
Milk protein (%)	Control	3.65	3.58	3.60	3.33	3.37
	SE	0.300	0.286	0.264	0.187	0.133
	Yeast	3.72	3.67	3.54	3.48	3.52
	SE	0.292	0.233	0.214	0.261	0.180
Milk lactose (%)	Control	4.77	4.69	4.77	4.52	4.60
	SE	0.141	0.124	0.143	0.118	0.104
	Yeast	4.79	4.80	4.76	4.65	4.65
	SE	0.160	0.174	0.174	0.196	0.121
SCC	Control	276	248	294	278	192
	SE	694.7	459.3	397.9	365.3	122.5
	Yeast	80.5	95.5	138	256	196
	SE	56.80	48.09	41.4	363.9	143.3
MUN	Control	9.68	10.9	13.2	10.9	8.76
	SE	2.574	2.67	1.45	2.85	0.797
	Yeast	8.98	11.6	13.5	11.5	9.58
	SE	2.368	2.99	1.64	2.69	2.501

SCC – Somatic cell count; MUN – Milk urea nitrogen and SE – standard error

[1]Milk sample 1 = 21 September 2009, sample 2 = 5 October 2009, sample 3 = 19 October 2009, sample 4 = 2 November 2009, sample 5 = 16 November 2009.

Table A8 Literature review representing the authors study where yeast was supplemented.

No[1]	Authors	Product	Dose[2]	Ly/CY	CFU/g	Animal	DIM	Parity
1	Abd El-Ghani (2004)	-	3 6	CY	-	Zaraibi Goat	-	-
2	Andrighetto *et al.* (1993)	Lisomix	20 40	CY	-	Sheep	-	-
3	Angeles *et al.* (1998)	Yea-Sacc 1026	3	CY	1.00E+08	Ewe	-	-
4	Angeles *et al.* (1998)	Levucell	1	Ly	2.00E+10	Ewe	-	-
5	Arambel and Kent (1990)	Diamond V XP	90	CY	2.00E+06	Holstein cows	(+) 56	-
6	Arcos-García *et al*. (2000)	Yea-Sacc 1026 Levucell	3 1	CY Ly	1.00E+08 2.00E+10	Suffolk ewe	- -	- -
7	Bach *et al.* (2007)	Levucell CNCM I-1077	5	Ly	2.00E+09	Large breed	(+) 335±42	multi
8	Besong *et al.* (1996)	Liquid yeast	20 (%)	CY	-	Holstein cow	(mid)	-
9	Bruno *et al.* (2009)	A-Max XTRA	30	CY	-	Holstein	(+) 20 to 140	multi
10	Cooke *et al.* (2007)	Diamond V XP	56	CY	-	Holstein	(+) 182±40	multi
11	Dann *et al.* (2000)	Diamond V XP	60	CY	-	Jersey	-	primi & multi
12	Dawson *et al.* (1990)	killed yeast live yeast live yeast	0.002 9	CY Ly Ly	0 2.04E+09 2.04E+09	*In vitro* Jersey Steers	- -	- -

Table A8 continued

No	Authors	Product	Dose	Ly/ CY	CFU/g	Animal	DIM	Parity
13	Desnoyers *et al.* (2009a)	-	-	-	-	-	-	-
14	Desnoyers *et al.* (2009b)	SC, CBS, 493.94	10	Ly	2.20E+10	Saanen & Alpine dairy goats	(+) 40±4	-
15	Doreau and Jouany (1998)	CNCM I-1077	0.5	Ly	6.00E+10	Holstein cows	(+) 14	multi
16	Enjalbert *et al.* (1999)	Diamond V XP	50	CY	-	Holstein cows	-	multi
17	Erasmus *et al.* (1992)	Yea-Sacc 1026	10	CY	-	Holstein	(+) 56 to 94	-
18	Erasmus *et al.* (2005)	Diamond V XP	-	-	-	Holstein cow	(-)21	multi
19	Erdman and Sharma (1989)	Diamond V XP & NaHCO3 Diamond V XP	1% 1%	CY	-	Holstein cows	(+) 154	-
20	García *et al.* (2000)	Levucell Levucell and monensin (25 mg)	1 1	Ly	2.00E+10 2.00E+10	Suffolk sheep	-	-
21	Giger-Reverdin *et al.* (1996)	Levucell *S. cerevisiae* I-1077	0.2	Ly	1.10E+10	Dairy goats	(early)	-
22	Grochowska *et al.* (2009)	Biosaf SC 47 Diamond V XP	7 60	Ly CY	- -	Holstein cow	-	multi
23	Guedes *et al.* (2008)	Levucell SC 10 ME	0.3 1	Ly	1.00E+10	non-lactating cows	-	-

Table A8 Continued

No	Authors	Product	Dose	Ly/ CY	CFU/g	Animal	DIM	Parity
24	Haddad and Goussous (2005)	Diamond V XP	3 6	CY	-	Awassi Lambs	-	-
25	Harris *et al.* (1992)	Diamond V XP	57	CY	-	Holstein	(early to mid)	-
26	Hinman *et al.* (1998)	Diamond V XP	85	CY	-	Angus crossbred	-	-
27	Kalmus *et al.* (2009)	Yea-Sacc 1026	10	CY	-	Holstein	(-) 14 to (+) 98	multi
28	Kamalamma *et al.* (1996)	Yea-Sacc 1026	10	CY	-	Holstein cross & Jersey cross	(mid)	multi
29	Kamel *et al.* (2004)	Alexandria-Eqypt	11.25 22.5	-	-	Sheep	-	-
30	Kung *et al.* (1997)	Biomate Yeast Plus	20mg/10 gdiet	CY	3.50E+09	*In vitro*	-	-
			200 mg 10	CY		Holstein cow	(early)	-
			10 20	CY		Holstein cow	(mid)	-
31	Lascano and Heinrichs (2009)	Yea-Sacc 1026	±8	CY	-	Dairy Heifers	-	-
32	Lehloenya *et al.* (2008)	Diamond V XP	56	CY	-	AngusxHereford	-	-
33	Longuski *et al.* (2009)	Diamond V XP	56	CY	-	Holstein	(+) 96±14	multi

Table A8 Continued

No	Authors	Product	Dose	Ly/ CY	CFU/g	Animal	DIM	Parity
34	Marden *et al.* (2008)	Sc 47	5	Ly	-	Holstein	(early)	-
35	Mazek *et al.* (2008)	Yea-Sacc 1026	3 6	-	-	Istrian Sheep x Friesain dairy ewes	-	-
36	Miller-Webster *et al.* (2002)	Diamond V XP A-Max X yeast	57g/d/ 2.3mg/gDM 57g/d/ 2.3mg/gDM	CY CY	- -	*In vitro*	-	-
37	Mir and Mir (1994)		10	-	5.00E+09	Steer	-	-
38	Miranda *et al.* (1996)	Yea-Sacc 1026	10	CY	-	Holstein heifers	-	-
39	Moallem *et al*. (2009)	Biosaf SC 47	1 g/4 kg DMI	Ly	1.00E+11	Holstein	(+) 114+-54	primi & multi
40	Moloney and Drennan (1994)	Yea-Sacc	10	CY	6.50E+08	Fresian steers	-	-
41	Moloney and Drennan (1994)	Yea-Sacc	10	CY	1.01E+09	Fresian steers	-	-
42	Newbold *et al.* (1995)	NCYC 240 NCYC 694 NCYC1026 NCYC1088 Yea-Sacc NCYC 240 NCYC 1026 Yea-Sacc	500 mg/d 500 mg/d 500 mg/d 500 mg/d 500 mg/d 2 2 2	CY	6.57E+04	Rustec *In vitro* *In vivo* Sheep	- -	- -

Table A8 Continued

No	Authors	Product	Dose	Ly/ CY	CFU/g	Animal	DIM	Parity
43	Nocek *et al.* (2003)	DFM (yeast & *E. faecium*)	90	CY	5.00E+09	Holstein	(-) 21 to (+) 70	multi
44	Olson *et al*. (1994)	Diamond V XP	28.4	CY	-	Beef steer	-	-
45	Pinos-Rodríguez *et al.* (2008)	Levucell CNCM I-1077	1	Ly	2.00E+10	Holstein calve	-	-
		S. boulardii CNCM I-1079	1	Ly	2.00E+10			
46	Piva *et al*. (1993)	Thepax Dry	10	CY	1.00E+10	Holstein	(+) 105±2	-
47	Plata *et al.* (1994)	Yea-Sacc 1026	10	CY	-	Holstein steers	-	-
48	Putnam *et al.* (1997)	Yea-Sacc 1026	10	CY	-	Holstein	(early)	primi
49	Roa V *et al.* (1997)	Yea-Sacc 1026	10	CY	-	Holstein steers	-	-
50	Robinson (1997)	Diamond V mills XP	57	CY	-	Holstein cows	-	-
51	Robinson and Garrett (1999)	Diamond V mills XP	57	CY	4.00E+07	Holstein	-	primi multi

Table A8 Continued

No[1]	Authors	Product	Dose[2]	Ly/ CY	CFU/g	Animal	DIM	Parity
52	Schingoethe *et al.* (2004)	Diamond V XP	60	CY	-	Holstein cows	(+) 105	primi & multi
53	Shaver and Garrett (1997)	Diamond V XP	57	CY	-	Holstein	(+) 140	primi & multi
54	Soder and Holden (1999)	Biomate Yeast Plus yeast	20 15	CY	5.00E+09 -	Holstein	-	primi & multi
55	Stella *et al.* (2007)	Levucell CNCM I-1077 SC 20	0.2	Ly	2.00E+10	Saanen dairy goats	-	-
56	Swartz *et al.* (1994)	Western 2x-2-2-5 Cellcon	114 114	CY	4.60E+07 4.40E+08	Holstein cow	< (+)120	-
57	Thrune *et al.* (2009)	Levucell CNCM I-1077 SC 20	0.5	Ly	2.00E+10	Holstein	(+) 344±60	multi
58	White *et al.* (2008)	Diamond V XP	56	CY	-	Holstein	(+)179 to (+) 191	-
59	Wiedmeier *et al.* (1987)	Diamond V XP	90	CY	-	non-lactating Holstein	-	-
60	Williams *et al.* (1991)	Yea-sacc 1026	10	CY	5.00E+09	Holstein	(+) 49 to (+) 84	multi
61	Wohlt *et al.* (1991)	Biomate Yeast Plus	10	Ly	5.00E+09	Holstein	(-) 30 to (+) 126	primi
62	Wohlt *et al.* (1998)	Biomate Yeast Plus	10 20	CY	5.00E+09	Holstein	-	multi
63	Yoon and Stern (1996)	Diamond V XP	57	CY	-	Holstein cows	(+) 173	-

[1]**No represents the study number and corresponds to a fixed author throughout the tables**

[2] **Dose = g/day**

CFU = colony forming units; DIM = days in milk; Ly = live yeast; CY = yeast culture;(+)= postpartum; (-) = prepartum; multi = multiparous**; primi =** primiparous

Table A9 Literature review of the studies diet

No	Dose	Concentrate		Roughage		NE	Total diet composition					
		Resource-based	%	Resource-based	%		CP %		NDF %		ADF %	
							Cont	Yea	Cont	Yea	Cont	Yea
1	3 6	Maize,wheatbran & cottonseed oilcake	60	Alfalfa & wheat straw	40	-	-	-	-	-	-	-
2	20 40	Barley	50	Corn silage & ryegrass hay	50	-	15.2	I	40.9	I	22.2	I
3	3	-	-	-	-	-	10.2	10.2	60.6	57.8	28.7	25.3
4	1	-	-	-	-	-	10.2	10.2	60.6	59.8	28.7	29
5	90	Rolled barley	60	Alfalfa hay, alfalfa haylage & corn silage	40	1.69	16.5	I	47.4	I	29.4	I
6	3 1	Sorghum grain & wheat bran	50	Sugar cane tops	50	-	11.5	I	62.6	I	28.5	I
7	5	Corn grain	15	Ryegrass silage	85	-	-	-	-	-	-	-
8	20(%)	Mixed concentrate	45	Chopped alfalfa hay	55	-	-	-	-	-	-	-
9	30	Corn grain mix	54	Alfalfa hay, corn silage & wheat silage	46	2.81-2.85	-	-	-	-	-	-
10	56	Steam flaked corn based concentrate	43.1	Alfalfa hay, corn silage & whole cotton seed	56.9	-	18.0	18.0	38.9	39.1	17.5	18.0
11	60	Ground corn shelled	-	corn silage & chopped alfalfa hay	-	-	-	-	-	-	-	-
12	0.002	Cracked corn	22.5	Fescue hay	77.5	-	-	-	-	-	-	-
	9	Cracked corn	22.5	Fescue hay	77.5	-	-	-	-	-	-	-

Table A9 Continued

No	Dose	Concentrate		Roughage		NE	Total diet composition					
		Resource-based	%	Resource-based	%		CP %		NDF %		ADF %	
							Cont	Yea	Cont	Yea	Cont	Yea
13	-	-	-	-	-	-	16.1	I	34.5	I	-	-
14	10	Wheat, barley & maize	20	Lucerne hay, grass hay & sugarbeet pulp	80	-	-	-	39.9	I	21.4	I
			50	Grass hay & sugarbeet pulp	50	-	-	-	34.7	I	15.9	I
15	0.5	Wheat & barley	40	Corn silage	60	-	16.1	I	37.0	I	21.7	I
16	50	Wheat grain	32	Corn silage	67	1.65	13.0	I	33.7	I	-	-
17	10	-	65	Wheat straw & alfalfa hay	35	-	16.5	I	31.5	I	19.1	I
18		Flaked corn grain & rolled barley grain	62	Alfalfa hay	38	1.59	18.1	I	31.2	I	19.2	I
19	1% 1%	-	60	Corn silage	40	1.78	17.0	I I	-	- -	9.90	I I
20	1 1	Sorghum grain	50	Alfalfa hay	50	-	-	-	-	-	-	-
21	0.2	Barley maize	25	Alfalfa hay & sugar beet pulp silage	75	-	- -	- -	- -	- -	- -	- -
22	7 60	-	-	-	-	-	-	-	-	-	-	-
23	0.3 1	Barley, maize and wheat grain	52	Maize silage & meadow hay	58	-	13.4	I	37.4	I	20.2	I

Table A9 Continued

No	Dose	Concentrate		Roughage		NE	Total diet composition					
		Resource-based	%	Resource-based	%		CP %		NDF %		ADF %	
							Cont	Yea	Cont	Yea	Cont	Yea
24	3 6	barley grain and corn grain	80	Alfalfa hay & chopped wheat straw	20	-	16.1	I	24.4	I	13.0	I
25	57	Corn meal	50	Corn silage	50	-	-	-	-	-	-	-
26	85	Barley & potato	88	Alfalfa & corn silage	12	-	12.3	12.1	27.0	28.2	12.9	12.5
27	10	-	-	Grass silage	-	-	17.9	18.6	30.1	29.6	14.9	13.6
28	10	-	-	Finger millet straw	-	-	-	-	-	-	-	-
29	11.25 22.5	-	-	Berseem hay	100	-	-	-	55.4	I	38.2	I
30	20 mg/10 g diet 200 mg	-	50	Corn silage & alfalfa hay	50	-	-	-	-	-	-	-
	10	Wheat mildings & corn meal	50	Corn silage & chopped alfalfa hay	50	1.58	15.1	I	36.4	I	21.0	I
	10 20	Wheat mildings & corn meal	50	Corn silage & chopped alfalfa hay	50	1.69	17.0	I	41.3	I	23.7	I
31	±8	Ground corn	20,40, 60	Corn silage	80,60, 40	-	-	-	-	-	-	-
32	56	Ground corn	55.9	Alfalfa & sorghum-silage based	44.1	-	16.7	I	40.6	I	25.5	I
33	56	Ground corn grain High moisture corn	-	Corn & alfalfa silage	-	-	17.0	I	25.0	I	-	-

Table A9 Continued

No	Dose	Concentrate		Roughage		NE	Total diet composition					
							CP %		NDF %		ADF %	
		Resource-based	%	Resource-based	%		Cont	Yea	Cont	Yea	Cont	Yea
34	5	Wheat bran, corn grain & ground corn	43	Corn silage & dehydrated alfalfa	57	1.60	18.7	I	37.8	I	19.9	I
35	3 6	Corn & barley(1 kg)	-	Lucerne hay (0.3 kg) & graze	-	-	-	-	-	-	-	-
36	57g/d/ 2.3mg/gDM 57g/d/ 2.3mg/gDM	Ground corn	47	Corn silage & haylage	53	-	17.1	I	33.2	I	21.7	I
37	10		62	Alfalfa hay	38	-	-	-	-	-	-	-
38	10	Barley	60	Alfalfa hay	40	-	13.2	I	27.6	I	-	-
		Barley	40	Alfalfa hay	60	-	13.1	I	36.7	I	-	-
39	1g/4kg DMI	Ground corn grain	60	Wheat silage	40	1.75	16.5	I	31.7	I	16.0	I
40	10	Barley	40	Chopped barley straw (high fibre & low protein)	60	-	10.4	I	64.6	I	39.2	I
		Barley	80	Chopped barley straw (low fibre & high protein)	20	-	17.2	I	35.7	I	16.8	I
41	10	Barley	40	Chopped barley straw (high fibre & low protein)	60	-	10.4	I	64.6	I	39.2	I
		Barley	80	Chopped barley straw (low fibre & high protein)	20	-	17.2	I	35.7	I	16.8	I

Table A9 Continued

No	Dose	Concentrate		Roughage		NE	Total diet composition					
		Resource-based	%	Resource-based	%		CP %		NDF %		ADF %	
							Cont	Yea	Cont	Yea	Cont	Yea
42	-	Barley	50	Grass hay	50	-	14.8	I	51.5	I	24.4	I
43	90	Ground shell corn	55	Haylage & corn silage	45	1.86	18.6	I	29.5	I	17.6	I
44	28.4	-	-	Grazing mixed-grass prairie	-	-	-	-	-	-	-	-
45	1 1	Whole milk	-	Calf starter	-	-	19.3	I	32.9	I	11.6	I
46	10	Flaked corn	48	Corn silage & alfalfa hay	52	1.73	17.6	I	33.5	I	21.1	I
47	10	Bakery waste	60/40/20	Oats straw	40/60/80	-	-	-	-	-	-	-
48	10	Corn meal	56	Corn silage, haycrop silage and hay(legume)	44	1.74 1.71	16.1 18.8	I I	28.3 26.2	I I	17.2 16.9	I I
49	10	Sorghum grain	60	Alfalfa hay Alfalfa hay & cornstalk Alfalfa hay & coffee hulls	40	-	14.0	I	27.2 30.1 33.5	I	-	-
50	57	Ground barley and corn	47	Corn silage, timothy hay and timothy silage	53	1.61	14.3	I	32.5	I	19.3	I
51	57	Grain based	57	Corn & timothy silage	43	1.715	-	-	-	-	-	-

Table A9 Continued

No[1]	Dose[2]	Concentrate		Roughage		NE	Total diet composition					
		Resource-based	%	Resource-based	%		CP %		NDF %		ADF %	
							Cont	Yea	Cont	Yea	Cont	Yea
52	60	-	51	Corn silage & alfalfa hay	49	1.78	17.5	I	30.8	I	20.2	I
53	57	High moisture corn	34	Alfalfa silage	66	-	18.8	18.8	28.5	27.8	18.7	18.4
54	20 15	Corn & barley	53	Alfalfa haylage & corn silage	47	1.63	16.5	I I	33.2	I I	20.5	I I
55	0.2	-	47	Triticale silage, hay & dried beet pulp	53	-	15.5	15.6	38.1	37.9	-	-
56	114 114	Corn & oats	47	Corn silage, legume haylage and alfalfa hay	53	1.68	18	I	35.1	I	22.3	I
57	0.5	Corn grain	40	Alfalfa hay & corn silage	60	1.63	17.1	I	27.7	I	18.0	I
58	56	Steamed & rolled corn	34	corn silage & alfalfa hay	66	-	17.4	17.7	37.7	36.7	22.6	22.4
59	90	Rolled barley & wheat bran	50	chopped barley hay & alfalfa hay	50	-	13.3	13.3	42.8	42.7	25.0	25.0
60	10	Rolled barley	50	Hay	50	-	-	-	-	-	-	-
		Rolled barley	50	Straw	50		-	-	-	-	-	-
		Rolled barley	60	Hay	40		-	-	-	-	-	-
		Rolled barley	60	Straw	40		-	-	-	-	-	-
61	10	-	50	Corn silage and hay	50	-	18.0	I	-	-	-	-
62	10 & 20	-	-	-	-	-	-	-	-	-	-	-
63	57	Corn grain	50	Corn silage and alfalfa hay	50	1.57	15.2	I	32.3	I	17.7	I

[1]**No represents the study number and corresponds to a fixed author throughout the tables**

[2] **Dose = g/day**

NE=Nett energy (Mcal/kg DM); CP= Crude protein; NDF=Neutral detergent fibre; ADF= Acid detergent fibre; Ly = live yeast; CY = yeast culture; I= Identical to the control

Table A10 Literature review representing the dry matter intake, milk, fat-corrected milk yield and fat, protein and lactose milk percentages

No	Dose	DMI	DMI		MY	Milk yield		FCM		Milk fat%		Milk protein%		Milk lactose%	
		Unit	Cont	Yea	Unit	Cont	Yea	Cont	Yea	Cont	Yea	Cont	Yea	Cont	Yea
1	3 6	kg/d	1.09[a]	1.21[b] 1.25[b]	kg/d	0.98[a]	1.1[b] 1.15[b]	0.69[a] (7%)	0.774[b] 0.819[c]	4.22[a]	4.15[b] 4.27[c]	3.15[a]	3.05 2.98[b]	4.85	4.52 4.65
5	90	kg/d	21.9	21.8	kg/d	37.9	36.5	36.3 (3.5%)	35.5	3.33	3.37	2.97	2.94	5.13	5.03
8	20%	kg/d	23.6[a]	23.4[b] 20.9[b]	kg/d	23.4	23.4 23.5	-	- -	3.48	3.58 3.33	3.1	3.11 3.15	-	- -
9	30	kg/d	26	25.8	kg/d	42.2[a]	43.4[b]	-	-	3.58[a]	3.48[b]	2.83	2.81	4.83	4.84
10	56	kg/d	26.2	25.6	kg/d	37.4	38.2	-	-	3.87	4.11	3.19	3.11	4.89	4.84
11	60	kg/d	15.2	16.5	kg/d	22.9	23.5	-	-	4.27	4.44	3.64	3.78	4.93	4.99
13	-	g/kg BW	34.6[a]	35[b]	g/kg BW	46.5[a]	47.7[b]	-	-	3.8	3.85	3.2	3.19	-	-
17	10	kg/d	21.8	23.2	kg/d	18.9	20.1	-	-	3.19	3.19	3.41	3.38	-	-
18	-	kg/d	22.2	21.7	kg/d	36.3	37.9	-	-	3.74	3.8	3.03	3.02	4.76	4.88
19	1% 1%	kg/d	19.6	19.5 19	kg/d	26.3	25 25.6	23.2 (4%)	25 22.2	3.42	3.76 3.46	3.44	3.51 3.5	-	- -
21	0.2	kg	87.4 85.1	86 88.5	g/kg MW	0.137 0.166	0.177 0.178	0.147 (3.5%) 0.178	0.199 0.202	4.26 4.09	4.51 4.76	3.32 3.51	3.4 3.29	4.98 4.92	4.95 4.96

Table A10 Continued

No	Dose	DMI	DMI		MY	Milk yield		FCM		Milk fat%		Milk protein%		Milk lactose%	
		Unit	Cont	Yea	Unit	Cont	Yea	Cont	Yea	Cont	Yea	Cont	Yea	Cont	Yea
22	7 60	kg/d	20.7	21 21.4	kg/d	31.6	32.4 30.4	-	- -	4.39	4.34 4.25	3.12	3.17 3.1	-	- -
24	3 6	kg/d	1.041	1.106 1.051	-	-	-	-	-	-	-	-	-	-	-
25	57	kg/d	22.9[a]	22[b]	kg/d	25.3	26.5	-	-	3.43	3.38	3.12	3.03	-	-
26	85	kg/d	9.64	9.83	-	-	-	-	-	-	-	-	-	-	-
27	10	-	-	-	kg/d	30.7	32.7	-	-	-	-	-	-	-	-
28	10	kg/d	11.16	11.16	kg/d	9.78	9.76	9.36 (4%)	9.23	-	-	-	-	-	-
29	11.25 22.5	kg/d	1.406	1.501 1.498	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
30	20 mg 200 mg	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10	kg/d	20.8	19.6	kg/d	32.9	33.4	33.3 (3.5%)	33.4	3.58	3.5	3.13	3.1	-	-
	10 20	kg/d	22.8	22.8 22.8	kg/d	39.6	40.5 40.2	36.4	39.3 38	2.99	3.3 3.18	2.56	2.6 2.64	-	- -

Table A10 Continued

No	Dose	DMI	DMI		MY	Milk yield		FCM		Milk fat%		Milk protein%		Milk lactose%	
		Unit	Cont	Yea	Unit	Cont	Yea	Cont	Yea	Cont	Yea	Cont	Yea	Cont	Yea
33	56	kg/d	27.5 25.4	27.7 26	kg/d	43 43.6	42.6 44.8	41.6 (3.5%) 39.8[a] (3.5%)	41 43[b]	3.34 3.03	3.32 3.31	2.99 2.92	2.99 2.94	5.16 5.13	5.13 5.1
35	3 6	-	-	-	kg/d	0.929[a]	0.996 1.072[b]	0.94[a] (6.5%)	1.116[b] 1.221[b]	7.8	7.7 8	5.8	5.7 5.7	4.4	4.4 4.3
39	1g/4kg DMI	kg/d	24.1[a]	24.7[b]	kg/d	36.3[a]	37.8[b]	32.8a (4%)	34.8[b]	3.49	3.63	3.2	3.24	4.86[a]	4.91[b]
42	2 2 2	kg/d	1.5	1.5	-	-	-	-	-	-	-	-	-	-	-
43	90	kg/d	15.3	19.9	kg/d	34.93	36.17	na	na	3.67	3.82	3.4	3.52	-	-
45	1 1	kg/d	0.842[a]	0.911[b] 0.857[ab]	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -
46	10	kg/d	21.1	22.8	kg/d	25.4[a]	26.2[b]	21.6[a] (4%)	23.6[b]	3.25	3.54	3.38	3.4	-	-
47	10	kg/d	8.41	8.49	-	-	-	-	-	-	-	-	-	-	-
48	10	kg/d kg/d	18.1 18.3	19.2 19	kg/d kg/d	31 31.6	32.1 31.9	26.5 (4%) 27.9	28.4 28	3.04 3.23	3.24 3.23	3.03 3.08	3.07 3.08	- -	- -

Table A10 Continued

No[1]	Dose[2]	DMI	DMI		MY	Milk yeild		FCM		Milk fat%		Milk protein%		Milk lactose%	
		Unit	Cont	Yea	Unit	Cont	Yea	Cont	Yea	Cont	Yea	Cont	Yea	Cont	Yea
50	57	kg/d	17.38	17.62	kg/d	34.09	34.65	-	-	4.17	4.33	3.26	3.19	4.51	4.6
51	57	kg/d	14.34 19.45	15.4 20.76	kg/d	25.36 38.6	27.81 40.35	- -	- -	3.88 3.88	3.59 3.82	3.16 3.05	3 3.05	4.63 4.49	4.66 4.5
52	60	kg/d	23.1	22.1	kg/d	34.9	35.4	31.2 (4%)	32	3.34	3.41	2.85	2.87	4.83	4.8
53	57	-	-	-	kg/d	36.7[a]	37.6[b]	34.5 (4%)	34.8	3.65[a]	3.55[b]	3.15[a]	3.13[b]	-	-
54	20 15	kg/d	22.1	21 22.8	kg/d	40.2	40.4 41.7	38 (4%)	35.8 41.5	3.92	3.82 4.02	3.15	3.13 3.1	-	- -
55	0.2	kg/d	2.35[a]	2.71[b]	kg/d	2.08[a]	2.38[b]	-	-	4.46[a]	4.32[b]	3.65	3.65	4.99	4.94
56	114 114	kg/d	-	- -	kg/d	31.8	31.8 31.6	32.8 (3.5%)	32.4 32.4	3.69	3.67 3.76	3.15	3.12 3.17	-	- -
57	0.5	kg/d	16.9	16.7	-	-	-	-	-	-	-	-	-	-	-
58	56	kg/d	26.2	26.4	kg/d	41.9	42.0	42.4 (3.5%)	43	3.61[a]	3.69[b]	3.21[a]	3.19[b]	-	-
60	10	kg/d kg/d kg/d kg/d	18.1 15.7 17.3 17.8	18.8 16.5 19.6 18.7	kg/d kg/d kg/d kg/d	23.4 22.5 23.3 23.3	23.3 21.5 27.4 23.5	21.1 21.7 21.5 19.4	20.6 21.2 24.5 22.8	3.44 3.78 3.45 3.19	3.35 3.81 3.26 3.66	-	-	-	-
61	10	kg/d	19.2	18.5	kg/d	26	27.2	-	-	3.96	3.91	3.23	3.12	-	-
62	10 20	kg/d	24.1[a]	25.4[b] 25.1[ab]	kg/d	40.5	41.8 42.8	38.8	42.6 41.9	3.24	3.64 3.43	3.01[a]	2.99[ab] 2.83[b]	-	- -

[1]**No represents the study number and corresponds to a fixed author throughout the tables**

[2] **Dose = g/day**

DMI= Dry matter intake; MY = Milk yield; FCM = Fat-corrected milk yield; Cont= Control; Yea= Yeast

[a,b] **Means in the same study with different superscripts differ (P < 0.05)**

Table A11 Literature review representing the acetic, propionic, butyric valeric and lactic acid percentages

No	Dose	Acetic acid%		Propionic acid%		Butyric acid%		Valeric acid%		Lactic acid %	
		Cont	Yea	Cont	Yea	Cont	Yea	Cont	Yea	Cont	Yea
2	20	62.3	63.5	21.7	21	12.4	11.5	1.5	1.9	-	-
	40		64.5		20		11.5		2		-
3	3	66.6	67.4	21.3	21.4	11.8	11.2	-	-	-	-
4	1	66.6	67.5	21.3	21.1	11.8	11.4	-	-	-	-
6	3	66.6	66.6	22.2	22.2	11.3	11.2	-	-	-	-
	1		67.3		20.4		12.3		-		-
8	20%	65.38	64.09	18.16[a]	19.21[b]	11.87	11.8	1.83	1.99	-	-
			61.33		21.36[b]		12.69		2.09		-
12	0.002 (*In vitro*)	62.7	63	20.1[a]	20.1[ab]	11.6	11.7	1.4	1.4	-	-
			62.3		20.9[b]		11.4		1.4		-
	9	72.3	72	15.2	15.1	7.3	7.6	2.5[a]	3.7[b]	-	-
15	0.5	60.1	61.3	20.7	19.8	14.6	14.6	-	-	-	-
16	50	61.13	61.03	20.9	22.38	12.96	11.71	1.93	1.68	-	-
17	10	57.7	55.2	25.3	27	12.9	13.7	2.6	2.7	1.93[a] (peak)	1.73[b]
18	-	65.9	68	29.6	35.1	11.1	11.9	2.3	2.8	-	-
20	1	71.4[a]	71[ac]	18.9[a]	18.4[ac]	9.5	10.6	-	-	-	-
	1		66.3[b]		24.3[b]		9.2				
23	0.3	57.5[a]	57.85[b]	22.15[a]	23.98[b]	10.95[a]	11.73[b]	-	-	2.1[a]	0.83[b]
	1		62.73[b]		31.7[b]		12.3[b]				0.73[b]
30	20 mg/10 g diet	55.1	54.1	22.9	23	15.3	15.3	3.2[a]	3.2[ac]	-	-
	200 mg		54.9		22.4		14.5		3.1[b]		

Table A11 Continued

No	Dose	Acetic acid%		Propionic acid%		Butyric acid%		Valeric acid%		Lactic acid %	
		Cont	Yea	Cont	Yea	Cont	Yea	Cont	Yea	Cont	Yea
31	±8	60.98	61.83	19.19	19.37	14.25	12.81	2.58	2.84	-	-
32	56	66.9	66.3	17.8	18.1	10.6	11.3	1.29	1.36	0.26 mM	0 mM
33	56	65.4	66.1	26.1	25.5	13.9	15.1	2.06	2.04	-	-
		66.3	67.6	28.5	27.6	15.1	18.6	2.2	2.13	-	-
34	5	53.2[a]	59.1[b]	18[a]	25.8[b]	106	10.2	-	-	16.5[a]	5.4[b]
36	-	57.3[a]	47.1[b]	20.4[a]	32[b]	18.1	16	2.6[a]	3.7[b]	-	-
	-		53.2[c]		23.6[c]		18.7		3[b]		
38	10	71.4	71.7	16.2	15.9	12	12.2	-	-	-	-
		72.4	72.8	15.4	16.3	11.6	10.3	-	-	-	-
39	1 g/4 kg DMI	-	-	-	-	-	-	-	-	-	-
40	10	68	69.1	17.7	16.9	11.5	11.1	2.8	2.6	-	-
		61.6	62.5	17.6	18.4	16.7	15.3	4.1	3.9	-	-
42	500 mg/d	27.8 (mmol/day)	28.2	7.1 (mmol/day)	7.8	5.4 (mmol/day)	5.5	-	-	0.31m (mmol/dL)	0.44
	500 mg/d		32.4		7.8		5.4		-		0.56
	500 mg/d		32.8		8.7		7.3		-		0.41
	500 mg/d		33.5		9		6.7		-		0.58
	500 mg/d		25.8		7.7		5		-		0.47
	2		63.8		17.8		14.5		-		1.29
	2	64.2	63.6	20	19.7	12.3	13.1	-	-	1.32	1.2
	2		62.7		20.6		13		-		1.32

Table A11 Continued

No[1]	Dose[2]	Acetic acid%		Propionic acid%		Butyric acid%		Valeric acid%		Lactic acid %	
		Cont	Yea	Cont	Yea	Cont	Yea	Cont	Yea	Cont	Yea
44	28.4	-	-	-	-	-	-	-	-	-	-
45	1	54.2[a]	54.3[ab]	-	-	-	-	-	-	-	-
	1		57[b]		-	-	-		-	-	-
46	10	60.2	63.7	24.3	23.3	11.1	10.3	-	-	-	-
47	10	60.3	58	20.7	22.2	18.9	19.6	-	-	-	-
48	10	67.9	64.8	38.5	35.5	13.3	12.2	2.6	2.1	2	2.5
		67.6	63.6	35.9	37.9	13.4	12.9	2.9	3.2	1.9	1.9
49	10	63.39	65.31	21.4	22.9	15.21	11.79	-	-	-	-
		66.6	67.51	19.96	18.7	13.43	13.79	-	-	-	-
		65.76	65.54	21.08	19.4	13.15[a]	15.23[b]	-	-	-	-
57	0.5	67.5	67	18.2	18.1	9.7[a]	10.4[b]	-	-	-	-
59	90	69.3	68.8	14	13.8	12.4	13	-	-	-	-
60	10	-	-	-	-	8.58 meq/L	6.96	-	-	3.55 mM	1.43 mM
63	57	63.5	63.9	19.6	19.3	12.9	12.7	1.48	1.49	-	-

[1]No represents the study number and corresponds to a fixed author throughout the tables

[2] Dose = g/day

Cont= Control; Yea= Yeast

[a,b] Means in the same study with different superscripts differ (P < 0.05)

Table A12 Literature review representing the ruminal pH, ammonia nitrogen and volatile fatty acid concentrations

No	Dose	Rumen pH		NH3-N (mg/dL)		VFA C (mM/L)	
		Cont	Yea	Cont	Yea	Cont	Yea
1	3 6	6.52	6.54 6.57	21.12	19.37 19.71	95.07	97.4 102.53
2	20 40	6.44[a]	6.08[b] 6.13[b]	24.4	22.5 24.1	97.7[a]	108.2[b] 111.7[b]
3	3	6.81	6.51	11.01	10.16	82.8	87.9
4	1	6.81	6.61	11.01	10.71	82.8	94.5
6	3 1	6.05[a]	5.85[b] 5.96[c]	9.24[ab]	10.5[a] 9.38[b]	97.3[ab]	105.6[a] 107.6[b]
7	5	5.49a	6.05b	-	-	-	-
8	20%	6.23	6.14 6.13	-	- -	117.7	124 125.8
12	0.002 (*In vitro*)	6.65	6.61 6.75	36.7	36.1 34.1	157.8	160 143.1
	9	7.04	6.97	-	-	68.8	73.2
13	-	6.31[a]	6.34[b]	-	-	95.2[a] (mM)	97.3[b] (mM)
14	10	6.16 6.12	6.16 6.14	- -	- -	- -	- -
15	0.5	6.31	6.35	10.16	10.73	76.67	81.18
16	50	6.34	6.31	10.16	8.91	82.75	93
17	10	5.99	6	10.7	9.6	-	-
18	-	5.79	5.69	12.99	10.81	-	-
20	1 1	6.45	6.51 6.42	-	- -	61.8	91.3 79.7
23	0.3 1	6.41[a]	6.51[b] 6.55[b]	21.65	22.15 23.98	90.5[a]	94.53[ab] 106.73[b]

Table A12 Continued

No	Dose	Rumen pH		NH3-N (mg/dL)		VFA C (mM/L)	
		Cont	Yea	Cont	Yea	Cont	Yea
30	20 mg/10 g diet	6.15	6.14	10.39[ab]	10.4[a]	113.3	115.4
	200 mg		6.2		12.44[b]		114
31	8	6.08	6.06	5.73[a]	3.41[b]	113.87[a]	123.66[b]
32	56	6.37	6.32	14.32	13.86	112	114
33	56	6.04	6.04	7.11	8.07	110.7	112.2
		5.9	5.91	6.42	7.42	115.5	119.3
34	5	5.94[a]	6.14[b]	-	-	53.2[a]	59.1[b]
36	-	6.3[ab]	6.17[a]	5.53	7.46	-	-
	-		6.37[b]		8.27		-
38	10	6.11	6.24	10.4	12.9	75.6	81
		6.53	6.48	13.4	12.7	78.2	76.6
39	1 g/4 kg DMI	6.54	6.67	15.19[a]	12.61[b]	-	-
40	10	7.33	7.34	11.33	12.35	79.6	81.4
		6.91	6.99	21.02	19.02	110.3	107.3
42	500 mg/d	7.1	7.2	43.8	48.5	-	-
	500 mg/d		7.2		62.2		-
	500 mg/d		7.2		66.6		-
	500 mg/d		7.1		63.6		-
	500 mg/d		7.2		52		-
	2	6.46	6.53	17.1	17	107	109
	2		6.56		16.4		102
	2		6.53		16.4		105

Table A12 Continued

No[1]	Dose[2]	Rumen pH		NH3-N (mg/dL)		VFA C (mM/L)	
		Cont	Yea	Cont	Yea	Cont	Yea
44	28.4	6.2	6.2	-	-	-	-
45	1	5.6	5.4	-	-	81.5	112.6
	1		5.3		-		103.7
46	10	7.2	7.08	18.5	16	480 (mg/dL)	509
47	10	5.96	5.98	-	-	49.8 (mM)	54
48	10	6.04	6.05	7.48	8.05	-	-
		6	6	10.82	10.99	-	-
49	10	5.94	6.34	27.7[a]	36.3[b]	74.3[a]	88.2[b]
		6.06	6.48	28.2[a]	36.5[b]	53.6	63.1
		5.92	6.36	26.9[a]	36.4[b]	51.7[a]	69.6[b]
57	0.5	6.32[a]	6.53[b]	13.6	14.4	122.4	107.3
59	90	6.34	6.34	18.21	18.72	64.6	66.4
60	10	-	-	-	-	78	73
63	57	5.96	5.98	12.9	15	124.9	120.4

[1]No represents the study number and corresponds to a fixed author throughout the tables

[2] Dose = g/day

Cont= Control; Yea= Yeast; NH3-N=Ammonia nitrogen; VFA= Volatile fatty acids

[a,b] Means in the same study with different superscripts differ (P < 0.05)

Table A13 Literature review representing the dry matter, neutral detergent, organic matter and crude protein digestibility

No	Dose	Digestibility %							
		DM		NDF		OM		CP	
		Cont	Yea	Cont	Yea	Cont	Yea	Cont	Yea
1	3 6	tt65.84	66.24 64.24	tt61.32[a] (CF)	65.24[b] 66.21[b]	tt71.65	72.21 72.14	tt69.67	72.41 70.91
2	20 40	tt73.7	71.3 73.1	64.1	60.2 64.3	77.2	74.9 76.8	77.2	75.6 77.7
5	90	-	-	tt40.6	35.8	-	-	tt57.1	56.9
10	56	tt62.36	60.86	tt40.5	40.71	-	-	tt67.51	63.44
15	0.5	tt68.8	69.8	tt46.9	47.6	tt71.1	72.2	-	-
16	50	r40.3	r38.62	32.92	33.04	-	-	-	-
17	10	tt69.4	69.3	tt50.4	50.5	-	-	tt72.5[a]	74.5[b]
20	1 1	tt0.72	0.71 0.73	tt0.67	0.62 0.64	-	- -	-	- -
23	0.3 1	-	-	rL24.7[a]/H39.3	rL25.7[b]/H40.2 rL30.6[c]/H41	-	- -	-	- -
24	3 6	tt63.2[a]	67.6[b] 66.9[b]	tt52.1[a]	56.1[b] 55.7[b]	tt64.5[a]	68.3[b] 67.5[b]	tt58.9[a]	65.3[b] 64.8[b]
25	57	tt66.42	67.43	tt52.33	47.86	-	-	62.33	63.01

Table A13 Continued

No[1]	Dose[2]	Digestibility							
		DM		NDF		OM		CP	
		Cont	Yea	Cont	Yea	Cont	Yea	Cont	Yea
26	85	91.89	92.49	58.69	56.13	-	-	74.87	73.23
32	56	-	-	57.5	66.4	45.8	58.5	56.1	66.5
34	5	tt59	64	tt29.6[a]	t41.6[b]	tt62.2	t66.6	-	-
36	57g/d/ 2.3mg/gDM	66.6[a]	71.6[b]	44.5	44.8	49.7	51.6	-	-
	57g/d/ 2.3mg/gDM		69[b]		46.6		49.3		-
38	10	-	-	r22.2	53.7	-	-	-	-
		-	-	r56.9	59.7	-	-	-	-
39	1 g/4 kg DMI	tt61.7	62.4	tt43.9	44.8	tt58.3	58.9	tt62.9	61.7
41	10	tt56.5	56.2	tt47.8	47.9	tt59.4	59.1	tt66.4	61.1
		tt72.4	73	tt46.3	46.8	tt74.7	75.3	tt76.1	77.2
47	10	-	-	r48.6 (72h)	60.45	-	-	-	-
49	10	-	-	r46.6[a]	55[b]	-	-	r85.7[a]	90.2[b]
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
58	56	tt59.5	59.5	tt34.6	37.8	-	-	-	-
59	90	tt77	79.1	-	-	-	-	tt79.5	82.2
61	10	tt72.4	72.8	tt55.3	55.5	-	-	tt73.8	75.4
62	10	74.1	75.6	57.6	61	-	-	-	-
	20		74.8		57.9	-	-	-	-
63	57	-	-	-	-	-	-	r42.2	46

[1]No represents the study number and corresponds to a fixed author throughout the tables

[2] Dose = g/day

DM= Dry matter; NDF= Neutral detergent fibre; OM= Organic matter; CP= Crude protein; CF= Crude fibre Cont= Control; Yea= Yeast; NH3-N=Ammonia nitrogen; VFA= Volatile fatty acids; tt= total tract; r= ruminal

[a,b] Means in the same study with different superscripts differ (P < 0.05)