Effects of *Brevibacillus laterosporus* and live yeast on rumen fermentation, nutrient digestibility and microbial protein synthesis

by

RASAQ ADEMOLA ADELEKE

Submitted in accordance with the requirements
for the degree of

MASTER OF SCIENCE

in Agriculture

at the

University of South Africa

Supervisor: Dr Claude Mukengela Muya

Co-supervisor: Prof. Khanyisile R. Mbatha

November 2016

DECLARATION OF ORIGINALITY

I certify that the work presented in this dissertation is to the best of my knowledge and belief, original, except as acknowledged in the text. This dissertation has not been submitted, either in whole or in part, for a degree at this or other university. I also certify that I have complied with the rules, requirements, procedures and policy of the university.

Signed: _____

RASAQ ADEMOLA ADELEKE

Date: 20 November 2016

DEDICATION

This is dedicated to Almighty God, who is the source of my strength and inspiration. Also to the memory of my late parents and my first teachers, Prince Hussain Ayodeji Jimoh-Adeleke and Princess Memunat Aderonke Ebe Jimoh-Adeleke. They both taught me how to read and write at home and provided constant inspiration, guidance, spiritual support and motivation since the beginning of my life on earth. I pray that their souls continue to rest in perfect peace (AMEN).

ACKNOWLEDGEMENTS

I thank Almighty God, the most gracious and most merciful who in His infinite mercy has kept me this far. He blessed me with good health, talent and made it possible for me to achieve this goal which is another phase of my life.

I wish to express my profound gratitude and honor to my supervisor, Dr. Claude Mukengela Muya, who took time out of no time for his valuable guidance, unfairly interest, untiring help, constructive criticism, fruitful discussion, and most importantly continuous encouragement during the conduct of my research work and completion of my write-up. In addition, great thanks to my co-supervisor, Prof. Khanyisile R. Mbatha for her attention, valuable and fruitful discussions and most importantly continuous encouragement and valuable suggestions.

I am very thankful to all the entire staff and students of the Department of Agriculture and Animal Health (University of South Africa) and Agricultural Research Council (Animal Production Institution, Irene, South Africa for allowing me to use their facilities during this study. Thank you and God bless you all.

I would like to express my sincere appreciation to all people who stand out most notably in my mind as contributing to the intent of this research through their support, encouragement and guidance.

My late father who passed on during this study, it was indeed a great lost. His advice, unlimited support and encouragement have guided me this far. Rest in peace Dad, I really miss you.

My deep appreciation goes to my ever-loving brothers, Dr. Adeyinka Adeleke and Dr. Adegbola Adeleke for their moral, financial and spiritual support. I pray that Almighty God will continue to provide for you, protect you and your families. In addition, to my ever supportive other brothers, sisters and their families, thank you so much for the care, advice and spiritual support. God bless you all.

Doyinsola Mufuliat Niniola (my loving wife), her love, understanding, spiritual support and patience gave hope that the future is certainly bright for both of us. Am so fulfilled by meeting you and may God bless you more. Thank you so much for your love.

I wish to express my deepest appreciation and gratitude to my in-laws for their patience, hospitality, prayer and support. May God continue to bless and provide for you all. Thank you all.

To all my cousins and friends, the Oladejo's, Prince Abideen Ladigbolu, Dr Abass, Prof. Cyril, Raimi Ramon, Dr Billy, Dr Raji, Dr Yusuf, Alhaji Ayo Adeniyi, Hon. Yinka Taiwo, Adekoya Ife, Yusuf Mariam, Ogundiran Femi Jr, Taiwo, Kennyslim, Oladokun Lanre, Dr Ajayi, Mrs. Adeyemi, Dr Fajemisin, Osuntola Oluwaseyi and to others that I did not mention by names. All of you have been so helpful in all my activities both at home and on farm.

My acknowledgements will be incomplete if I fail to appreciate my research colleagues and staff of Nutrition Laboratory, Agricultural Research Council – Animal Production Institution (South Africa) for their pain, joy and encouragement given during the course of the research and to all individual whom have contributed directly or indirectly to the successful of this study. Thank you and God bless all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

This study investigated the effects of *Brevibacillus laterosporus* and live yeast (LY) on rumen fermentation, nutrient digestibility and microbial protein synthesis. The basal diet was a total mixed ration formulated to fulfil the minimum nutrient requirement of early lactating 600 kg Holstein cow producing 40kg of milk with 3.5 % fat and 3.3 % protein using CPM-dairy software (NRC, 2001). Treatments were: T_1 (Control: basal diet with no additive), T_2 (Basal diet + *Brevibacillus laterosporus*), T_3 (Basal diet + Live yeast), and T_4 (Basal diet + *Brevibacillus laterosporus* + Live yeast). *In situ* degradation, *in vitro* batch fermentation were performed. Data obtained were subjected to analysis of variance (ANOVA) using PROC GLM (SAS Institute, 2009). The effective dry matter (DM) degradability evaluated at low (0.02) and medium (0.05) ruminal passage rate (ED1 and ED2) were higher ($p<0.05$) in T_1 compared to T_2 and T_3, but did not differ ($p>0.05$) between T_2, T_3 and T_4, and between T_1 and T_4. When evaluated at fast passage rate (0.08) the effective DM degradability (ED3) was higher ($p<0.05$) in T_1 compared to T_3 and T_4, but did not differ ($p>0.05$) between T_1 and T_2. The difference in ammonia nitrogen production was observed only between T_1 and T_2, and was higher ($p<0.05$) in T_1. The total VFA's concentration was higher ($p<0.05$) in T_3 compared to the control. All additives decreased the molar percentage of acetate ($P<0.05$). The concentration of acetate was lower ($p<0.05$) in T_3 and T_4 compared to control. Propionate concentration was higher ($p<0.05$) in T_3 and T_4 compared to other treatments and lower ($p<0.05$) in the control compared to the rest of treatments. Butyrate concentration was higher ($p<0.05$) in T_2 and T_4 compared to the rest of the treatments, and lower ($p<0.05$) in T_3 than other treatments. The microbial protein synthesis measured as purine derivate done on residues was higher ($p<0.05$) for T_3 compared to T_1 and T_2, but did not differ between T1, T_2 and T_4, and between T3 and T4. These results showed that the two additives have different individual effects on DM and CP degradability, but also associative effects in some fermentation parameters such as propionate production.

Keywords: *Brevibacillus laterosporus*, live yeast, feed additive, nutrient digestibility, rumen fermentation, microbial protein, propionate, acetate, butyrate, lactating cow, volatile fatty acid, degradability, *in vitro*

LISTE OF ABBREVIATIONS

AA	Amino acid
ADF	Acid detergent fiber
ADY	Active dry yeast
ANOVA	Analysis of variance
AO	*Aspergillus oryzae*
ARC-API	Agricultural Research Council- Animal Production Institute
ATP	Adenosine triphosphate
BCS	Body condition score
B-HBA	B-Hydro-xybutric acid
BL	*Brevibacillus laterosporus*
CH_4	Methane
CP	Crude protein
CRC	Controlled-release capsule
CSPB	Canoe-shaped parasporal body
DFM	Direct fed microbial
DM	Dry matter
DMI	Dry matter intake
ED	Effective degradability
FAO	Food and Agriculture Organization
FCR	Feed conversion ratios
FDA	Food and Drug Administration
FME	Fermentable metabolisable energy
IOP	Ionophore
LYC	Live yeast culture
ME	Metabolisable energy
MPS	Microbial protein synthesis
NAN	Non-ammonia nitrogen
NDF	Neutral detergent fiber
NDFd	Neutral detergent fiber degradation

NEFA	Non-esterified fatty acids
NPN	Non-protein nitrogen
NRC	National Research Council
OM	Organic matter
PCR	Polymerase chain reaction
RDP	Ruminally degradable protein
RUP	Ruminally undegraded protein
SARA	Sub-acute ruminal acidosis
SC	*Saccharomyces cerevisiae*
SPSS	Statistical Package of Social Sciences
TMR	Total mixed ratio
US	United States
VFA	Volatile fatty acids
YC	Yeast culture

CHAPTER 1

INTRODUCTION

Antibiotics feed additives have successfully been used to manipulate rumen fermentation and improve ruminant productivity (Santra and Karim, 2003). Public concerns with respect to the utilization of antibiotics in livestock production have expanded due to the development of multidrug-resistant bacteria which is transferable to humans (Silbergeld *et al*., 2008). The use of Direct Fed Microbial (DFM) in animal feed is regarded as potential alternatives to antibiotics as rumen modifier and for control of specific enteric pathogens (Vila *et al*., 2009). A number of studies (Retta, 2016; Tadesse, 2014; DiLorenzo, 2011; Choi *et al*., 2012; Khampa *et al*., 2007) have been performed with the aim to increase ruminant performance by manipulating the rumen ecology, increasing digestibility and nutrient metabolism. Previous studies have reported enhanced animal performance with DFM products and promoted less usage of antibiotics (Wallace *et al*., 1994; Guedes *et al*., 2008; Wallace *et al*., 2008). These studies showed that benefits of dietary supplementation with DFM to dairy animal include the body weight gain and increased milk production.

Yeast products have been used in animal production industry for several years and have shown high potential to promote growth of rumen bacteria as well as stimulation of cellulolytic and lactate-utilizing bacteria (Chaucheyras-Durand *et al*., 2008). The latter author indicated that this stimulation of bacterial growth with yeast cells is accomplished by the removal of oxygen in the ruminal fluid, which prevents the toxicity to the ruminal anaerobic microbes. In addition, yeast culture provides branched-chain fatty acids and vitamin B which stimulate cellulolytic bacteria (Weidmeier *et al*., 1987).

Another important DFM candidate *is Bacillus.* This is a bacterial genus that is abundant in soil and contains several species that produce many types of antibiotics (Wu *et al*., 2005). Antibiotics from *Bacillus* are mainly active against gram-positive bacteria but also inhibit the growth of gram-negative bacteria, yeast and fungi. *Bacillus* species produce antibiotics that have a peptidic nature (Hassi *et al*., 2012**;** Kleinkauf *et al*., 1990). *Brevibacillus laterosporus* is an example of *Bacillus* which is known to reduce nitrates to nitrites (Bioscienceportal, 2013). This nitrate

reducing bacteria can be utilized as probiotic to prevent the accumulation of nitrite when sodium nitrate is utilized to decrease *in vitro* methane outflows (Sakthivel *et al*., 2012; Pillanatham *et al*., 2012). Synergetic and associative effects of additives are reported to benefits animal host and advantages include balanced microflora, growth and general performance (Chiedza *et al*., 2014). The mode of DFM action differs according to their composition (Chaucheyras-Durand *et al*., 2008), whilst animal responses to dietary supplementation with DFM might depend, on the diet composition. Complementary effects of feed additives have been reported in few reviews (Yang *et al*., 2015), but there has not been any study that combined the use of *Brevibacillus laterosporus* and live yeast (*Saccharomyces cerevisiae*).

It was hypothesised that adequate protein degradability may be produced and rumen fermentation may be improved by combining *B. laterosporus* and *S. cerevisiae* through synergistic effects. Therefore, the objective of this study is to evaluate the effects of the addition of *B. laterosporus*, *S. cerevisiae* and their combination to ruminant's diet on rumen ammonia and volatile fatty acids production, and degradability of dry matter and crude protein *in vitro.*

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In this chapter, the importance of feed additives is viewed in terms of its contribution to the energy gained by the dairy cow during early lactation period, production of milk, control of disease and food security. The role of feed additives in manipulating the rumen metabolism of the cow and the management of the total mixed ration (TMR) is also reviewed. This chapter also focuses on the challenges affecting rumen and the effects of changing the rumen metabolism process in early lactating in other to alter the energy used by the cow. Furthermore, this chapter reviews the antibiotics and feed additives used such as the bacteria and the live yeast with their effects to the rumen.

2.2 Feeding and managing the dairy cow

Feeding options available to high producing cows keeps challenging dairy farmers and nutritionists. Dairy profit margins vary in response to the yearly shift in the cost of milk prices and feed costs. Feed costs represent the largest input cost to produce milk (estimated to be almost 70 %) (Alvaro, 2010). An accomplished feeding system is aimed at improving milk yield, produce desirable milk components, maximize rumen microbial yield, stimulate dry matter intake and produce key nutrients for mammary gland synthesis (Hutjens, 1991).

2.2.1 Rumen fermentation during early lactating dairy cow

There will be a better use of feed nutrient if there is proper management for early lactating dairy cows and this will help in high milk production and reduce body weight (BW) loss. This is as a result of the effects on the energy consumed during this stage to produce high yield of milk it has on the dairy cow's body weight (Daryl *et al*., 2011). The end-products of fermentation are volatile fatty acids (VFA) which are imbued through the rumen wall and

oxidized to provide energy to the host animal (Goff, 2003). These VFAs are imbued through the rumen wall and oxidized for energy generation (Besden *et al*., 2013). Propionic acid is the energetically richer volatile fatty acid amongst all others. Propionate enters the tricarboxylic acid cycle and replenishes oxaloacetate, the main substrate for gluconeogenesis and energy generation (Richardson et al., 1976). It is recognised that energy intake must not be compromised during the transition period, and that meeting the energy demands of lactation is one of the basic physiological functions that must be sustained (Goff, 2001). Manipulation of rumen fermentation for more energy is the main purpose of feeding management during the early period of lactation because dairy cows in early lactation are susceptible to negative energy balance. Consequently, the cow will not consume enough nutrients to meet the energy demands of lactation (Kononoff, 2016).

2.2.2 Nutrient requirement of the lactating dairy cow

2.2.2.1 Energy requirements of lactating dairy cow

Dairy cows need energy for maintenance, especially during early lactation period to maintain the peak period of milk production and BW maintenance. Out of the energy, cows require to manage all their bodily functions. The 50-80 % of this energy comes from VFAs derived by fermentation of feed carbohydrates in the rumen, with the rest generated from carbohydrates, proteins and fats that escape rumen degradation (Moran, 2005). Ruminant energy requirements and feed energy supplies are generally expressed in terms of metabolisable energy (ME). This energy is obtainable by the cow after accounting for losses in digestion, gases and urine whereas fermentable metabolisable energy (FME) is the proportion of ME that is potentially available in the rumen (ADHB, 2016). Imbalance of the main energy sources can cause problems that can lead to metabolic diseases such as displaced abomasum (Herdt, 2014).

2.2.2.2 Protein requirement of lactating dairy cow

Amino acids (AA) required by lactating dairy cows are supplied via microbial and undegraded feed protein flowing from the rumen (Cyriac, 2009). Approximately 59 % of non-ammonia nitrogen reaching the duodenum is supplied by microbial crude protein (CP) produced in the rumen (Clark *et al*., 1992) using degradable protein. Dairy cows require supplemental sources of ruminally undegraded protein (RUP) and ruminally degradable protein (RDP). The RUP will reach the small intestine, where they will be degraded and absorbed. The RDP will be degraded in the rumen to meet microbial needs. Strategies focused on enhancing microbial protein flow to the intestine by meeting ruminal RDP requirements will reduce undegraded protein needs of lactating dairy cows. Ruminal bacteria use dietary protein degradation products such as ammonia (Allison, 1969), peptides and AA to support growth and protein synthesis (Argyle and Baldwin, 1989). The significant net recycling of blood urea into the rumen supplements dietary degradable nitrogen sources (Lapierre and Lobley, 2001, Remond *et al*., 2002) and may act to buffer ammonia concentrations in the rumen when low RDP diets are fed. Ruminal microbes are thought to have an ammonia requirement of 5 mg/dl, which corresponds to 13 % dietary CP (Fessenden, 2013). When ruminally available nitrogen is deficient, degradation of organic matter (OM) can be reduced due to inhibition of fibre digesting bacteria (Cyriac, 2009). Reductions in fibre digestion can lead to reductions in dry matter intake (DMI), energy supply, and milk production (Allen, 2000, Kalscheur *et al*., 2006).

Klusmeyer *et al*., (1990) discovered no changes in microbial growth or microbial nitrogen flow from the rumen when dairy cows were fed 11 % CP diets (5.7 % RDP) bringing about ruminal ammonia concentrations of 2.5 mg/dl. Utilizing *in-vitro* techniques Argyle and Baldwin (1989) discovered that growth of mixed microbial populations was maximized at AA and peptides concentrations of 10 mg/L. Feeding a lactating dairy cow with 14.5 % CP diets, a ruminal peptide concentration of 54 mg/L at 16 h after feeding will be produced (Chen *et al*., 1987). Thereafter it was then suggested that diets below 14.5 % CP (that is 7-8 % dietary RDP) will produce adequate ammonia, AA, and peptides that will maximize microbial growth. However, the NRC (2001) predicted a higher requirement (9.5 % of DM

as RDP) using a regression approach that was used to evaluate milk and milk protein responses to concentrations of RDP and RUP in the dietary DM (Cyriac, 2009). Ruminal degradable protein requirements must be based on microbial nitrogen flow out of the rumen in dairy cows (NRC, 2001).

2.2.3 Protein metabolism in the rumen

It was discovered that microbial synthesis in the rumen provides majority of protein used by the lactating ruminants for maintenance and milk production (Broderic, 1980). Thus, an increase in microbial protein formation is an ideal way to improve utilization of dietary CP.

An understanding of ruminal protein metabolism is required to reduce ammonia (NH_3) emission into the environment while maintaining milk production in cows (Cyriac, 2009). The dietary CP content is the product of ruminal degradable (non-protein nitrogen and true protein) and undegraded proteins that show separate and distinct functions (Varga, 2010). The ruminal undegraded protein that escapes degradation by ruminal microbes is available for metabolism in the intestine. Ruminal bacteria and protozoa play essential roles in feed degradation in the rumen (Wallace *et al*., 1995; Santra and Karim, 2003). The microbial organism attached to inaugurated feed in the rumen is 80 % (Craig *et al*., 1987). Degradable protein will be transformed into peptides and amino acid by cell bound microbial proteases, when it is outside the bacterial cell (Brock *et al*., 1982). These will be attracted into the cell where peptidases degrade peptides into amino acid. This could be either used to make microbial protein or could be deaminated to keto acids and finally to ammonia and VFAs (Tamminga, 1979).

Microbial protein production, ruminal digestion, energy and protein availability to the cow will be affected if the RDP fed to the cow is below requirements (Clark *et al*., 1992; Stokes *et al*., 1991). Thus, it is important to provide sufficient RDP to meet requirements of ruminal microbial organisms.

However, Gardner and Park, (1973) were of the view that reducing dietary protein from 15.5 % to 13.2 % will decrease milk production significantly. Additionally, decline in RDP may not necessarily lead to reductions in metabolizable protein availability because reductions in microbial nitrogen flow can be offset by increases in RUP flow (Santos *et al*., 1998). However, feeding RDP is less expensive than feeding RUP. Accordingly, it is important to comprehend least RDP required to maximize microbial protein stream out of rumen and maintain milk production.

2.2.4 Feeding total mixed ration

Nutrition management is an essential procedure in the dairy herd and it has been of great concern to the dairy farmers (FAO, 2016). Feeding dairy cows with a diet that will enable them to produce high rate of milk and provide energy to maintain their BW is necessary (Kellems, 2016). This is to be taken into consideration for the benefit of both condition of animals and the income of dairy farmers. Feeding a balanced total mixed ration (TMR) at all times ensures that a dairy cow achieve maximum performance (Yi Zheng, 2013; Alanna and Jud, 2015). This allows cows to consume optimal energy requirements and maintain physical or dietary fibre characteristics that are required for appropriate rumen function. Furthermore, this creates a more stable and ideal environment for the rumen microbes and increased nitrogen utilization, including non-protein nitrogen (Alanna and Jud, 2015). The TMR has all required dietary components included in a single feedstuff resulting in nutritional advantage over other feeding systems (Herdt, 2014). Thus, fibre and non-fibre ration components are delivered in uniform proportions throughout the feeding period. Therefore, rumen pH changes are reduced and healthy rumen conditions are promoted, even at relatively high rates of energy intake (Herdt, 2014). Different approaches can be employed in the TMR systems for different animal groups (Ishler et al., 1996). They can be formulated to suit each physiological stages such as different phases of lactation period. Adequate management of TMR systems requires accurate weight of each dietary item and a mixer capable of incorporating forages and concentrates into a uniform product (Herdt, 2014). Increase in feed utilization can be expected compared to other types of feeds fed

separately, twice daily. When a TMR is mixed properly, a cow cannot consume significantly more or less of a forage or concentrate.

In TMR fed dairy cattle, the incidence of digestive and metabolic problems is often decreased resulting in reduction of medical costs (Ishler et al., 1996). Milk production has been improved to be as high as 5 % when dairy cows are fed the TMR, compared to conventional rations (Alanna and Jud, 2015).

2.3 The rumen dynamic

2.3.1 Characteristics of the rumen environment

The rumen is the major site of fermentation, making up over 65 % of the volume of an adult cow (ADHB, 2016). The fermentation process of ingested feed was used in breaking down rumen microbes (Lee, 2008; de Ondarza, 2000). Ammonia, VFAs and a variety of long chain fatty acids was produced by breakdown of degradable feed materials (Minson, 1990; de Ondarza, 2000). The importance of ammonia is detected in its use as a source of nitrogen for microbial growth and sources of energy for the cow that is absorption of VFAs from the rumen (Lee, 2008). This increase the availability of energy content of the diet in the rumen in the form of sugar and starch stimulates papillae growth which improves VFA absorption (ADHB, 2016). The energy inherent in the feed available to the animal is approximately 70-85 % and this is because of rumen fermentation which avails good use to be made of fibrous feeds that could not be digested. Out of this energy, 6-15 % is commonly lost as gases (mainly methane) and 6-7 % as heat (de Ondarza, 2000).

Growth of the rumen microbes and their fermentation process depends on several characteristics on the rumen (Russell, 1988). There will be under development of microbes, disorder in digestion, and ultimately decreased in production of milk if there is a change in the rumen environment (Russell, 1988).

2.3.2 Rumen microbes

Breakdown of plant materials are done by introduction of rumen microorganisms, of which their microbial protein often represents the main source of protein for the ruminants (Belanche, 2012). The most important limiting factors for microbial growth are energy (E) and nitrogen (N) which are available in the rumen (Firkins, 1996), while microbial accessibility to nutrients and nitrogen degradation rate are vital to enhance rumen fermentation.

The three main groups of rumen microbes that speed up the fermentation in the rumen are; Firstly, bacteria that are more than 2000 species, 99.5 % are obligate anaerobes which assist in the digestion of sugars, starch, fibre, and protein in ruminants (Wikivet, 2008; Moran, 2005). Secondly, protozoa are large, unicellular organisms that ingest and digest bacteria, starch granules, and some fibre affected by diets consumed. Lastly, a small fraction of the rumen microbial population is present in fungi. They split open plant fibres making them more easily digested by the bacteria.

2.4 Rumen fermentation

Rumen bacteria represent a significant and varied microbial group which have been classed according to their metabolic activities viz: fibrolytic e.g. *Fibrobacter succinogenes*; amylolytic e.g. *Streptococcus bovis*; proteolytic e.g. *Prevotella spp*.; lipolytic e.g. *Anaerovibrio lipolytica*; lactate producers e.g. *S. bovis*; and lactate consumers e.g. *Megasphaera elsdenii* (Belanche *et al*., 2012). The role of rumen protozoa are of different ways in which they are able to degrade fibre and as well as in bacterial predation which has a harmful effect of regarding nitrogen utilization (Firkins, 1996; Demeyer and Fievez, 2000).

2.4.1 Methane and volatile fatty acids

One of the major end products of anaerobic fermentation of feeds in the rumen is methane. The production of methane in the rumen constitutes a significant loss of energy for the host animal and contributing to global warming (Moss *et al*., 2000). Many attempts have been made to reduce rumen methanogenesis using feed additives e.g. ionophores, halogen compounds, unsaturated fatty acids and organic acids. However, depression of fibre digestion or a reduction of protozoa growth is adverse effects simultaneously produced by some of these substances (Demeyer and Fievez, 2000).

Specific alteration of microflora in a host may have beneficial effects on animal production by alteration of ruminal flora which results in production of changes in the proportions of VFAs during ruminal digestion (Reinhardt, 2013). Through the digestion process, microbial protein and energy or VFAs that can be used by the animals are produced (Kaufman *et al*., 1980). The rumen microbes are made up of three primary VFAs: acetate (CH_3COOH), propionate (C_2H_5COOH), and butyrate (C_3H_7COOH) (Basden *et al*., 2013). Large amounts of propionate are derived from grain fermentation while acetate is derived primarily from the fermentation of fibre (Ishler *et al*., 1996). Volatile fatty acids constitute a major source of energy for the animal which are end-products of feed fermentation in the rumen, and are imbibed through the rumen wall (de Ondarza, 2000).

Volatile fatty acids are produced from the microbial fermentation of carbohydrates which are both structural (that is neutral detergent fibre) and non-structural (that is sugars and starches) (Ishler *et al*., 1996). The VFAs can provide up to 80 % of total energy needed by the animal and their descending order in regard of primary abundance is acetic, propionic, butyric, isobutyric, valeric, isovaleric, and traces of various other acids (Ishler *et al*., 1996).

Acetic acid makes up 50 to 60 % of the total VFAs and it dominates a high forage diet (Ishler *et al*., 1996). The main precursor for lipogenesis in adipose tissue is fatty acid synthesis and this is achieved by using acetate (Ishler *et al*., 1996). Thus, to maintain acceptable quantities of milk fat, production of adequate levels of acetate in the rumen are

needed (Hoffman, 2012). More acetic acid results in higher milk fat content. Likewise, when acetic acid in the rumen is low due to a lack of digestion, butterfat is lower (Hoffman, 2012). Also, this can happen when fed a diet that are high in heat-treated starch such as pelleting, steam crimping, or steam flaking as well as heavy concentrate diet. Depressed acetic acid can also occur due to high intakes of oil (Ishler *et al*., 1996). Propionic acid may contain 18 – 20 % of the total VFAs and reaches its peak concentration in a high grain diet (Ishler *et al*., 1996). Blood glucose which provides energy in the liver and which is used in lactose or milk sugar synthesis are due to the conversion of propionic acid. Energy is been provided to the rumen wall by butyric acid which make up 12 – 18 % of the total VFAs. When immersed through the rumen epithelium, it is largely converted to ketones. The proportion of VFAs can be determined by the level of the methanogenic presence and diet in the rumen (Ishler *et al*., 1996).

It was aforementioned that energy from VFAs and microbial protein that can be used by the animals is produced through the digestion process (Kaufman *et al*., 1980). However, VFAs are the principal output of ruminant digestion (Janssen, 2010) and the rumen microbes constitutes mainly three primary VFAs; this includes acetate, propionate, and butyrate. Acetate is produced primarily from the fermentation of fibre (Ishler *et al*., 1996). Volatile fatty acids are actually from the rumen microbes waste products. However, the waste products are absorbs by cow from her rumen and uses them as the main source of energy (Janssen, 2010; de Ondarza, 2000).

According to Adams *et al*., (1981), there was no effect on the yeast culture. On contrast, it was observed that there was a stimulation in VFA and proportion propionate production, which had effects on acetate, or even a rise in the proportion of acetate (Harrison *et al*., 1988; Dawson *et al*., 1990; Newbold *et al*., 1990; Mutsvangwa *et al*., 1992). Williams *et al*., (1990) found that steers fed with yeast culture has a lower ruminal total VFA's concentration. In contrast Andrighetto *et al*., (1993), Kumar *et al*., (1994) and Dutta *et al*., (2001) reported that there was a higher mean molar concentration of VFA's in the rumen liquor of animals fed with yeast culture. Nevertheless, Kopecny *et al*., (1989) found that

there was no effect on ruminal VFA production when the animals were fed with *Lactobacillus acidophilus, Butyrovibrio fibrosolven* along with *Streptococcus bovis*.

2.5 Microbial protein synthesis

Microbial protein serves as a source of protein for the ruminants (Moran, 2005). Dietary protein that escapes degradation in the rumen and microbial protein synthesized in the reticulo-rumen is the source of protein for ruminants (Ishler *et al*., 1996). These are essential in maintaining a balance ruminal pH. Most of dietary protein entering the rumen is degraded by the micro-organisms. The extent of protein degradation varies with types of protein, treatments and the time spent in the rumen (Samaniego, 1996). Dipeptides and amino acids are from the breakdown of oligopeptides which was released Proteolytic digestion. Amino acids are further hydrolysed to organic acids, ammonia and carbon dioxide (Jouany, 1991).

The micro-organisms utilize ammonia, amino acids and small peptides to produce microbial protein (Samaniego, 1996). In addition, ammonia and probably some of the amino acids not utilized by the microbes are absorbed through the rumen wall, and carried to the liver in the blood stream. In the liver, these compounds (mainly the ammonia) are converted into urea (Samaniego, 1996). Urea is hydrolysed to ammonia and re-utilised by the microbes to produce microbial protein (Zhongyan Lu, 2013). The microbial protein produced is of high quality and the amino acid profile is moderately the same to that of milk and meat. Therefore, microbial protein can be easily converted to meat and milk. (de Ondarza, 2000).

Microbial fermentation in the rumen enables the ruminant to utilize poor quality forage or non-protein nitrogen, which could not be utilized by the host animal (Samaniego, 1996). Microbial origin is the source of most or all protein arriving to the small intestine (Ishler *et al*., 1996). Microbial origin can contribute from 0.42 to 0.93 of the total protein available to the host animal (Djouvinov and Todonov, 1994). The bodies of the microbes grown in the rumen are moved to the cow intestine and contain a big proportion of the diet and a bigger proportion of the protein supply.

2.6 Feed additive in dairy cows

Feed ingredient is a part of feed additives which are capable of causing desirable response of animal in a non-nutrient role for example growth, pH shift and/ or metabolic modifier (Hutjens, 1991). The factors to be put into consideration to ascertain if the feed additives ought to be utilized are available research, economic return, field responses, and anticipated response (Hutjens, 1991). When a feed additive is included in the diet of dairy cows, the response performance are increase in milk yield such as milk persistency, higher milk component such as fat or protein, higher dry matter intake, stimulate rumen microbial synthesis of VFAs production. Others include increase digestion, stabilize rumen environment and pH, improve growth, minimize weight loss, reduction of the effect of heat stress and improve health e.g. less ketosis, reduce acidosis and improve immune response.

2.6.1 Antimicrobial activity of feed additives

Lactating dairy cows require a tremendous enormous amount of nutrients in order to support basic life function referred to as maintenance and in the production of milk (Donna, 2010). Protein and energy requirements of a dairy cow increase tremendously as milk production increases (Moran, 2005). Milk production solely results in the largest change in energy needs. For these reasons, feed additives such as antibiotic are needed to enhance the performance of this type of affected animal. One of such antibiotics is ionophores (Reinhardt, 2013).

2.6.1.1 Reduced use of antibiotics and alternatives

There is an increasing public concern about the utilization of antibiotics in livestock production due to the development of multidrug-resistant bacteria which is transferable to humans such as meat and milk consumed from animal products (Kristy, 2014). The United States of America have signed on for an in-feed antibiotic bans for food safety concerns. In 2009, for country like the United States, it was calculated that the total quantity of the antibiotics was over 80 % and traded per year. Approximately 13,000 tonnes of these

antibiotics are used in production of livestock. This made them rank the best producer and user of antibiotics in the world. It has never been more important in China which fed livestock with more than half of the 200,000 tonnes of antibiotics produced. China have been on look for alternative means to improve animal live ability and there performance (Kristy, 2014). In many countries, antibiotics are freely available but in western countries, antibiotics can only be used under a veterinarian's supervision (Barrow, 2000).

Data showing the quantity of antibiotics used in livestock production are scarce in South Africa, and there is no information about the patterns of antibiotic consumption in food animals (Henton *et al*., 2011). The limited information on quantities of antibiotics used for specific purposes in agriculture and human medicine is not surprising and this is as a result of lack of information on the total quantity of antibiotics produced (Moyane *et al*., 2013). Out of all available antibiotics used in livestock production in South Africa, approximately 29 % was reported (Eagar, 2008). These are like premixes which represent a large percentage of all registered antimicrobials. Picard and Sinthumule (2002) and Eagar (2008) reported that antibiotics on weight basis (as measured in the market) is most frequently used as growth promoters, treating and preventing diseases in poultry and pigs. One of the four growth promoters banned in Europe is tylosin and this was the most extensively sold antibiotic in South Africa (Eagar, 2008). After tylosin, tetracyclines, sulphonamides and penicillins follow, respectively (Henton *et al*. 2011). As in other part of the world, the use of antibiotics feed additives might become also a commercial barrier for the African meat and milk products, which requires alternative.

Interest in exploring harmless alternatives to chemical feed additives in ruminant livestock has been renewed from the experience deduced from the increasing public concerns against the utilization of chemical residues in animal-derived foods and pressures of antibiotic-resistant bacteria (Patra *et al*., 2009). The utilization of antibiotics has been for years in animal research and is used for a number of reasons (Landers *et al*., 2012). This includes reduction of faecal carriage of *Salmonella*, chemotherapy of *Salmonella* and other bacterial infections (including *E. coli* and *Mycoplasma*), milk production enhancer and energy boost in lactating dairy cow and growth promotion. The use of antibiotics is being restricted by

regulations without veterinary prescription and each country varies with the antibiotics they use (Maron *et al*., 2013).

There is an issue regarding the use of antibiotics in animal feed to act as growth promoters (Butaye *et al*., 2003). Large numbers of healthy animals are administered with low concentrations of antibiotics for long periods to increase the rate and efficiency of growth (Graham *et al*., 2007). These low levels of antibiotics are below the minimum inhibitory concentration of most pathogens (Sandergen, 2007). Antibiotic resistance in micro-organisms has been linked to the continuous use of antibiotics which could be transmitted from animals to humans (Marshall *et al*., 2011; Wenger, 2012). However, short-term application of antibiotics reduces this risk. Barrow (2000) discussed a number of issues concerning the use of antibiotics:

- Resistance can be monitored by using sentinel bacteria such as *E. coli* and gram-positive microorganisms. It would be more useful to study omnipresent bacteria than studying pathogen resistance which might not always be present.
- Thought should be given to a restriction of antibiotics being used prophylactically, because of the evolutionary pressures being exerted by antibiotics, with only therapeutic use being allowed.
- Antibiotics should perhaps not be used to reduce the intestinal carriage of food-borne pathogens.

In addition to animal health and economics, the use of antibiotics remains an important public health issue. The countries concerned as well as those countries which do not currently regard this issue as being important, should address the issues discussed above (Barrow, 2000).

Du Toit (2011) reported that a new performance enhancer is needed in order to produce food cheaply and help animals realize their full genetic potential. The traits that are necessary for good economic returns are low FCR (feed conversion ratios), high daily weight gains, and shorter fattening times (Nobo *et al*., 2012). The consumers have become accustomed to cheap foods which are produced under ethically acceptable conditions in terms of animal

welfare and health (Harper *et al*., 2002). Consumers will no longer accept antibiotics that do not adhere to these criteria (Mellor, 2000). In North America, nearly half of all antibiotics used end up in livestock and poultry feeds. The quantity of antibiotics utilized for weight gain in food production for animal has been reduced by legislation in parts of Europe (Reid and Friendship, 2002).

It is the dairy producer's ultimate goal to apply consumer-friendly and cost-effective strategies for feed consumed (Hruby and Cowieson, 2006). Mellor (2000) reported that in an attempt to smoothen the transition from cheap food to "safe" food, the gap is already populated by alternatives. The public is beginning to demand that this transition is achieved by a "natural" route. Examples of this natural route are from feed additives which include bacteria (*Bacillus*) and LYC. All such products must comply with certain standards and regulations. According to Natasha (2011), these alternative products must not be toxic to the animal or their human handlers, not to promote Salmonella or give rise to environmental pollution (Mellor, 2000). Over the years, strategies for improvement in animal health, productivity, and microbial food safety over the use of antibiotics have been explored (Joerger, 2003). Furthermore, this suggests that bacteria inhibit the antibiotics such as *Brevibacillus laterosporus* could be used as an alternative to antibiotics as earlier mentioned.

2.6.2 Direct fed microbial

Empirical observations suggested that some live microorganisms in feeds might positively affect animal performance in different types of production systems have been the major basis for the utilization of microbial preparations (Beev *et al*., 2007). From history, large-scale applications of live microorganisms in feeds were not common (Denev, 2007).

In addition, utilizing preparations containing live microorganisms as feed supplement for ruminant is of significant interest (Dawson, 2002). The real idea of administering microorganisms to animals is associated with the feeding of large quantities of "beneficial" microbes to livestock when they were "stressed" or ill (Denev *et al*., 2007). Microbial

products used in this way were originally called “probiotics” or products “for life” (Beev *et al*., 2007). The term “probiotic” implied a curative nature (Kung, 2006). It is noteworthy that claims by a product to reduce mortality, increase production (Increase in milk production or dry matter intake) and improve health cannot be made of any product unless its safety and efficacy have been documented and approved by government regulatory agencies (US is a case to study) (Denev *et al*., 2007).

Thus, the regulatory agencies and feed industries had accepted the more generic term which is “Direct-Fed Microbial” (DFM) to describe microbial-based feed additives so as to overcome this requirement (Beev *et al*., 2007). Furthermore, some microorganisms that were accepted for use in animal feeds was developed (Denev *et al*., 2007). According to Fuller (1989), some of the major hypotheses on how DFM may benefit animals can be found in a good discussion. One of the commonest explanations for improving the health of the animal when ruminants are fed with DFM depicts that beneficial microbes compete with potential pathogens which prevents their establishment. It was suggested by Denev (1996, 2006) that DFM may also generate antimicrobial end products such as acids which limit the growth of pathogens. In addition to this, feeding DFM to ruminants led to metabolism of toxic compounds and production of stimulatory substances (Denev *et al*., 2007).

The process of digestion in ruminant produced as a result of fermentation provided by the rumen microbial flora and by chemical reaction (Santra and Karim, 2003). During the last decade, the rumen as well as intestinal microbial flora balance has been identified as main factors to manipulate in order to obtain the best growth performance of the animals (Santra and Karim, 2003; Thulasi *et al*., 2013). This microbial flora has an impact on the animal's performance although their equilibrium is constantly been threatened by proliferation of microbes that are not desirable which can cause damage to the health and the animals’ performance (Thulasi *et al*., 2013). Therefore, the use of live microbial cultures (probiotics) as natural feed additives for enhancing rumen metabolic activity and overall animal production is being tried nowadays. Supplementing different probiotics (fungi/yeast and bacteria) resulted in improved nutrient status and productivity of the ruminants under certain conditions (Santra and Karim, 2003).

The US Food and Drug Administration (FDA) define DFM as a source of live (viable) naturally occurring microorganisms which includes bacteria and yeast (Miles and Bootwalla, 1991). The frequently utilized probiotics that are used for animal feeding are divided into two major categories which are bacterial origin and yeast origin.

The effects of probiotics are shown more in the fastest growing animals which reduce with age (Soren *et al*., 2013). According to Santra and Karim, (2003), the utilization of probiotics in farm animals increased growth, improved the efficiency of feed conversion, better absorption of nutrients which is due to the control of gut epithelial cell proliferation and differentiation, improved carbohydrate and calcium metabolism, and also, synthesis of vitamins.

2.7 *Bacillus*

The genus *Bacillus* is comprised of gram-positive, rod-shaped, spore-forming bacteria that generate a diverse array of antimicrobial compounds of particular interest in the ability of certain strains to produce antifungal compounds (Tewelde, 2004). Such organism has the potential for application in agriculture where they can be employed as bio control agents against selected plant pathogenic fungi (Ji *et al*., 2013; Pertot *et al*., 2015; Tewelde, 2004).

2.7.1 Mode of action of *Bacillus*

Parker (1974) reported that probiotics contributed to intestinal microbial balance. Fuller, 1989 reported that the contribution to intestinal microbial balance by probiotics was beneficial to the host animal. According to Lee *et al.* (2010), it was observed that the spore forming *Bacillus spp* made the environment of the gut to be less conducive to colonization by pathogenic bacteria and this is by competing with them for mucosal attachment and nutrients as well as improving nutrient uptake through villi development. Also, it was observed that *Bacillus spp* lowers the pH through acid fermentation which creates positive environment for beneficial bacteria e.g. *Lactobacilli* and this have been shown to reduce amounts of pathogenic bacteria for example *Salmonella*, *E. coli*, *Campylobacter* and *Clostridium* (Kirsty, 2014). In contrast to some of other probiotics, *Bacillus* strains can also resist heat and high pressure which help them survive the hostile steam conditioning and pelleting process routinely used in the feed industry. There is also strong evidence that suggest that multi-strain *Bacillus* are more effective than single strain equivalents (Kirsty, 2014).

2.7.2 Brevibacillus laterosporus

There has been a lot research about probiotics and its relation to ruminant (Uyeno *et al*., 2015; Wallace and Newbold, 1992). Probiotics can replace a number of beneficial bacteria that reside in the rumen of a dairy cow to actively promote health and wellness of the dairy cow (Rifat *et al*., 2016; Yirga, 2015). Probiotics do this by populating the large rumen with "associate bacteria" that can feed or adapt on other microorganisms that the ruminant does or does not need (Rusell *et al*., 2003). Some of these other bacteria are often potentially harmful or useless. It is suggested by the goal of this study to affect knowledge of previous study on probiotics used in ruminant dairy cow to increase number of good bacteria while decreasing the number of bad bacteria (Thomas, 2016) and associate effect of bacteria with other feed additive.

Brevibacillus laterosporus, a bacterium depicted by the production of a unique canoe-shaped lamellar body which is been attached to one side of the spore is a natural inhabitant of water, soil and insects. *Brevibacillus laterosporus* Laubach that is a rod-shaped, endospore-forming bacterium is morphologically characterized through the production of a typical canoe-shaped parasporal body (CSPB) firmly attached to one side of the spore (Oliveira *et al*., 2004). This determines its lateral position in the sporangium. Probiotics are ubiquitous species that can be isolated from a wide range of materials including soil (Oliveira *et al*., 2004), fresh water (Laubach *et al*., 1916) and sea water (Suslova *et al*., 2012). Furthermore, probiotics can be found in milk (Varadaraj *et al*., 1993), honey (Iurlina *et al*., 2005), insect bodies (White, 1912), animal hide and wool (Chen *et al*., 2012), quails (Bagherzadeh *et al*., 2012). In addition, it can be traced in leaf surfaces (Roy *et al*., 2006), locust beans (Sarkar *et al*., 2002), compost (Adegunloye *et al*., 2007) and starchy foods (Fangio *et al*., 2010). *Brevibacillus laterosporus* can be found as commercial products from various sources depending on its usefulness and can be used in industries like livestock.

2.7.2.1 Use of *Brevibacillus laterosporus*

The general idea of introducing beneficial microorganisms in to the ruminant is not a new practice**.** This has been in existence for a while from many producers and veterinarians. This includes utilization of rumen fluid from healthy animals by inoculating sick ruminants specifically those that have been off feed. This is in promises of inducing normal rumen environment and improving dry matter intakes (Denev, 1996). However, based on this concept aforementioned, there is absence of commercial products and uncontrolled research studies on the efficacy of this practice. In contrast, with more specification on applications, there are bacterial based DFM that are on sale for utilization in ruminant diets. *Lactobacillus* spp. as one of the most common microorganisms utilised are often contained in all these products. Various species of *Bacillus, Bifidobacterium, and Enterococcus* are other commonly utilised bacteria (Denev, 1996).

Effects of most bacterial-based DFM are in the gut not in the rumen probably because of its beneficial (Denev *et al*., 2000; Denev, 2006). For example, bacteriocins produced by *Brevibacillus laterosporus* SA14 produce lactic acid (Somsap. *et al*., 2013). The DFM ruminants were carried out initially, this includes applications for cattle being transported, and young calves fed milk (Jenny *et al*., 1991; Hutchenson *et al*., 1980). These animals have immature microbial ecosystems in their guts and are easily stressed (Vandevoorde *et al*., 1991).

Beauchemin *et al*., (2003); Beev *et al*., (2007); Seo *et al*., (2010) and Uyeno, (2015) have documented reports on the positive effects on feeding bacterial DFM to lactating dairy cows. The best candidates for such products would be high producing cows in early lactation stage. This could be for the reasons that cows which are in negative energy balance and has diets that contain highly fermentable carbohydrates that sometimes lead to acidosis (Jaquette *et al*., 1988 and Ware *et al*., 1988). Supplementation of *B. laterosporus* may be useful in the different aspect of livestock production. Recently, the potential of the bacterium *B. lateropsorus* as an emerging entomopathogen against the house fly has been highlighted (Ruiu *et al*., 2014).

The introduction of multifunctional microbial control agents e.g. *B. laterosporus* used in animal production systems may provide additional contribution to the prospect of a more integrated approach to farm development. A wider view arising from the presented concepts includes the use of feed additives producing beneficial (probiotic) effects on animals (ruminant) and at the same time in directly contributing to contain insect pests developing in manure and plant parasites in amended soil. Current and future research findings toward this direction will support an eco-sustainable vision of the farm considered as a whole (Ruiu *et al*., 2014).

2.7.2.2 Mode of action of *Brevibacillus laterosporus*

The first important step in the digestion of ruminant involves fermentation in the rumen (Auclair, 2001). Many changes have been reported using bacteria in the rumen. Fermentation by the rumen microbial flora and chemical reaction are reasons for the occurrence for the digestion process in ruminants (Santra and Karim, 2003). Bacteria are very beneficial in the rumen. Among all these various bacteria used as feed additives, idea known about the mode of action are of little. The modes of action of these bacteria (*B. laterosporus*) are believed to inhibit the mode of action of ionophores antibiotics. *Brevibacilus laterosporurus* reduce gram positive bacteria resulting in greater energetic efficiency (lower CH_4 and A:P ratio). It also encourages better protein utilisation through less peptidolysis and amino acids deamination (Eramus *et al*., 2009). The application of these bacteria is assumed to manipulate the rumen fermentation and increase the proportion of propionic at the expense of the rest of acids found in VFA. *Brevibacillus laterosporus* is known to reduce nitrates (NO_3) to nitrites (NO_2). The data showed that when sodium nitrate is used in reducing in vitro methane emissions, nitrate reducing bacteria can be utilised as probiotic to avert the accumulation of nitrite (Chiedza *et al*., 2014).

2.8 Live Yeast (*Saccharomyces cerevisiae*)

Several reports (Lila *et al*., 2004; Jouany, 2001; Alshaikh *et al*., 2002; Tricarico *et al*., 2006) revealed that improvement of microbial activities, beneficial changes in activity and numbers of rumen microbes are of the features of positive effects on YC. *Saccharomyces cerevisiae,* the main mode of action that YC mostly derived from and this attracted attention from a number of researchers in the world because it improves livestock performance. Thus, they beneficially modify microbial activities, fermentative and digestive functions in the rumen can be due it addition of YC supplements (Denev *et al*., 2007).

Dietary supplementation of microbial additives, such as live yeasts is used to manipulate rumen fermentation and may improve digestive efficiency in ruminants (Wallace *et al*.,

1994). For example, the amount of total ruminal anaerobes (Girard, 1997; Newbold *et al*., 1991; Dawson *et al*., 1990; Jouany, 2001) and cellulolytic bacteria (Girard, 1997; Harrison *et al*., 1988; Jouany, 2001) have been increased with YC. It is because of the current trend of consumers for choosing natural and organic alternatives that make chemicals less favoured compared to microbial performance promoters. Yeasts are naturally found in the rumen. Using yeasts as feed additives require continuous daily supplementation because at optimal 25 ºC (Lund, 1974), the rumen temperature does not promote their growth.

Animal's response to dietary yeast supplements depends on dosage, feed management practices, type of microorganism, and composition of basal diet (Newbold *et al*., 1995). Products from yeast that are available commercially differ in number of live cells, strain and species, and growth medium of the microorganism's (Erasmus *et al*., 1992). Therefore, effect on a given dietary-induced rumen environment depends on different types of yeast (Callaway and Martin, 1997). As a result of this, test on the effectiveness of industrially produced strains on rumen fermentation and animal performance need to be done. Influence related to the ruminal function might be due to the primary mechanism through which yeasts affect animal performance (Dawson *et al*., 1990; Chaucheyras-Durand *et al*., 2008). dry matter intake (Wohlt *et al*., 1991; Erasmus *et al*., 1992) and milk yield (Erasmus *et al*., 1992; Williams *et al*., 1991; Piva *et al*., 1993), as well as overall performance response of dairy cows might be able to improve due to supplementing dairy cows with live yeast strains.

According to Girard (1997), the cellulolytic activities of the rumen microorganisms obviously can be improved by yeast culture (YC). This improves digestion of fibre, improve use of starch supplied in the feeding ration, reduce the concentration of oxygen in rumen fluid and increases the total number of rumen microorganisms. In this way the rate of VFAs production is influenced (inhibit) and improves the intensity of digestion and stability of rumen environment increased. In addition, direct stimulation of rumen fungi from YC might improve digestion of fibre (Chaucheryas et al., 1995). In steers fed straw-based diets, YC increased the number of rumen protozoa and NDF digestion (Plata et al., 1994). It has

been reported that in the presence of methanogens and YC stimulate acetogenic bacteria (Chaucheryas et al., 1995), which can leads to efficiency in ruminal fermentation.

It has been demonstrated that, for stimulating microbial populations specifically for the growth and activity of fibre degrading bacteria, the potential of *S. cerevisiae* strain CNCM I-1077 is utilised (Mosoni *et al*., 2007; Guedes *et al*., 2008; Chaucheyras-Durand and Fonty, 2001; Michalet-Doreau *et al*., 1997). Strengthening the reducing power of rumen fluid and stabilizing rumen pH by the aptitude of live yeast supplements could improve total tract dietary fibre digestibility (Wallace, 1996; Dawson, 1992; Marden *et al*., 2008; Williams and Newbold, 1990; Newbold *et al*., 1996; Jouany, 2001).

The documentation of specific ability of yeast culture preparations to stimulate the ruminal bacteria growth and specifically increased the concentration of the groups of useful bacteria in the rumen have been well reported (Jouany, 2001; Dawson and Tricarico, 2002). One of the most continuously measured responses to YC in the rumen has been from increased concentrations of cellulolytic bacteria and presence of the total anaerobic bacteria in the rumen (Harrison *et al*., 1988; Dawson *et al*., 1990; Wiedmeier *et al*., 1987; Newbold and Wallace, I992; Jouany, 2001; Girard, 1997). However, other studies have suggested that proteolytic bacteria (Yoon and Stern, 1996), lactic acid-utilizing bacteria growth (Edwards, 1991; Girard, 1997; Jouany, 2001), and bacteria that convert molecular hydrogen to acetate in the rumen can be enhanced from preparations of YC (Chaucheyras *et al*., 1995).

2.8.1 Beneficial effect of yeast supplementation

Bach *et al*., (2007) reported that the loose housed lactating cows increase in average rumen pH, that is, average maximum pH by 0.5 units and average minimum pH by 0.3 units. These cows were supplemented with live yeast strain SC I-1077. The results showed that the eating behaviour of the animals changes significantly. Cows not supplemented have a longer inter-meal interval of 4.32 h than cows with 3.32 h that are supplemented with live yeast. Rumen

pH recovery and the beneficial effect of live yeast on pH stabilization could be as a result of induced changes presence eating behaviour. De Ondarza *et al*., (2010) in a multi-study analysis**,** discovered that supplementation of a live yeast SC I-1077 improves milk yield by 1.15 kg per day (live yeast of 34.19 kg per day against control value of 33.04 kg per day). In spite of this, there was no observed effect on dry matter intake. It seems that live yeast has an effect on intake pattern rather than on intake per se. As a result of this, improve on the feed efficiency is enhanced by the presence of live yeast.

Jouany (2001) and Williams *et al*., (1991), demonstrated that the beneficial effects on lactic acid concentrations from the Yea-Sacc®1026 are of high concentrate diets in the rumen. Consequently, high energy diets fed to animals that decreased lactic acid concentrations are associated with higher ruminal pH characterised by much more stable ruminal fermentation. Due to these alterations, it is expected that improved digestion can be provided from ruminal fermentations, and improved intake could also be reflected. Prevention of accumulation of lactic acid in the rumen is due to ability of Yea-Sacc®1026. This suggests that viable yeast helps to overcome ruminal dysfunctions associated with the utilisation of high-energy diets. This practiced in both fast growing beef and dairy cattle. Girard, (1997) and Jouany, (2001), suggested that lower lactic acid concentrations in the rumen are not a result of direct inhibition of starch-digesting lactate producers but likely due to growth enhanced and lactic acid-utilizing bacteria activities. Live yeast culture (LYC) is able to scavenge excess oxygen (Newbold *et al*., 1996; Jouany, 2001) and this might improve ruminal fermentation. This is due to more conjusive environment for rumen anaerobic bacteria is created. On rumen fermentation, there are specific strains of *S. cerevisiae* that has stimulatory effects.

2.8.2 Modes of action of live yeast

The popularity of fungal DFM as additives in ruminant diets has been in existence for many years. Many of these fungal additives are available which enhance the fermentation in rumen, LYC (*Saccharomyces cerevisiae*) (Denev, 1996). The study by Guedes *et al*., 2008 indicated that *in situ* NDF degradation (NDFd) of corn silage and other feeds could be increased by the addition of *S. cerevisiae* I-1077. In addition, study noted the importance of

the yeast effects experimented on lactate concentration and pH. Moreover, it recommended that the effectiveness of the yeast to intensify the NDFd is not the only distinctive feature associated to a pH stabilisation effect.

Studies have also suggested that overall stimulation of beneficial ruminal bacteria were involved from more basic mechanisms (Girard, 1996, 1997; Girard and Dawson, 1994, 1995). In addition, these resulted in the isolation of a group of small, nitrogen-containing compounds then stimulation of bacteria occurred through logarithmic growth and thus stimulates microbial activities. These stimulatory compounds have basic chemical features that are consistent with biologically and those of small active peptides. This demonstration occurred in small peptides that are under stimulatory activities with pure cultures of ruminal bacteria (Girard, 1996). Stimulating the growth of representative fibre digesting bacteria from the rumen and similar stimulatory effects have also shown as a result from synthetic tryptophan-containing peptides. Individual amino acids are not associated with these stimulatory activities. Aforementioned occurred at concentrations level that was considerably below those that would suggest that these compounds are limiting nutrients (Denev et al., 2007). Alternatively, these compounds seem to stimulate beneficial ruminal bacteria as a result of metabolic trigger and thus enter into an exponential growth phase. Observed effects of YC in the rumen can be explain better from this stimulatory activities towards specific strains of ruminal bacteria (Beev et al., 2007)

2.8.3 Use of live yeast in dairy cow

Yeast culture products and *Aspergillus oryzae* fungal fermentation (Aguilar, 2013) extracts are widely used to improve the performance of livestock due to their act of modifying ruminal fermentation and stimulation of ruminal bacterial, protozoa and fungal growth (Erasmus *et al*., 1992; Williams *et al*., 1991). Higher activity of rumen microbes will enhance the digestibility of dietary fibre (Williams and Newbold, 1990), and lead to higher dry matter intake (DMI). Feed intake is therefore considered as fungal feed additive driven. Numerous factors are recognised to influence appetite, specifically to those related to *Saccharomyces cerevisiae* (Aguilar, 2013) and AO supplementations in ruminants rations

are palatability, level of fibre digestion, protein status and digesta flow rate (Yoon and Stern, 1996**).** The enhanced DMI, which may drive production responses to microbial feed additives, is most likely due to an improved rate of breakdown of feedstuffs in the rumen. Increased DMI and consequently yield of milk and milk components between 2 and 5% are effects from the addition of yeasts to ruminant diets but slightly reduced feed efficiency (Kung *et al*., 1997; Robinson and Erasmus, 2009; Williams *et al*., 1991).

2.8.4 Effects of yeast on protein degradability

Significant advancement has been reported in yeast fed animals for digestibility of DM, CP, fibre and OM (Huber, 1997; Angeles *et al*., 1998). It has also being reported that in yeast fed animals, higher retention of nitrogen and energy contrarily, the results have been from response influenced by the type of diet, variable effects, utilisation of microbial strain and physiological state of the animals (Santra and Karim, 2003). Animal productivity and nutrient digestibility are influenced by basal diet of the yeast culture fed animals (Moloney and Drennan, 1994; Williams and Newbold, 1990). Fallon and Harte (1987) described that YC does not increased growth performance and nutrient digestibility of calves fed a non-starch based concentrate (corn gluten) but does in a starch-based concentrate (barley) fed to calves. In addition, Williams *et al*., (1991) suggested that in diets comprising a high proportion of the readily fermentable carbohydrate such as barley based concentrate, the effect of yeast culture will likely to be greatest. Contrarily the effects aforementioned, Williams *et al*., (1990) and Harrison *et al*., (1988) did not observe any yeast culture supplementation effect to the animals on ruminal nutrient digestibility. Reducing the hind gut digestion, increasing the ruminal digestion and site of digestion might be affected due to inclusion of yeast in animal's diet so that the overall tract digestibility seems the same as control (Williams *et al*., 1990). However, nutrient digestibility in yeast fed animals was better reported by Pandey *et al*., (2001), Panda *et al*., (1995) and Widmeier *et al*., (1987).
Supplementation of diets with *L. acidophilus* and *S. cervisiae* in goats has led to a substantial improvement in the crude fibre and crude protein digestibility (Sharma and Malik, 1992). Abu-Tarboush *et al*., (1996) also reported that diet containing culture of *L. acidophilus* fed to Holstein calves has no significant effect on apparent digestibility of DM,

OM, CP, ADF and gross energy. Further study is encouraged in the area of using yeast and bacteria as a feed additive.

2.9 Chapter summary

The review of literature in this chapter reveals that antibiotics have contributed significantly to the lactating dairy cow development, especially in the energy performance, increased milk production and reduction in diseases. However, antibiotic have been a great concern to the consumer because of its transferable resistance to the human. In addition, antibiotics have not only affected the consumer but its host itself. It is essential to determine the type and quantity of the antibiotics that the animal supposed to consume. Due to risks related with using them, these antibiotics were banned in some countries and alternatives are needed. Generally, feed additives contribute to the performance and control of lactating dairy cow by providing the rumen bacteria, protozoa and fungi needed for their fermentation process.

Several factors have contributed both positively and negatively to the changes in rumen environment (Bauman *et al*., 2000; Kohn *et al*., 2000; Fernando *et al*., 2010). These factors include rumen pH, rumen fermentation, nutrient digestibility and microbial protein synthesis. To ensure the alternative for antibiotics are good enough to promote growth and consumer free disease, the option has to be performing the benefit the antibiotics offer but not as resistance to humans. One of the alternatives to these antibiotics is assumed to be from bacillus family which is *B. laterosporus*. These bacteria can act as antibiotics used as feed additives in early lactating dairy cow and have no effect on the consumer and the host itself.

The literature focused on the challenges affecting early lactating dairy cow. It also reveals the importance of feed additives and their mode of actions. Problems encountered include low milk production, energy lost and cost of production. If the nutritionist and dairy farmers are to increase their profit margin, enhance their production and assure consumer of their products, certain management practice must be in place. The literature reveals the process by

which the antibiotic works and what the alternatives can offer. With adequate and proper introduction of this *B. laterosporus*, live yeast and their associate effects can help the dairy farmers reduce their vulnerability to some of the challenges affecting them. Interest in feed additives will be sustain, continue and added influence idea from new research results, profit margins and publicizing (Hutjens, 2005).

CHAPTER 3

MATERIALS AND METHODS

The experiments were carried out at the Animal Research Council - Animal Production Institute (ARC-API), Irene, South Africa during the year 2015. Both ARC-API (APIEC15/038) and the College of Agriculture and Environmental Sciences, (2015/CAES/066) animal ethics committees, approved the use of the cannulated dairy cattle for rumen fluid.

3.1 Diet and treatments

The basal diet (Table 3.1 and 3.2) was formulated to fulfil the minimum nutrient requirement of an early lactating 600 kg Holstein cow producing 40 kg of milk with 3.5 % fat and 3.3 % protein using CPM-dairy software (NRC, 2001). The dietary treatments were:

T_1: Control: basal diet with no additive
T_2: Basal diet + *B. laterosporus* (0.5g/kg of feed)
T_3: Basal diet + Live yeast (*Saccharomyces cervicae*: 0.25 g/kg of feed)
T_4: Basal diet + 0.5 g *B. laterosporus* + 33 mg Live yeast per kg feed.

Levucell, the live yeast product (VITAM, 142 South Street, Centurion, 0157, South Africa) contained 10^8 cfu/g of *Saccharomyces cervicae* and the *B. laterosporus*-containing product (Bioworx / (Pty) Ltd, Meiring Naude road, Pretoria, 2608, South Afrrica) contained 0.5 x 10^8 cfu spore. Treatments 2, 3 and 4 were prepared by adding the specific quantity of the additives to the basal diet.

Table 3.1 - Ingredient of the basal total mixed ration

Ingredients (%)	
Corn silage	26.8
Alfalfa silage	13.2
Ground corn	20.8
Hominy	4.0
Soybean hulls	8.9
Wheat	7.0
Soybean meal, 44 % crude protein	12.1
Distillers grains	5.0
Limestone	1.08
Magnesium oxide	0.08
Trace mineral salt	0.49
Trace nutrient premix[1]	0.60

[1]Contained copper sulphate, sodium selenate, zinc sulphate, biotin, and vitamins A, D, and E and was formulated to provide 9 mg of Cu, 13 mg of Zn, 1 mg of biotin, 0.3 mg of Se, 3,600 IU of vitamin A, 1,080 IU of vitamin D, and 20 IU of vitamin E/kg of TMR.

Table 3.2 - Chemical composition of the diets (dry matter (DM) basis)

Item	
Dry matter	69.5
Organic matter, % dry matter	94.0
Crude protein, % dry matter	17.1
Neutral detergent fibre, % dry matter	33.7
Forage neutral detergent fibre, % dry matter	16.9
Starch, % dry matter	28.0
Non-fibre carbohydrates, % dry matter	38.8
Net energy for lactation, [1] Mcal/kg	1.6
Calcium, % dry matter	1.00
Phosphorus, % dry matter	0.36
Magnesium, % dry matter	0.25
Potassium, % dry matter	1.26

[1]Calculated using NRC (2001)

3.2 Collection of rumen fluid and buffer solution

Ruminal fluid was obtained from a ruminally cannulated lactating Holstein cow that was fed a total mixed ration (TMR). The ruminal content was collected 2 h after feeding. This was squeezed through four layers of cheese cloth into pre-warmed flasks and approximately 50 g of inoculum added before being transported to the laboratory (Ding *et al*., 2005). The transfer of rumen fluid was done as quickly as possible into thermos vacuum to keep them warm. The pH was measured immediately from the sample collected. The rumen fluid with inoculum was blended (Waring blender; Waring Products) at high speed for 10 seconds and placed in a 39 °C water bath in other to maintain the temperature of the rumen fluid prior to the next stage of usage. The blending action was to shift associated particulate in microbes and check a representative microbial population for the *in vitro* fermentation (Holden, 1999). The rumen inoculum was divided into two pre-warmed flasks under constant

purging with CO_2 and mixing. One of the separated rumen inoculum was utilised for Daisy techniques and the second was utilised for *in vitro* batch fermentation. A buffer solution used for all incubations was prepared as showed in Table 3.3.

Table 3.3 - Composition of the buffer

Macro mineral	
Distilled water	1000
Na_2HPO_4 anhydrous (g)	5.7
KH_2PO_4 anydrous (g)	6.2
$MgSO_4.7H_2O$ (g)	0.59
NaCl (g)	2.22
Micro mineral	
Distilled water (ml)	100
$CaCl_2.2H_2O$ (g)	13.2
$MnCl_2.4H_2O$ (g)	10
$CoCl_2.6H_2O$ (g)	1
$FeCl_3.6H_2O$ (g)	8
Buffer solution	
Distilled water (ml)	1000
NH_4HCO_3 (g)	4
$NaHCO_3$ (g)	35
Reducing solution	
Distilled water (ml)	100
Cysteine hydrochloric acid (g)	0.625
KOH pellets (g)	10
$Na_2S.9H_2O$ (g)	0.625

3.3 In situ degradation

The technique used for determination of conventional *in vitro* digestibility followed with the Galyean (1997) modification of the Tilley and Terry (1963) was used. Multi-layer polyethylene polyester bags (ANKOM® F57 filter bag, ANKOM® Technology Corp., Fairport, NY, USA) with the porosity of 30 µm (ANKOM Technology Corporation, 1997) and an incubator (Daisy^{II}; Ankom Technology Corporation, Fairport, NY, USA) were utilized.

A sample size of each treatment used was 0.5 g per bag with 24 bags per incubation jar. Each run contained three replicates by treatment (12 samples) as well as two standards and two blank bags. Samples were heat sealed (Heat sealer #1915; ANKOM Technology Corporation, Fairport, NY, USA) in bags, placed in jars, and incubated for 0, 2, 4, 8, 12, 24, 36 and 48 h at 39 °C in a buffer-inoculum solution (Vogel *et al*., 1999; Holden, 1999). The nylon bags were then removed from the jars afterwards, rinsed three times using cold tap water (Holden, 1999) and soaked in acetone for 5 min, air-dried, then put in an oven at 60 °C for 48 h. Thereafter, the samples were cooled in a desiccator, and weighed. The residuals were taken to laboratory for further analyses.

The disappearance of crude protein (CP) and dry matter (DM) from the bags was calculated from the respective amount remaining after ruminal incubation. *In situ* degradability kinetics for DM and CP were evaluated by the exponential model (Orskov and McDonald, 1979) as $p = a + b\ (1\text{-}e^{-ct})$ using SAS (2009).

The model assumes there is a soluble fraction “a”, a potentially degradable insoluble fraction “b”, and a constant “c” that represents the degradation rate of the fraction “b” per unit of time. Estimation parameters of “a”, “b”, and “c” were obtained by adjusting the model by the nonlinear regression procedure NLIN. The effective degradability of DM and CP (Bhargava and Orskov, 1987) was determined as:

$ED = (a + b\ c) / (c + k)$

Effective degradability ED1, ED2 and ED3 were calculated with an essential solid outflow rate from the rumen of 0.02, 0.05 and 0.08, which are effective degradability (k), respectively representing low, medium, and high intake levels, respectively (Agricultural Research Council, 1984).

3.4 *In vitro* batch fermentation

The effects of additives on rumen microbial fermentation were evaluated in *in vitro* batch fermentation of diet. The buffered rumen fluid was transferred in 250 ml serum bottle with 0.5 g of the diet ground through a 2 mm screen. Incubation was then carried out in a shaking water bath at 39 °C. All fermentations were performed for four time periods (0, 12, 24 and 48 h) with three replicates per time (n = 12).

Ten millilitres were collected from all bottles after each incubation time. The pH was measured immediately and samples were stored at -20 °C pending analysis. Later, ruminal fluid was thawed, centrifuged (15,000 x g, 4 °C for 15 min) and analysed for ammonia nitrogen and VFA. The pH was measured with a standard pH meter, ammonia nitrogen was measured by phenol-hypochlorite reaction as described by Weatherburn (1967) and VFA analysed by gas chromatography (Hofirek and Haas, 2001).

3.5 Determination of microbial protein synthesis

The microbial protein synthesis was determined according to the method of Zinn and Owens (1982) as purine derivatives. Briefly, 0.25 g digested residue was weighed into a 25 mm width screw-cap Pyrex tube and 2.5 ml perchloric acid (70 % A.R.) was added. The mixture was covered and incubated in water bath at 90-95 °C for one hour. After cooling, tubes were opened, the pellets were broken using glass rod and 17.5 ml of 0.0285 M ammonium phosphate ($NH_4H_2PO_4$) was added. Tubes were returned to water bath at the same temperature for 30 min. After incubation, tubes were cooled then the contents were filtered twice through Whatman No. 4 filter paper. One ml of the filtrate was then transferred into 15 ml tube before adding 0.5 ml of silver nitrate (0.4 M) and 8.5 ml of ammonium phosphate

(0.2 M). The tubes were screwed and allowed to stand overnight at 4 °C. The tubes were then centrifuged at 4000 rpm for 15 min and supernatant fraction was discarded with care not to disturb the pellet. The pellets were then broken with glass rod, washed with 5 ml of the pH 2 distilled water (with sulphuric acid) and again centrifuged at 4000 rpm for 15 min (at 4 °C). The supernatant was discarded, the pellets were broken again with glass rod and suspended in 10 ml of 0.5 N hydrochloric acid (HCl). The remaining was mixed thoroughly using a vortex and transferred into 25 mm width screw cap tube then placed in the water bath at 90-95 °C for 30 min. After cooling, the tubes were centrifuged at 4000 rpm for 15 min (at 4 °C) and the absorbance reading of the supernatant fraction was recorded at 260 nm against 0.5 N hydrochloric acid using spectrophotometer. A standard of 0.05 g yeast RNA (93 % CP), treated as described above but diluted according to AOAC (1995) just before the incubation in the water bath using 0.5 N HCl as diluent.

3.6 Chemical analysis

Feed samples and residues after digestion were milled and sieved through 2 mm sieve and chemical analyses were performed on a dry matter basis. The dry matter (DM) contents were determined by oven drying at 60 °C for 48 h. Crude protein was determined according to the method of AOAC (Association of Official Analytical Chemists, 2000) procedure 968.06 and ether extract (EE) according to AOAC, (2000) procedure 920.39. Calcium (Ca), potassium (K) and magnesium (Mg) was determined according to AOAC, (2000) using a Perkin Elmer Atomic Spectrophotometer. Phosphorus (P) was assayed according to the method of AOAC (2000) procedure 965.17. The neutral detergent fibre (NDF) and acid detergent fibre (ADF) were dtermined by using ANKOM$^{200/220}$ Fibre analyzer (ANKOM® Technology Corp., Fairport, NY, USA). The NDF component was determined on 0.5 g of each original sample into separate F57 ANKOM or nylon fibre analysis bags and their relative residues after incubation as described by the manufacturers. The bags were heat-sealed and NDF determined using the method of Van Soest *et al*. (1991). The sodium sulphite anhydrous (Na_2SO_3) was added to the NDF solution during extraction and heat-stable α-amylase was added during rinsing with warm water. ADF was also determined using the method of Van Soest *et al*. (1991)

3.7 Statistical analysis

3.7.1 Dry matter and crude protein degradability, and rumen ammonia and volatile fatty acid

The data on DM and CP degradability, rumen ammonia and VFA were subjected to one way ANOVA using PROC GLM (SAS Institute, 2009). Least squares means for all treatments were reported and significance tested at $P < 0.05$.

The statistical model was:

$Y_{it} = \mu + \alpha_i + \beta_t + T_{it} + e_{cit}$,

where

Y_{it} = an observation value obtained from treatment i at time t;

μ = overall mean for the population;

α_i = fixed effect of treatment i, where i = T_1, T_2, T_3 or T_4;

β_t = fixed effect of time t

T_{it} = fixed interaction of effect of treatment i and time t;

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

3.7.2 Effective degradability and microbial protein synthesis

Data on effective degradability and microbial protein synthesis was subjected to ANOVA using PROC GLM (SAS Institute, 2009). The statistical model used was

$Y_i = \mu + T_i + e_i$

Where Y_i = observation value for treatment i

μ = overall mean of the population,

T_i = fixed effect of the i^{th} treatment (T_1, T_2, T_3 OR T_4),

e_i = error associated with each observation

Significance was declared at $p < 0.05$

CHAPTER 4

RESULTS

4.1 Dry matter and crude protein degradability as affected by *Brevibacillus laterosporus,* live yeast and their combination *in vitro*

The disappearance of diet DM and CP from the bags were evaluated by *in vitro* method and results are presented in Table 4.1 and 4.2, respectively.

Table 4.1 - The *in situ* rumen degradation characteristics of dry matter in control diets (T1), diets supplemented with *Brevibacillus laterosporus* (T2), live yeast (T3), *Brevibacillus laterosporus* and live yeast (T4)

Treatments/	**T_1**	**T_2**	**T_3**	**T_4**	**SEM**
A	40.4	36.6	36.7	38.2	7.85
B	33.9	20.0	21.5	23.2	10.00
C	0.13	0.14	0.08	0.11	0.08
ED1	59.4^{a}	54.1^{b}	53.6^{b}	56.0^{ab}	4.27
ED2	54.5^{a}	51.5^{b}	49.6^{b}	52.1^{ab}	2.22
ED3	52.2^{a}	49.5^{ab}	47.1^{b}	49.8^{ab}	4.22

abc means treatments in the same row with different superscripts differ ($p < 0.05$).
A means the rapidly soluble fraction (%),
B means the potentially degradable fraction (%),
C means the constant rate of disappearance of B
ED1, ED2 and ED3: the effective degradation (at k = 0.02, 0.05 and 0.08 respectively*)*

The rapidly degradable fraction of DM (A), the potential degradable fraction (B), and the rate of disappearance of B (C) did not differ ($p>0.05$) between treatments and averaged 38.0, 24.7 and 0.11, respectively (Table 4.1). The effective DM degradability evaluated at low (0.02) and medium (0.05) ruminal passage rate (ED1 and ED2) were higher ($p<0.05$) for T_1 compared to T_2 and T_3, but did not differ significantly ($p>0.05$) between T_2, T_3 and T_4, and between T_1 and T_4. When evaluated at fast passage rate (0.08) the effective DM

degradability (ED3) was higher ($p<0.05$) in T_1 compared to T_3, but did not differ ($p>0.05$) between T_1, T_2 and T_4

Table 4.2 - The rumen degradation characteristics of crude protein in control diet (T1), diets supplemented with *Brevibacillus laterosporus* (T2), live yeast (T3), Brevibacillus laterosporus and live yeast (T4)

Treatments/	**T_1**	**T_2**	**T_3**	**T_4**	**SEM**
A	46.23^{a}	34.22^{ab}	30.34^{b}	44.63^{a}	11.78
B	26.21	31.90	34.12	25.46	6.58
C	0.29	0.10	0.16	0.12	0.13
ED1 (0.02)	68.80	60.19	59.87	59.53	9.89
ED2 (0.05)	65.15	54.80	55.62	56.41	10.18
ED3 (0.08)	62.69	51.37	52.69	54.71	9.98

abc meanstreatments in the same row with different superscripts differ ($p<0.05$).
A means the rapidly soluble fraction (%),
B means the potentially degradable fraction (%),
C means the constant rate of disappearance of B
ED1, ED2 and ED3: the effective degradation (at k = 0.02, 0.05 and 0.08 respectively)

The rapidly degradable fraction of CP (A) ranged from 30.34 for T_3 to 46.23 for T_1 and was higher for T_1 and T_4 compared to T_3 (Table 4.2). No differences in rapidly degradable fraction of CP ($p>0.05$) were observed between T_1, T_2 and T_3, also between T_3 and T_4. The potential degradable fraction (B) and the constant of disappearance of B (C) were the same ($p>0.05$) in all treatments, averaging 6.59 and 0.32 respectively. No differences ($p>0.05$) were observed for ED1, ED2 and ED3 between all the treatments, but ED2 tended ($p<0.10$) to be higher for the control compared to T_2 ($p<0.10$) and ED3 tended ($p<0.10$) to be higher for T_1 compared to T_2.

4.2 Effects of *Brevibacillus laterosporus,* live yeast and their combination *in vitro* on ammonia nitrogen and volatile fatty acids

The difference in ammonia nitrogen production was observed only between T_1 and T_2, and was higher ($p<0.05$) in T_1 than T_2 (Table 4.3). The total VFA's concentration was higher

($p<0.05$) in T_3 compared to the control (T_1). The concentration of acetate was lower ($p<0.05$) in to T_3 and T_4 compared to control. Propionate concentration was significantly higher ($p<0.05$) in T_3 and T_4 compared to other treatments and lower ($p<0.05$) in the control compared to the rest of treatments. Iso-butyrate and iso-valerate concentration was both higher ($p<0.05$) in T_1 and T_2 when compared to T_3 and T_4. Butyrate concentration was significantly higher ($p<0.05$) in T_2 and T_4 compared to the rest of the treatments, and lower ($p<0.05$) in T_3 than other treatments, and higher in T_1 compared to T_3. Valerate concentration did not differ ($p>0.05$) between treatments T_1 and T_2, where it was higher than the rest of treatments.

Table 4.3 - The in vitro rumen volatile fatty acids production in control diet (T1), diets supplemented with *Brevibacillus laterosporus* (T2), live yeast (T3), *Brevibacillus laterosporus* and live yeast (T4)

Treatments/	**T_1**	**T_2**	**T_3**	**T_4**	**SEM**
NH_3 , mg/L	5.50^{a}	4.16^{b}	4.88^{ab}	4.91^{ab}	0.72
Total VFA, mmol	105.51^{b}	112.21^{ab}	116.65^{a}	112.38^{ab}	7.17
Acetate, %	63.82^{a}	60.25^{ab}	62.29^{b}	59.58^{b}	1.69
Propionate, %	17.75^{c}	20.33^{b}	23.95^{a}	23.49^{a}	4.05
Iso-butyrate, %	1.27^{a}	1.21^{a}	0.11^{b}	0.09^{b}	0.01
Butyrate, %	12.47^{b}	13.57^{a}	10.53^{c}	13.65^{a}	1.03
Iso-valerate, %	1.52^{a}	1.62^{a}	0.86^{b}	0.81^{b}	0.01
Valerate, %	2.85^{a}	2.83^{a}	2.27^{b}	2.06^{c}	0.05

abc means treatments in the same row with different superscripts differ ($p<0.05$).

Changes in major volatile fatty acids (acetate, propionate and butyrate) as affected by additives relative to the control were evaluated and presented in Figure 4.1, 4.2 and 4.3, respectively. All additives decreased ($p<0.05$) the molar percentage of acetate (Table 4.3; Figure 4.1), with the addition of *B. laterosporus* (T_3) decreasing least, but the % decrease did not differ ($p>0.05$) between T_2 and T_4. There was an increase ($p<0.05$) in propionate with all additives compared to the control. The percentage increase ranged from 14.5 % for

T_2 to 32 % for T_3 (Figure 4.2), but did not differ ($p>0.05$) between T_3 and T_4. Treatment 2 and T_4 similarly ($p>0.5$) increased butyrate concentration while T_3 decreased it.

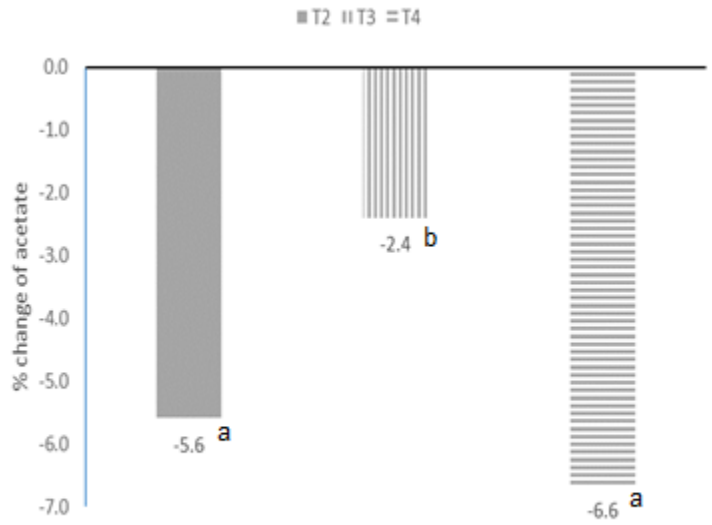

Figure 4.1 - Percentage (%) changes of acetate in total volatile fatty acid as affected by *Brevibacillus laterosporus* (T2), live yeast (T3), *Brevibacillus laterosporus* and live yeast (T4).

[ab] means treatments with different superscripts within each incubation time differ ($p<0.05$).

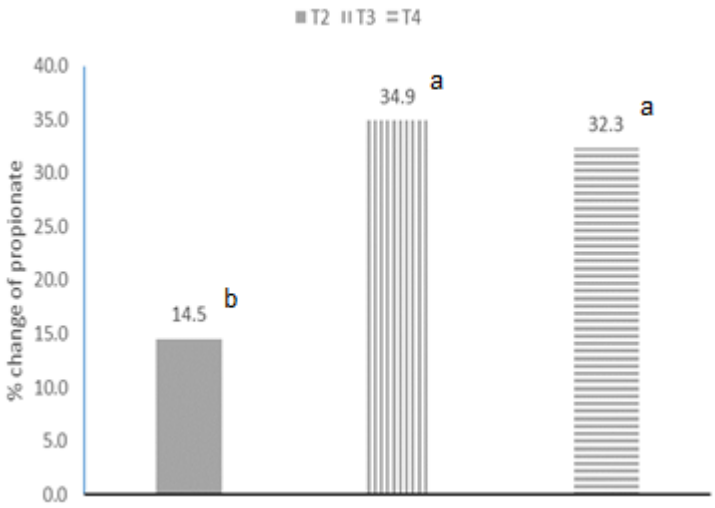

Figure 4.2 - Percentage (%) changes of propionate in total volatile fatty acid as affected by *Brevibacillus laterosporus* (T2), live yeast (T3), *Brevibacillus laterosporus* and live yeast (T4).

[ab] means treatments with different superscripts within each incubation time differ ($p<0.05$).

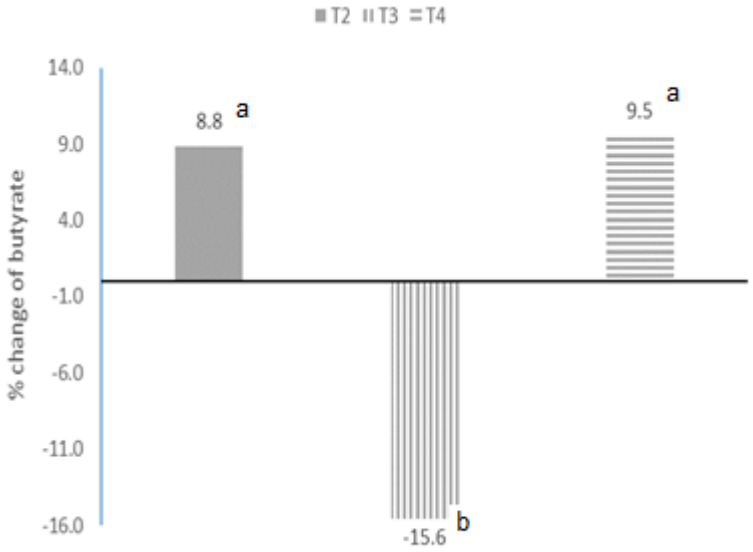

Figure 4.3 - Percentage (%) changes of butyrate in total volatile fatty acid as affected by *Brevibacillus laterosporus* (T2), live yeast (T3), *Brevibacillus laterosporus* and live yeast (T4).

[ab] means treatments with different superscripts within each incubation time differ ($p<0.05$).

4.2.1 The change in ammonia nitrogen and volatile fatty acids overtime as affected by *Brevibacillus laterosporus*, live yeast and their combination *in vitro*

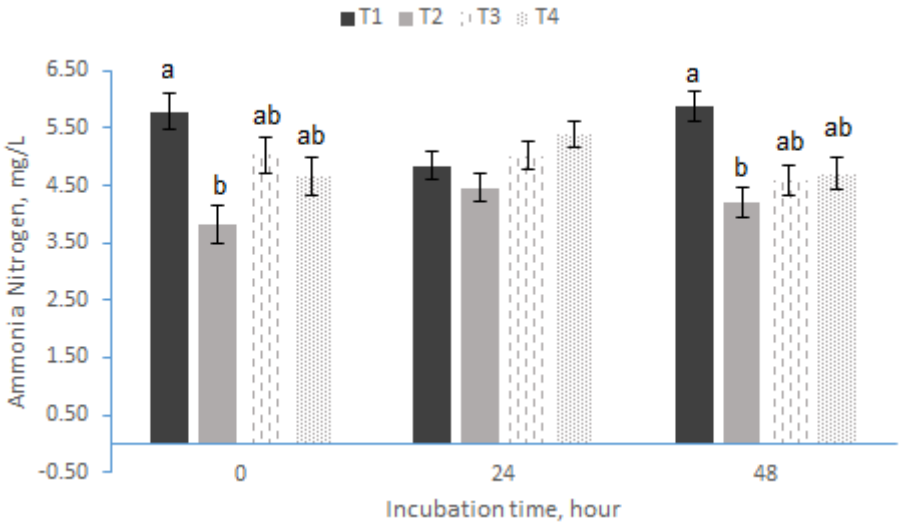

Figure 4.4 - Change in ammonia overtime as affected by no additive (T1), (control; T1), addition of *Brevibacillus laterosporus* (T2), live yeast (T3), *Brevibacillus laterosporus* and live yeast (T4). Error bar indicates the standard error of means.

[abc] means treatments with different superscripts within each incubation time differ ($p<0.05$).

At 0 and 48 h, ammonia nitrogen concentration was higher in T_1 compared to T_2, and these two treatments did not differ with the rest of treatments (Figure 4.4). At 24 h, the concentration of ammonia nitrogen did not differ between all the treatments.

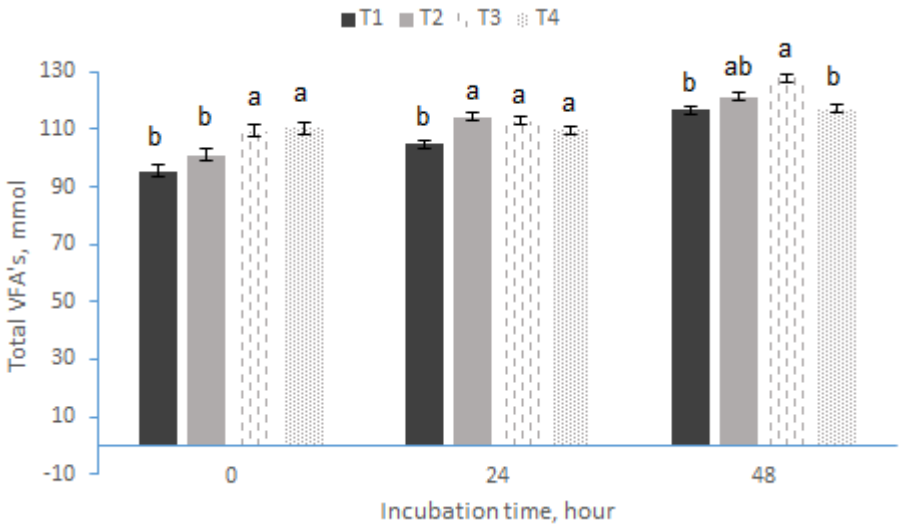

Figure 4.5 - Change in total VFAs overtime as affected by no additive (T1), (control; T1), addition of *Brevibacillus laterosporus* (T2), live yeast (T3), *Brevibacillus laterosporus* and live yeast (T4). Error bar indicates the standard error of means.
[abc] means treatments with different superscripts within each incubation time differ ($p<0.05$).

At 0 h, there was no difference in total VFA's between T_1 and T_2 and between T_3 and T_4. The T_1 and T_2 were lower than T_3 and T_4 (Figure 4.5). At 24 h T_1 was lower than T_2, T_3 and T_3. No difference was found between T_2, T_3 and T_4. At 48 h total VFA's was higher for T_3 compared to T_1 and T_4, with no differences between T_2 and T_3 and between T_1, T_2 and T_4.

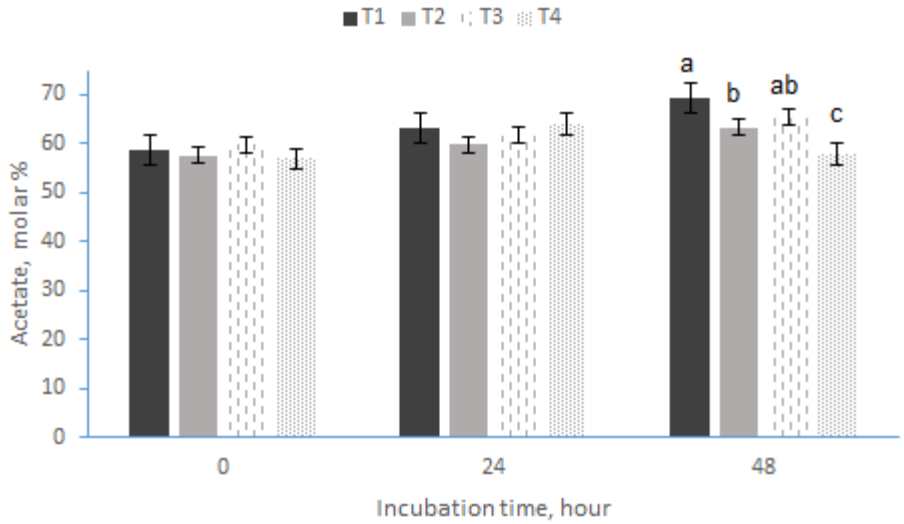

Figure 4.6 - Change in ruminal acetate overtime as affected by no additive (T1), (control; T1), addition of *Brevibacillus laterosporus* (T2), live yeast (T3), *Brevibacillus laterosporus* and live yeast (T4). Error bar indicates the standard error of means.
[abc] means treatments with different superscripts within each incubation time differ ($p<0.05$).

No differences in acetate molar proportion were observed at 0 and 24 h (Figure 4.6). At 48 h acetate was higher in T_1 compared to T_2 and T_4, but no difference was observed between T_1 and T_3 and between T_2 and T_3. At 48 h the molar proportion of acetate was the lowest in T_4 than the rest of treatments.

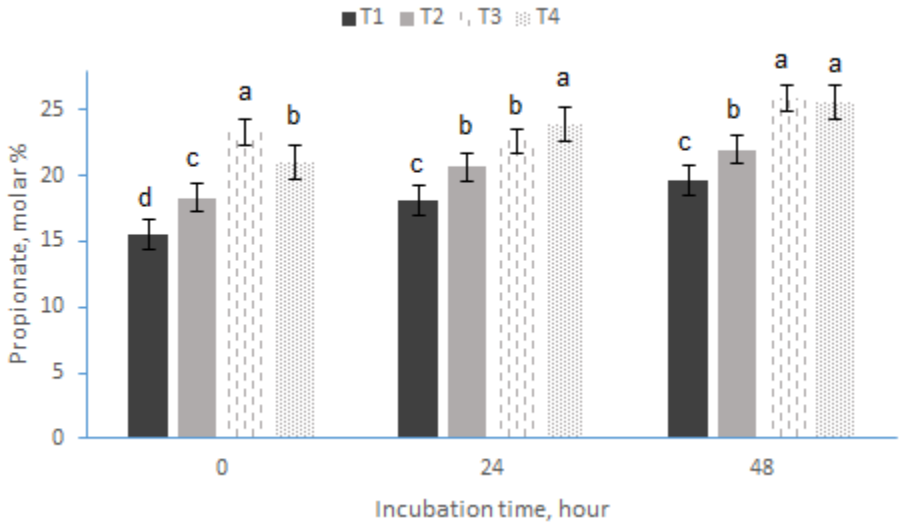

Figure 4.7 - Change in ruminal propionate overtime as affected by no additive (T1), (control; T1), addition of *Brevibacillus laterosporus* (T2), live yeast (T3), *Brevibacillus laterosporus* and live yeast (T4). Error bar indicates the standard error of means.

[abc] means treatments with different superscripts within each incubation time differ ($p<0.05$).

At 0 h, the molar proportion of propionate was higher in order of $T_3 > T_4 > T_2 > T_1$ and there was significant difference ($p<0.05$). At 24 and 48 h the proportion was higher in order for $T_4 > T_3 > T_2 > T_1$, with no significant difference ($p<0.05$) between T_2 and T_3 at 24 h. No significant difference ($p<0.05$) was observed between T_4 and T_3 at 48 h.

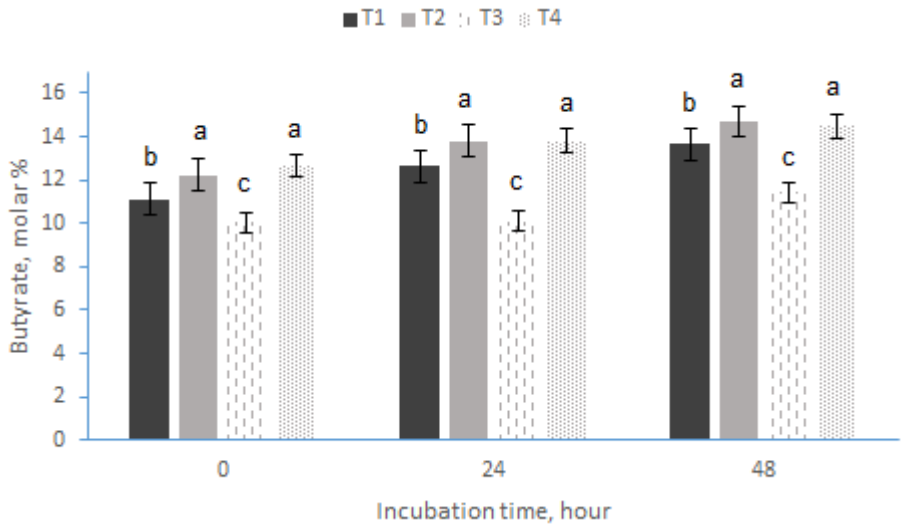

Figure 4.8 - Change in ruminal butyrate overtime as affected by no additive (T1), (control; T1), addition of *Brevibacillus laterosporus* (T2), live yeast (T3), *Brevibacillus laterosporus* and live yeast (T4). Error bar indicates the standard error of means.

[abc] means treatments with different superscripts within each incubation time differ ($p<0.05$).

At all three incubation times (0, 24 and 48 h), the molar proportions of butyrate were higher in T_2 and T_4 compared to T_1 and T_3, and lower in T_3 compared to the rest of treatment (Figure 4.8). No differences in molar proportion of butyrate were observed between T_2 and T_4.

4.3. Effects of *Brevibacillus laterosporus*, live yeast and their combination in vitro on microbial protein synthesis

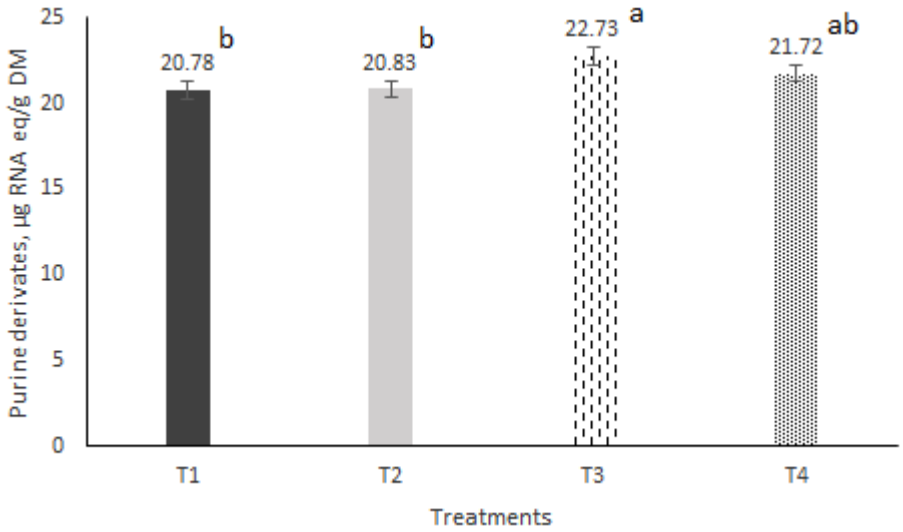

Figure 4.9 - Microbial protein synthesis measured as purine derivates content on the diet incubated without additive (control; T1), diet supplemented with *Brevibacillus laterosporus* (T2), live yeast (T3), or *Brevibacillus laterosporus* and live yeast (T4) after 48 h

[abc] means treatments with the same superscripts do not differ ($p>0.05$).

The microbial protein synthesis (MPS) measured as purine derivates done on residues was higher for T_3 compared to T_1 and T_2, but did not differ between T_1, T_2 and T_4, and between T_3 and T_4 (Figure 4.9).

CHAPTER 5

DISCUSSION

The genus bacillus has been use in recent years as probiotics for both human as emerging foods or as supplements for diet and in animal as feeds to prevent a gastrointestinal infection that was used extensively in the poultry and aquaculture industries (Hong *et al*., 2004). The mode of action of *B. laterosporus* and other bacillus is exerted through its ability to produce various antibiotics (Cotter *et al*., 2005; Heng *et al*., 2007), which inhibit a range of other bacteria. In turkey, inclusion of bacillus as direct fed microbial has prevented bacterial translocation, digesta viscosity and reduction in bone mineralization (Latore *et al*., 2014).

Bacillus species are not inhabitant of the gastrointestinal tract (Sanders *et al*., 2003) and limited studies where they were used as feed additives in dairy cows are available. Addition of bacillus species was reported to increase cellulotic digesting bacterial numbers, increase fibre digestion, enhance utilisation of lactic acid by other bacteria and stabilise rumen pH (Fuller, 1989). Jadamus *et al*. (2009) reported that *Brevibacillus cereus* germinates rapidly in broiler chicken and piglets, which condition is a prerequisite for probiotic effects. This was an indication that germination that the probiotic strain was metabolically active in the intestine.

5.1 Dry matter and crude protein degradability as affected by *Brevibacillus laterosporus,* live yeast and their combination *in vitro*

In the current study, the dietary addition of *B. laterosporus*, live yeast and their combination did not affect the rumen degradability parameters of feed dry matter (DM) (Table 4.1). However, the small numerically changes observed with all additives on these parameters induced significant decrease in the effective degradability of the DM at all rumen passage rates. Change in DM digestibility has been associated with increased number of cellulolytic bacteria (Chaucheyras-Durand and Fonty, 2001; Mosoni *et al*. 2007; Inal *et al*, 2009). The mode of action of *B. laterosporus* in the rumen is not well described and has not been much investigated. The present results suggested that *B. laterosporus* did not affect rumen CP

degradability. However, it was expected that the increase in these cellulolytic bacteria with live yeast (LY) as it has been reported in many previous studies could lead to increased DM digestibility. The observed decrease on DM digestibility is unclear and did not agree with previous studies where LY increased DM digestibility (Chaucheyras-Durand and Fonty, 2001; Mosoni *et al*. 2007; Inal *et al*, 2009). There was variability in the effectiveness of the yeast and this is influenced by the diet composition and the strain. Then its supplementation in the ruminant diet might improve digestion of fibre, cellulolytic bacteria and numbers of anaerobic (Inal *et al*. 2010). Addition of LY to the gnotobiotically reared lambs as indicated by Fonty (2001) showed that it enhanced the activities of fibre digesting enzymes which tend to improve *in situ* dry matter degradation of wheat straw and accelerated the establishment of cellulolytic bacteria in the rumen.

The CP degradability was decreased with the addition of live yeast alone (Table 4.2). This can be ascribed to the negative effect on bacteria from LY such as *Streptococcus bovis* and proteinase activities (Chaucheyras-Durand *et al*., 2005), which may decrease the rate of degradation of peptides, and increase the amount of rumen undegraded protein available. Although the tendency to decrease the effective degradability of CP observed with addition of BL is a positive response for ruminant diets, it is difficult to explain. This can be associated with observed decrease in ammonia production and is usually attributed to the ability of additive to protect protein for the action of rumen microbes.

5.2. Effects of *Brevibacillus laterosporus*, live yeast and their combination *in vitro* on ammonia nitrogen and volatile fatty acids

Only addition of *B. laterosporus* decreased ruminal ammonia nitrogen production, but this effect was not expressed when combined with live yeast, suggesting an antagonism between the two additives for the action on ammonia (Table 4.3). The opposite antagonism action was observed on VFA as it was increased with the addition of live yeast alone but not when combined with *B. laterosporus*. It is clear from these observations that when *Brevibacillus laterosporus* and live yeast were combined, the competition of one of the two additives prevented the other from expressing its effects.

Addition of live yeast alone and in combination of *Brevibacillus Laterosporus* with live yeast decreased rumen acetate in agreement with Kowalik *et al*. (2012). This result might be due to the growth inhibition in cellulolytic bacteria population, which is associated with reduced methane production (Michalet-Doreau and Moran, 2010). In contrast, supplementation with LY in the present study increased propionate. Erasmus *et al*. (2005) did not observe change in acetate when LY was added, but propionate production tended to be improved. Structural carbohydrates undergo fermentation by cellulolytic bacteria and this produces acetate while non-structural carbohydrates undergo fermentation by amylolytic bacteria which produce propionate (Inal *et al*., 2010). Generally, LY stimulate the increase of propionate at the expense of acetate (Mokatse *et al*., 2014). The decrease in acetate observed in the present of live yeast agrees with Kowalik *et al*. (2012).

Although the decrease in acetate with addition of *B. laterosporus* was only numerical, it resulted in increased propionate (Table 4.3). The effect of LY and BL on acetate was observed from only 48 h suggested the need of sufficient time for adaptation of ruminal microbial population. Rusell *et al*., (2003) reported that the increased propionate at the expense of acetate was observed when additives such as monensin was believed to be caused by their ability to inhibit gram positive bacteria favouring gram negative bacteria due to the differences in their cell membrane structures. The structure of gram negative bacteria outer membrane make them impermeable to large molecules such as ionophores and are resistant to ionophore action (Callaway et al., 1999), while gram positive bacteria are lacking the complex outer membrane, and are usually sensitive to ionophores (Rusell *et al*., 2003). Gram positive bacteria produce acetate, butyrate, lactate and ammonia and gram negative bacteria produce propionate and succinate (Bagg, 1997). Like action of ionophores, Samsop *et al*. (2014) indicated that the bacteriocin produced by *B. laterosporus* showed more effect against to gram positive bacteria than gram negative bacteria, which can explain the increased propionate in the present study.

Addition of *B. laterosporus* alone and in combination with live yeast increased butyrate while live yeast on its own decreased it (Figure 4.3). Together with the results on the

percentage change of major VFAs, these observations suggested that dietary addition of these two additives as well as their combination increase rumen production of propionate and butyrate (except live yeast for butyrate) at the expense of acetate (Figure 4.2). All additives showed their increasing effects on propionate from the beginning to the end of the incubation. The changes in favour of propionate are of great benefit for dairy cow, especially during the early lactation period. When produced, propionate replenishes oxaloacetate, the main substrate for gluconeogenesis and energy generation (Richardson *et al*., 1976). The contribution of propionate to net glucose release ranges from 50 to 60 % (Reynolds *et al*., 2003). As a result, more glucose is produced for lactose synthesis in the mammary gland, which increases milk production (Van der Werf *et al*., 1998). The increase in rumen propionate is generally associated with a decrease in methane production in the rumen, which is suggested to be responsible for 60 % of the improved energy utilization of animal fed ionophores (Wedegaertner and Johnson, 2013). As for propionate, addition of *B. laterosporus* and the combination of both live yeast and *B. laterosporus* treatments increased butyrate at all three incubation times.

5.3. Effects of *Brevibacillus laterosporus*, live yeast and their combination *in vitro* on microbial protein synthesis

The increase in microbial protein synthesis (MPS) with addition with LY discovered in this study was in agreement with previous studies (Zelenac *et al*., 1994; Thripaty and Karim, 2009) and can be explained by the decrease in CP degradability. This indicated a decrease in the rate of degradation of peptides as also reported by Alshaikh *et al*., (2002) with yeast culture. It can be related to the observed decrease in ammonia concentration in this study, which might have led to more ammonia-N incorporated into ruminal microbial proteins (Carro *et al*., 1992). There was no change in microbial nitrogen production expressed as purine derivative or in N digestion with BL because its supplementation had no effects on CP metabolism. However, when BL was combined with live yeast, it was increase. Change in MPS is associated with nitrogen digestion in the rumen (Yang *et al*., 2003). The deficiency of BL effects on MPS can be explained through the deficiency of change in CP degradability discovered in this study. The increase in MPS when LY was added was not

clear. Addition of LY in this study showed a decrease in CP degradability, which was supposed to result in a decrease of MPS because of less peptides and other products of protein degradation available to be captured by microbes to form microbial proteins. However, there are several other possible reasons for the increased MPS. One of them is the release of reducing sugars, which can likely make more nutrients available to be utilized by the microorganisms (McAllister *et al*., 2001). It also favours an increased glycocalyx production which was product of bacteria that allows adhesion in between bacteria and substrate (Bala *et al*. 2009).

CHAPTER 6

CONCLUSION

The results from this study indicated that *B. laterosporus* does not affect rumen CP degradability. Despite the known ability of LY to increase cellulolytic bacteria, addition of LY decreased DM digestibility which is difficult to explain, but increased CP degradability. Both *B. laterosporus* and live yeast changed the pattern of rumen fermentation, reducing acetate and increasing propionate, which is a benefit for ruminants. The effects of the two additives (*B. laterosporus* and live yeast) on acetate were observed from only at 48 h suggesting sufficient time-frame for adaptation of ruminal microbial population. Addition of LY increased MPS, which was associated with the decrease in CP degradability, indicated a decrease in the rate of degradation of peptides. More investigations are needed with different diets to confirm these effects and improve the knowledge on the mode of action of BL as additive for ruminants.

REFERENCES

Abu-Tarboush H. M., Al-Saiady M. Y. and Keir El-Din A. H. (1996). Evaluation of Diet Containing Lactobacillus on Performance, Faecal Coliform and Lactobacillus of Young Dairy Calves. Anim. Feed Sci. Technol. 57:39-49.

Adams D. C., Galyean M. L., Kiesling H. E., Wallace J. D., and Finker M. D. (1981). Influence of Viable Yeast Culture, Sodium Bicarbonate and Monensin on Liquid Dilution Rate, Rumen Fermentation and Feedlot Performance of Growing Steers and Digestibility in Lambs. J. Anim. Sci. 53:780-789.

Adegunloye D. V., Adetuyi, F. C., Akinyosoye F. A., Doyeni M. O. (2007). Microbial analysis of Compost using Cowdung as Booster. Pak. J. Nutr. 6:506-510.

Agricultural Research Council (1984). The Nutrient Requirements of Ruminant Livestock. Supplement No. 1. Commonwealth Agricultural Bureaux, Wallingford, UK.

Aguilar A. (2013).Yeast Products in Feed: What, Why, where and When? Published on 10 April 2013. http://www.progressivedairy.com/topics/feed-nutrition/yeast-products-in-feed-what-why-where-and-when. Accessed on 25 June 2015.

Belanche A.; Doreau M., Edwards J. E., Moorby J. M., Pinloche E. and Newbold C.J. (2012). Shifts in the Rumen Microbiota Due to the Type of Carbohydrate and Level of Protein Ingested by Dairy Cattle Are Associated with Changes in Rumen Fermentation. J. Nutr. 142(9):1684-1692.

Allen M. S. (2000). Effects of Diet on Short-Term Regulation of Feed Intake by Lactating Dairy Cattle. J. Dairy Sci. 83(7):1598-1624.

Allison, M. J. (1969). Biosynthesis of Amino Acids by Ruminal Microorganisms. J. Anim. Sci. 29:797-807.

Alshaikh M. A., Alsiadi M. Y., Zahran S. M., Mogawer H. H., and Aalshowime T. A. (2002.) Effect of Feeding Yeast Culture from different Sources on the Performance

of Lactating Holstein Cow in Saudi Arabia. Asian-Australas. J. Anim. Sci. 15:352–356.

Andrighetto I., Bailoni L., Cozzi G., and Berzaghi P. (1993). Effect of Yeast Culture Addition on Digestion in Sheep Fed a High Concentrate Diet. Small Rum. Res. 12:27-34.

Angeles C, S. C., Mendoza M, G. D., Cobos P, M. A., Crosby G, M. M., & Castrejón P, F. A. (1998). Comparison of two commercial yeast cultures (Saccharomyces cerevisiae) on ruminal fermentation and digestion in sheep fed on corn-stover diet. Small Rum. Res. 31(1):45-50.

AOAC International. (1995). Official Methods of Analysis of AOAC International. Vols. 16th Edition. Arlington, VA, USA.

AOAC. (2000). Official Methods of Analysis. 17th Edition. Washington: Association of Analytical Chemists. Arlington, VA, USA.

Argyle, J. L., and Baldwin R. L. (1989). Effects of Amino Acids and Peptides on Rumen Microbial Growth Yields. J. Dairy Sci. 72(8):2017-2027.

Bach A., Calsamiglia S., and Stern M. D. (2005). Nitrogen Metabolism in the Rumen. J. Dairy Sci. 88: E9-21E.

Bach A., Iglesias C., Devant M. (2007). Daily Rumen pH Pattern of Loose Housed Dairy Cattle as Affected by Feeding Pattern and Live Yeast Supplementation. Anim. Feed Sci. Techno. 136:146-153.

Bagg R., (1997). Mode of Action of Ionophores in Lactating Dairy Cattle. In: Proceedings of the Symposium on Usefulness of Ionophores in Lactating Dairy Cattle. Ontario Vetenary College, Guelph, Canada, pp. 13–21.

Bagherzadeh Kasmani F., Karimi Torshizi M. A., Allameh A., Shariatmadari F. A. (2012). Novel Aflatoxin-Binding Bacillus Probiotic: Performance, Serum Biochemistry, and Immunological Parameters in Japanese Quail. Poult. Sci., 91:1846-1853.

Bala P., Malik R., and Srinivas B. (2009). Effect of Fortifying Concentrate Supplement with Fibrolytic Enzymes on Nutrient Utilization, Milk Yield and Composition in Lactating Goats. J. Anim. Sci. 80:265-272.

Barrow P. A. (2000). The Paratyphoid Salmonellae Diseases of Poultry: World Trade and Public Health Implications. Scientific and Technical Revue. Office International Épizooties.1Sci. Tech. Rev. 9(2):351-375.

Bauman, D. E., Baumgard, L. H., Corl, B. A., & Griinari, D. J. (2000). Biosynthesis of conjugated linoleic acid in ruminants. J. Anim. Sci. 77(E-Suppl): 1-15.

Beev G., Todorov, P., and Tchobanova S. (2007). Yeast cultures in ruminant nutrition. Bulg. J. Agric. Sci. 13:357-374.

Brock F. M., Forsberg C. W., and Buchanan-Smith J. G. (1982). Proteolytic Activity of Rumen Microorganisms and Effects of Proteinase Inhibitors. Appl. Environ. Microbiol. 44:561– 569.

Broderick G. A., Kang J. H. (1980). Automated Simultaneous Determination of Ammonia and Total Amino Acids in Ruminal Fluid and *in vitro* Media. J. Dairy Sci. 63(1):64-75.

Butaye P., Devriese L. A., Haesebrouck F. (2003). Antimicrobial Growth Promoters Used in Animal Feed: Effects of Less Well Known Antibiotics on Gram-Positive Bacteria. Clin. Microbiol. Rev. 16(2): 175–188.

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

Callaway T. R., Adams K. A. and Russell J. B. (1999). The Ability of Low G+C Gram-Positive' Ruminal Bacteria to Resist Monensin and Counteract Potassium Depletion. Curr. Microbiol. 39: 225-230.

Carro M. D., Lebzien P., Rohr K. (1992). Influence of Yeast Culture on the *in vitro* Fermentation (Rusitec) of Diets Containing Variable Portions of Concentrates. Anim Feed Sci Technol. 37: 209-220.

Chaucheryras F., Fonty G., Bertin G., and Gouet P. **(**1995). *In vitro* Utilization by a Ruminal Acetogenic Bacterium Cultivated Alone or in Association with an Archea methanogen is Stimulated by a Probiotic Strain of *Saccharomyces cerevisiae.* Appl. Environ. Microbiol., 61: 3466-3467.

Chaucheyras-Durand F., Fonty G. (2001). Establishment of Cellulolytic Bacteria and Development of Fermentative Activities in the Rumen of Gnotobiotically-Reared Lambs Receiving the Microbial Additive Saccharomyces cerevisiae CNCM I-1077. Reprod. Nutr. Dev. 41:57-68.

Chaucheyras-Durand, F., Masseglia, S. and Fonty, G. 2005. Effect of the microbial feed additive *Saccharomyces cerevisiae* CNCM I-1077 on protein and peptide degrading activities of rumen bacteria grown *in vitro*. Curr. Microbiol. 50:96-101.

Chaucheyras-Durand F., Walker N. D., Bach A. (2008). Effects of Active Dry Yeasts on the Rumen Microbial Ecosystem: Past, Present and Future. Anim. Feed Sci. Tech. 145:5-26.

Chen Y., Gao, H., Zhang Y., Deng M., Wu Z., Zhu L., Duan Q., Xu B., Liang C., Yue Z., Xiao X. (2012). Analysis of the Bacterial Diversity Existing on Animal Hide and Wool: Development of a Preliminary PCR-restriction Fragment Length Polymorphism Fingerprint Database for Identifying Isolates. J. AOAC Int. 95:1750-1754.

Chiedza I. M., Sangbuem C., David T. M., Hong-gu L., Nag-Jin C. (2014). Effect of Encapsulating Nitrate in Sesame Gum on i*n vitro* Rumen Fermentation Parameters. Asian-Australas. J. Anim. Sci. 27(11):1577-1583.

Clark, J. H., Klusmeyer T. H., and Cameron M. R. (1992). Microbial Protein Synthesis and Flows of Nitrogen Fractions to the Duodenum of Dairy Cows. J. Dairy Sci. 75:2304–2323.

Cotter, P.D., Hill, C., and Ross, R.P. (2005). Bacteriocins: Developing Innate Immunity for Food. Nat. Rev. Microbiol 3:777–788.

Craig W. M., Broderick G. A., and Ricker D. B. (1987). Quantitation of Microorganisms Associated with the Particulate Phase of Ruminal Ingesta. J. Nutr. 117(1):56-62.

Cyriac J. (2009). Lowering Ruminally Degradable Protein in Lactating Dairy Cow Diets. PhD thesis, Virginia Polytechnic Institute and State University, Blacksburg, USA.

Daryl Maulfair, Jud Heinrichs, and Virginia Ishler. (2011). Feed Efficiency for Lactating Dairy Cows and Its Relationship to Income Over Feed Costs. Department of Dairy and Animal Science, Bob VanSaun, Penn State and Rebecca White, Penn State Reviewers Extension.psu.edu.

Dawson K. A. **(**2002). Manipulating Rumen Microbial Population to Improve Animal Productivity. Proceedings Intermountain Nutrition Conference „Animal Nutrition, Health and Profit", Utah State University, USA, pp 1-22.

Dawson K. A. and Tricarico J. 2002. The Evolution of Yeast Cultures-20 Years of Research. In: Navigating from Niche Markets to Mainstream. Proceedings of Alltech's European, Middle Eastern and African Lecture Tour, pp 26-43.

Dawson K. A., Neuman K. E., and Boling J. A. (1990). Effects of Microbial Supplements Containing Yeast and *Lactobacilli* on Roughage-Fed Ruminal Microbial Activities. J. Anim. Sci. 68 (10):3392-3398.

De Ondarza M. B. (2000). The Stomach of the Dairy cow. Milkproduction.com. http://www.milkproduction.com/Library/Scientific-articles/Animal-health/The-stomach-of-the-dairy-cow/ accessed on 23 June 2015.

De Ondarza M. B., Sniffen C. J., Dussert L., Chevaux E., Sullivan J., and Walker N. D. (2012). Multiple-Study Analysis of the Effect of Live Yeast on Milk Yield, Milk Component Content and Yield, and Feed Efficiency. Prof. Anim. Sci. 26:661-666.

Demeyer D. I. and Fievez V. (2000). Ruminants et Environnement: la Me´thanogene` se (Ruminants and Environment: Methanogenesis). Ann. Zootech. 49:95–112.

Denev S. A., Peeva T., Radulova P., Stancheva N., Staykova G., Beev G., Todorova P., and Tchobanova S. (2007). Yeast Cultures in Ruminant Nutrition. Bulg. J. Agric. Sci. 13: 357-374.

Denev S. A., Suzuki I., Kimoto H. **(**2000). Role of Lactobacilli in Human and Animal Health. Anim. Sci. J. 71(6): 549-562.

Denev S.A**.** (1996). Probiotics-Past, Present and Future. Bulg. J. Agric. Sci. 2:445-474.

Denev S.A. **(**2006). Role of *Lactobacilli* in Gastrointestinal Ecosystem. Bulg. J. Agric. Sci. 12(1):63-114.

Dierick N. A., Vervaecke I. J., Decupypere J. A., and Henderickx H. K. (1986). Influence of The Gut Flora and Some Growth Promoting Feed Additives on Nitrogen Metabolism in Pigs. I. Studies *In vivo*. Livest. Prod. Sci. 14: 161-176.

Ding J., Zhou Z. M., Ren L. P., and Meng Q. X. (2005). Effect of Monensin and Live Yeast Supplementation on Growth Performance, Nutrient Digestibility, Carcass Characteristics and Ruminal Fermentation Parameters in Lambs Fed Steam-flaked Corn-based Diets. State Key Laboratory of Animal Nutrition, College of Animal Science and Technology China Agricultural University, Beijing 100094, China.

Djouvinov D. S. and Todorov, N. A. (1994). Influence of dry matter intake and passage rate on microbial protein synthesis in the rumen of sheep and its estimation by cannulation and a non-invasive method. Anim. Feed Sci. Technol. 48(3-4): 289–304

Doreau M., Jouany J. **(**1998). Effect of a *Saccharomyces cerevisiae* on Nutrient Digestion in Lactating Dairy Cows. J. Dairy Sci. 81:214–3221.

Dutta T. K., Kundu S. S., and Sharma D. D. (2001). Potential of Probiotic Supplementation on *in vitro* Rumen Fermentation and 35S Incorporation in Microbial Protein. Indian J. Anim. Nutr. 18:227-234.

Eager H. A. (2008). A Survey of Antimicrobial Usage in Animals in South Africa with Specific Reference to Food Animals. MVSc Thesis, University of Pretoria, Pretoria, South Africa.

Edwards I. E. **(**1991). Practical Uses of Yeast Culture in Beef Production: Insight into its Mode of Action. In: Biotechnology in the Feed Industry, Vol.VI. Alltech Technical Publications, Nicholasville, KY. 45-49.

El Hassan S. M., Newbold C. J., Edwards I. E., Topps J. H. and Wallace R. J. (1996). Effect of Yeast Culture on Rumen Fermentation, Microbial Protein Flow from the Rumen and Live-weight Gain in Bulls given High Cereal Diets. Anim. Sci. 62:(1):43- 48.

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

Erasmus L. J., Robinson P. H., Ahmadi A., Hinders R., Garret J. E. (2005). Influence or Pre-partum and Post-partum Supplementation with Live Yeast and Monensin or both on Ruminal Fermentation and Performance of Multiparous Dairy Cows. Anim. Feed Sci. Techn. 122:219-239.

Fallon R. J., and Harte F. J. (1987). The Effect of Yeast Culture Inclusion in the Concentrate Diet on Calf Performances. J. Dairy Sci. 70 (Supp. 1) 126 (Abstr.).

Fangio M. F., Roura S. I., Fritz R. (2010). Isolation and Identification of Bacillus spp. and Related Genera from Different Starchy Foods. J. Food Sci. 75:M218-M221.

FAO. (2016). Dairy Production and Products. http://www.fao.org/agriculture/dairy-gateway/milk-production/farm-practices/en/#.VsbseOYYODQ. Accessed on 14 July 2017.

Feng P., Hunt C. W., Pritchard G. T and Julien W. E. (1996). Effect of Enzyme Preparations on In Situ and In vitro Degradation and *In vitro* Digestive Characteristics of Mature Cool-Season Grass Forage in Beef Steers. J. Anim. Sci. 74:1349–1357.

Fessenden S. W. (2013). Effects of Bismuth Subsalicylate and Beta Extract of Hops (*Humulus lupulus*) on *In vitro* Fermentation with Ruminal Microbes. MSc thesis. University of Minnesota, USA.

Firkins J. L. (1996). Maximizing Microbial Protein Synthesis in the Rumen. J. Nutr. 126:S1347–54.

Firkins J. L., Berger L. L., Merchen N. R., Fahey G. C. Jr., Nelson D. R. (1986). Effects of Feed Intake and Protein Degradability on Ruminal Characteristics and Site of Digestion in Steers. J. Dairy Sci. 69(8):2111-2123.

Fonty G., Chaucheyras Durand F. (2006). Effects and Modes of Action of Live Yeasts in the Rumen. Biologia 61 (6) 741-750.

Fuller R. (1989). A Review: Probiotics in Man and Animals. J. Appl. Bacteriol. 66:365-378.

Galyean M. L., (1997). Laboratory Procedures in Animal Nutrition Research, 12th ed. West Texas A and M University, Division of Agriculture and Texas A and M Research and Extension Center, Amarillo, TX, USA.

Gardner R. W. and Park R. L. (1973). Protein Requirements of Cows Fed High Concentrate Rations. J. Dairy Sci. 56(3):390-394.

Girard I. D., **(**1996). Charactetization of Stimulatory Activities from Saccharomyces cerevisiae on the Growth and Activities of Ruminal Bacteria. Ph.D. Dissertation. University of Kentucky, Lexington, USA

Girard I. D. **(**1997). Characterization of Stimulatory Activities of *Saccharomyces cerevisiae 1026* on the Growth and Metabolism of Ruminal Bacteria. In: Alltech's 13th Annual Symposium Biotechnology in the Feed Industry", Lexington, Kentucky, USA, pp. 45.

Girard I. D. and Dawson K. A., **(**1994). Effects of Yeast Culture on the Growth of Representative Ruminal Bacteria. J. Anim. Sci., **77** (Suppl. l): 300.

Girard I. D. and Dawson K. A., **(**1995). Stimulatory Activities from Low-molecular Weight Fractions Derived from *Saccharomyces cerevisiae* Strain1026.23rd Biennial Conference on Rumen Function, Chicago, Illinois, pp. 23.

Goering H. K. and Van Soest P. J. (1988). Forage Fiber Analyses (Apparatus, reagents, procedures and some applications). Agriculture Handbook No. 379. A.R.S., USA.

Gomes M. J., Hovell F. D., Chen X. B., Nengomasha E.M. and Firkemariam D. (1994). The effect of starch supplementation of straw on microbial protein supply in sheep Anim. Feed Sci. Technol. 49:277-286.

Graham J. P., Boland J. J., Silbergeld, E. (2007). Growth Promoting Antibiotics in Food Animal Production: An Economic Analysis. Public Health Rep. 122(1):79–87.

Green B. L., McBride B. W., Sandals D., Leslie K. E., Bagg R., Dick P. (1999). The Impact of a Monensin Controlled Released Capsule on Subclinical Ketosis in the Transition Dairy Cow. J. Dairy Sci. 82:333–342.

Guedes C. M., Gonçalves D., Rodrigues M. A. M., Dias-da-Silva A. (2008). Effects of a *Saccharomyces cerevisiae* Yeast on Ruminal Fermentation and Fibre Degradation of Maize Silages in Cows. Anim. Feed Sci. Tech. 145:27-40.

Harper G. C., Makatouni A. (2002). Consumer Perception of Organic Food Production and Farm Animal Welfare. Brit. Food J. 104(3/4/5):287-299.

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

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

Heng N. C., Tagg J. R., and Tompkins G. R. (2007). Competence-dependent Bacteriocin Production by *Streptococcus gordonii* DL1 (Challis). J Bacteriol 189:1468–1472.

Herdt T. H. (2000). Ruminant Adaptation to Negative Energy Balance. Vet. Clin. North Am. Food Anim. Pract. 16:215–230.

Henton M. M., Eagar H. A., Swan G. E., van Vuuren M. (2011). Part VI. Antibiotic Management and Resistance in Livestock Production. S. Afr. Med. J. 101(8):583-586.

Hoffman Karen. (2012). Managing Dairy Nutrition for the Organic Herd: Assessing the Feeding Program Organic. USDA Natural Resources Conservation Service.

Hofirek B., Haas D. (2001). Comparative Studies of Ruminal Fluid Collected by Oral Tube or by Puncture of the Caudoventral Ruminal Sac. Acta Vet. Brno 70:27-33.

Holden L. A., (1999). Comparison of Methods of *In vitro* Dry Matter Digestibility for Ten Feeds. J. Dairy Sci. 82:1791–1794.

Hong H. A., Duc L. H., and Cutting S. M. (2005). The Use of Bacterial Spore Formers as Probiotics. FEMS Microbiol. Rev. 29(4):813-835.

Hoover W. H. and Stokes, S. R. (1991). Balancing carbohydrates and proteins for optimum rumen microbial yield. J. Dairy Sci.74:3630-3645.

Hruby M., Cowieson A. J. (2006). The Role of Enzymes and Betaine in Antibiotic Growth Promoter Free Nutrition. Antimicrobial Growth Promoters: Where Do We Go from Here?, D. Barug, J. de Long, A. K. Kies, and M. W. A. Verstegen, ed. Wageningen Acad. Publ., Wageningen, the Netherlands. pp 269 – 287.

Huber, J. T. (1997). Probiotics in cattle. In Probiotics 2 (pp. 162-186). Springer Netherlands.

Hutchenson D. P., Cole N. A., Keaton W., Graham G., Dunlap R., and Pitman K. **(**1980). The Use of Living, Non-freeze Dried *Lactobacillus acidophilus* Culture for Receiving Feedlot Calves. Proc. West. Sec. Amer. Soc. Anim. Sci. 31: 213-215.

Hutjens M. F. (1991). Feed Additives. Vet. Clin. North Am. Food. Anim. Pract. 7(2):525-540.

Hutjens, M. (2005). Feed Efficiency and its Impact on Large Herds. Proc. Southwest Nutr. Conf. pp. 186-191.

Inal F., Gurbuz E., Coskun B., Alatas M. S., Çitil O. B., Polat E. S., Şeker E., Özcan C. (2010). The Effects of Live Yeast Culture (*Saccharomyces cerevisiae*) on Rumen Fermentation and Nutrient Degradability in Yearling Lambs. Kafkas Univ Vet Fak Derg. 16: 799-804.

Iurlina M. O., Fritz R. (2005). Characterization of Microorganisms in Argentinean Honeys from Different Sources. Int. J. Food Microbiol. 105:297-304.

Jadamus A., Vahjen W. and Simon O. (2001). Growth Behavior of a Spore Forming Probiotic Strain in the Gastrointestinal Tract of Broiler Chickens and Piglets. Arch Anim Nutr 54:1–17.

Janssen P. H. (2010). Review: Influence of Hydrogen on Rumen Methane Formation and Fermentation Balances Through Microbial Growth Kinetics and Fermentation Thermodynamics. Anim. Feed Sci. Technol. 160:1–22.

Jaquette, R. D., Dennis R. J., Coalson J. A., Ware D. R., Manfredi E. T., and Read P. L. (1988). Effect of Feeding Viable *Lactobacillus acidophilus* (BT1386) on the Performance of Lactating Dairy Cows. J. Dairy Sci. 1 **(**Suppl. 1): 219.

Jenny B. F., Vandijk H. J., and Collins J. A. **(**1991). Performance and Fecal Flora of Calves Fed a *Bacillus subtilis* Concentrate. J. Dairy Sci. **7**4:1968-1973.

Ji S. H., Paul N. C., Deng J. X., Kim Y. S., Yun B.-S., Yu S. H. (2013). Biocontrol Activity of *Bacillus* amyloliquefaciens CNU114001 against Fungal Plant Diseases. Mycobiol. 41(4):234–242.

Joerger R. D. (2003). Alternatives to Antibiotics: Bacteriocins, Antimicrobial Peptides and Bacteriophages. Poult. Sci. 82:640-647.

Jordan E. R. and Fourdraine R. H. (1993). Characterization of the Management Practices of the Top Milk Producing Herds in the Country. J. Dairy Sci. 76:3247-3256.

Jouany J. P. (1991). Rumen Microbial Metabolism and Ruminant Digestion. Institut Nationa de la Recherche Agronomique, Paris.

Jouany J. P. **(**2001). 20 Years of Research and Now More Relevant than Ever- the Coming of Age of Yeast Cultures in Ruminant Diets. In: Responding to a Changing Agricultural Landscape. Alltech's European, Middle Eastern and African Lecture Tour, pp. 44 - 69.

Jouany J. P., Morgavi D. P. (2007). Use of 'Natural' Products as Alternatives to Antibiotic Feed Additives in Ruminant Production. Animal 1:1443-1466.

Joy A. D., Samuel J. J. (1997). Effect of Probiotic Supplementation on the Performance of Broilers. J Vet Anim Sci 28:10–14.

Kalscheur K. F., Baldwin R. L., Glenn B. P., and Kohn R. A. (2006). Milk Production of Dairy Cows Fed Differing Concentrations of Rumen-degraded Protein. J. Dairy Sci. 89(1): 249-259.

Kaufman W., Hagemeister H., and Durksen G. (1980). Adaptation to changes in dietary composition level and frequency of feeding. In: Digestive Physiology and Metabolism in Ruminants, ed. Y. Ruckebusch and P. Thivend. Westport, Ct.: AVI Publishing. pp. 587- 602.

Kellems, R. O. (2016). Optimizing Dairy Feeding Programmes. ftp://193.43.36.92/upload/Agrippa/519_en.doc. Accessed on 2 June 2015.

Kirsty K. (2014). Probiotics and enzymes: A good combination http://www.allaboutfeed.net/Nutrition/Feed-Additives/. Access on 14 June 2015

Kleinkauf H., Von Döhren H. (1990). Nonribosomal Biosynthesis of Peptide Antibiotics. Euro. J. Biochem: 192(1):1–15.

Klusmeyer, T. H., McCarthy R. D., Jr., Clark J. H., and Nelson D. R. (1990). Effects of Source and Amount of Protein on Ruminal Fermentation and Passage of Nutrients to the Small Intestine of Lactating Cows. J. Dairy Sci. 73(12):3526-3537.

Kononoff P. J. (2016). http://www.thedairysite.com/articles/703/nutritional-management-of-the-highproducing-dairy-cow-in-the-21st-century. Accessed on 5 May 2015

Kopecny J., Simunek J., Kalacnjuk G. I., Savka O. G., Gerasimiv M. G., and Leskovic B. (1989). Testing the Probiotic Effect of Selected Rumen Bacteria. Zivocisna Vyroba. 34:205-214.

Kowalik B., Skomiał J., Pająk J. J., Taciak M., Majewska M., Bełżecki G. (2012). Population of Ciliates, Rumen Fermentation Indicators and Biochemical Parameters of Blood Serum in Heifers Fed Diets Supplemented with Yeast (*Saccharomyces cerevisiae*) Preparation. Anim. Sci. Pap. Rep. 30(4):329-338.

Kumar U., Sareen U. K., and Singh S. (1994). Effect of *Saccharomyces cervisiae* Yeast Culture Supplement on Ruminal Metabolism in Buffalo Calves Given a High Concentrate Diet. Anim. Prod. 59:209-215.

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

Landers T. F., Cohen B., Wittum T. E., Larson E. L. (2012). A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential. Public Health Rep. 127(1):4–22.

Lapierre, H., and Lobley G. E. (2001). Nitrogen Recycling in the Ruminant: A Review. J. Dairy Sci. 84:E223-E236.

Latorre J. D., Hernandez-Velasco X., Kogut M. H., Vicente J. L., Wolfenden R., Wolfenden A., and Tellez G. (2014). Role of a *Bacillus subtilis* Direct-Fed Microbial on Digesta Viscosity, Bacterial Translocation, and Bone Mineralization in Turkey Poults Fed with a Rye-based Diet. Front. Vet. Sci. 1:26 . doi:10.3389/ fvets.2014.00026

Laubach A. C. (1916). Studies on Aerobic, Sporebearing, Non Pathogenic Bacteria. Spore Bearing Organism in Water. J. Bacteriol. 1:505-512.

Lee Rinehart. (2008). Ruminant Nutrition for Graziers. NCAT Agriculture Specialist. Paul Driscoll, Editor, Amy Smith, Production, IP318 ,Slot 52, Version 030308. www.attra.ncat.org/attra-pub/PDF/ruminant.pdf.

Lila Z. A., Mohammed N., Yasui T., Kurokawa Y., Kanda S., and Itabashi H. **(**2004). Effects of Twin Strain of *Saccharomyces cerevisiae* Live Cells on Mixed Ruminal Microorganism Fermentation in vitro. J. Anim. Sci. 82: 1847–1854.

Lozano M. C, Trujillo M. (2012). Chemical Residues in Animal Food Products: An Issue of Public Health. In J. Maddock (Ed.), Public Health - Methodology, Environmental and Systems Issues, InTech DOI:10.5772/2678. pp. 163-188.

Lund A. (1974). Yeast and Moulds in the Bovine Rumen. J. Gen. Microbiol. 81: 453-462.

Lynch H.A., and Martin S. A. (2002). Effects of *Saccharomyces* Culture and *Saccharomyces cerevisiae* Live Cells on In vitro Mixed Ruminal Microoganism Fermentation. J. Dairy Sci. 85(10):2603-2608.

Manickam R., Viswanathan K., Mohan M. (1994). Effect of Probiotics in Broiler Performance. Indian Vet. J. 71:737–739.

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

Maron D. F., Smith T. J. S., Nachman K. E. (2013). Restrictions on Antimicrobial Use in Food Animalproduction: An international Regulatory and Economic Survey. Globalization and Health. http://www.globalizationandhealth.com/content/9/1/48. Accessed on 15 May 2015

Marshall B. M., Levy S. B. (2011). Food Animals and Antimicrobials: Impacts on Human Health. Clin. Microbiol. Rev. 24(4):718–733.

McAllister, T.A., Hristov, A.N., Beauchemin, K.A., Rode, L.M. and Cheng, K-J., 2001. Enzymes in ruminant diets. In: Enzymes in Farm Animal Nutrition. Eds. Bedford, M.R. and Partridge, G.G., CAB Inter. pp. 273-298.

Mellor S. (2000). Nutraceuticals–alternatives to antibiotics. Wrld Poult. 16(2):30-33.

Metwally A. and Windisch W. (2015). Effect of Inactivated Yeast on Rumen Dry Matter Degradation and Fermentation of Low Concentrate Feed. Afr. J. of Agric. Res. 10(53):4888-4895.

Michalet-Doreau, B., and Morand D. (1996). Effect of Yeast Culture, *Saccharomyces cerevisiae*, on Ruminal Fermentation during Adaptation to High Concentrate Feeding. Ann. Zootech. 45:337-350.

Michalet-Doreau B., Morand D., Martin C. (1997). Effect of the Microbial Additive Levucell SC CNCM I-1077 on Microbial Activity in the Rumen during Stepwise Adaptation of Sheep to High Concentrate Diet. Reprod. Nutri. Dev. 88:82-85.

Miles R. D. and Bootwalla S. M. (1991). Direct Fed Microbials in Animal Production. In: Direct Fed Microbials in Animal Production. Natl. Feed Ingred. Assoc., West Des Moines, IA.

Minson, Dennis J. (1990). Forage in Ruminant Nutrition. Academic Press, Inc., NY.

Mokatse B., Muya M. C., Acheampong-Boateng O., and Nherera F.V. *In vitro* Rumen Fermentation and Gas Production as Affected by Addition of Live Yeast and

Monensin in Low and High Concentrate Diets. Proc 47th Conf. South African Society of Animal Science, 6-8 July 2014, University of Pretoria, Pretoria.

Moloney A. P., Drennan M. J., (1994). The Influence of the Basal Diet on the Effects of Yeast Culture on Ruminal Fermentation and Digestibility in Steers. Anim. Feed Sci. Tech. 50:55-73.

Mosoni P., Chaucheyras-Durand F., Bera-Maillet C., Forano E. (2007). Quantification by Real-Time PCR of Cellulolytic Bacteria in the Rumen of Sheep after Supplementation of a Forage Diet with Readily Fermentable Carbohydrates: Effect of a Yeast Additive. J. Appl. Microbiol. 103:2676-2685.

Moss A. R., Jouany J. P. and Newbold J. (2000). Methane Production by Ruminants: Its Contribution to Global Warming. Ann Zootech 49:231–253.

Moyane J. N., Jideani A. I. O., Aiyegoro O. A. (2013). Antibiotics Usage in Food-Producing Animals in South Africa and Impact on Human: Antibiotic Resistance. Afri. J. Microbiol. Res. 7(24):2990-2997.

Mutsvangwa T., Edwards I. E., Topps J. H., and Paterson G. F. M. (1992). The Effect of Dietary Inclusion of Yeast Culture (Yea-Sac) on Pattern of Rumen Fermentation, Food Intake and Growth of Intensively Fed Bulls. Anim. Prod. 55:35-40.

National Research Council (1989). Nutrient Requirements of Dairy Cattle, Sixth ed. Natl. Acad. Sci., Washington, DC, USA.

National Research Council. (2001). Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Newbold C. J. and Wallace R. J. **(**1992). The Effect of Yeast and Distillery By-Products on the Fermentation in the Rumen Simulation Technique. Anim. Prod., 54: 504. Abstract.

Newbold C. J., Brock R., and Wallace R. J. (1991). Influence of Autoclaved or Irradiated Aspergillus oryzae Fermentation Extract on Fermentation in the Rumen Simulation Technique (Rusitec). J. Agric. Sci. Camb. 116:159-162.

Newbold C. J., Wallace R. J. and McIntosh F. M. **(**1996). Mode of Action of the Yeast *Saccharomyces cerevisiae* as a Feed Additive for Ruminants. Brit. J. Nutr. 76:249-261.

Newbold C. J., Wallace R. J., Chen X. B., and McIntosh F. **(**1995). Different Strains of Saccharomyces cerevisiae differ in their Effects on Ruminal Bacterial Numbers *In vitro* and in Sheep. J. Anim. Sci. 73:1811-1818.

Newbold C. J., Williams P. E. V., Walker A., and Wallace R. J. (1990). The Effect of Yeast Culture on Yeast Numbers and Fermentation in the Rumen Ecosystem. Proc. Nutr. Soc. UK, 49:47A. Abstract.

Nobo G., Moreki J. C., Nsoso S. J. (2012). Feed Intake, Body Weight, Average Daily Gain, Feed Conversion Ratio and Carcass Characteristics of Helmeted Guinea Fowl Fed Varying Levels of Phane Meal (Imbrasia belina) as Replacement of Fishmeal under Intensive System. Int. J. Poult. Sci. 11(6):378-384.

Oeztuerk H. (2009). Effects of Live and Autoclaved Yeast Cultures on Fermentation i*n vitro*. J. Anim. Feed Sci. 18(1):142-150.

Oeztuerk H., Schroeder B., Beyerbach M. and Breves G. (2005). Influence of Living and Autoclaved Yeasts of *Saccharomyces boulardii* on *in vitro* Ruminal Microbial Metabolism. J. Dairy Sci. 88:2594-2600.

Oliveira E. J., Rabinovitch L., Monnerat R. G., Passos L. K., Zahner V. (2004). Molecular Characterization of Brevibacillus laterosporus and its Potential Use in Biological Control. Appl. Environ. Microbiol. 70:6657-6664.

Olson K C., Caton J. S., Kirby D. R and Norton P. L. (1994). Influence of Yeast Culture Supplementation and Advancing Season on Steers Grazing Mixed-grass Prairie in

the Northern Great Plains: II. Ruminal Fermentation, Site of Digestion, and Microbial Efficiency. J. Anim. Sci. 72:2158–2170.

Olson K. C., Caton J. S., Kirby D. R., and Norton P. L (1994a). Influence of Yeast Culture Supplementation and Advancing Season on Steers Grazing Mixed-grass Prairie in the Northern Great Plains: I. Dietary Composition, Intake, and *In situ* nutrient Disappearance. J. Anim. Sci. 72:2149–2157.

Opsi F., Fortina R., Tassone S., Bodas R., and Lopez S. (2012). Effects of Inactivated and Live Cells of *Saccharomyces cerevisiae* on In vitro Ruminal Fermentation of Diets with Different Forage: Concentrate Ratio. J. Agric. Sci. 150:271–283.

Orskov E. R. and McDonald I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. (Cambridge) 92:499-503

Panda A. K., Singh R., and Pathak N. N. (1995). Effect of Dietary Inclusion of *Saccharomyces cervisiae* on Growth Performance of Crossbred Calves. J. Appl. Anim. Res. 7:195-200.

Panda A. K., Rao S. V. R., Raju M. V. L. N., Sharma S. R. (2006). Dietary Supplementation of *Lactobacillus Sporogenes* on Performance and Serum Biochemico-lipid Profile of Broiler Chickens. J. Poult. Sci 43:235–240.

Pandey P., and Agarwal I. S. (2001). Nutrient Utilization and Growth Response in Crossbred Calves Fed Antibiotic and Probiotic Supplement Diet. Indian J. Anim. Nutr. 18:15-18.

Parker R. B. (1974). Probiotics in other Half of the Antibiotics Story. Anim. Nutr. Health 29:4-8.

Pathak A. K. (2008). Various Factors Affecting Microbial Protein Synthesis in the Rumen (Review). J. Vet. World 1(6):186-189.

Patra A. K. and Saxena J. (2009). The Effect and Mode of Action of Saponins on the Microbial Populations and Fermentation in the Rumen and Ruminant Production. Department of Animal Nutrition, West Bengal University of Animal and Fishery Sciences, Belgachia, Kolkata, India,Coskata, 4575 Weaver Parkway, Warrenville, Illinois 60555, USA. Nutr. Res. Rev. 22:2042-19.

Picard J A., Sinthumule E. (2002). Antimicrobial Database Report 2002. Pretoria: University of Pretoria.

Pillanatham C. S., Devki N. K., Neeta A. and Lal C. C. (2012). Effects of Sodium Nitrate and Nitrate Reducing Bacteria on *In vitro* Methane Production and Fermentation with Buffalo Rumen Liquor. Asian–Australas J Anim Sci. 25 (6):812-817.

Piva G., Belladona S., Fusconi G., Sicbaldi F. (1993). Effects of Yeast on Dairy Cows Performance, Ruminal Fermentation, Blood Components, and Milk Manufacturing Properties. J. Dairy Sci. 7: 2717-2722.

Plaizier J. C., Martin A., Duffield T., Bagg R., Dick P., McBride B. W. (2001). Effect of Prepartum Administration of Monensin in a Controlled-Release Capsule on Apparent Digestibilities and Nitrogen Utlilization in Transition Dairy Cows. J. Dairy Sci. 83: 2918–2925.

Plata F. P., Mendoza G. D., Barcena- Gama J. R. and Gonzalez S. M. (1994). Effect of a Yeast Culture (*Saccharomyces cerevisiae*) on Neutral Detergent Fiber Digestion in Steers Fed Oat Straw Based Diets. Anim. Feed Sci. Tech., 49: 203-210.

Reid G. and Friendship R. (2002). Alternatives to Antibiotic Use: Probiotics for the Gut. Animal Biotechnology, 13(1), 97-112.

Antimicrobial Feed Additives. Merck Manuals, The Merck veterinary manual. http://www.merckvetmanual.com/mvm/pharmacology/growth_promotants_and_production_enhancers/antimicrobial_feed_additives.html. Accessed on 18 November 2015.

Remond D., Noziere P., and Poncet C. (2002). Effect of Time of Starch Supply to the Rumen on the Dynamics of Urea and Ammonia Net Flux Across the Rumen Wall of Sheep. Anim Res. 51(1):3-13.

Reynolds C. K., Aikman P. C, Lupodi B., Humphries D. J., and Beever D. E. (2003). Splanchnic Metabolism of Dairy Cows during the Transition from Late Gestation through Early Lactation. J. Dairy Sci. 86:1201-1217.

Richardson L. F., Raun A. P., Potte E. L., Coaley C. O., and Rathmarcher R.P. (1976). Effects of Monensin on Rumen Fermentation *In vitro* and *In vivo*. J. Anim. Sci. 43:657-664.

Rifat U. K., Shabana N., Kuldeep D., Karthik K., Ruchi T., Mutassim M. A., Ibrahim A. A., and Arshad Z. (2016). Direct-Fed Microbial: Beneficial Applications, Modes of Action and Prospects as a Safe Tool for Enhancing Ruminant Production and Safeguarding Health. Inter. J. Pharmacol. 12:220-231.

Robinson P. H. and Erasmus L. J. (2009). Effects of Analyzable Diet Components on Responses of Lactating Dairy Cows to *Saccharomyces cerevisiae* Based Yeast Products: A Systematic Review of the Literature. Anim. Feed Sci. Technol. 149:185-198.

Roy D. K., Singh G. P., Sahay A., Sahay D. N., Suryanarayana N. (2006). Leaf Surface Microflora for Tasar Crop Improvement. Indian Silk 45:19-21.

Ruiu L., Satta A., and Floris I. (2014). Administration of *Brevibacillus laterosporus* Spores as a Poultry Feed Additive to Inhibit House Fly Development in Faeces: A New Eco-sustainable Concept. Dipartimento di Agraria, University of Sassari, via E. De Nicola, 07100 Sassari, Italy. Poult. Sci. 93:519–526.

Russell J. B., Houlihan A. J. (2003). Ionophore Resistance of Ruminal Bacteria and its Potential Impact on Human Health. FEMS Microbiol. Rev. 27(1):65-74.

Samaniego Marcial Antonio (1996). Comparison of Methods for the Measurement of Digesta Flow and Microbial Protein Supply in Sheep. Facultad de Ingenieria Agronomica, University of Aberdeen.

Sandegren L. (2014). Selection of Antibiotic Resistance at Very Low Antibiotic Concentrations. Upsala Journal of Medical Sciences, 119(2), 103–107.http://doi.org/10.3109/03009734.2014.904457.

Sanders M. E., Morelli L., and Tompkins T. A. (2003). Sporeformers as Human Probiotics: *Bacillus*, *Sporolactobacillus*, and *Brevibacillus*. Compr. Rev. in Food Sci. and Food Saf. 2(3):101-110.

Santos F. A., Santos J. E., Theurer C. B., and Huber J. T. (1998). Effects of Rumen Undegradable Protein on Dairy Cow Performance: A 12-year Literature Review. J. Dairy Sci. 81:3182–3213.

Santra A. and Karim S. A. (2003). Rumen Manipulation to Improve Animal Productivity. Central Sheep and Wool Research Institute, Avikanagar - 304 501, Rajasthan, India.

Sarkar P. K., Hasenack B., Nout, M. J. R. (2002). Diversity and Functionality of Bacillus and Related Genera Isolated from Spontaneously Fermented Soybeans (Indian Kinema) and Locust Beans (African Soumbala). Int. J. Food Microbiol. 77:175-186.

Sharma D. D. and Malik R. (1992). Probiotic Supplementation in Animal Feed. In: Proceeding of National Symposium on Role of Amino Acid and Feed Supplementation in Animal Feeds, Trivendum, India.

Silbergeld E. K., Graham J., Price L. B. (2008). Industrial Food Animal Production, Antimicrobial Resistance, and Human Health. Annu. Rev. Public Health 29:151-69.

Singh S., Sharma V. P., Panwar V. S. (1999). Influence of the Levels of Probiotic and Energy on Mortality and Economics of Broilers in Summer. Indian J. Anim. Sci. 69:830–831.

Somsap On-anong, and Monthon Lertcanawanichakul. (2013). Characteristic and the Mode of Action of Bacteriocin Produced by *Brevibacillus laterosporus* SA14 which Isolated from the Air. J. Agric. Technol. 9(5):1319-1331.

omsap On-anong, Bangrak Phuwado, bhoopong Phuangthip, and Lertcanawanichakal. (2014). Antibacterial Activity and Purification of Bacteriocin Produced by *Brevibacillus laterosporus* SA14. Agriculture Technology and Biological Sciences Walailak. J. Sci. Technol. 13(1):55-65.

Soren N. M., Tripathi M. K., Bhatt, R. S. (2013). Effect of Yeast Supplementation on the Growth Performance of Malpura lambs. Trop. Anim. Health Prod. 45:547-554.

Statistics Analysis Systems. (2009). SAS User's guide: Statistics Version 8 Statistical Software. SAS Institute Inc. Cary NC., USA.

Stokes S. R., Hoover W. H., Miller T. K., and Manski R. P. (1991). Impact of Carbohydrate and Protein Levels on Bacterial Metabolism in Continuous Culture. J. Dairy Sci. 74: 860-870.

Suslova M. Y., Lipko I. A., Mamaeva E. V., Parfenova V. V. (2012). Diversity of Cultivable Bacteria Isolated from the Water Column and Bottom Sediments of the Kara Sea Shelf. Microbiol. 81:484-491.

Tamminga S. (1979). Protein Degradation in the Fore Stomachs of Ruminants. J. Anim Sci. 49(6):1615-1630.

Thripaty M. K. and Karim S. A. (2009). Intake, Growth, Ciliate Protozoa and Extra Cellular Microbial Enzyme Status on Lamb of Different Yeast Culture Feeding. Poceeding of XIe International Symposium on Ruminant Physiology. Wageningen Academic Publisher. 6700. AE Wageningen, Netherland.

Tilley J. M. A and Terry R. A. (1963). A Two-stage Technique for the *In vitro* Digestion of Forage Crops. J. Brit. Grassl. Soc. 18:104-111.

Tricarico J. M., Harrison G. A., and Johnston J. D. **(**2006). Modeling Yea-Sacc ®1026 Effects on Ruminal Function and Performance in Lactating Dairy Cattle within the Framework of the CPM Dairy Ration Analyzer. Proceedings of the 22nd Annual Symposium Nutritional Biotechnology in the Feed and Food Industries" (Suppl. 1), Lexington, KY, USA.

Uyeno Y., Shigemori, S., & Shimosato, T. (2015). Effect of Probiotics/Prebiotics on Cattle Health and Productivity. Microbes and Environments, 30(2):126–132.

Van der Werf J.H.J., L.J. Jonker, and J.K. Oldenbroek. (1998). Effect of Monensin on Milk Production by Holstein and Jersey Cows. J Dairy Sci. 81:427-433.

Van Soest P. J., Robertson J. B., and Lewis B. A. (1991). Methods for Dietary Fiber, Neutral Detergent Fiber, and Non-starch Polysaccharides in Relation to Animal Nutrition. J. Dairy Sci. 74:3583-3597.

Van Soest, P. J., Robertson J. B., and Lewis B. A. (1991). Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *J. Dairy Sci.* 74:3583-3597.

Vandevoorde L. Christianens H., and Verstraete W. **(**1991). *In vitro* Appraisal of the Probiotic Value of Intestinal lactobacilli. *World.* J. Microbiol. Biotechnol. 7**:**587-592.

Varadaraj M. C., Devi N., Keshava N., Manjrekar S. P. (1993). Antimicrobial Activity of Neutralized Extracellular Culture Filtrates of Lactic Acid Bacteria Isolated from a Cultured Indian Milk Product ('Dahi'). Int. J. Food Microbiol. 20:259-267.

Varga Gabriella A. (2010). Why Use Metabolizable Protein for Ration Balancing? Department of Dairy and Animal Science, The Pennsylvania State University, USA.

Vila B., Fontgibell A., Badiola I., Esteve-Garcia E., Jimenez G., Castillo M., and Brufau J. (2009). Reduction of *Salmonella enterica* var. Enteritidis Colonization and Invasion by *Bacillus cereus* var. toyoi Inclusion in Poultry Feeds. Poult. Sci. 88(5):975–979.

Vogel K. P., Petersen J. F., Masterson S. D., Toy J. J. (1999). Evaluation of a Filter Bag System for NDF, ADF, and IVDMD Forage Analysis. Crop Sci. 39: 276–279.

Wallace R. J. (1994). Ruminal Microbiology, Biotechnology and Ruminant Nutrition. Progress and Problems. J. Anim. Sci. 72:2992-3003.

Wallace R. J. (1996). The Mode of Action of Yeast Culture in Modifying Rumen Fermentation. In: Proceedings of Alltech's 12th Annual Symposium on Biotechnology in the Feed Industry. Nottingham University Press, Loughborough, Leics. UK., pp. 332-338.

Wallace R. J., Colombatto D., Robinson P. H. (2008). Enzymes, Direct-Fed Microbials and Plant Extracts in Ruminant Nutrition. Anim. Feed Sci. Tech. 145:1-4.

Wallace R. J., & Newbold, C. J. (1992). Probiotics for Ruminants. In Probiotics (pp. 317-353). Springer, Netherlands.

Wallace, R. J. and Newbold, C. J. (1995). Microbial Feed Additives for Ruminants. In: Fuller, R., Heidt, P.J., Rusch, V., Van DerWaaij, D. (Eds.), Probiotics: Prospects of Use in Opportunistic Infections. Institute for Microbiology and Biochemistry, Herborn-Dill, Germany, pp. 101–125.

Ware D. R., Read P. L., and Manfredi E. T. (1988). Lactation Performance of Two Large Dairy Herds Fed *Lactobacillus acidophilus* Strain BT 1386. J. Dairy Sci. 71 (Suppl. 1): 219. Abstract

Weatherburn M. W. (1967). Phenol-hypochlorite Reaction for Determination of Ammonia. Anal. Chem. 39:971-974.

Wedegaertner T. C. and Johnson D. E. (2013). Monensin Effects on Digestiblity, Methanogenesis and Heat Increment of a Cracked Corn-silage Diet Fed to Steers. J Anim. Sci. 57:168-177.

Wegener H. C. Antibiotic Resistance—Linking Human and Animal Health. In: Institute of Medicine (US). Improving Food Safety through a One Health Approach: Workshop Summary. Washington (DC): National Academies Press (US); 2012. A15.

White G. F. (1912). The Cause of European Foulbrood. US Dep. Agric. Bur. Entomol. 157:1-15.

Wiedmeier R. D., Arambel M. J., and Walters J. L. (1987). Effects of Yeast Culture and *Aspergillus oryzae* Fermentation Extract on Ruminal Characteristics and Nutrient Digestion. J. Dairy Sci. 70:2063-2068.

Williams, P. E. V. and Newbold C. J. (1990). Rumen Probiosis: The Effect of Novel Microorganisms on Ruminal Fermentation and Ruminant Productivity. In: Recent Advances in Animal Nutrition (Ed. W. Haresign and D. J. A. Cole). Butterworths, London, England.

Williams P. E. V., Tait C. A. G., Innes G. M., and Newbold C. J. (1991). Effects of the Inclusion of Yeast Culture (*Saccharomyces cerevisiae* Plus Growth Medium) in the Diet of Dairy Cows on Milk Yield and Forage Degradation and Fermentation Patterns in the Rumen of Steers. J. Anim. Sci. 69:3016- 3026.

Wohlt J. E., Finkelstein A. D., and Chung C. H. **(**1991). Yeast Culture to Improve Intake, Nutrient Digestibility, and Performance by Dairy Cattle During Early Lactation. J. Dairy Sci. **7**4: 1395-1400.

Wu S., Jia S., Sun D., Chen M., Chen X., Zhong J., Huan L. (2005). Purification and Characterization of Tow Novel Antimicrobial Peptides Subpeptin JM4-A and Subpeptin JM4-B Produced by *Bacillus subtilus* JM4, Curr. Microbiol; 51(5):292–296.

Wu X. Y., Walker M., Vanselow B., Chao R. L., Chin J. (2007). Characterization of Mesophilic Bacilli in Faeces of Feedlot Cattle. J. Appl. Microbiol. 102:872–879.

Yang C., Chowdhury M. A. K., Hou Y., Gong J. (2015). Phytogenic Compounds as Alternatives to In-Feed Antibiotics: Potentials and Challenges in Application. Pathogens 4:137-156.

Yang W. Z., and Beauchemin K. A. (2003). Effects of Physically Effective NDF on Rumen Fermentation and Nutrient Digestion of Dairy Cows Fed Diets Based on Barley or Corn Silages. J. Dairy Sci. 86(Suppl. 1) 1:340.

Yi Zheng (2013). Adoption of Total Mixed Ration Practice and Profitability: The Case of Ontario Dairy Farms. Faculty of Food, Agricultural and Resource Economics, The University of Guelph, Canada.

Yirga H. (2015). The Use of Probiotics in Animal Nutrition. J. Prob. Health doi:10.4172/2329-8901.1000132.

Yoon I. K and Stern M. D. (1996). Effects of *Saccharomyces cerevisiae* and *Aspergillus oryzae* on Ruminal Fermentation in Dairy Cows. J. Dairy Sci. 79:411-417.

Yoon, I. K., and Stern M. D. (1995). Influence of Direct-Fed Microbials on Ruminal Microbial Fermentation and Performance of Ruminants: A Review. Asian-Australas. J. Anim. Sci. 8:533–555.

Zelenac, I, Jalc D., Kmet V., Siroka P. (1994). Influence of Diet and Yeast Supplement on *in vitro* Ruminal Characteristics. Anim. Feed Sci. 49:211- 221.

Zhongyan Lu. (2013). Urea Transport in Sheep Rumen Epithelium In vitro: Modulation by luminal Ammonia and pH. Fachbereich Biologie, Chemie, Pharmazie, der Freien Universität Berlin, Germany.

Zinn R. A., and Owens F. N. (1982). Rapid Procedure for Quantifying Nucleic Acid Content of Digesta. In: F. N. Owens (Ed.). Protein Requirements for Cattle: Symposium. Oklahoma State Univ. MP: pp 26-30.