

**MILK YIELD AND QUALITY, NITROGEN METABOLISM AND RUMEN FERMENTATION
PARAMETERS IN DAIRY COWS FED DIFFERENT LEVELS OF DIETARY
CONCENTRATE AND LIVE YEAST**

BY

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DECLARATION

I, Nomthandazo Petronella Shabangu declare that this dissertation is submitted in fulfilment of the requirements for the Master of Science in Agriculture (Animal Science) degree at the University of Venda. It is hereby submitted by me and has not been submitted for a degree at this or any other university before. I further declare that all sources have been duly acknowledged.

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DEDICATION

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ABSTRACT

The overall objective of this study was to investigate the effects of level of dietary concentrate and live yeast (LY) on milk yield, milk composition, rumen fermentation and nitrogen metabolism in lactating dairy cows. Four primiparous Holstein dairy cows in early lactation (average weight 500 ± 9 kg and 20 days in milk (DIM)) were used in a 4 x 4 Latin Square design for a period of 44 days. The animals had seven days of adaptation to the treatments and four days for measurements. The treatments were, Low concentrate to forage (C:F) diet (40:60) with no additive, High C:F diet (60:40) with no additive, High C:F diet with LY and Low C:F diet with LY. Cows were milked at 06h00 and 16h00 daily and milk samples were analysed for fat, protein, lactose milk urea nitrogen (MUN) and somatic cell count (SCC). Proximate analysis of feed samples was done and daily feed intake was recorded. Weight and body condition score were determined at beginning and end of every experimental period. Faecal and urinary nitrogen (N) were determined. *In vitro* batch fermentation was conducted to determine ruminal fermentation kinetics. Data generated from the feeding trial was analysed for a 4 x 4 Latin square design (LSD) using the PROC MIXED procedure of SAS (2009) and data for the *in vitro* trial was subjected to ANOVA using PROC GLM (SAS Institute, 2009) for a complete randomized design. Addition of LY affected only dry matter intake (DMI) ($P < 0.05$), which effect was pronounced when cows were fed low (40:60) C:F diet resulting in better feed efficiency (FE). Cows fed high C:F diet consumed more feed, produced more milk with high fat and protein content ($P < 0.05$). Both LY and C:F reduced ($P < 0.05$) N intake as result of low DMI, but reduced ($P < 0.05$) N excretion in manure. Addition of LY decreased ruminal ammonia and increased total VFA's ($P < 0.05$). The effects on ammonia suggest a better utilisation of diet proteins and probably more incorporation of products of CP degradation into microbial proteins, which support the observed reduced manure N excretion. The opposite was observed with high C:F diet, which increased ammonia and decreased total VFA's. Propionate and butyrate were increased and decreased, respectively by high C:F diet. Addition of LY reduced SCC and MUN compared to control. The effects of LY were better pronounced on most parameters at low C:F diet. It is therefore recommended that the effects of LY be tested at low C:F on a larger scale of animals over longer periods to observe its effect of the rest of the parameters.

Key words: diet, concentrate, yeast, nitrogen, milk yield, quality.

TABLE OF CONTENTS

DECLARATION	i
ACKNOWLEDGEMENTS	ii
DEDICATION	iiiiv
ABSTRACT	ivv
LIST OF TABLES	viiiviii
LIST OF FIGURES	viiiix
CHAPTER 1: INTRODUCTION	1
1.1. Background	1
1.2. Problem statement	2
1.3. Justification	3
1.4. Objective	3
1.5. Specific objectives	3
1.6. Hypothesis: H ₀	4
CHAPTER 2: LITERATURE REVIEW	5
2.1. Introduction	5
2.2. Feeding early lactating dairy cows	6
2.3. Forage and concentrate feeding in early lactating dairy cows	7
2.4. Feed additives in dairy cows.	8
2.4.1 Ionophores	9
2.4.2. Direct fed microbial	9
2.4.2.1. Bacterial direct fed microbial	1110
2.4.2.2. Fungal direct fed microbial	11
2.5. Yeast products	11
2.5.1. Live yeast and yeast culture	12
2.5.2. Active dry yeast	12
2.5.3. Yeast mechanism of action	1312
2.6. Effects of live yeast on dairy cows	1413
2.6.1. Effects on dry matter intake	1413
2.6.2. Effects on milk yield and composition	14
2.6.3. Effects on rumen microorganisms	1514
2.6.4. Effects of live yeast on rumen fermentation	1615
2.6.4.1. Effects on volatile fatty acid production	1615
2.6.4.2. Effects on rumen pH	16
2.7. Effect of live yeast on nitrogen metabolism	1817

CHAPTER 3. EFFECTS OF DIETARY CONCENTRATE AND LIVE YEAST ON MILK YIELD AND COMPOSITION	20
3.1. INTRODUCTION.....	20
3.2. MATERIAL AND METHODS	21
3.2.1. Study site	21
3.2.2. Cows, diets and experimental design	21
3.2.3. Sampling and analysis.....	21
3.2.3.1. Feed intake.....	21
3.2.3.2. Body condition score and body weight measurements	21
3.2.3.3. Milk production and composition	22
3.2.3.4. Nitrogen metabolism.....	22
3.2.3.5. Determination of nitrogen in urine	22
3.2.3.6. Chemical analysis	22
3.2.3.7. Calculations.....	23
3.2.3.8. Statistical analysis.....	23
3.4. DISCUSSIONS	31
3.4.1 Feed intake, milk yield and milk composition.....	31
3.4.2 Body weight and body condition score	33
3.5. CONCLUSIONS	34
CHAPTER 4. <i>IN VITRO</i> DETERMINATION OF THE EFFECTS OF DIETARY CONCENTRATE AND LIVE YEAST ON RUMINAL AMMONIA AND VOLATILE FATTY ACIDS	35
4.1. INTRODUCTION.....	35
4.2. MATERIALS AND METHODS	37
4.2.1. Collection and preparation of rumen fluid.....	37
4.2.2. <i>In vitro</i> batch fermentation	38
4.2.3. Sampling and analysis.....	38
4.2.5 Statistical analysis	38
4.3. RESULTS	40
4.4. DISCUSSIONS.....	44
4.5. CONCLUSION	45
CHAPETR 5: GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS...	46
REFERENCES	48
ANNEXURES.....	63 62

LIST OF TABLES

Table 3.1.	Ingredient composition (g/kg of dry matter) of experimental diets	25
Table 3.2	The effect of dietary concentrate level and live yeast supplementation on feed intake, milk yield and composition, somatic cell count and feed efficiency	26
Table 3.3.	The effect of dietary concentrate level and live yeast supplementation on body weight and body condition score	27
Table 3.4.	The effect of dietary concentrate level and live yeast supplementation on faecal and urinary nitrogen	29
Table 4.1.	Composition of the <i>in vitro</i> buffer solution	37
Table 4.2.	Contrast of effect of dietary concentrate level and live yeast supplementation on ammonia nitrogen and volatile fatty acids	42

LIST OF FIGURES

Figure 2.1.	Possible sites targeted by feed additives to improve nitrogen metabolism in the rumen	19
Figure 3.1	Faecal Nitrogen (N) as percentage of N intake. ^{ab} Means with different superscripts differ (P<0.05)	29
Figure 3.2	Urinary Nitrogen (N) as percentage of N intake. ^{ab} Means with different superscripts differ (P<0.05)	30
Figure 3.3.	Relationship between N intake (g/d) and urinary N (g/d)	30
Figure 4.1	The change in % of ruminal major VFA's as affected by live yeast in low and high C:F diets	43

LIST OF ABBREVIATIONS

AA	Amino acid
ADF	Acid detergent fiber
ADYs	Active dry yeast
AOAC	Association of official Analytical Chemists
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
GHG	Greenhouse gases
LY	Live yeast
N	Nitrogen
NDF	Neutral detergent fiber
RDP	Rumen degradable protein
RUP	Rumen undegradable protein
VFA	Volatile fatty acids
YC	Yeast culture
YP	Yeast products

CHAPTER 1: INTRODUCTION

1.1. Background

The rumen microbes are mainly bacteria, protozoa and fungi. Bacteria are the major group involved in ruminant's feed digestion. The dairy cows require amino acids (AA) from microbial protein synthesis and requirements depend on the age of the animal and level of milk production. As milk yield increases, amino acid requirements increase accordingly (Hopkins and Whitlow, 2007). Ruminal degradation of protein from dietary feed ingredients is one of the most important factors influencing intestinal amino acid supply to ruminants (Stern *et al.*, 2006). These AA must come from either microbial protein synthesized in the rumen or from undegraded dietary protein (UDP) in the rumen (Hopkins and Whitlow, 2007). Microbial protein synthesis in the rumen provides the majority of protein supplied to the small intestine of ruminants, accounting for 50 to 80% of total absorbable protein (Stern *et al.*, 2006). Carbohydrates are the main source of energy for bacteria, although they can also be used as carbon skeletons for protein synthesis in combination with ammonia (Stern *et al.*, 2006). Rumen microbes incorporate rumen degradable protein (RDP) into microbial protein by utilising readily fermentable carbohydrates (both fibrous and non-fibrous) as energy sources along with ammonia. When energy is limiting, microbial protein synthesis is also limited. Readily fermentable carbohydrates such as starch or sugars are more effective than other sources such as cellulose in promoting microbial growth (Stern and Hoover, 1979). Diets limited in energy should be supplemented to aid microbial protein supply to the small intestines to assure adequate supply of AA for vital biological processes.

During normal feeding, before protein is used for microbial protein synthesis in the rumen, large part of protein passes through the ammonia pool (Hristov *et al.*, 2005). Conversion of dietary protein to ammonia, and overall ammonia utilization in the rumen is depending on readily fermentable carbohydrate (RFC) availability (Russell *et al.*, 1993). When carbohydrate availability is higher, as it is in high concentrate diets, amino nitrogen (N) is incorporated into microbial protein, reducing ammonia production (Russell *et al.*, 1993; Hristov *et al.* (2005). Conversely, when rate of protein degradation exceeds the rate of carbohydrate fermentation, large quantities of N can be lost as ammonia (Nocek and Russell, 1988). Feed additives such as live yeast (LY) represent an interesting tool to improve feed efficiency and performance (Chaucheyras-Durand 2014), because they benefit from a natural and well-accepted image by the consumer, as they are not involved in health disorders and do not have any detrimental impact on the environment. Nowadays, major species used are *Saccharomyces boulardii* and *Saccharomyces cerevisiae* yeasts (Duarte *et al.*, 2012). The use of probiotics in animal feeding

provides the opportunity to promote animal production while reducing pollution from livestock manure excretions. This is important due to the changing climate that requires livestock production enterprises to produce their products with low contribution to greenhouse gas emissions. Probiotics are live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance (FEFANA, 2005). The application of probiotics provides a potential alternative strategy to the traditional practice of sub therapeutic antibiotic use (Yirga, 2015). Probiotics are designed to encourage certain strains of bacteria in the gut at the expense of less desirable bacteria (McDonalds *et al.*, 2010). Feeding probiotics may promote the growth of the gut bacteria there by improving the microbial profile in the gut (Quigley, 2011). Probiotics act extensively on the gut mainly based on competitive exclusion, bacterial antagonism and immune modulation (Hughes & Heritage, 2002; Steiner, 2009). Currently, there is a growing interest concerning the use of probiotics in animal nutrition but there is still a need to clarify the effectiveness of probiotics and to define their mechanism of action broadly in the gut.

1.2. Problem statement

The increase in grain prices has forced producers to look for strategies to reduce production cost. The wide-scale use of conventional nitrogen supplements such as fishmeal, soyabean meal and cottonseed meal, by most smallholder farmers is constrained by the high cost (Baloyi *et al.*, 2014). This increase has led to farmers feeding more forage than concentrate because forage is easily available and affordable. Providing more roughage than concentrate in the diets has its disadvantages like causing rumen dysfunctions and not providing adequate nutrients to the animal. If there is not enough fermentable carbohydrate available in the rumen to use the free ammonia, then rumen ammonia levels increase. The excess ammonia is absorbed through the wall of the rumen and travels to the liver, where it is metabolized to urea (which is less toxic than ammonia) (Hopkins and Whitlow, 2007). It costs the cow approximately 7.3 kcal of energy for each gram of ammonia that is converted to urea in the liver and this is energy that could be used for milk production (Hopkins and Whitlow, 2007). Therefore, ways to increase nitrogen utilization by dairy cows should be investigated.

Animal production and emission from animal manure contributes to air and water pollution (USEPA, 2004). In the agricultural industry, livestock production seems to be one of the major contributors to nonpoint N pollution from animal manure excretions. Therefore, ways to reduce the animal footprint in nonpoint N pollution are vital for investigations and nutrition is one way to reduce N emission from animal manure.

1.3. Justification

Conversion of dietary protein to ammonia and overall utilization in the rumen is dependent on the carbohydrate availability (Russel *et al.*, 1993). When the carbohydrate availability is higher, amino N is incorporated into microbial protein, reducing ammonia production (Russel *et al.*, 1993; Hristov *et al.*, 2005). Live yeast is reported to decrease the rate of degradation of peptides, which may reduce ammonia wastage and may increase the amount of rumen undegradable protein (RUP) available to the cow (Chaucheyras-Durand *et al.*, 2005). This can be explained by its negative effect on *S. bovis* and protease activities. Improving the efficiency of microbial capture of ammonia in the rumen by increasing carbohydrate availability is likely to reduce urinary N losses and subsequently gaseous and emissions from cattle manure. In addition, the effect of LY on *S. bovis* and protease activities would reduce protein degradation and reduce urinary N losses. However, the addition of LY to a diet with low ruminally fermentable energy might enhance the utilization of ammonia for microbial protein synthesis, decrease urinary N losses, and reduce the ammonia emitting potential of dairy manure.

There is increasing concern of the role of livestock on climate change thus nutritional strategies that aim at decreasing N loss in the rumen are becoming of high interest and the use of yeast promises good results. Therefore the aim of this study is to contribute towards mitigation of climate change through reduction of nitrogen in the rumen. There is a paucity of data on the effects of LY on milk production and rumen fermentation parameters that could improve animal performance through improving N metabolism in dairy cattle.

1.4. Objective

The overall objective of this study was to evaluate the effects of dietary concentrate level and supplementation with LY on milk yield, milk composition and rumen fermentation parameters of early lactating dairy cows.

1.5. Specific objectives

The objectives are to determine the effects of dietary concentrate level and LY on;

1. Milk yield and quality,
2. Urinary and faecal N excretion,
3. Nitrogen metabolism,
4. Ruminal pH, total VFA and molar proportion VFA.

1.6. Hypothesis: H_0

It is hypothesised that the addition of live yeast to diets with low ruminally fermentable energy would;

- Will not influence milk yield and composition
- Will not decrease urinary and faecal N losses
- Will not increase N metabolism
- Will not reduce rumen pH and increase total VFA and molar proportion VFA.

CHAPTER 2: LITERATURE REVIEW

2.1. Introduction

The production of milk and milk products is highly affected by the nutrition and health of the animals. Feed costs accounts for 45-60% of the total cost of milk production (Ishler *et al.*, 2006). Forage availability and nutritive quality are frequently lacking in livestock nutrition (Ayad *et al.*, 2013). Finding strategies to raise dairy cattle economically and efficiently is important in order to increase dairy industry profitability (Lascano *et al.*, 2009). The dairy industry is faced by a lot of challenges like the rising feed cost, variable market prices, less predictable farm incomes, but with an increasing demand for safe, high quality beef and dairy products. Animal production and performance can be increased through improved animal nutrition, while taking into consideration the profit margins of the production.

Feed additives and growth promoters have been used for ages (Beeson & Perry, 1952) and based on a growing concern over the use of antibiotics and other growth promoters in the animal feed industry, interest in the effects of microbial feed additives on animal performance has increased (Ayad *et al.*, 2013). Lately, with increased consumer's concern about safety, quality of animal products and also environmental issues, the current purpose of using these "natural" additives is not only to increase productivity, but to limit excretion of pollutants (Chaucheyras-Durand *et al.*, 2008). Yeast products have been widely utilized as feed additives for ruminant animals (Enjalbert *et al.*, 1999; Chaucheyras-Durand & Fonty, 2001; ABD EL-Ghani, 2004; Kamel *et al.*, 2004; Robinson & Erasmus 2009; Bruno *et al.*, 2009). Products containing *Sacchomyces cerevisiae* have been used to improve daily gain and milk production in ruminants (Wallace, 1994). Live yeast addition was reported to increase nutritional value of poor quality forages and high grain diets (Arambel & Kent, 1990). All these reports on yeast have highlighted health and production benefits with yeast used as a feed additive in animal production.

Yeast products (YP) (active dry yeast, live yeast & culture yeast) are increasingly being used in ruminant nutrition as feed additives to improve feed efficiency and performance, and at the same time, to prevent health disorders (Chaucheyras-Durand *et al.*, 2008). These products are particularly useful in high-producing ruminants whose digestive microbial balance can be altered by high-dietary energy input. In order to achieve maximum production, increasing feed intake is critical, but difficult to achieve especially in early lactation due to physiological changes in the dairy cow, which cause negative energy balance. During this period, increasing

energy density of feed become necessary, and this is usually achieved with addition of feed additives such as live yeast.

2.2. Feeding early lactating dairy cows

The increase in milk yield of dairy cows requires the use of large amounts of concentrates that are rich in energy and crude protein (CP) to meet their nutrient requirements (Cabrita *et al.*, 2009). Ruminant diets should be formulated to provide adequate CP and energy because the two nutrients have a substantial impact on production (Weiss, 2002), this means feeding forages along with grains (concentrates). According to the National Research Council (NRC) (2001), the diet of a fresh Holstein cow in early lactation should have 19.5% CP, and this dietary protein should have 10.5% readily degradable protein (RDP) and 7% RUP. The protein must be well balanced to meet the RDP requirements to feed the rumen microbes for the requirements of high milk production. The requirements of absorbable proteins imply that more dietary protein escapes rumen degradation with the risk that ruminal NH_3 concentrations is sufficient to support rumen microbial CP production (Julien *et al.*, 2015), this is likely to cause a depression in fiber degradation, resulting in decreased DMI and energy supply to the animal.

Dietary carbohydrate is composed of neutral detergent fibre (NDF) and non-fibre fractions, which collectively constitute 65% to 75% of the diets of lactating dairy cattle. Non-fibre carbohydrates (NFC) may provide 30 to 45% of the diet on a dry matter (DM) basis (Hall *et al.*, 2010). Fiber and carbohydrates should be included at the a rate of NDF 25-33%, ADF 17-21% and NFC 36-44% (NRC, 2001). This has led to the modern feeding strategies evolving from primary forage-based to progressively more readily fermentable carbohydrates feedstuffs in dairy rations to meet the energy content requirement in the diet (Madern *et al.*, 2008). This strategy results in an increase in non-structural carbohydrate (NSC) supply which can favour the occurrence of ruminal acidosis due to a production of large quantities of lactate in the rumen and precipitating depressed fiber degradation (Julien *et al.*, 2015). It is critical to balance the NSC and RDP to maintain optimal rumen function and the increase in production, because an imbalance of NSC to RDP results in uncoupled fermentation and a consequent reduction in ruminal microbial activities. In emphasizing the balance of NSC to RDP, Russel *et al.* (1993) stated that NH_3 utilisation in the rumen is intrinsically related to carbohydrate availability, meaning that ruminal proteolysis and deamination will proceed to complete conversion of dietary protein to NH_3 if carbohydrate availability is low. Ammonia production decreases when the carbohydrate availability increases, thus ruminal N will be incorporated into microbial protein and not wasted as urea in urine. Minerals also form a vital part of the

cows ration and dietary Calcium, P and Mg should be 0.74%, 0.385% and 0.36%, respectively (NRC, 2001)

Addition of cereal or grain to ruminant diets maximises production which is also good for farm profit. Non-structural carbohydrates are an essential part of diets for high yielding dairy cows, with grains being the most important sources of NSC. Non-structural carbohydrates provided energy at the animal level and supply rumen microbes with energy needed for capture of degraded feed protein into microbial protein. Block (2006) reported that microbial production appears to be maximised when the carbohydrates (RFC) are provided along with protein (RDP), providing both energy and N for microbial growth. In support, Russel *et al.*, (1993) reported that increasing carbohydrate fermentation rate increase microbial efficiency, therefore increasing protein also increase. The increase in microbial protein results in the increment of milk produced by the cow.

2.3. Forage and concentrate feeding in early lactating dairy cows

The consumption of forage should be controlled in early lactation since cows are in a negative energy balance, this should be done to avoid cows consuming too much forage leaving no room for concentrate consumption which are necessary to meet energy requirements for production (Adesogan, 2009). Concentrates are an important part of the cows ration and they provide for energy requirements. The amount of concentrate mix fed depends on; i) the amount of forage consumed, ii) amount of milk produced and iii) composition of the milk produced (Adesogan, 2009). Concentrates must be fed at 60% to avoid changes in proportion of ruminal VFA which occurs at concentrates levels above 60%. The change in ruminal VFA proportion results in the reduction of milk fat (Adesogan, 2009). The concentrate to forage ratio (C:F) ratio varies from time to time depending on the stage of lactation as the nutritional needs of the cows changes.

2.3.1. Effect of dietary concentrate on feed intake

Dietary characteristics influence milk yield and composition (Min *et al.*, 2005). Low-protein diets may have a negative effect on milk production as this type of a diet reduces DMI. If an average lactating cow diet has around 17% CP, diets with CP at or below 16% can be considered 'low-protein'. These kinds of diets may or may not be deficient in metabolizable protein (MP), which is the unit used by NRC (NRC, 2001) to define protein requirements. There are well-established positive responses in milk yield that accompany increases in dry matter intake (DMI) of dairy cows (Hristov *et al.*, 2005) and, given that DMI responds positively to

increased dietary CP concentration (Newbold *et al.*, 1995). It follows that a significant component of the milk yield response to dietary CP (Ipharraguerre and Clark, 2005) resides in improvements in voluntary intake.

2.3.2. Effect of dietary concentrate on milk yield and composition

In ruminant animals, increasing the dietary protein content in the ration enhances DMI, milk yield and BW, but the responses are greatly according to the type and level of protein supplements (Ipharraguerre and Clark, 2005). Producers often feed high CP rations to ensure a sufficient supply of MP for maximal milk and protein production of dairy cows (Colmenero & Broderick, 2006). However, other studies have reported no improvement in milk protein production with the increase in dietary CP in the diet (Leonardo & Armentano, 2003). Reports on the effects of LY on milk composition have been quite variable no effects (Bruno *et al.*, 2009), to decreasing effects (Robertson and Erasmus, 2009) and increasing effects in some parameters like energy corrected milk, milk fat %, solids-non-fat, lactose and milk true proteins (Bagheri *et al.*, 2009; Bruno *et al.*, 2009). In goats, high dietary CP diet (65%) depressed milk yield in late lactation compared with 50% CP diet (Goatsch *et al.*, 2001). However, these results did not support similar response of dietary CP and energy levels with dairy cows (NRC, 2001). Rations with more than 60% of concentrate may result in changes in the proportions of VFAs, which may in turn result in a reduction in milk fat. Milk yield is negatively affected by low dietary CP diets and this is through the reduction in DMI with low CP diets.

2.4. Feed additives in dairy cows.

Adesogan, (2009), defined feed additives as typically non-nutritive compounds or additives added to the diet to improve dietary nutrient utilization, enhances performance, minimise the risk of metabolic diseases and curtail adverse impacts of diets on the rumen environment. Feed additives are required in the rumen as it is the most important organ in the digestive system and optimising its function means booting animal performance and increasing production. Rumen function problems may reduce feed intake, digestion and animal health thus lowering animal performance which can affect farm profit as well. Feed additives are then required to exert the following effects; i) modulate ruminal pH, ii) reduce the risk of the development of metabolic disorders, iii) enhance rumen development in neonatal animals iv) improve the efficiency of ruminal N utilization and v) increase the level and efficiency of animal performance (Adesogan, 2009). Feed additives support nutrient intake and digestion and are important to maintain the animal in a healthy but high productive state.

2.4.1 Ionophores

Ionophores are organic compounds mainly from *Streptococcus* species that facilitate selective transportation of ions across the outer cell membrane (Adesogan, 2009). According to Kennelly *et al.* (1998), ionophores function primarily in the rumen and improve the efficiency of production by influencing energy metabolism and nitrogen metabolism. As well, ionophores tend to reduce the incidence of lactic acidosis, bloat, and ketosis through their effects on rumen fermentation (Kennelly *et al.*, 1998). Ionophores exert their effects via their influence on rumen microbial populations and metabolism. Gram negative (-) bacteria tend to be resistant to ionophores. Gram positive (+) bacteria are more sensitive to ionophores due to the structure of their cell membrane (Bagg, 1997). Ionophores alter the movement of ions across the cell membrane of gram (+) bacteria forcing the bacteria to expend energy to maintain intracellular pH and ion balance (Bagg, 1997). This energy expenditure reduces the bacteria's ability for growth and reproduction, leading to their eventual death. The resultant change in the ratio of gram (-) to gram (+) bacteria favours the production of propionate and succinate and reduces acetate, butyrate, hydrogen, ammonia, and lactic acid (Kennelly *et al.*, 1998), the effects therefore favour milk production.

Ionophores are extensively used in many divisions of the poultry and ruminants industries to aid animal performance. Commonly used ionophores include Monensin sodium, Lasalocid Sodium, Salinomycin and Laidlomycin Propionate Potassium. Monensin as an ionophore was first approved in 1971 for use in broilers to control coccidiosis (McGuffey *et al.*, 2001), and by 1975 gained approval from the Food and Drug Administration of the USA for use in confined cattle to improve feed efficiency. Monensin is the most commonly used of all ionophores and is a polyether antibiotic which is orally fed as a sodium salt (Yang *et al.*, 2007), it is a carboxylic ionophore produced by a naturally occurring strain of *Streptococcus cinnamonensis*. The most consistent and well documented rumen fermentation alteration observed with feeding ionophores is the increased molar proportion of propionate with a concurrent decrease in the molar proportions of acetate and butyrate (Erasmus *et al.*, 2005), and this increase in VFA production enhances the energy available to the host animal.

2.4.2. Direct fed microbial

There is increasing pressure for livestock producers to minimise the use of antibiotics as feed additives and growth promoters in food animals (Jacela *et al.*, 2010). This has led to the increased use of feed additives containing live microorganisms and (or) their metabolites to alter rumen fermentation and improve animal performance in response to demands for using

more “natural” growth-promoting substances (Sontakke, 2012). The original concept of administering microorganisms to animals involved the feeding of large amounts of “beneficial” microbes to livestock when they were “stressed” or ill (Denev *et al.*, 2007). Microbial products used in this manner were originally called “probiotics”, or products “for life”. Fuller (1989) later defined these beneficial microorganisms as direct-fed microbials (DFM). In the year 1995 the definition was narrowed to “a source of live, natural occurring microorganisms” (Yoon & Stern, 1995). In the last two decades DFM were later defined as “alive, natural microorganisms that have been used to improve digestive function of livestock” (Krehbiel *et al.*, 2003; Yang *et al.*, 2004). Over the years the definition of DFM has been very broad because it needs to cater for the specific and non-specific bacterial and fungal species used. There are three main categories of DFM; 1) Bacterial, 2) fungal and 3) a combination of both.

Probiotics are live cultures of organisms that can beneficially affect the host animal by enhancing the microbial balance in the gut. Probiotics are utilised as feed additives to improve the balance of bacteria in the gut (Jacela *et al.*, 2010). A probiotic must possess the following traits to be an effective feed additive; i) Stability and ability to survive in feed, ii). Ability to replicate after passage through the stomach, and the iii). Ability to block the effects of harmful microorganisms (Sulabo *et al.*, 2007). The ability of probiotics to alter rumen fermentation makes them the ideal tool to improve rumen function which is the basis of animal nutrition in the ruminant animal. Manipulating rumen function through feed supplements is a nutritional strategy to increase feed efficiency, growth and production.

Prebiotics are non-digestible feed substances that selectively stimulate the growth of bacterial favourable species in the gut and therefore benefit the host animal (Jacela *et al.*, 2010). These substances are derived primarily from non-digestible oligosaccharides and they provide readily available substrates for normal bacteria to grow because they are not absorbed by the host animal (Zimmermann *et al.*, 2001). Probiotics generally used as feed additives are oligofructose, fructooligosaccharide and inulin. However, research studies conducted on these probiotics has in the past resulted in contradicting results regarding the beneficial aspects (Smiricky-Tjardes *et al.*, 2003; Loh *et al.*, 2006). The biological importance of probiotics to the animal are improved digestion, stimulation of gastrointestinal immunity and increased resistance to infectious diseases in the gut (Kommera *et al.*, 2006). Prebiotics are of plant origin and nourishes good bacteria in the colon of the host animals which benefits the health and digestion of the animal. In this way prebiotics play a vital role in aiding animal production and performance.

2.4.2.1. Bacterial direct fed microbial

Many microorganisms are used in DFM formulations and the commonly used microbes include *Lactobacillus acidophilus*, *L. casei*, *Enterococcus diacetylactis* and *Bacillus subtilis*. The most common used bacterial organisms in DFM products for ruminants are *Lactobacilli*. These organisms appear to have a better effect in the lower gut than in the rumen fermentation (Ware *et al.*, 1988). Their mode of action depends on many factors including dosages, feeding time, feeding frequencies and strains of the DFM used (Elghandour *et al.*, 2014). Puniya *et al.* (2015) reported how some of the DFM act within the rumen while others impact the gastrointestinal tract. Bacterial DFM strains may be classified as Lactic acid utilising bacteria (LAB), Lactic acid producing bacteria (LUB) or other microorganisms.

2.4.2.2. Fungal direct fed microbial

The commonly used fungal DFM are *Saccharomyces cerevisiae* (yeast) and *Aspergillus oryzae* (Elghandour *et al.*, 2014; Puniya *et al.*, 2015). These DFM have been extensively used in ruminants for improving performance and normalizing rumen fermentation (Elghandour *et al.*, 2015). Many mechanisms of action have been explained to how these DFM exert their effects on ruminal fermentation and improvement of animal performance. Direct fed microbials have been suggested to provide a source of exogenous enzymes and B-vitamins or may supply other unidentified growth promoting factors (Kung, 1997).

2.5. Yeast products

Yeasts are unicellular eukaryotic fungal micro-organisms and their properties are completely different from those of bacteria (Sontakke, 2012). *Saccharomyces cerevisiae* (Yeast) is of high industrial importance due to its ability to convert sugars into ethanol and carbon dioxide. It is widely used in the baking, brewing, distillery and liquid fuel industries, as well as in agriculture as an animal feed additive. Probiotic yeast cultures based on *Saccharomyces cerevisiae* are currently well accepted and widely used in ruminant diets (Throne *et al.*, 2009; Sontakke, 2012; Ayad *et al.*, 2013). They have a high nutritive value, and are rich in enzymes, fatty acids, vitamin B complex, unknown growth factors and amino acids (Sontakke, 2012). The main purpose of using such additives in ruminant diets is to prevent rumen flora disorders and disturbances, especially those associated with the consumption of high energy concentrates (Sontakke, 2012).

Various strains of yeast products have elicited different responses in the cow, but published literature has not shown conclusive evidence that supplementation is beneficial at all times (Yoon & Stern, 1995; Erasmus *et al.*, 2005). Microbial additives of probiotics such as *Saccharomyces cerevisiae* products have been widely used in ruminant nutrition to manipulate rumen fermentation and improve animal performance (Bruno *et al.*, 2009). Yeast culture supplements contain some viable *S.cerevisiae* cells, while the yeast live cell supplements contain higher amounts of yeast with a minimal amount of carrier. Live yeast cells and yeast culture have similar effects on ruminal fermentation (Lynch & Marthin, 2002). Newbold *et al.* (1995) noted that strains of yeast differed in their ability to increase the number of viable bacteria *in vitro* (Lila *et al.*, 2004; Chaucheyras-Durant *et al.*, 2005) and *in vivo*, and responses to yeast were highly variable and apparently influenced by the compositions of the diet. To date, the most consistent positive effects of YP have been reported to have biological importance on rumen microbial activity in young ruminants, stabilisation of rumen pH and prevention of acidosis, as well as stimulation of growth and activity of fibre-degrading bacteria (Chaucheyras-Durand *et al.*, 2008).

2.5.1. Live yeast and yeast culture

Live yeast products contain only viable yeast (De Ondarza *et al.*, 2011). Live yeast products are differentiated according to their particular strain of *S. cerevisiae* (SC). Yeast culture (YC) products contain the media in which the yeast was grown, the metabolites made by the yeast cell during a manufacturer's fermentation process and dead yeast cells (De Ondarza *et al.*, 2011). Mašek *et al.* (2008) reported that YC is produced by fermenting selected liquid and cereal grain raw ingredients with baker's yeast (*S. cerevisiae*) and drying the entire culture medium without destroying components associated with the yeast, such as B vitamins and other fermentation products.

2.5.2. Active dry yeast

Active dry yeast products (ADYs) is yeast that has its biomass dried to preserve cell viability and metabolic activity and, in some products, the cells are mixed together with their fermentation medium (Chaucheyras-Durand *et al.*, 2008). ADYs are well accepted as having beneficial effects in livestock production. These products are generally characterised by a high concentration of viable cells (>10 billion cfu/g) similarly to LY, with the most common species being *S.cerevisiae*. In dairy ruminants, ADYs have been reported to improve animal performance, the most consistent effects being an increase in dry matter (DM) intake and milk production (El-Ghani, 2004; Sniffen *et al.*, 2004; Jouany, 2006; Stella *et al.*, 2007). Again, in

beef cattle or young ruminants, growth parameters (average daily gain & BCS) have been reported to be improved by daily ADY supplementation in several studies (Lesmeister *et al.*, 2004; Galvao *et al.*, 2005).

2.5.3. Yeast mechanism of action

The effects, modes and mechanisms of action of yeast as additives on rumen micro biota have been extensively investigated over two decades now. Several mechanisms have been described, mostly from *in vitro* studies but also from *in vivo* studies with live animal (Chaucheyras-Durand *et al.*, 2008). Three main effects that have been identified (Chaucheyras-Durand *et al.*, 2008), are: (1) improvement of rumen maturity by favouring microbial establishment, (2) stabilisation of ruminal pH and interactions with lactate-metabolising bacteria, and (3) increase of fibre degradation and interactions with plant cell wall degrading microorganisms. However, Robinson & Erasmus (2009) reported the mechanisms to explain why yeast products could stimulate DM intake and productivity in growing and lactating cattle.

The oldest hypothesis is that the yeasts are able to grow, at least for a short period of time, in the rumen thereby directly enhancing fiber digestion and/or producing nutrients that stimulate growth of rumen bacteria, which do the bulk of the fiber digestion (Robinson & Erasmus, 2009). The alternate mechanism is that it is the yeast culture itself, which is created in the yeast fermentation process, provides a mixture of micro-nutrients which stimulates bacterial growth in the rumen thereby facilitating increased fermentation of fiber and/or utilization of the end-products of fiber fermentation to prevent their accumulation in the rumen (Robinson & Erasmus, 2009). The rumen is an anaerobic chamber, and its microbial population's survive at an oxygen free environment. Live yeast's has an oxygen scavenging ability, and this lowers the redox potential (Chaucheyras-Durand and Fonty, 2002), which should improve growing conditions for the anaerobic rumen microbes, especially the cellulolytics, which are more sensitive to the presence of oxygen in their environment. This growth of yeast in the rumen utilizes the trace amounts of dissolved oxygen, particularly at the interface of the cellulolytic bacteria and fiber, thereby stimulating growth of rumen bacteria, to which oxygen is toxic (De Ondarza *et al.*, 2011). Yeasts utilize nutrients, such as lactic acid and if allowed to accumulate in the rumen, could suppress bacterial growth and/or suppress DM intake by driving rumen pH down. Accumulation of lactic acid in the rumen is associated with rumen dysfunctions like bloat and acidosis which are detrimental to the animal's health and milk production. Live yeast on the other hand as reported for its negative action on *S. bovis* would suppress the rise of rumen pH caused by lactic acid level rise in the rumen.

2.6. Effects of live yeast on dairy cows

2.6.1. Effects on dry matter intake

Yeast products have been reported to have a variety of biological importance to cow performance and rumen fermentation dynamics, but their role in increasing DMI is of major concern since increase in DMI is associated with increased milk production or animal performance. However, diet composition has been suggested as a factor that influences the variability of responses when *S. cerevisiae* (SC) is fed to ruminant animals (Ghasemi *et al.*, 2012). Many investigations on the effect of YP have been done to check its effect on DMI, and quite a number of authors (Williams *et al.*, 1991; Shaver and Garrett, 1995; McCoy *et al.*, 1997; Sanchez *et al.*, 1997; Bruno *et al.*, 2009) agree that in commercial dairy herd trials, the use of YP as feed additive increased DMI. Bruno *et al.* (2009) reported that feeding a yeast culture of *S. cerevisiae* improved yields of milk and milk components in heat-stressed multiparous Holstein cows. This means that YP as feed additives have more beneficial effects in this manner.

2.6.2. Effects on milk yield and composition

Milk production has been documented vastly to be increased by addition of YC as a feed additive by a number of authors (Williams *et al.*, 1991; Shaver & Garrett, 1995; McCoy *et al.*, 1997; Sanchez *et al.*, 1997; Bruno *et al.*, 2009). Live yeast increased milk yield and feed efficiency across multiple experiments, possibly due to improved rumen function or reduced nitrogen waste (De ondarza *et al.*, 2011). In a study done by Mašek *et al.* (2008), a significant increase in total milk yield and morning milk yield in dairy sheep fed yeast as an additive was reported.

Addition of YC to diets has been shown to increase volatile fatty acid (VFA) concentration (Mutsvangwa *et al.*, 1992) and propionate molar proportion (Harrison *et al.*, 1988), and to decrease lactic acid content of ruminal fluid (Erasmus *et al.*, 1992). Milk fat was found to be significantly increased in the cows fed the LY diet but milk composition was not affected (Mašek *et al.*, 2008). The level of concentrate in the diet, stage of lactation of the animals and environmental temperature fluctuations may have contributions to the increase or decrease in the response of the LY cells in the diet (Mašek *et al.*, 2008). Bagheri *et al.* (2009) documented an improvement in nutrient digestibility and numerical increase in fat-corrected-milk (FCM) and energy-corrected-milk (ECM). Compared to cows not given YC as a supplement in the

diet, Bruno *et al.* (2009), found an improvement in milk true protein, solids-not-fat (SNF) and lactose from cows fed YC as a supplement. Milk composition is very important as consumer's preference for high fat milk has changed to high protein milk. With yeast being reported to increase certain milk components, its addition to dairy cow's rations will in feeding according to the desired milk components. Not all yeast strains favours the same response, therefore strain selection is important.

2.6.3. Effects on rumen microorganisms

It has been reported that cellulolytic rumen microorganism's establishment is more rapid in calves and lambs receiving *S. cerevisiae* daily (Sontakke, 2012), and also the cellulolytic population of animals supplemented with *S. cerevisiae* is more stable than that of animals not receiving supplement. Previous studies done (Chaucheyras-Durand & Fonty, 2001; Chaucheyras-Durand & Fonty, 2002), have shown that protozoa appeared earlier in calves supplemented with *S. cerevisiae*, this is because protozoa appears in the rumen once the bacterial population is present and the *S. cerevisiae* stimulates the growth of rumen cellulolytic bacteria by providing a mixture of micronutrients that stimulate bacterial growth (Sontakke, 2012; Robinson and Erasmus, 2009). Basically, the maturation of the microbial ecosystem is accelerated in the presence of the yeast (Sontakke, 2012). Post-prandial variations of pH in sheep were reported from sheep fed LY deist's (Mathieu *et al.*, 1996). Wiedmeier *et al.* (1987) reported an increase in the numbers of cellulolytic bacteria and ruminal degradation of fibre (Chademana and Offer, 1990) and this is in agreement with reports from other authors (Chaucheyras-Durand and Fonty, 2001; De Ondarza *et al.*, 2011), who also reported positive results.

Chaucheyras-Durand and Fonty (2001), noted that the addition of LY to the diet of gnotobiotically reared lambs showed that it accelerated the establishment of rumen cellulolytic bacteria, enhanced the activity of fiber digesting enzymes and tended to increase *in situ* dry matter degradation of wheat straw. A number of scientific investigators (Newbold *et al.* 1996; Beauchemin *et al.*, 2003), are in agreement that yeast addition improves the development of rumen lactate-consuming bacteria, prevents accumulation of lactate, and rumen pH drops. Yeast stimulates lactate users, increasing their numbers and serves as a competitor with lactate producers by utilizing lactic acid, which if allowed to accumulate in the rumen, may suppress bacterial growth and DIM by lowering rumen pH (Nisbet and Martin, 1991; Sontakke, 2012). Lactic acid is the primary cause of acidosis in lactating dairy cattle, reducing its concentration can have a significant effect on the pH (Sontakke, 2012), rumen pH fermentation is beneficial to cellulolytic microorganisms which are sensitive to acid. Live yeast favours

cellulolytic microorganisms by having a negative on lactic metabolising bacteria and maintaining rumen pH stability favours optimum rumen function.

2.6.4. Effects of live yeast on rumen fermentation

2.6.4.1. Effects on volatile fatty acid production

Ruminal propionate is the single most important substrate for gluconeogenesis in lactating dairy cows (Drackley *et al.*, 2001). Estimates of Seal and Reynolds (1993) indicated that propionate supplies 32-73% of the energy demands (glucose). Rumen microbial populations hydrolyze and ferment dietary compounds into volatile fatty acids (VFAs), whose amounts drive pH evolution. Moreover, lactic acid is a common product of carbohydrate fermentation, produced by bacterial species such as *S.bovis*, *Selenomonas ruminantium*, *Lactobacillus sp*, etc. *S. bovis* is considered as a major contributor in lactate production from high fermentable. Changes in ruminal fermentation and digestion are thought to be responsible for these improved performances when using YC as feed additives (Enjalbert *et al.*, 1999). Addition of YC to diets has been shown to increase volatile fatty acid (VFA) concentration (Mutsvangwa *et al.*, 1992) and propionate molar proportion (Harrison *et al.*, 1988), and to decrease lactic acid content of ruminal fluid (Erasmus *et al.*, 1992). A study conducted by Enjalbert *et al.* (1999) is in agreement with this findings, they found YC to have significantly increased molar percentage of propionate and decreased the acetate: propionate ratio before feeding.

Yeasts decrease the partial pressure of O₂ in the rumen and favour a fermentation pathway that will be less acidic. The conversion of propionate to lactate and vice-versa is a function of the partial pressure of O₂ in the rumen. In situations where partial pressure of O₂ is low, the lactate to propionate reaction is favoured and in situations where the partial pressure of O₂ is high the propionate to lactate reaction is favoured (Sontakke, 2012). Proportion of concentrates in the diet may influence effect of YC on ruminal digestion or performance, the greatest improvements being, encountered with high concentrate diets (Williams *et al.*, 1991; Carro *et al.*, 1992). However, results may depend upon the strain of YC (Newbold *et al.*, 1995). On the other hand, responses can be affected by the time of sampling when separate meals are fed (Williams *et al.*, 1991). With LY being reported to increase VFA's dairy cows this suggest why LY supplemented cows have increase milk production, the dairy farmer's most important objective is to increase milk yield which increases profit for the enterprise.

2.6.4.2. Effectson rumen pH

According to Chaucheyras-Durand *et al.*,(2012), rumen pH is a function of DMI, where it becomes below 6 when the DMI exceeds 3.8% of body weight and usually high producing animals with alleviated nutritional requirements become more prone to pH drop. Dairy cows in early lactation are usually at a negative energy balance, have alleviated nutritional needs and are more at risk to ruminal pH drop. The quality of feed consumed directly matters also, as where the pH drops below 6 when the rumen digested starch accounts for greater than 40% of the consumed diet.

Ruminant diets must be formulated to meet CP plus energy requirements because these two nutrients have an important impact on production (Weiss, 2002). Dairy cows are often fed high concentrated diets by addition of cereal or grain to their diets and forage is usually limited in the ration. However, this puts dairy cows at higher risk of metabolic disorders as this diet types may produce a large quantity of lactate. Cows fed high concentrate diets will have a lower rumen pH, acetate, and butyrate concentrations, where else propionate concentration will rise (Chaucheyras-Durand *et al.*, 2012). When the rumen acidity is alleviated with a buffer, total VFA production increases and so does milk yield and milk fat content especially to cow fed high concentrate diets.

Incorporation of LY to diets of ruminants is thought to help reduce ruminal lactate (Marden *et al.*, 2008), and enhance NH₃-N utilisation by rumen microbes (Galip, 2006). High fiber diets will not sustain elevated propionate production which negatively impacts milk lactose synthesis and overall milk yield (Chaucheyras-Durand *et al.*, 2012). Quick microbial fermentation results in increased VFA concentrations in the rumen which contributes to lower rumen pH (Chaucheyras-Durand *et al.*, 2008). The decline in rumen pH out favours lactate producing bacterial species at the expense of lactate consuming bacteria. Martin *et al.*, (2006), reported that when the rumen pH is low, microbial diversities is compromised as the protozoa numbers declines.

Live yeast has been reported to stabilise rumen pH (Robinson & Erasmus, 2009) and also has interactions with lactate metabolising bacteria (Chaucheyras-Durand *et al.*, 2008). This is explained by its effect on *S. bovis* and protease activities. Stimulation and growth of lactate utilizing bacteria such as *M. eldenii* or *S. ruminatum* were observed *in vitro* in the presence of different yeast (Nisbet and Martin, 1991; Newbold *et al.*, 1996), this was through a supply of different growth factors and organic acids essential for the lactate-fermenting bacteria. A number of investigators have reported the effect of LY on ruminal pH stabilisation in different animals an *in vitro* (William *et al.*, 1991; Lynch & Martin, 2002; Lila, 2004; Marden *et al.*, 2008). With this positive reports on LY's ability to maintain rumen pH stability and adequate balance

between soluble N and carbohydrates supply can enhance microbial growth. Meaning that more digested carbohydrates would be incorporated into microbial protein and not wasted as urea which cost the cow a lot of energy to convert (NH_3 to urea) and therefore this energy would be utilised for milk production.

2.7. Effect of live yeast on nitrogen metabolism

Yeast products based on *S. cerevisiae* strain have been used with variable success to favourable modify the rumen environment and promote microbial growth in ruminants (Hristova *et al.*, 2012). Yeasts are aerobes and are unlikely to proliferate in the rumen environment and only negligible amount of yeast (live or not) are reportedly beneficial to the rumen microbial population (Beauchamp *et al.*, 2003). Mwenya *et al.* (2005), found yeast culture to have decreased the rumen methane production in sheep. Enteric methane pollution is the major cause of pollution from livestock production and yeast's N metabolising effect promises to reduce nonpoint N pollution from ruminant farms.

According to Wallace *et al.* (1997), most of protein are rapidly degraded by bacteria and protozoa into peptides, AAs and NH_3 in the rumen. A part of NH_3 is converted into microbial protein which represents an essential source of N for ruminant animals and part is recycled in the form of urea by the animal. Meanwhile, an important proportion of NH_3 produced in the rumen is excreted and represents N loss of 20-25% of dietary N intake (Leng & Nolan, 1984), this portion contributes to the undesired greenhouse gases (GHG) emissions from livestock. Data documenting the effects of LY on N metabolism to reduce NH_3 concentration in the rumen are very limited and inconsistent. This is mainly due to the nature of the diet, dosage of LY in diet and physiological stage of animals used in experiments.

The effect of YP to stimulate rumen bacteria numbers may increase the rate of substrate fermentation and microbial protein synthesis (Wallace & Newbold, 2006). Figure 1 shows how LY decreases the rate of peptide degradation which may reduce NH_3 wastage and can increase the amount of RUP available for the cow (Chaucherus-Durand *et al.*, 2005; Jouany & Morgavi, 2007). Ammonia concentrations in the rumen can decrease with LY inclusion in ruminant diets as its addition can improve the efficiency of microbial capture of NH_3 by increasing carbohydrate availability. This is likely to reduce urinary and faecal N losses to the environment and may lower nonpoint N pollution from ruminants.

The mechanism of yeast's action on N metabolism by a competition between LY cells and bacteria for energy supply and its direct inhibitory effect on yeast small peptides on targeted

peptidases suggest how best yeast increase N utilisation in ruminants. However, dietary level of soluble N is a key parameter that drives the production response of yeast (Sniffen *et al.*, 2004). Therefore, with an adequate balance between soluble N and carbohydrates supply, *S. cerevisiae* could enhance microbial growth and decrease N loss from ruminants (Fonty & Chaucherus-Duran, 2006). More studies should be carried out on LY's effect on ruminal NH_3 concentration since previous data reported shows promising effects.

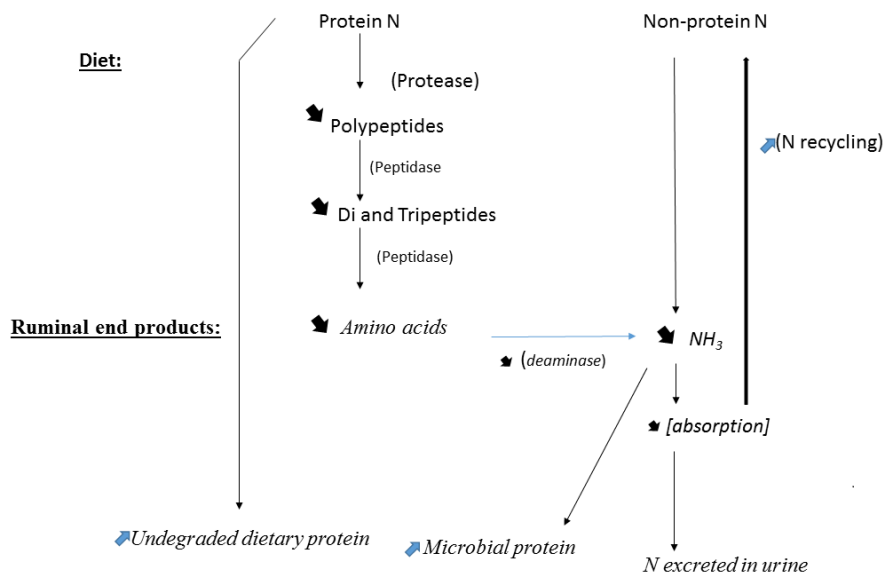


Figure 2.1. Possible sites targeted by feed additives to improve nitrogen metabolism in the rumen (⬇: decrease of rumen function; ⬆: increase of rumen function) (Jouany & Morgavi, 2007).

CHAPTER 3. EFFECTS OF DIETARY CONCENTRATE AND LIVE YEAST ON MILK YIELD AND COMPOSITION

3.1. INTRODUCTION

In many regions of the tropics and sub-tropics, feeds for ruminants are primarily based on natural pasture resources and/or crop residues (Sanh *et al.*, 2002), these natural pastures decrease in nutritive value as they mature or during the dry season. Attempts to improve the quality or quantity of nutrient supplies through grass and legume cultivation are constrained by variable rainfall and several other factors, the emphasis of research strategies in such circumstances has generally been directed at improvement of efficiency in milk production by genetic improvement of cows rather than nutritional means (Cronje, 2000). Feed additives on the other hand provide improvement for low quality feeds and their availability is not affected by environmental conditions but are cost implicated. The economic advantage of probiotics depends on its relevant prices and on the levels of milk production of the dairy cows (Vibhute *et al.*, 2011).

Limitations of high quality forage is a worldwide epidemic in the livestock industry. Dairy cows are often fed high concentrate diets with low quality forages putting dairy cows at higher risks of metabolic disorders like ketosis and sub-acute ruminal acidosis (SARA) (Zhang *et al.*, 2013). The health and performance of dairy cows depends on the intake of digestible and metabolisable nutrients from feeds. Certain amounts of fibre from roughage are necessary for rumination and rumen processes (Meyer *et al.*, 2001; Meyer *et al.*, 2002). But normally roughage is less digestible than concentrate and cannot provide enough energy for high yielding cows. However the addition of LY in feed rations stimulates the bacteria's growth which digest cellulose and hemicellulose as *Fibrobacter succinogens* and *Ruminococcus ssp.*, this increase fiber digestion in the rumen and may result in higher consumption of organic matter and consequently increase milk production (Vieira *et al.*, 2014). Live yeast aids digestions of poor quality forages by its beneficial effects on rumen microbes and providing nutrients for the microbes. The specific objective of this study was therefore to determine the effects of dietary concentrate level and LY on milk yield, milk composition and nitrogen metabolism early lactating dairy cattle.

3.2. MATERIAL AND METHODS

3.2.1. Study site

The study was conducted at the Dairy Section Unit of the Animal Production Institute (Irene) of the Agricultural Research Council of South Africa (2589' S and 2820' E) Pretoria, at 1475m altitude and average annual minimum temperature of 14°C and annual maximum temperature of 24°C.

3.2.2. Cows, diets and experimental design

Four fresh primiparous Holstein dairy cows (350-450kg), were used immediately after calving in a 4 x 4 Latin square design with 11-d periods. The first seven days of each period were used to adapt the cows to treatments, and the remaining 4 d were used to collect data. The four experimental treatments were in a 2 x 2 factorial arrangement: Low C:F diet with no additive, Low C:F diet with live yeast, high C:F diet with no additive and high C:F diet with live yeast. Live yeast was added to the two basal (high and low C:F diet) TMR's (Table 3.1) at 2g/d per animal. Animals were housed individually in 5mx7m pens and had access to a dirt exercise lot. The animals were fed only their assigned experimental diets offered *ad libitum* once a day in the morning at 08h00. The feeding rate was adjusted to yield orts of about 5-10% intake. The basal diet was formulated to meet the requirements of Holstein cow diet using CNCPS ver 6.1 of the Agriculture Modeling and Training System (AMTS. Cattle, USA).

3.2.3. Sampling and analysis

3.2.3.1. Feed intake

Feed allocated and orts were recorded daily at 8:00 am before feeding. Feed intake was calculated as the difference between feed offered and feed refusals.

3.2.3.2. Body condition score and body weight measurements

Body condition score (BCS) and body weight were determined at assignment and termination of the period (day 1 and day 11). Cows were scored using the 5-point BCS scale of (one: very thin & five: very fat) (Wildman *et al.*, 1982).

3.2.3.3. Milk production and composition

Cows were milked twice at 06h00 and 16h00 in a twelve-point DeLaval herringbone parlour equipped with an Alpro Herd Management System (DeLaval. (Pty) Ltd, Heilbron, 9650, South Africa). Milk production from each cow was recorded on a daily basis. Composite milk samples were prepared from consecutive morning and afternoon milking once for all the days of measurements. A mastitis test cup was used to check for mastitis daily before feeding. The Broad Spectrum Microtabs® II milk preservative was used to preserve the milk samples pre analysis. Milk analysis was done by means of a System 4000 Infrared Analyser (Foss Electric, Hillerod, Denmark) at Lacto Lab (Pty) Ltd, Main road, Irene. Milk was analysed for fat, protein, lactose, milk urea nitrogen and SCC.

3.2.3.4. Nitrogen metabolism

Faecal grab samples were done daily by hand collection directly from the rectum using a gloved hand for the 4d of measurements and weighed. A 100 g/kg sub-sample of the faecal sample from each cow will be collected and stored daily. Approximately ± 50 g of the faeces was dried at 60°C for 48h for DM determination (Muya *et al.*, 2011). The remaining faecal sample were bulked for each cow for each period and kept at 4°C for organic matter (OM) and N determination.

3.2.3.5. Determination of nitrogen in urine

Spot samples of 50 mL of urine were obtained from all cows at the 10th day of each experimental period (Freitas-Júnior *et al.*, 2014), four hours after the morning feeding, by vulva massage stimulation into plastic buckets with approximately 100 ml of 10% sulphuric acid (H₂SO₄) to maintain a final pH below 4. A sub-sample of 50 ml was taken and stored at -20°C prior to analysis for N. Analyses of N in the urine was performed through commercial kit (Labtest Diagnóstica, Uréia CE, Lagoa Santa, Minas Gerais) via enzymatic colorimetric method.

3.2.3.6. Chemical analysis

Samples of TMR ration and orts, within a cow, were collected daily, frozen and composited by treatment. The DM contents of TMR and orts were determined by oven drying at 60°C for 48 hours (Muya *et al.*, 2011). Dried TMR and orts were ground (through a 2mm screen) and analysed for organic matter (OM), ether extract (EE), calcium (Ca), potassium (K), magnesium

(Mg), phosphorus (P) (AOAC, 2000). Ash and OM contents were determined as described by AOAC (2000). Aluminium pans were dried in an oven at 100°C for 15 minutes then cooled in desiccator, weighed and recorded. Two gram of feed samples were added to the pans, weighed and recorded. The pan plus feed samples were placed in an oven at 100°C for 12 hours then cooled in desiccator, weighed and recorded. After recording the pans plus sample were placed in a muffle furnace at 500°C for three hours to determine ash. After the three hours, the furnace was cooled for eight hours, the samples were cooled in a desiccator, weighed and recorded. Ash was calculated as %Ash (dmb) = (Ash weight/ Dry weight) x 100. Organic matter was then calculated as %OM (dmb) = %DM - %Ash (dmb). Crude fibre (CF), P and EE was determined according to AOAC (2000). Feed efficiency (FE) was calculated as FE = (energy corrected milk x DMI). CP was determined using the Kjeldahl method, through calculations using the N value. Ca, K and Mn were determined according to Giron (1973) using the Perkin Elmer Atomic Spectrophotometer (USA). Neutral detergent fiber was determined using the heat-stable α -amylase (sigma-Aldrich co. LTD., Gillingham, UK, no. A-1278) with sodium sulphite (Van Soest *et al.*, 1991). The ADF was determined according to Goering and Van Soest (1970) as modified by Van Soest (1991) using the Fibretech system equipment (Tecator LTD, Thornbury, Bristol, UK). Non fiber carbohydrate (NFC) was calculated from the assayed nutrients as NFC = 100 - (CP + Fat + Ash + NDF) (Hall, 1998).

3.2.3.7. Calculations

Total Manure excretion (Me) was determined by using the equations of Nennich *et al.* (2005) based on DMI and urine excretion as described by Nennich *et al.* (2006) based on MUN.

Manure excretion (Me), kg day⁻¹ = [DMI x 2.63 (\pm 0.10)] + 9.4 (\pm 2.8)

Urine excretion, kg day⁻¹ = [MUN x 0.563 (\pm 0.115)] + 17.1 (\pm 2.0)

3.2.3.8. Statistical analysis

Data on DM intake (DMI), milk yield and milk contents were analysed for a 4 x 4 Latin square using the MIXED procedure in SAS software (SAS, 2000). Least square means are reported and differences (P < 0.05) are indicated by different subscripts in the table. The Turkey's test was used to compare the treatment means.

The model was;

$$Y_{ijkl} = \mu + C_i + L_j + A_k + P_l + (C \times L)_{ij} + e_{ijkl}$$

Where;

Y_{ij} = observation value for measurements

μ = overall mean of the population

C_i = i^{th} effect of dietary concentrate

L_j = j^{th} effect of live yeast

A_k = k^{th} cow effect

P_l = l^{th} period effect

$(C \times L)_{ij}$ = fixed effect of effect of dietary concentrate i and live yeast j ;

e_{ij} = error associated with the measurement taken

Linear regression were also performed to evaluate relationship between N intake and N excreted in urine and faeces, using MINITAB (2012).

3.3. RESULTS

The chemical composition of the diets are summarised in Table 3.1.

Table 3.1: Chemical composition (g/kgDM) of experimental diets.

Item	60:40 C:F	40:60 C:F
Dry matter, (g/kg)	695	598
Organic matter	940	933
Crude protein	173	173
Readily undegradable protein	372	331
Neutral detergent fiber	337	303
Forage NDF	169	254
Starch	280	293
NFC	388	416
NEL, ¹ Mcal/kg	16	162
Calcium	10	9.8
Phosphorus	0.36	0.35
Magnesium	0.25	0.25
Potassium	1.26	1.40

¹Calculated using National Research Council (2001);C:F: concentrate to forage ratio; NDF: neutral detergent fiber; NFC: nonfiber carbohydrates; NEL; net energy for lactation; 60:40: 60 % concentrate and 40 % forage; 40:60: 40% concentrate and 60% forage; DM: dry matter.

The results on effects of dietary C:F and addition of LY on feed intake, milk yield and composition, somatic cell count and feed efficiency are presented in Table 3.2. Cows fed low C:F diet supplemented with LY had lower DMI and lower milk yield, but greater FE compared to cows in all other groups ($P<0.05$). Milk fat yield was lower ($P<0.05$) for cows fed low C:F diet supplemented with LY compared to cow fed high C:F with no LY. These cows had also lower protein yield ($P<0.05$) compared to all other groups. No differences in milk fat %, milk lactose (% and kg/d), MUN, SCC and energy corrected milk (ECM) were observed between groups.

Dry matter intake, milk yield, milk protein (% and Kg/d) as well as ECM were higher ($P<0.05$) in high C:F compared to low C:F diet. No effects of dietary C:F were observed on milk fat and milk lactose and SCC ($P>0.05$). Addition of live yeast decreased ($P<0.05$) cow's DMI, tended to decrease ECM and milk protein yield, tended to increase FE ($P<0.10$), but did not affect ($P>0.05$) milk yield and composition and SCC. There was an interaction ($P=0.03$) C:F x LY on FE (data not shown). Diet C:F and LY did not affect MUN.

The results on body weight (BW) and body condition score (BCS) as affected by dietary concentrate level and live yeast supplementation are presented in Table 3.3.

Table 3.2: The effect of dietary concentrate level and live yeast supplementation on feed intake, milk yield and composition, somatic cell count and feed efficiency

C:F	LY	DMI	MY	Milk quality						MUN	SCC	ECM	FE
				Milk fat, %	Milk fat yield, kg/d	Milk protein yield, %	Milk protein yield, kg/d	Milk lactose yield, %	Milk lactose yield, kg/d				
60:40	C	22.3 ^a	26.5 ^a	3.9	1.04 ^a	2.9 ^a	0.79 ^a	4.9	1.31	16.6 ^a	5631	27.8	1.29 ^b
	LY	21.1 ^a	26.1 ^a	3.6	0.93 ^{ab}	2.9 ^a	0.76 ^{ab}	5.2	1.36	12.3 ^b	5319	26	1.26 ^b
40:60	C	20.3 ^a	25 ^a	3.6	0.9 ^{ab}	2.8 ^b	0.7 ^b	5.6	1.37	13.4 ^{ab}	5975	25	1.25 ^b
	LY	15.1 ^b	23.3 ^b	3.5	0.82 ^b	2.7 ^b	0.64 ^c	4.9	1.14	13.8 ^{ab}	5531	22.9	1.59 ^a
SEM		0.58	0.38	0.12	0.03	0.02	0.01	0.17	0.04	0.82	418	0.59	0.04
Concentrate to forage means													
60:40		21.7 ^a	26.3 ^a	3.8	0.99	2.9 ^a	0.77 ^a	5.1	1.33	14.4	5475	26.9 ^a	1.3
40:60		17.7 ^b	24.1 ^b	3.6	0.86	2.8 ^b	0.67 ^b	5.2	1.26	13.6	5753	23.9 ^b	1.4
SEM		0.58	0.38	0.12	0.03	0.02	0.01	0.17	0.04	0.82	418	0.59	0.04
Live yeast means													
C		21.3 ^a	25.7	3.76	0.97	2.89	0.74	5.26	1.34	15	5803	26.4	1.27
LY		18.1 ^b	24.7	3.56	0.87	2.82	0.7	5.05	1.25	13	5425	24.4	1.42
SEM		0.58	0.38	0.12	0.03	0.02	0.01	0.17	0.04	0.82	418	0.59	0.04
Significance													
C:F		**	**	NS	NS	**	**	NS	NS	NS	NS	**	NS
LY		**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C:F x LY		*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SEM		0.58	0.38	0.12	0.03	0.02	0.01	0.17	0.04	0.82	418	0.59	0.04

^{ab} Means within a section of a with different superscripts differ (P<0.05) for comparison between groups; *significant effect (P<0.01); ** significant effect (P<0.001); *** significant effect (P<0.0001); NS:non-significant (P>0.05); SEM: Standard error of mean; ³LY: Live yeast; ²C: Non-supplemented diet; SCC: Somatic cell count; ECM: Energy-corrected milk = ((0.327x kg milk)+(12.95x kg fat)+(7.2x kg protein)) (Orth, 1992). FE: Feed efficiency; FE = ECM/DMI; C:F: concentrate to forage ratio; MY: milk yield; DMI: dry matter intake.

Table 3.3: The effect of dietary concentrate level and live yeast supplementation on body weight and body condition score

Concentrate	LY	Initial BW, kg	Final BW, kg	BW change, kg	BCS change
40:60	C	438	456	18.5	0.6
	LY	440	440	-0.7	0.6
60:40	C	430	452	22.2	-0.6
	LY	430	427	-3.5	0.06
SEM		8.72	7.27	7.49	0.04
Concentrate to forage means					
60:40		430	439	9.5	0.00
40:60		439	448	8.9	0.06
SEM		8.72	7.27	7.49	0.04
Live yeast means					
C		435	433	-2	0.06
LY		434	454	20.3	0.06
SEM		8.72	7.27	7.49	0.04
Significance					
C:F		NS	NS	NS	NS
LY		NS	NS	NS	NS
C:F x LY		NS	NS	NS	NS
SEM		8.72	7.27	7.49	0.04

^{ab} Means within a section of a with different superscripts differ ($P < 0.05$) for comparison between groups; *significant effect ($P < 0.01$); ** significant effect ($P < 0.001$); *** significant effect ($P < 0.0001$); NS: Effects non-significant ($P > 0.05$); SEM: Standard error of means; NS:non-significant ($P > 0.05$) for interaction; SEM: Standard error of mean; ³LY: Live yeast; ²C: Non-supplemented diet; C:F concentrate to forage ratio/ dietary concentrate level

Body weight and BCS were not affected by both the dietary addition of live yeast and dietary C:F ($P>0.05$) and averaged 444 kg and 2.45, respectively. Dietary C:F and LY did not affect BW and BCS. Body weight and BCS did not differ between cows fed low C:F diet with LY and cows fed high concentrate diet with no LY.

The results on nitrogen metabolism as affected by dietary concentrate level and live yeast supplementation are presented in Table 3.4. Intake and excretion of N were affected by Dietary C:F and addition of LY. Increasing dietary C:F increased Intake of N, but also its excretion in urine, faeces and in total manure. The opposite was observed with addition of LY, which decreased intake of N and its excretion in urine, faeces and in total manure. Intake of nitrogen was lower ($P<0.05$) for cows fed low C:F diet with LY compared to the rest of the groups. Faecal N was higher for cows fed high C:F with no LY compared to cows fed high C:F with LY and cows fed low C:F with no LY. Urinary N and total manure N were lower for cows fed high C:F with no LY compared to the rest of groups.

Faecal and urinary N excretion were evaluated as percentage of N intake and results are presented in Figures 3.1 and 3.2, respectively. Addition of LY did not affect ($P>0.05$) faecal N excretion for cows fed low C:F diet, but decreased it ($P<0.05$) for cows fed high C:F diet (Figure 3.1). Cows fed high C:F diet excreted more ($P<0.05$) N in faeces compared to low and high C:F diets supplemented with LY. Addition of LY decreased urinary N for cows fed low C:F diet, but did not have effect on cows fed high C:F diet (Figure 3.2). Urinary N excretion was higher for cows fed low C:F diet without LY compared to the rest of the groups.

The linear regressions to determine whether the change in urinary and faecal N were due to the amount of N intake indicated a strong positive relationship ($R^2=0.61$, $P<0.001$) between N intake and urinary N, and the equation was:

Urinary N (g/d) = 14.4 + 0.32 N intake (g/d) (Figure 3.3).

Table 3.4: The effect of dietary concentrate level and live yeast supplementation on faecal and urinary nitrogen

Concentrate	LY	N intake, g/d	N in faeces, g/d	N in urine, g/d	N in manure, g/d	N retained/g
40:60	C	563 ^a	169 ^b	217 ^b	422 ^b	141 ^b
	LY	437 ^b	232 ^{ab}	142 ^b	276 ^b	161 ^b
60:40	C	625 ^a	248 ^a	225 ^a	432 ^a	217 ^a
	LY	592 ^a	153 ^b	361 ^b	231 ^b	231 ^b
SEM		16.4	16.8	18.3	35.2	10.6
Concentrate to forage means						
60:40		608 ^a	232 ^a	206 ^a	393 ^a	224 ^a
40:60		500 ^b	169 ^b	179 ^b	349 ^b	151 ^b
SEM		16.4	16.8	18.3	35.2	10.6
Live yeast means						
C		514 ^b	153 ^b	165 ^b	318 ^b	196 ^b
LY		594 ^a	248 ^a	267 ^a	427 ^a	179 ^a
SEM		16.4	16.8	18.3	35.2	10.6
Significance						
C:F		**	**	**	**	NS
LY		**	NS	NS	NS	**
C:F x LY		NS	NS	NS	NS	NS
SEM		16.4	16.8	18.3	35.2	10.6

^{ab} Means within a section of a column with different superscripts differ ($P < 0.05$) for comparison between groups; * significant effect ($P < 0.01$); ** significant effect ($P < 0.001$); *** significant effect ($P < 0.0001$); NS: Effects non-significant ($P > 0.05$); SEM: Standard error of mean; NS: Effect non-significant ($P > 0.05$) for interaction; SEM: Standard error of mean; ¹LY: Live yeast; ²C: Non-supplemented diet; C:F: concentrate to forage ratio.

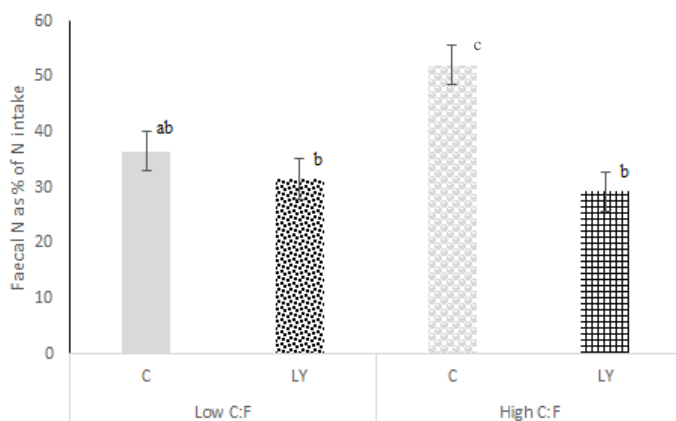


Figure 3.1: Faecal Nitrogen (N) as percentage of N intake.
^{abc}Means with different superscripts differ ($P < 0.05$).

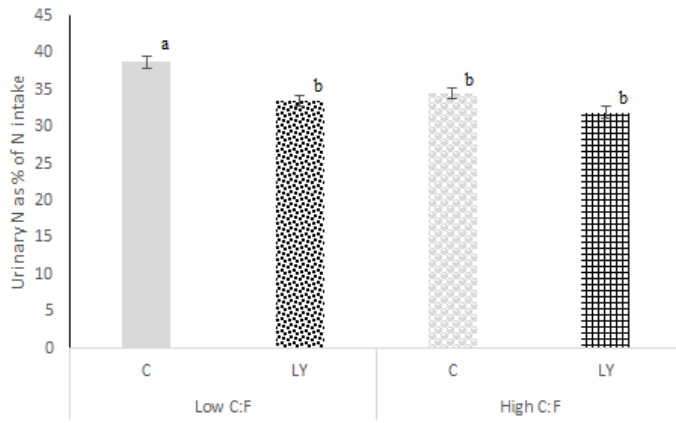


Figure 3.2: Urinary Nitrogen (N) as percentage of N intake.
^{ab}Means with different superscripts differ ($P < 0.05$).

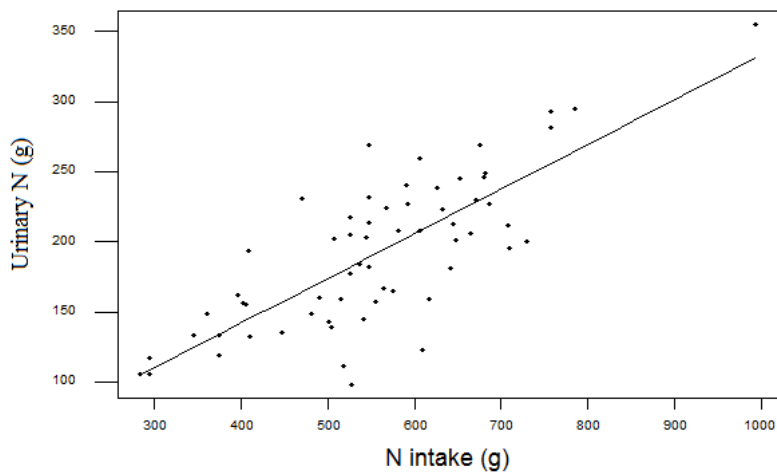


Figure 3.3: Relationship between N intake (g/d) and urinary N (g/d)

3.4. DISCUSSIONS

3.4.1 Feed intake, milk yield and milk composition

Reports on the effects of yeast on DMI have been very inconsistent and variable over the years and diet composition and origin plays a great role in the variation, therefore it has been very difficult to conclude on the effects on yeast on DMI. Yeast culture provides soluble growth factors that stimulate growth of cellulolytic bacteria and cellulose digestion (Callaway & Martin, 1997), which can aid DMI improvements. In the present study, the average DMI ranged from 15.1 for cows receiving LY in low C:F to 22.3 kg/d for cows fed high C:F with no LY. The decreased effect of LY on DMI is in agreement with a number of previous studies (Alshaikh *et al.*, 2001; Erasmus *et al.*, 2005; Kudrna *et al.*, 2007; Titi *et al.*, 2008; Cikoruglu *et al.*, 2010). While some studies reported an increased DMI (Dann *et al.*, 2000; Garg *et al.*, 2000; Guedes *et al.*, 2008; Bagheri *et al.*, 2009), others (Bruno *et al.*, 2009; Bagheri *et al.*, 2009; De Ondarza *et al.*, 2010; Yalcin *et al.*, 2011; Zhang *et al.*, 2013) did not observe any response. Although numerous findings reported no effect of live yeast on DMI, some researchers documented positive effects of live yeast on DMI, while some trials reported no response (Bruno *et al.*, 2009; Bagheri *et al.*, 2009; De Ondarza *et al.*, 2010; Yalcin *et al.*, 2011;). Although yeast supplementation is reported to cause a number of effects in the rumen, including increased pH (Beauchemin *et al.*, 2003; Sontakke, 2012), increased number of cellulolytic bacteria (Chauchryus-Durant & Foty, 2002; De Ondarza *et al.*, 2011), VFA concentrations (Enjalbert, 1999; Mutsvangwa *et al.*, 1992) and increased rate or extent of ruminal fiber degradation (Robenson & Erasmus, 2009). The DMI with addition of LY observed in this study may be due to low microbial population with enough soluble growth factors such as organic acids and AA (Dann *et al.*, 2000).

The effect of yeast on DMI is dependent on the status of the energy balance of the cows, where when (<70 days in milk), yeast is expected to increase DMI in early lactation, when cows are under negative energy balance (Zhang *et al.*, 2013), which was not observed in the current study. The response of DMI to yeast is believed to be related to a number of other factors. Guedes *et al.* (2008), indicated that the influence of yeast on fiber degradation are dependent on the inherent digestibility of dietary forages, with the largest DMI improvements reported in cows fed low-quality forages. The effect of yeast on fiber degrading bacteria is also influenced by the number of cells of the fiber degrading bacterial in the yeast and the inclusion rate/dosage of yeast supplemented. In our study, the level of yeast was very low compared to dosage levels which were found to be effective on DMI improvements with yeast supplementation. A number of investigators reported the dosage level of 20g/day to be

beneficial in DMI improvement (Wohlt *et al.*, 1998; Konyves *et al.*, 2005; Vibhute *et al.*, 2011). Wohlt *et al.* (1998), documented that an additional 10g/day of yeast culture at 29 days in lactation added to 10g/day of yeast culture fed from 30 days prepartum to 28 days in lactation increased DMI from the 5-18 week of lactation, this suggest that higher levels of yeast supplementation are likely to improve DMI. In high dosage levels, yeast cells are available in abundance to attach to the feed particles and therefore be available in the rumen in adequate amounts to provide effects to the rumen microbes. Feed efficiency values were similar for all the treatments and were in the range to be expected for early lactation cows (Hutjens, 2005), and although DMI was decreased with LY, feed efficiency (FE) was numerically increased. More recent reports are in support of the current study that yeast had no significant influence on FE (Yalcin *et al.*, 2011; Szucs *et al.*, 2013).

Milk yield ranged from 23.3 to 26.1 kg/d for the LY supplemented and 20.3 to 26.5 kg/d for the unsupplemented diet. Addition of LY did not have an influence on milk production, which is in agreement with previous studies (Dann *et al.*, 2000; Alshaikh *et al.*, 2001; Schingoethe *et al.*, 2004; Erasmus *et al.*, 2005; Kalmus *et al.*, 2009;; Yalcin *et al.*, 2011; Bayat *et al.*, 2015;), but contradicts others (Kudrna *et al.*, 2007; Vibhute *et al.*, 2011; Szucs *et al.*, 2013) that indicated an increase. This discrepancies could be well associated with differences in breed type, parity, stage of lactation, diet composition, yeast product used and length of the study period used in the different experiments. Supplementation with LY increased milk yield only when the diet was high in C:F, which was associated with higher intake of DM by cows in this group.

In the current study, milk fat (yield and percentage) was not affected by yeast supplementation, which supports recent investigations (Dann *et al.*, 2000; De Ondarza *et al.*, 2010; Szucs *et al.*, 2013; Bayat *et al.*, 2015). Milk fat responses of dairy cows to yeast supplementation have been quite variable. Other reports indicated milk fat increase (Alshaik *et al.*, 2001; Zhang *et al.*, 2013; Ayad *et al.*, 2013), or numerical changes (Bhageri *et al.*, 2009; Bruno *et al.*, 2009; Vibhute *et al.*, 2011). Lack of milk fat yield response might be an indicator of no effects of cellulolytic bacteria, and could be an indicator that the stimulation of fiber-digesting ruminal bacteria was sufficient for milk synthesis in the current study (Arambel & Kent, 1990).

As for other milk components, response on milk protein (yield and percentage) to LY varied (Kudrna *et al.*, 2007). The lack of effect of LY on milk protein is in agreement with some authors (Dann *et al.*, 2000; Nocek *et al.*, 2011; Bayat *et al.*, 2015) but not with other authors (Bruno *et al.*, 2009; Maarmouri *et al.*, 2012) who reported an increase in milk protein percentage when a direct fed microbial was supplemented. Other studies reported a decrease (Kalmus *et al.*, 2009; Majdoub-Mathlouthi *et al.*, 2009; De Ondarza *et al.*, 2010; Szucs *et al.*, 2013) in milk

protein in response to addition of LY. Improvement of milk protein yield and percentage has also been widely demonstrated (Bruno *et al.*, 2009; Zhang *et al.*, 2013; Maarmouri *et al.*, 2014).

As for milk protein, milk lactose was not affected by addition with LY, supporting some reports (Erasmus *et al.*, 2005; Kudrna *et al.*, 2007; Bruno *et al.*, 2009; ; Nocek *et al.*, 2011; Bayat *et al.*, 2015). Szucs *et al.* (2013) and Yalcin *et al.* (2011) observed and increase in milk lactose with LY. Lactose concentration in milk usually is not affected or is only slightly altered by dietary manipulation (Casper *et al.* 1990; Kim *et al.* 1991), which is confirmed in the present study.

3.4.2 Body weight and body condition score

The lack of effects of LY on BW and BCS are in agreement with findings of Lacsano *et al.* (2009) and Yalcin *et al.* (2011), respectively. Reports on BW have not always been consistent as other investigators reported a decrease in BW (Dann *et al.*, 2000; Erasmus *et al.*, 2005), there is a paucity of data reporting the increase in BW with yeast. The low BW for the cows supplemented with LY can be associated with the low DMI. These results are indicative of a higher mobilisation of endogenous reserves for cows supplemented with LY, suggesting a greater requirement of energy. Several studies also previously reported similar results to our finding on BCS (Schingoethe *et al.*, 2004; Bruno *et al.*, 2009; Bahgeri *et al.*, 2009; Kalmus *et al.*, 2009; Yalcin *et al.*, 2011). These authors documented a BCS range of 2.3-2.5 which are in consisted to our study. An decrease in BCS has been also reported (Dann *et al.*, 2000), while others reported yeast supplemented cows to have a better effect on BCS from the last 2 weeks prepartum to 6 weeks postpartum and also yeast was found to minimise weight loss around calving (Ayad *et al.*, 2013).

It has been well documented that LY supplementation alters ruminal VFA concentrations by decreasing the molar proportion of acetate and butyrate and increases the molar proportion of propionate (Mutsvangwa *et al.*, 1992; Enjalbert, 1999). Increasing the amount of propionate produced in the rumen provides more glucose for the cow, resulting in increased blood sugar levels, improved energy balance and decreases loss of BCS (Erasmus *et al.*, 2005). The improvement of energy balance resulting from increased propionate in the current study helped cows to maintain BCS. The results from a study conducted by Enjalbert *et al.* (1999), indicates that modifications of ruminal fermentation due to YC addition are time dependent when the diet is fed twice daily. This is may be one of the reasons and possible explanations to why contradictory literature is available on the effects of YP on animal performance and

rumen fermentation dynamics. Feeding frequency increases DMI and this might be why other studies have found an increased in weight or BCS while studies' results differed. In the current study cows were only fed once, which can be reason enough to why there was no improvements with yeast supplementation.

3.4.2. Nitrogen metabolism

The effects of dietary concentrate level and addition of LY on DMI resulted in high and low N intake with high C:F and LY, respectively, but supplementation with LY showed lower excretion of N in urine and faeces, suggesting improved ruminal digestion of CP (Yoon & Stern, 1995; Miller-Webster *et al.*, 2014). Urinary N or total N losses have been rarely investigated with yeast products (Hristove, 2012). Live yeast had an increasing effect on intake N and faecal N on the low C:F diets (Table 3.4.), but on the rest of the other parameters LY had a decreasing effect on both high and low C:F diets. The low levels of N excreted in manure caused by LY supplementation might suggest that N microbial metabolism in the rumen was enhanced with LY addition. Since in the rumen, most of the dietary proteins are rapidly degraded by bacteria and protozoa into peptides, AA and ammonia (Wallace *et al.*, 1997). Microbial conversion of peptides and AA into ammonia in the rumen is unfavourable to the host animal, because energy is required for microbial protein synthesis, and not all ammonia is incorporated into protein (Wallace *et al.*, 1997). Live yeast is also reported to decrease the rate of degradation of peptides, which may reduce ammonia wastage and may increase the amount of RUP available to the cow (Chaucheyras-Durand *et al.*, 2005). In addition, the effect of LY on *S. bovis* and protease activities would reduce protein degradation and reduce urinary N losses as in the current study. This has also been proven in gnotobiologically-reared lambs, in the rumen of new born lambs and in adult ruminants (Kumar *et al.*, 1994; Chaucherus-Durand and Fonty, 2001; Chaucherus-Durand and Fonty, 2002).

3.5. CONCLUSIONS

Addition of LY affected only DMI, which effect was pronounced when cows were fed low (40:60) C:F diet resulting in better FE. Cows fed high C:F diet consumed more feed, produced more milk with high fat and protein content. Live yeast reduced N intake as a result of low DMI, but reduced N excretion in manure.

CHAPTER 4. *IN VITRO* DETERMINATION OF THE EFFECTS OF DIETARY CONCENTRATE AND LIVE YEAST ON RUMINAL AMMONIA AND VOLATILE FATTY ACIDS

4.1. INTRODUCTION

The dairy industry is continuously searching for environmentally friendly alternatives to enhance production or prevent and treat disease conditions of livestock without the use of antibiotics (Throne *et al.*, 2009). For some decades now, ruminant nutritionists along with microbiologist have been interested in manipulating the microbial ecosystem of the rumen to improve production efficiency by ruminants (Galip, 2006). According to Williams *et al.* (1991), inclusion of YC in ruminant diets produce a range of beneficial effects in the rumen including increased pH, reduced ruminal concentrations of VFA's and acetate to propionate ratio (A:P), reduced methane production, increased in total number of microorganisms and cellulolytic bacteria.

The main purpose of using yeast in ruminant diets is to prevent rumen micro flora disorders and disturbances, especially those associated with high energy concentrate diets (Sontake, 2012). Dairy cows are usually fed high concentrate diets because of their nutritional requirements especially in their early stages of lactation. The main goals of rumen microbial studies are to improve feed utilization, animal health and performance, which may be achieved by facilitated desirable fermentation while minimising ruminal degradation and excluding pathogens (Elghandour *et al.*, 2015). Live yeast provides this benefits by having effects on lactate metabolising bacteria, fiber degrading microorganisms and interaction with cell wall degrading microorganisms (Chaucheyras-Durand *et al.*, 2008).

Ruminant diets mostly contain about 300g/kg of cellulose and hemicellulose and their degradation is sometimes limited because plant cell wall polymers are insoluble, structurally complex and are not totally physically accessible (Forsberg *et al.*, 2000; Chaucheyras-Durand *et al.*, 2008). Live yeast has been reported to influence the growth and activities of fiber degrading microorganisms and cellulolytic bacteria in the rumen (Sontake, 2012). Lactic acid accumulation in the rumen is associated with drop in pH. The drop in pH is usually due to diets having readily fermentable carbohydrates and leads to reduction in the number of cellulolytic bacteria (Williamset *al.*, 1991). Yeast stimulates lactate users, increase their numbers and serves as a competitor with lactate producers (Sontake, 2012). This can be explained by its effect on *S. bovis*, since lactic acid is the primary cause of acidosis in dairy cattle. Therefore, reducing ammonia concentration will have a significant beneficial effect on ruminal pH taking

into consideration the effect of live yeast on cell wall and hemicellulose degradation and its ability to stabilize rumen pH, LY promises to be an ideal additive to maintain optimum rumen function through its ability to alter rumen fermentation. The specific objectives of the current study were to determine rumen pH, NH_3 , total VFA and molar proportion VFA *in vitro*.

4.2. MATERIALS AND METHODS

4.2.1. Collection and preparation of rumen fluid

Rumen liquor was collected from a Holstein rumen fistulated cow and rumen content was squeezed through a two layer cheese cloth into pre-warmed flasks and a small amount of inoculum (Table 4.1) was added. The flasks were completely filled with rumen liquor before being capped to keep the anaerobic milieu while they were transported to the laboratory. The rumen fluid with inoculum was blended in pre-warmed industrial blender (Warning Commercial ® Heavy Duty Blender, Warning ® Corporation, New Hartford, CT, USA), at a low speed for 10 seconds to free bacteria that may be attached to solids (Goering & Van Soest, 1970). The rumen fluid was then filtered through a two layer cheese cloth into beakers and maintained at 39 °C in the water bath while being flushed with carbon dioxide (CO₂) to sustain anaerobic condition.

Table 4.1. Composition of *in vitro* inoculum

Macro mineral	Reagents	1L volume
	Distilled water	1000
	Na ₂ HPO ₄ anhydrous (g)	5.7
	KH ₂ PO ₄ anhydrous (g)	6.2
	MgSO ₄ ·7H ₂ O	0.59
	NaCl (g)	2.22
Micro mineral	Reagents	100ml volume
	Distilled water	100
	CaCl ₂ ·2H ₂ O (g)	13.2
	MnCl ₂ ·4H ₂ O (g)	10
	CoCl ₂ ·6H ₂ O	1
	FeCl ₃ ·6H ₂ O	8
Buffer solution	Reagents	1L volume
	Distilled water	1000
	Na ₄ HCO ₃ (g)	4
	NaHCO ₃ (g)	35
Reducing solution	Reagents	100ml volume
	Distilled water	100
	Cysteine Hydrochloric acid (g)	0.625
	KOH pellets (g)	10
	Na Sulphide no hydrate (g)	0.625

4.2.2. *In vitro* batch fermentation

The effects of treatments on rumen microbial fermentation were evaluated in *in vitro* batch fermentation according to Lila *et al* (2004). The dietary treatments (Low C:F diet with no additive, Low C:F diet with live yeast, high C:F diet with no additive and high C:F diet with live yeast) were used for the feeding trial and were used as the substrates for *in vitro* fermentation. The feed was dried at 65°C and grounded in particles of 1 mm. Feed samples of 0.5±0.005 g were weighed into bottles containing a magnetic stirrer each. These bottles were then flushed with CO₂ after adding 40 ml of reduced buffer solution each bottle. The bottles were closed and placed in a water bath at 39°C until the medium was reduced, after which bottles were re-opened and 10 ml of rumen fluid added while flushing with CO₂. The bottles were tightly closed with rubber stoppers, crimp sealed to contain the gas pressure and placed in a 39 °C water bath and periodically mixed. All fermentations were performed for 3 time periods (0, 24 and 48 h) with three replicates per time (n = 9). The mixture (Table 4.1) was stored and flushed with O₂-free carbon dioxide then sealed. All fermentation tubes were then incubated at 39°C for 48 hours.

4.2.3. Sampling and analysis

Ten millilitres samples of ruminal fluid were collected from all bottles after each incubation time. The pH was measured immediately and sample were stored at -20 °C pending analysis. For analysis the samples of ruminal fluid were thawed, centrifuged (15,000 x g, 4 °C, 15 min) and analysed for NH₃. Ammonia was determined by serie-automated colorimetry (O'Dell, 1993). The sample was buffered at a pH of 9.5 with a borate buffer in order to decrease hydrolysis of cyanates and organic nitrogen compounds, and was distilled into a solution of boric acid. Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue colour formed was intensified with sodium nitroprusside and measured calorimetrically and ammonia-N was determined according to Broderick and Kang (1980). The VFAs were determined with a Varian 3300 flame ionization detector (FID) gas chromatograph (Varian Associates, Inc., Palo Alto, CA, USA) by the procedure of Suzuki and Lund (1980).

4.2.5 Statistical analysis

The data on pH, NH₃, total VFA and molar proportion of VFA was subjected to ANOVA using PROC GLM (SAS, 2000) for a complete randomized design. Least squares means for all treatments were reported, and significance was tested at $P < 0.05$. The Turkey's test was used

to compare the treatment means.

The statistical model measure:

$$Y_{it} = \mu + \alpha_i + \beta_t + (\alpha \times \beta)_{it} + e_{it}$$

Where;

Y_{it} = an observation value for pH, ammonia, total VFA and molar proportion of individual VFA obtained from treatment i at time t ;

μ = overall mean for the population;

α_i = fixed effect of treatment i , where i = 40:60 diet with no LY, 40:60 diet with LY, 60:40 diet with no LY or 60:40 diet with LY;

β_t = fixed effect of time t

$(\alpha\beta)_{it}$ = fixed interaction of effect of treatment i and time t ;

e_{it} = error associated with each Y_{it} .

Statistical differences were declared at $P \leq 0.05$ and differences between were considered as a trend toward significance.

And

4.3. RESULTS

The results on pH, ammonia and volatile fatty acids as affected by dietary concentrate level and LY supplementation are presented in Table 4.1. Ruminal pH on the supplemented treatments did not differ statistically to the unsupplemented diets on both low and high C:F. However, LY tended to numerically increase rumen pH compared to the control (6.29 and 6.26 respectively). Ruminal ammonia in the unsupplemented and supplemented group of cows fed low C:F diet did not differ, and was lower compared to unsupplemented and supplemented groups of cows fed high C:F. Total VFA's was higher in the supplemented group fed high C:F compared to unsupplemented of cow fed high C:F diet. Acetate was higher in the unsupplemented group fed low C:F compared to supplemented group fed low C:F diet. Propionate and butyrate did not differ between supplemented and supplemented group fed high C:F where it was higher and lower, respectively, compared to unsupplemented and supplemented group of cows fed low C:F diets. For cows fed low C:F diets, the unsupplemented group had higher and lower in acetate and butyrate, respectively compared to the supplemented group. Iso-valerate was lower in supplemented group of cow fed high C:F compared to cows fed lower C:F. The acetate to propionate ratio (A:P) did not differ between the supplemented group in both low and high C:F. These two groups had higher A:P compared to the unsupplemented group fed low C:F diet. No difference in A:P was found between the unsupplemented and supplemented groups fed high C:F diet. The interaction between LY and C:F for propionate, iso-valerate and A:P resulted on propionate being increased ($P < 0.05$) by LY in low C:F diet, but decreased ($P < 0.05$) in high C:F diet. Iso-valerate was increased (0.05) by LY in low C:F and decreased ($P < 0.05$) in high C:F diet. Supplementation with LY increased ($P < 0.05$) A:P ratio in high C:F, but decreased ($P < 0.05$) it low C:F diet. Rumen pH was not affected by LY on both dietary concentrations, also control did not differ from treatment with not being influenced. This is in accordance with other reports who found no influence with LY treatment (Enjalbert *et al.*, 1998; Bagheri *et al.*, 2009; Ghasemi *et al.*, 2012), but contrary to others who reported an increase in rumen pH (Giger-Reverdin *et al.*, 2004; Thrune *et al.*, 2009).

Effects of supplementation with LY were observed on all rumen parameters except for iso-butyrate, valerate and A:P ratio, while effects of C:F were observed on all rumen parameters evaluated except total VFA and acetate. Ruminal NH_3 and propionate were higher ($P < 0.05$) in high C:F diet compared to low C:F diet on both the supplemented and unsupplemented groups. The remaining affected parameters were higher on the low C:F diets compared to the high C:F diet (Iso-butyrate, Butyrate, iso-valerate and valerate). Addition of LY reduced

ruminal ammonia, increased total VFA's and Iso-valerate, but did not affect the molar percentage of acetate in the rumen. Iso-butyrate and valerate were not affected by LY, but propionate and butyrate tended to be increased with LY. As for high C:F, addition of LY also decreased the A:P ratio. There were interactions between LY and C:F for propionate, iso-valerate and A:P.

As shown in Figure 4.1, effect of LY on major VFAs were more pronounced in Low C:F than high C:F diets., Acetate was decrease by 5.5% in low C:F diet, but was slightly increase in high C:F diet. Propionate and butyrate were increase by 3.26 and 1.27 % in high C:F diet, respectively, but were slightly decreased and increased, respectively in high C:F diets.

Table 4.2: Contrast of effect of dietary concentrate level and live yeast supplementation on ammonia nitrogen and volatile fatty acids

Concentrate	LY	pH	NH ₃ , mg/l	Total VFA	Acetate, molar %	Propionate, molar%	Iso But, molar, g/l	Butyrate, molar, g/l	Iso Val, molar, g/l	Valerate molar, g/l	A:P
40:60	C	6.26	5.49 ^c	107 ^{ab}	61.5 ^a	20.3 ^c	1.3 ^a	14.3 ^b	1.1 ^b	1.5 ^{ab}	2.04 ^c
	LY	6.34	5.33 ^c	114 ^{ab}	55.9 ^b	23.6 ^b	1.34 ^a	15.6 ^a	1.34 ^a	1.9 ^a	1.67 ^a
60:40	C	6.26	7.58 ^a	98.9 ^b	58 ^{ab}	27.6 ^b	0.09 ^c	11.1 ^c	0.9 ^c	1.3 ^{bc}	2.14 ^b
	LY	6.26	6.33 ^b	118 ^a	59.2 ^{ab}	27 ^a	0.11 ^b	11.3 ^c	0.96 ^c	1.15 ^c	2.2 ^{ab}
SEM		0.03	0.19	3.28	0.89	0.64	0.1	0.38	0.06	0.05	0.08
Concentrate to forage means											
60:40		6.26	6.9 ^a	108	59	27.4 ^a	0.1 ^b	11.2 ^b	0.9 ^b	1.2 ^b	2.2
40:60		6.30	5.4 ^b	111	58.7	22 ^b	1.3 ^a	15 ^a	1.4 ^a	1.6 ^a	2.7
SEM		0.03	0.19	3.28	0.89	0.64	0.1	0.38	0.06	0.05	0.08
Live yeast means											
C		6.26	5.8 ^b	116 ^a	57.4	25.4	0.73	13.5	1.4 ^a	1.4	2.3 ^b
LY		6.29	6.5 ^a	103 ^b	60.2	24	0.7	12.7	1.0 ^b	1.4	2.6 ^a
SEM		0.03	0.19	3.28	0.89	0.64	0.1	0.38	0.06	0.05	0.08
Significance											
C:F		NS	*	NS	NS	NS	NS	NS	***	NS	***
LY		NS	***	NS	NS	***	***	***	***	*	***
C:F x LY		NS	*	NS	NS	NS	NS	NS	***	NS	***
SEM		0.03	0.19	3.28	0.89	0.64	0.1	0.38	0.06	0.05	0.08

^{abc} Means within a section of a with different superscripts differ (P<0.05) for comparison between group; *significant effect (P<0.01); ** significant effect (P<0.001); *** significant effect (P<0.0001); NS: Effects non-significant (P>0.05); SEM: Standard error of mean. NS: non-significant (P>0.05) for interaction; SEM: Standard error of mean; ³LY: Live yeast; ²C: Non-supplemented diet; C:F: concentrate to forage ratio; VFA: volatile fatty acids; A-P: acetate to propionate ratio.

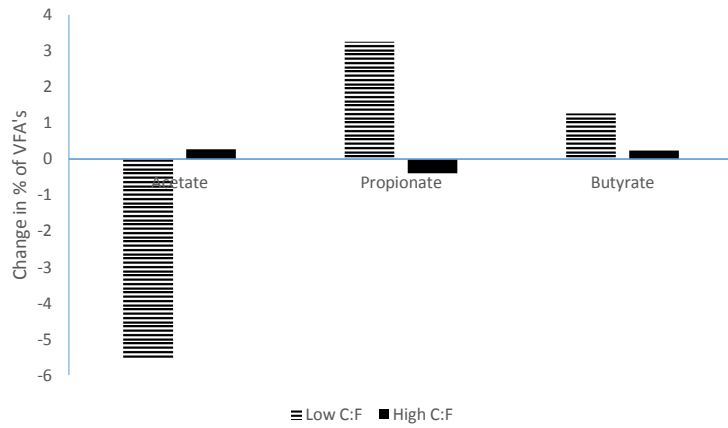


Figure 4.1: The change in % of ruminal acetate, propionate and butyrate as affected by live yeast in low and high C:F diets

4.4. DISCUSSIONS

In vitro effects of *S. cerevisiae* on rumen fermentation parameters have been widely documented and they have varied over the years (Enjalbert *et al.*, 1999; Alshaikh *et al.*, 2001; Chaucheyras-Durand *et al.*, 2005; Dolezal *et al.* 2010; Bayat *et al.*, 2015). Rumen pH was not affected by LY on both dietary concentrations. Dietary concentration did not influence rumen pH on both the supplemented and unsupplemented diets. This is in accordance with other reports who found no influence with LY treatment (Enjalbert *et al.*, 1998; Bagheri *et al.*, 2009; Ghasemi *et al.*, 2012), but contrary to others who reported an increase in rumen pH (Giger-Reverdin *et al.*, 2004; Thrune *et al.*, 2009). The decrease in concentration of rumen ammonia observed in the present study with LY supplementation on both high and low C:F diets is in agreement with some reports (Enjalbert *et al.*, 1999; Alshaikh *et al.*, 2001, Agle *et al.*, 2010; Hristove *et al.*, 2012), but contradict with others (Oestruerk *et al.*, 2009; Dolezal *et al.*, 2010) who reported an increase. Other authors indicated that supplementation with LY did not affect ruminal ammonia concentrations (Lila *et al.*, 2004; Erasmus *et al.*, 2005; Thrune *et al.*, 2009; Bayat *et al.*, 2015). The reduction in rumen ammonia concentration is believed to be a result of increased incorporation of ammonia into microbial proteins due to microbial activity (Williams & Newbold, 1990, Carro *et al.*, 1992; Chaucheyras-Durand *et al.*, 2005). Chaucheyras-Durand *et al.* (2005) indicated that *S. bovis* and protease activities decrease the rate of degradation of peptides, which also contribute to reduced ammonia wastage while increasing the amount of rumen undegraded protein available to the cow.

The reduction of ammonia concentration with LY was more pronounced in high C:F compared to the low C:F diet, as also reported by Hristove *et al.*, (2012). Such a decrease in ruminal ammonia concentration has been suggested to be due to a lower degradation of dietary proteins, as attested by higher duodenal flow of undegraded feed N *in vivo* (Putnam *et al.*, 1997). This confirms previous observations (Hristove *et al.*, 2005), that provision of fermentable carbohydrates can reduce ammonia production or enhance microbial capture of released ammonia in the rumen. However, some investigators found the ammonia concentration to increase with increasing dietary concentrate level in the diet (Moorby *et al.*, 2006), while others found no effect of ruminal ammonia concentration (Yang *et al.*, 2004).

In general, LY has been reported to increase VFA proportions in the rumen, but results have been variable and inconsistent. The increase in VFA observed in the present study in both high and low C:Fis similar to some previous studies (Dolezal *et al.*, 2010; Guedes *et al.*, 2008; Desnoyes *et al.*, 2009; Sullivan *et al.*, 2012), but differ from others who reported a decrease in total VFA (Konyves *et al.*, 2005; Thrune *et al.*, 2009). In some studies LY did not influence total VFA production in the rumen (Hristove *et al.*, 2012; Bayat *et al.*, 2015). The increase in

total VFA production will enhance energy availability to the host, suggesting an increase efficiency of energy utilization (Erasmus *et al.*, 2005).

As observed in the present study, a stimulation in the proportion of propionate at the expense of acetate was also observed by Newbold *et al.* (1990). This decreasing effect of acetate in favour of propionate indicates more energetically efficient rumen fermentation. In accordance to other reporters (Oeztruek *et al.*, 2009; Chung *et al.*, 2011; Sullivan *et al.*, 2011), addition of LY increased butyrate and iso-valerate molar proportions (Lila *et al.*, 2004; Marden *et al.*, 2008; Krizova *et al.*, 2011). These rumen modifications can be dependent on the diet composition. Putnam *et al.* (1997) observed an increase concentration of iso-butyrate with low C:F diets but decreased concentrations on the high C:F diets. The A:P ratio was found greater in both low and high C:F with LY supplementation, this is in accordance to others (Konyeves *et al.*, 2009; Ferrerato *et al.*, 2012), but contrary to some who reported a decrease in A:P ration with LY addition (Oeztrek *et al.*, 2009; Miller-Wenster *et al.*, 2014). However, a recent report found no influence on A:P ratio with LY supplementation (Bayat *et al.*, 2015).

4.5. CONCLUSION

Addition of LY decreased ruminal ammonia and increased total VFA's, with no effects on the rest of ruminal parameters. These effects on ammonia suggest a better utilisation of diet proteins and probably more incorporation of products of CP degradation into microbial proteins, which support the observed reduced manure N excretion. The opposite was observed with high C:F diet, which increased ammonia and decreased total VFA's. Propionate and butyrate concentration were increased and decreased, respectively by high C:F diet.

CHAPETR 5: GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

Cows supplemented with LY consumed less DM with no change in milk yield and ECM, which resulted in better FE for cows fed 40:60 C:F diets. Supplementation with LY decreased ruminal ammonia nitrogen and increased total VFA's. The decreased ammonia concentration was associated with reduced manure nitrogen suggesting more ammonia-N incorporated into ruminal microbial proteins which also benefit the dairy cow by increasing the amount of rumen undegraded protein available to the cow. This improved efficiency of dry matter and protein utilisation is of great benefit in early lactating cows, since cows cannot consume enough energy from reduced feed intake to support the rapidly increasing milk yield.

However, ammonia production in the presence of yeast can also be dependent on the availability of amino acids and could also be associated with other characteristics of the diet. While live yeast did not affect propionate, high C:F diet increased propionate. This particular change in VFA's suggests more energy for production. High concentrate diet as proven the importance of high density nutrient content of the diet, which will continue to play a major role in improving performance of early lactating dairy cows. Results of the present study indicate that LY and diet composition will continue to play an important role in ruminant nutrition and that their effects of on rumen fermentation are substrate dependent.

Feeding early lactating cows LY at 40:60 C:F resulted in better FE, which suggested that the effect of LY was better pronounced at the low C:F diet compared to the high C:F. This was also seen in the *in vitro* trial where addition of LY at 40:60 C:F decreased ruminal ammonia concentrations and increased total VFA's suggesting a better utilization of dietary proteins and more incorporation of products of CP into microbial proteins. This is evident by the reduced manure N excretion.

Feed additives and rumen modifiers such as yeast cultures and probiotics are increasingly playing an important role in helping to prevent metabolic disturbances and stabilise rumen environment. Live yeast alters ruminal fermentation in a specific way, and its effects can be dictated by different scenarios such as concentrate: forage ratio of the ruminant diet. For more pronounced effects of LY in early lactating cow's diets, it is recommended that LY is added to low C:F 40:60 diets in order to increase the production potential of cows, since LY at low C:F diets increased FE, decreased ruminal ammonia and increased total VFA concentration.

It also recommended the effects of LY at 40:60 C:F is tested on a larger scale of animals with longer periods as in the current study the experimental period was too short which might have

caused omission of some effects with prolonged time. The number of animals was also a limiting factor as the 4 x 4 LSD was used. Future studies on LY, must focus on the dosage level of LY at low C:F to better understand its effects and use a greater number of animals over a long experimental period.

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ANNEXURES

ANOVA MILK YIELD (MY), DMI, MILK FAT, MILK PROTEIN, MILK LACTOSE, MUN, SCC, ECM AND FE

Dependent Variable: MY MY

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	97.2967187	32.4322396	4.02	0.0113
Error	60	483.5718750	8.0595313		
Corrected Total	63	580.8685938			

R-Square	Coeff Var	Root MSE	MY Mean
0.167502	11.26351	2.838931	25.20469

Source	DF	Type III SS	Mean Square	F Value	Pr > F
C_to_F	1	72.46265625	72.46265625	8.99	0.0039
LY	1	17.74515625	17.74515625	2.20	0.1431
C_to_F*LY	1	7.08890625	7.08890625	0.88	0.3521

Dependent Variable: DIM DIM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	477.598673	159.199558	10.60	<.0001
Error	60	901.164733	15.019412		
Corrected Total	63	1378.763406			

R-Square	Coeff Var	Root MSE	DIM Mean
0.346396	19.65607	3.875489	19.71650

Source	DF	Type III SS	Mean Square	F Value	Pr > F
C_to_F	1	256.5715186	256.5715186	17.08	0.0001
LY	1	160.6264738	160.6264738	10.69	0.0018
C_to_F*LY	1	60.4006809	60.4006809	4.02	0.0494

Dependent Variable: ECM ECM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	205.697705	68.565902	3.42	0.0228
Error	60	1202.090894	20.034848		

Corrected Total 63 1407.788599

R-Square Coeff Var Root MSE ECM Mean
0.146114 17.59436 4.476030 25.44015

Source	DF	Type III SS	Mean Square	F Value	Pr > F
C_to_F	1	143.6680720	143.6680720	7.17	0.0095
LY	1	61.4971983	61.4971983	3.07	0.0849
C_to_F*LY	1	0.5324348	0.5324348	0.03	0.8711

Dependent Variable: FE FE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1.27914355	0.42638118	3.89	0.0132
Error	60	6.58491484	0.10974858		
Corrected Total	63	7.86405840			

R-Square Coeff Var Root MSE FE Mean
0.162657 24.55834 0.331283 1.348964

Source	DF	Type III SS	Mean Square	F Value	Pr > F
C_to_F	1	0.33913947	0.33913947	3.09	0.0839
LY	1	0.36876080	0.36876080	3.36	0.0718
C_to_F*LY	1	0.57124328	0.57124328	5.21	0.0261

Dependent Variable: Fat kg Fat kg

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.40010396	0.13336799	1.77	0.1618
Error	60	4.51167479	0.07519458		
Corrected Total	63	4.91177875			

R-Square Coeff Var Root MSE Fat_kg Mean
0.081458 29.60194 0.274216 0.926346

Source	DF	Type III SS	Mean Square	F Value	Pr > F
C_to_F	1	0.24249838	0.24249838	3.22	0.0776
LY	1	0.15580092	0.15580092	2.07	0.1552
C_to_F*LY	1	0.00180466	0.00180466	0.02	0.8774

17:03 Thursday, August 1,

2013 7

The GLM Procedure

Dependent Variable: Protein kg Protein kg

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.19253720	0.06417907	6.42	0.0008
Error	60	0.59980274	0.00999671		
Corrected Total	63	0.79233994			

R-Square	Coeff Var	Root MSE	Protein_kg Mean
0.242998	13.83846	0.099984	0.722505

Source	DF	Type III SS	Mean Square	F Value	Pr > F
C_to_F	1	0.15399738	0.15399738	15.40	0.0002
LY	1	0.03531017	0.03531017	3.53	0.0650
C_to_F*LY	1	0.00322965	0.00322965	0.32	0.5719

ANOVA FOR BODY WEIGHT AND BCS

Dependent Variable: Weight Weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1449.34375	483.11458	0.44	0.7230
Error	28	30422.62500	1086.52232		
Corrected Total	31	31871.96875			

R-Square	Coeff Var	Root MSE	Weight Mean
0.045474	7.499453	32.96244	439.5313

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Conc_level	1	770.2812500	770.2812500	0.71	0.4069
LY	1	621.2812500	621.2812500	0.57	0.4559
Conc_level*LY	1	57.7812500	57.7812500	0.05	0.8193

Dependent Variable: BCS BCS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.14843750	0.04947917	1.08	0.3731
Error	28	1.28125000	0.04575893		

Corrected Total	31	1.42968750			
	R-Square	Coeff Var	Root MSE	BCS Mean	
	0.103825	8.832552	0.213913	2.421875	
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Conc_level	1	0.07031250	0.07031250	1.54	0.2254
LY	1	0.00781250	0.00781250	0.17	0.6826
Conc_level*LY	1	0.07031250	0.07031250	1.54	0.2254

ANOVA NITROGEN INTAKE AND EXCRETION

Dependent Variable: N intake_g N intake (g)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	323603.509	107867.836	8.43	<.0001
Error	60	767414.007	12790.233		
Corrected Total	63	1091017.515			
	R-Square	Coeff Var	Root MSE	N_intake__g_ Mean	
	0.296607	20.39100	113.0939	554.6266	
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Additive	1	101669.0710	101669.0710	7.95	0.0065
C_F	1	187790.5948	187790.5948	14.68	0.0003

C_F*Additive	1	34143.8429	34143.8429	2.67	0.1075
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Dependent Variable: N in feaces g N in feaces (g)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	217388.904	72462.968	4.71	0.0051
Error	60	922757.292	15379.288		
Corrected Total	63	1140146.196			

R-Square	Coeff Var	Root MSE	N_in_feaces_g Mean
0.190668	61.71114	124.0133	200.9576

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Additive	1	144631.3485	144631.3485	9.40	0.0032
C_F	1	63347.2000	63347.2000	4.12	0.0468
C_F*Additive	1	9410.3554	9410.3554	0.61	0.4372

Dependent Variable: N in urine g N in urine (g)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	65720.8857	21906.9619	11.31	<.0001
Error	58	112381.8275	1937.6177		
Corrected Total	61	178102.7131			

R-Square	Coeff Var	Root MSE	N_in_urine_g Mean
0.369006	22.90176	44.01838	192.2053

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Additive	1	48529.47427	48529.47427	25.05	<.0001
C_F	1	11385.47482	11385.47482	5.88	0.0185
C_F*Additive	1	5591.77169	5591.77169	2.89	0.0947

Dependent Variable: N in manure g N in manure (g)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	239304.3669	79768.1223	11.10	<.0001
Error	58	416797.9310	7186.1712		
Corrected Total	61	656102.2979			

R-Square Coeff Var Root MSE N_in_manure__g_ Mean
 0.364736 22.85260 84.77129 370.9481

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Additive	1	181671.5767	181671.5767	25.28	<.0001
C_F	1	34692.6168	34692.6168	4.83	0.0320
C_F*Additive	1	21671.5899	21671.5899	3.02	0.0878

ANOVA pH, AMMONIA AND VFA

Dependent Variable: pH pH

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.03609956	0.01203319	0.31	0.8145

Error	32	1.22275152	0.03821099
Corrected Total	35	1.25885108	

R-Square	Coeff Var	Root MSE	pH Mean
0.028677	3.111986	0.195476	6.281401

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Additive	1	0.01119303	0.01119303	0.29	0.5921
C_F	1	0.01245327	0.01245327	0.33	0.5721
C_F*Additive	1	0.01245327	0.01245327	0.33	0.5721

14:28 Monday, July 1, 2013

3

The GLM Procedure
Least Squares Means

Additive	pH LSMEAN	HO:LSMean1=
		LSMean2
		Pr > t
C	6.26376812	0.5921
LY	6.29903382	

14:28 Monday, July 1, 2013

4

The GLM Procedure
Least Squares Means

C_F	pH LSMEAN	HO:LSMean1=
		LSMean2
		Pr > t
40 60	6.30000000	0.5721
60 40	6.26280193	

14:28 Monday, July 1, 2013

5

The GLM Procedure
Least Squares Means

C_F	Additive	pH LSMEAN	LSMEAN Number
40 60	C	6.26376812	1
40 60	LY	6.33623188	2
60 40	C	6.26376812	3
60 40	LY	6.26183575	4

Least Squares Means for effect C_F*Additive
Pr > |t| for HO: LSMean(i)=LSMean(j)

Dependent Variable: pH

i/j	1	2	3	4
			0.4374	1.0000
				0.9834

2	0.4374		0.4374	0.4254
3	1.0000	0.4374		0.9834
4	0.9834	0.4254	0.9834	

Dependent Variable: NH₃ NH₃

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	28.54555556	9.51518519	14.79	<.0001
Error	32	20.58444444	0.64326389		
Corrected Total	35	49.13000000			

R-Square	Coeff Var	Root MSE	NH ₃ Mean
0.581021	12.97095	0.802037	6.183333

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Additive	1	4.41000000	4.41000000	6.86	0.0146
C_F	1	21.46777778	21.46777778	33.37	<.0001
C_F*Additive	1	2.66777778	2.66777778	4.15	0.0500

Dependent Variable: Tot VFA Tot VFA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	2084.72936	694.90979	1.94	0.1428
Error	32	11457.64587	358.05143		
Corrected Total	35	13542.37523			

R-Square	Coeff Var	Root MSE	Tot_VFA Mean
0.153941	17.20776	18.92225	109.9635

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Additive	1	1642.154052	1642.154052	4.59	0.0399
C_F	1	41.634756	41.634756	0.12	0.7353
C_F*Additive	1	400.940552	400.940552	1.12	0.2979

Dependent Variable: Acet Acet

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	138.7341238	46.2447079	1.73	0.1811

Error	32	856.6494804	26.7702963
Corrected Total	35	995.3836042	

R-Square	Coeff Var	Root MSE	Acet Mean
0.139378	8.789872	5.174002	58.86322

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Additive	1	62.32049878	62.32049878	2.33	0.1369
C_F	1	1.07122500	1.07122500	0.04	0.8427
C_F*Additive	1	75.34240000	75.34240000	2.81	0.1032

Dependent Variable: Prop Prop

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	313.5757982	104.5252661	16.30	<.0001
Error	32	205.2330813	6.4135338		
Corrected Total	35	518.8088796			

R-Square	Coeff Var	Root MSE	Prop Mean
0.604415	10.25639	2.532496	24.69189

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Additive	1	18.5416360	18.5416360	2.89	0.0988
C_F	1	264.8647751	264.8647751	41.30	<.0001
C_F*Additive	1	30.1693871	30.1693871	4.70	0.0376

Dependent Variable: Iso but Iso but

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	13.16447184	4.38815728	282.42	<.0001
Error	32	0.49720424	0.01553763		
Corrected Total	35	13.66167608			

R-Square	Coeff Var	Root MSE	Iso_but Mean
0.963606	17.46118	0.124650	0.713869

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Additive	1	0.00962688	0.00962688	0.62	0.4370
C_F	1	13.15355735	13.15355735	846.56	<.0001

C_F*Additive 1 0.00128761 0.00128761 0.08 0.7753

Dependent Variable: But But

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	134.2003290	44.7334430	32.48	<.0001
Error	32	44.0759936	1.3773748		
Corrected Total	35	178.2763226			

R-Square 0.752766
 Coeff Var 8.961220
 Root MSE 1.173616
 But Mean 13.09661

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Additive	1	5.1287151	5.1287151	3.72	0.0626
C_F	1	126.6825284	126.6825284	91.97	<.0001
C_F*Additive	1	2.3890854	2.3890854	1.73	0.1972

Dependent Variable: Iso val Iso val

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	4.76162789	1.58720930	103.38	<.0001
Error	32	0.49131511	0.01535360		
Corrected Total	35	5.25294300			

R-Square 0.906469
 Coeff Var 10.37914
 Root MSE 0.123910
 Iso_val Mean 1.193833

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Additive	1	1.33864900	1.33864900	87.19	<.0001
C_F	1	2.48272544	2.48272544	161.70	<.0001
C_F*Additive	1	0.94025344	0.94025344	61.24	<.0001

Dependent Variable: Val Val

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1.38914919	0.46304973	6.86	0.0011
Error	32	2.16078511	0.06752453		
Corrected Total	35	3.54993431			

R-Square	Coeff Var	Root MSE	Val Mean
0.391317	18.54303	0.259855	1.401361

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Additive	1	0.00268669	0.00268669	0.04	0.8432
C_F	1	1.12890625	1.12890625	16.72	0.0003
C_F*Additive	1	0.25755625	0.25755625	3.81	0.0596

Dependent Variable: A_P A/P

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	4.64086341	1.54695447	44.95	<.0001
Error	32	1.10122650	0.03441333		
Corrected Total	35	5.74208990			

R-Square	Coeff Var	Root MSE	A_P Mean
0.808219	7.620127	0.185508	2.434452

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Additive	1	0.82222456	0.82222456	23.89	<.0001
C_F	1	2.62135107	2.62135107	76.17	<.0001
C_F*Additive	1	1.19728778	1.19728778	34.79	<.0001

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