

Effects of Yeast based Direct Fed Microbial Supplementation on the Performance of High Producing Dairy Cows

By

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DECLARATION

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

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SUMMARY

Effects of Yeast based Direct Fed Microbials on the Performance of High Producing Dairy Cows

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The use of direct fed microbials (DFM) is common in dairy rations worldwide but, due to variability in animal responses, more research is needed in order to demonstrate their efficacy. These products need to be tested in the environment they are going to be used in because of potential interactions of responses with feeding conditions. The objective of this study was to determine the effect of two yeast (*S. cerevisiae*) based feed additives on the production response of high producing dairy cows.

The study consisted of three high producing Holstein cow pens (\pm 315 cows/pen) that were used in a 3 x 3 Latin square design experiment. The three experimental treatments were: 1) Basal total mixed ration (Control), 2) Control supplemented with the 'DV XPC' yeast culture (14 g/cow/d), 3) Control supplemented with the Yeasture DFM (10 g/cow/d). The experimental periods were 28 days with the last 7 days for data collection.

Milk yield was higher for the Yeasture treatment ($P = 0.01$) compared to the control. With the increased milk yield there was also an increase in milk true protein ($P = 0.01$), lactose ($P = 0.01$) and energy ($P = 0.02$) outputs as well as a tendency for milk fat ($P = 0.07$) to increase. Milk yield and yield of milk components, milk energy output and levels of milk components were not impacted by feeding DV XPC. Total NE output for both the treatments vs. Control was increased, DV XPC ($P = 0.01$) and Yeasture ($P < 0.01$). The increased total NE output for Yeasture was all due to the increase in milk energy output while for DV XPC it was due to numerical increases in milk and BCS energy.

Neither treatment impacted the NE_L density of the diets compared to the control. Total tract apparent digestibility of OM tended to be lower ($P = 0.08$) for the DV XPC treatment while the total tract apparent OM digestibility for the Yeasture treatment was lower ($P = 0.02$) compared to the control. Total tract apparent CP digestibility followed the same trend, where the DV XPC tended ($P = 0.05$) to be lower while for the Yeasture treatment it was ($P < 0.01$) lower compared to the control. Total tract apparent aNDFom and starch digestibility was not affected by treatment and there was no effect of treatment on MCP flow from the rumen compared to the control. Blood plasma AA concentrations showed that the total EAA concentrations tended to be higher ($P = 0.07$) with the Yeasture compared to the control, and was mainly driven by an increase in threonine ($P = 0.03$), tryptophan ($P = 0.02$), valine ($P = 0.08$) and histidine ($P = 0.06$). Although total NEAA did not differ when Yeasture was fed compared to the control, there was an increase in concentrations of glycine ($P = 0.04$), asparagine ($P = 0.03$), tyrosine ($P = 0.05$), serine ($P = 0.07$), proline ($P = 0.06$) and taurine ($P = 0.07$). Feeding DV XPC had no impact on plasma concentrations of any AA.

Results suggest that there was no substantive impact of either yeast additive on rumen fermentation, mainly due to the lack of treatment impacts on aNDFom digestibility and MCP outflow, but suggest that a post ruminal effect occurred with Yeasture where GIT health was improved, as well as nutrient absorption efficiency, mainly due to the reduced total tract apparent digestion of CP and OM as well as increased plasma AA concentrations. This results shows that more research are needed on the effects of yeast based DFM's on post-ruminal digestive efficiency due, perhaps, to probiotic like effects of the components of the yeast products.

LIST OF ABBREVIATIONS

AA	Amino acids
AAS	Atomic absorption spectrometry
ADF	Acid detergent fiber
ADG	Average daily gain
ADICP	Acid detergent insoluble crude protein
ADIN	Acid detergent insoluble nitrogen
AL	Allantoin
aNDFom	NDF free of residual ash and assayed with an alpha amylase
BCS	Body condition score
BW	Body weight
Ca	Calcium
CA	California
CFU	Colony forming units
Cl	Chloride
Co	Cobalt
CP	Crude protein
Cu	Copper
d	Day
DDGS	Dried distillers grains with solubles
DFM	Direct fed microbial
DHIA	Dairy Herd Improvement Association
DIM	Days in milk
DM	Dry matter
DNA	Deoxyribonucleic acid
EAA	Essential amino acids
EE	Ether extract
FDA	Food and Drug Administration of the USA
Fe	Iron
g	Gram
GI	Gastrointestinal
GIT	Gastrointestinal tract
GRAS	Generally Recognised As Safe
h	Hour
H ₂	Hydrogen
H ₂ O	Water
HPLC	High Performance Liquid Chromatography
HQ	High quality
IA	Iowa
ICP-AES	Inductively coupled plasma by atomic emission spectrometry
IFN	Interferons
IL	Interleukin
IR	Infra-red

IVOMD	<i>In vitro</i> organic matter digestibility
K	Potassium
Kg	Kilogram
KJ	Kilojoules
LD ₅₀	Lethal dose 50%
LQ	Low quality
MA	Massachusetts
MCP	Microbial crude protein
ME	Metabolisable energy
Mg	Magnesium
mg	Milligram
MJ	Megajoules
Mn	Manganese
Mo	Molybdenum
MQ	Medium quality
MUN	Milk urea nitrogen
N	Nitrogen
Na	Sodium
NAN	Non ammonia nitrogen
NDF	Neutral detergent fiber
NEAA	Non-essential amino acids
NEact	NE activity requirements
NEFA	Non-esterified fatty acids
NE _L	Net Energy for lactation
NE _m	Net Energy for maintenance
NH	New Hampshire
NH ₃	Ammonia
NJ	New Jersey
NPN	Non-protein nitrogen
NRC	National Research Council of the USA
°C	Degree Celsius
OM	Organic matter
P	Phosphorus
PA	Pennsylvania
PD	Purine derivative
RFI	Residual feed intake
SAS	Statistical analysis system
SCC	Somatic cell count
SD	Standard deviation
Se	Selenium
SEM	Standard error of the mean
SG	Specific gravity
Temp	Temperature
TGF – β	Transforming growth factor β

Th	T-helper cells
TMR	Total mixed ration
TNF	Tumour necrosis factor
TX	Texas
USA	United States of America
VFA	Volatile fatty acids
WI	Wisconsin
Wt	Weight
Yst	Yeasture
Zn	Zinc

Yeast definitions:

Live yeasts: A product that contains live yeast cells. Viability of yeast cells is claimed (*e.g.*, Levucell).

Yeast cultures: Fermented yeast products, with dead yeast cells. Viability of yeast cells is not claimed (*e.g.*, DV XPC).

Yeast based DFM: A product that contains live yeast cells but includes a bacterial culture. Viability of live yeast cells is claimed (*e.g.*, Yeasture).

Yeast based product: A product that contains either live or dead yeast cells but also contains high concentrations of yeast extracts such as MOS and β -Glucans (*e.g.*, Vi-Cor Celmanac).

Appendix Table A2 contains common yeast products that are commercially available whereas appendix Table A3 shows how the products used in the articles used to compare to the products used in the trial are classified for comparison reasons.

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CHAPTER 1

GENERAL INTRODUCTION

Although there are several commercial yeast (*Saccharomyces cerevisiae*) based products, as well as a lot of research that has been completed on effects of supplementing yeast based products to ruminants, results vary (Robinson and Erasmus, 2008). Dairy nutritionists and dairy producers need more information on the effects of yeast based products on animal production in order to make informed decisions about the most efficacious option for their dairy operations. Many claims have been made about the impacts of yeast based products on ruminant animal performance, including (Chaucheyras-Durand *et al.*, 2012):

- Improved feed intake.
- Improved feed efficiency.
- Improved rumen fiber fermentation.
- Improved rumen microbial protein synthesis.
- Improved milk yield.
- Improved rumen pH.
- Improved digestion.

Rumen fermentation processes play a key part in ruminant nutrition (Van Soest, 1994). The extent of interactions among the microbial populations in the rumen is so complex that many pathways remain unknown (Russell, 2002). Thus ruminant nutritionists and rumen microbiologists have the same objective, namely to increase nutrient utilization within the rumen. Thus the rumen microbial ecosystem is one of the most extensively studied microbial ecosystems (Van Soest, 1994).

Concerns about use of antibiotics in livestock nutrition are well known due to the occurrence of antibiotic resistant bacteria that might represent a risk to human health (Barton, 2000). This concern led to the use of ionophores as growth stimulants in food animals being banned in the European Union in January 2006 (Regulation 1831/2002/EC) (DiLorenzo, 2010). Thus the search for safe, high quality and efficacious feed additives has led research to focus on natural alternative additives such as direct fed microbial's (DFM) and essential oils (Patra, 2011). The same debate has occurred on effects of methane, CO₂ and nitrous oxide greenhouse gas emissions, and natural rumen fermentation modifiers that can decrease the production of methane produced during enteric fermentation (Barton, 2000). Thus rumen microbial research has recently focused on use of natural feed additives and how they can be used to improve efficiency of animal production systems, whether by improving rumen fermentation, decreasing methane production, reducing nutritional stressors such as acidosis and bloat, improving post rumen gastrointestinal (GI) health as well as overall productivity of cows and profitability of dairy operations (Nagaraja, 2012).

General objectives of rumen fermentation manipulation are to enhance beneficial processes, to minimize, alter or eliminate inefficient processes and to minimize, alter or eliminate processes that are harmful to the host (Nagaraja *et al.*, 1997). The specific objective of this study was to determine effects of the two yeast (*S. cerevisiae*) based feed additives, that was not been extensively studied before, and their effects on dry matter (DM) intake, whole tract apparent digestibility, rumen fermentation, body condition score, milk yield and milk composition of high producing dairy cows in order to determine if it is beneficial to include these additives with limited research in the diets of high producing Holstein cows. The yeast products was chosen as there is limited research on their efficacy in animal production systems.

In the next chapter, a literature review on direct fed microbials (DFM), their mode of action and production responses will be presented, followed by chapters on the experimental materials and methods, results and discussion and finally a conclusion.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Feed additives are non-nutritive compounds added to diets at low levels to help animals utilize nutrients more efficiently thereby leading to increased performance, decreased risk of metabolic diseases such as rumen acidosis and bloat, as well as reducing collateral impacts on the environment (Adesogan, 2009). In ruminants, feed additives act in a number of ways by: regulating rumen pH and decreasing lactate accumulation in the rumen; stimulating rumen development in young ruminants; increasing rumen organic matter (OM) and fiber digestibility; and decreasing the risk of metabolic diseases (Newbold and Rode, 2006). In addition feed additives may also affect rumen energy and nitrogen (N) utilization (Alexander *et al.*, 2008). Rumen energy use is improved by decreasing methanogen numbers in the rumen and by decreasing the acetate: propionate ratio without lowering milk fat synthesis (Alexander *et al.*, 2008). Rumen N usage is improved by decreasing proteolysis, peptidolysis and amino acid (AA) deamination leading to lower N losses to the environment (Alexander *et al.*, 2008). Nitrogen usage is also improved by reducing the activity of rumen protozoa which can degrade and engulf beneficial bacteria and contribute to proteolysis and deamination while acting as a host for methanogenic bacteria (Adesogan, 2009). Feed additives may also increase synthesis of microbial protein by assisting coupling of rumen energy and protein metabolites which also leads to improved N efficiency (Wang *et al.*, 2001). The main objective of utilizing feed additives is generally to increase the level and efficiency of animal production. However, feed additives must be cost effective and approved by the appropriate regulatory authorities (Adesogan, 2009).

2.2 Rumen microbes

2.2.1 Introduction

The rumen microbial population is complex and includes Eubacteria (bacteria), Eukarya (protozoa and fungi) and Archaea (methanogens) (Nagaraja, 2012) of which bacteria contribute more than 95% of rumen microorganisms species (Brulc *et al.*, 2009), with the most common species being *Selenomonas ruminantium*, *Butyrivibrio fibrisolvens*, *Bacteroides amylophilus*, and *Bacteroides rumenicola* (Purser and Buechler, 1966). Methanogens were historically considered to be bacteria due to their morphological resemblance, but were moved to a new domain called archaea based on the evolutionary line and other distinct molecular features (Nagaraja, 2012).

Two types of protozoa occur in the rumen, namely flagellated and ciliated protozoa (Hobson and Stewart, 1997). Flagellated protozoa make no significant contribution to the rumen microbiological population as they contribute less than 10^3 cells/g of rumen content, but ciliated protozoa contribute a significant proportion of the microbial cell mass in the rumen and include a variety of morphological types that are grouped as entodiniomorphid or holotrichid ciliates (Nagaraja, 2012). Fungi are categorized by a two-stage life cycle, the

zoosporic stage consisting of actively motile spores that adhere to feed particles, germinate and develop into a vegetative stage consisting of a mycelia structure that is responsible for production of hydrolytic enzymes (Hobson and Stewart, 1997). Due to this two-stage life cycle, and the capability of the mycelial structures to grow on feed particles, it is not possible to accurately measure fungal cell mass in the rumen, but fungi in the rumen can contribute up to 10% of the rumen microbial mass based on indirect estimates (Hobson and Stewart, 1997).

The rumen contains bacteriophage viruses that infect bacteria and were first acknowledged by electron microscopic observations of rumen content. As many as 1011 phage particles have been counted per gram of rumen contents and more than 125 morphological types have been described, belonging to both the lytic and temperate types (Nagaraja, 2012). The majority of bacteriophages in the rumen are temperate (Nagaraja, 2012). Bacterial numbers and types in the rumen may be influenced by lytic phages. However, rumen population dynamics and the overall functional significance of bacteriophages in the rumen have not been fully determined.

Because microbes in the rumen share the same habitat and compete for the same substrates, there are a number of interactions which occur that can have positive and negative effects on the microbes and the host. For example, evidence to suggest transfer of genetic material between bacteria and ciliated protozoa in the rumen (Ricard *et al.*, 2006). Ciliates engulf and digest bacteria, and this leads to some of the deoxyribonucleic acid (DNA) being taken up by the ciliates and incorporated into their genome (Devillard *et al.*, 1999). The ecology of rumen microbes is based on a dynamic interaction between the host and the diet and is related to the vital functions of the animal, such as immune function, regulation of extracellular signaling and competition with pathogenic bacteria (Ricard *et al.*, 2006).

2.2.2 Microbial ecology and animal physiology

Microbes provide the host animal with vitamins, protein and energy (Nagaraja, 2012), and thus the rumen microbial composition can be linked to the host animal's meat and milk production. Some research has shown that significant differences occur in the rumen microbial profile between cattle with low residual feed intake (RFI) and those with a high RFI (Guan *et al.*, 2008). For example there were higher concentrations of the volatile fatty acids (VFA) butyrate and valerate in the rumen of more efficient cattle which suggests differences in microbial activity (Guan *et al.*, 2008). Although it is difficult to relate this to feed efficiency, butyrate may have a regulatory role in gene expression of bovine cells such as adipocytes (Li and Li, 2006). Cattle that have been selected for a lower RFI have been shown to produce less methane (Hegarty *et al.*, 2007), thereby showing that the microbial community structure within the rumen may differ depending on the efficiency with which they utilize nutrients.

2.2.3 Rumen pH and microbial activity

The pH of the rumen is probably the most important factor that affects microbial populations, activity and their fermentation products such as methane, acetate, propionate and lactate (Lana *et al.*, 1998). Among microbes, fungi and ciliated protozoa are more sensitive to

pH fluctuations compared to bacteria, but bacteria that degrade fiber, utilize lactate or produce methane are also sensitive to diurnal pH fluctuations (Nagaraja, 2012). The magnitude of the pH effect on the microbial population depends mostly on the duration of the suboptimal pH period (Nagaraja, 2012). The pH sensitivity of rumen bacteria is dependent on the pH gradient across the cell membrane and the ability of the bacterial cells to control its intracellular pH. Usually, in cattle fed once or twice a day, rumen pH decreases for 2 to 8 hours after feeding depending on diet composition, especially the level of rapidly fermentable carbohydrates (Nagaraja, 2012). The increase in rumen pH after this time is due to removal of volatile fatty acids (VFA) by absorption, saliva flow, the buffering capacity of feeds, and the rate of passage of rumen contents to the abomasum.

The effect of rumen pH on microbial activity depends on the extent of the decreased rumen pH and, perhaps more importantly, the length of either the optimal or suboptimal pH. It is difficult to design and perform *in vivo* studies to determine the period of suboptimal pH on rumen fermentation (Nagaraja, 2012), but batch culture and continuous culture *in vitro* systems have been used to explain pH effects on rumen fermentation (Calsamiglia *et al.*, 2008). Cerrato-Sanchez *et al.* (2008) showed that digestibility and concentrations of VFA and ammonia were not affected by maintaining a pH < 5.6 for 4 h or fluctuating the pH between 5.1 and 7.1 for 2 h/d, but were affected by maintaining a pH of 5.1 for 4 h. This shows that the effect of a low pH on rumen microbial activity is due to both pH and the length of time the rumen is under its optimal pH level. De Veth and Kolver (2001) showed that durations of 4 h at a pH of 5.4 was long enough to reduce digestibility of dry matter (DM), OM, and neutral detergent fiber (NDF). Periods of more than 8 h of a suboptimal pH (*e.g.*, 5.4) were required to decrease microbial protein synthesis, which suggests that a suboptimal pH may only affect the activity of the microbial mass, but not cell numbers (De Veth and Kolver, 2001). A reduction in rumen pH is normally associated with feeding diets rich in highly fermentable carbohydrates and changes in fermentation products depend on the availability of substrates in the rumen. Calsamiglia *et al.* (2008) varied forage to concentrate ratio to determine effects of pH on fermentation in a continuous culture system and demonstrated that pH was the major determinant of OM digestibility, NDF digestibility and butyrate concentrations. In contrast, they found that the VFA concentrations were affected by the combined effects of both pH and diet.

2.2.4 Rumen pH and fiber digestion

Rumen pH is the main factor affecting fiber digestion in the rumen and at a pH below 6 bacterial growth decreases thereby negatively affecting fiber digestion (Nagaraja, 2012). High producing dairy cows at peak production can have substantial periods when the rumen pH is below 6. Although prolonged exposure of cellulolytic bacteria to a low pH has little effect on cellulose digestion, the rumen pH needs to remain above 6 for long enough to ensure growth rates of bacteria exceed the passage rate of bacteria from the rumen (Nagaraja, 2012). Cows with a low rumen pH can maintain a normal population of cellulolytic bacteria (Palmonari *et al.*, 2010) and, even in cows with grain fed induced acidosis, the abundance of cellulolytic bacteria did not decline, which occurred only when severe sustained acidosis occurred (Khafipour *et al.*, 2009). Cellulolytic bacteria can provide metabolites to non-

cellulytic bacteria that are more acid tolerant, thereby helping to moderate pH and increase cellulose digestion. Fiber digestion only ceases at a pH below 5.3, but dairy cows seldom reach such low levels for a significant period of time (Nagaraja, 2012). There is evidence to suggest that very low rumen pH (<5.3) reduces binding of fibrolytic bacteria to feed particles thereby reducing fiber fermentation in the rumen (Mourino *et al.*, 2001).

2.3 Direct Fed Microbials

2.3.1 Introduction

Direct fed microbials (DFM), or probiotics, have been defined as living microbial feed supplements which positively affect the host animal once ingested by improving the intestinal microbial balance (Fuller, 1989). The term DFM or ‘probiotic’ has been used to describe enzyme preparations, viable microbial cultures and culture extracts, as well as various combinations thereof (Yoon and Stern, 1995), and can be differentiated between bacterial, fungal and yeast DFM (Seo *et al.*, 2010).

Direct fed microbial products are classified as ‘Generally Recognized As Safe’ (GRAS) products by the Food and Drug Administration (FDA) of the United States of America (USA) and some of these products have been used commercially in Europe (Adesogan, 2009). Such products are popular due to the negative public perception of products such as antibiotics and artificial growth stimulants which led to a growing interest in using ‘natural’ DFM in food animal production systems. Many animal producers and veterinarians inoculate sick ruminants with rumen fluid from healthy ones with the goal of stimulating normal rumen function. This led to the FDA requirements to feed manufacturers to use the classification DFM for these products (Miles and Bootwalla, 1991). All DFM products include viable cultures of either bacteria, fungal or yeast DFM.

The National Feed Ingredient Association of the USA, as well as the FDA, has set forth guidelines to regulate sales and statements of DFM products. For example producers and sellers of DFM products are not allowed to make therapeutic claims (Kung, 2011), and cannot claim to establish viable bacterial colonies in the gut and cannot claim to affect the function of the animal. Claims to decrease morbidity, reduce sick days, increase milk production or affect growth and feed intake cannot be done without a new animal drug application (Kung, 2011).

2.3.2 DFM Practical considerations

Direct fed microbial products exist in a variety of forms including boluses, capsules, granules powders and pastes, and can be mixed with feed or, administered in drinking water (Kung, 2011). The use of DFM in water must be managed closely since interactions with antibiotics, chlorine, flow rate, minerals and water temperature can affect the viability of the DFM organisms (Kung, 2011). Non-hydroscopic whey is often used as a carrier for bacterial DFM and is a good medium to initiate their growth. Bacterial DFM pastes are generally formulated with vegetable oils and inert gelling ingredients, while fungal products are generally formulated with grain by-products as carriers. There is not much information comparing the effectiveness of administering DFM in a single dose compared to continuous

daily dosing (Kung, 2011). Some DFM products are designed for one-time dosing while other products are designed for daily feeding. Lee and Botts (1988) reported that pulse-dosing or pulse-dosing with daily feeding of the *Streptococcus faecium* M74 strain resulted in improved performance of incoming feedlot cattle. The need for a bacterial DFM to attach and colonize the gut surfaces in order to have a beneficial effect is also unclear. In certain applications, the argument has been made that DFM organisms need only to produce its active component, without colonization of the gut, to be beneficial (Kung, 2011). Dose levels of bacterial DFM have varied extensively. Studies can be found where *L. acidophilus* has been fed at levels stretching from 10^6 to 10^{10} cfu/animal/d (Kung, 2011). Hutchenson *et al.* (1980) suggested that feeding more than 10^7 cfu/head/d may have led to reduced nutrient absorption due to overpopulation of the gastrointestinal tract (GIT). Orr *et al.* (1988) reported that feeding a continuous high dose of *L. acidophilus* to feeder calves (10^{10} cfu/head/d) had no influence on body weight gain, but reduced feed efficiency, compared to feeding a lower dose of 10^6 cfu/head/d.

Tolerance of DFM microorganisms to heat is important since many feeds are pelleted prior to feeding to animals. In general *Bifidobacterium*, *Lactobacillus*, *Streptococcus* and most yeast are destroyed by the heat of pelleting. In contrast, *Bacilli* species form stable endospores when conditions for growth are unfavorable and are very resilient to disinfectants, heat, moisture and pH. Thus, *Bacilli* are generally used in applications that require pelleting (Kung, 2011). Higher inclusion of DFM's can compensate for microbial loss during pelleting. Future improvements in encapsulation technology may allow use of heat sensitive organisms in pelleted feeds. Bacterial products may or may not be compatible with the use of antibiotics and thus care should be taken when formulations contain both DFM and antibiotics (Kung, 2011). Information on DFM and antibiotic compatibility ought to be available from the manufacturers. For example, some species of *bacilli* are sensitive to virginiamycin, and *lactobacilli* are sensitive to penicillin (Kung, 2011). Viability of DFM products has improved in recent years but it is sensible to adhere to manufacturer storage recommendations (Kung, 2011). For example, products should be kept away from heat, light and moisture.

In a review by Fuller (1989) a good probiotic is defined to be a microbial strain capable of exerting positive effects on the host animal production, be non-pathogenic and non-toxic, be present as viable cells (preferably in large numbers), should be capable of surviving metabolism in the gut environment (e.g., low pH) and should be stable and capable of remaining viable for long periods of storage under field conditions.

2.3.3 DFM in dairy Production

Bacterial probiotics have maximum efficacy in pre-ruminant calves, whereas fungal products have shown greater benefits in mature ruminants (Krehbiel *et al.*, 2003). Direct fed microbials have been extensively studied in neonatal dairy calves since young calves digest a substantial amount of the nutrients from the ration post ruminally, and may be at risk of intestinal proliferation of harmful organisms such as *E. coli*. Neonatal calves are often stressed in new environments, specifically after dehorning, transport, vaccination and weaning (Krehbiel *et al.*, 2003). Bacterial DFM such as *Bifidobacterium*, *Enterococcus*,

Lactobacillus, and *Streptococcus* have been widely studied when supplemented in young calf rations (Newman and Jacques, 1995). In young dairy calves the main goal of DFM products are helping to establish and maintain a healthy gastrointestinal (GI) microbial system because calves are adapted to solid feed quickly and can develop enteropathogens that results in diarrhea (Krehbiel *et al.*, 2003). With stressed calves, the intestinal microbial population is in transition to a ruminant and is thus sensitive to changes that occur due to stress. Abrupt changes in either the diet or the animal's environment can lead to GIT microbial changes (Savage, 1977). For example stress can lead to increased diarrhea in neonates, which is associated with a decrease in *Lactobacillus* bacteria in the gut (Tannock, 1983). Sandine (1979) reported higher fecal counts of *lactobacilli* coliforms in healthy animals, but reversed in stressed and diuretic animals. A successful rapid transition from liquid to calf starter by neonatal calves depends on prior development of the rumen epithelium as well as development of rumen fermentative capacity. For example dairy calves supplemented with yogurt that contained *L. acidophilus* showed tendencies to ruminate more at 30 d after calving compared to untreated calves, indicating that *L. acidophilus* may stimulate rumen development. Performance results of neonatal calves consuming bacterial DFM have been variable. Abu-Tarboush *et al.* (1996), Ellinger *et al.* (1978) and Morrill *et al.* (1977) all reported no improvement in daily weight gain of calves as a result of feeding *Lactobacilli*. In contrast, Bechman *et al.* (1977) reported an improved weight gain when *L. acidophilus* species was added to the milk or milk replacer. Feed efficiency is usually not altered by feeding DFM to young calves (Abu-Tarboush *et al.*, 1996). Improved health, and a reduction in the incidence and severity of diarrhea, is most likely a more significant response than improved rates of weight gain and feed efficiency of neonate pre-ruminant animals.

Limited research has evaluated the efficacy of bacterial DFM for lactating dairy cows but much more research has examined yeast based DFM. In general, increased milk yield has been the most consistent response, and changes in milk composition have been variable (Krehbiel *et al.*, 2003). Use of DFM in lactating dairy cows will be discussed in more detail in the remaining section of this review.

2.4 Bacterial DFM

2.4.1 Introduction

Bacterial DFM consist of a large group of bacteria species that includes the lactic acid producing bacteria, *Bifidobacterium lactis*, *Bifidobacterium longum*, *Bifidobacterium pseudolongum*, *Bifidobacterium thermophilum*, *Enterococcus faecalis*, *Enterococcus faecium*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus gallinarum*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Lactobacillus salivarius*, *Streptococcus bovis* and *Streptococcus faecium*; lactic acid utilizing bacteria *Megasphaera elsdenii*, *Propionibacterium acidipropionici*, *Propionibacterium freudenreichii*, *Propionibacterium jensenii* and *Propionibacterium shermanii* and other bacteria like *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus subtilis* and *Prevotella bryantii* (Seo *et al.*, 2010).

2.4.2 Modes of action of bacterial DFM

2.4.2.1 Bacterial DFM and Rumen Fermentation

Feeding bacterial based DFM to livestock was originally based on potentially beneficial post-ruminal effects. However, there have been indications that some bacterial DFM might also have positive effects in the rumen. Feeding probiotics to beef cattle is likely the second most common practice (after ionophores) in terms of acidosis prevention (DiLorenzo, 2010).

Lactate producing bacteria such as *Enterococcus* and *Lactobacillus* species might help avoid rumen acidosis in cattle (Nocek *et al.*, 2002) because the presence of these bacteria produces lactate which leads some rumen microorganisms to adapt to its presence in the rumen (Yoon and Stern, 1995), and/or stimulate growth of lactate utilizing bacteria (Beauchemin *et al.*, 2003) that in turn stimulates stabilization of rumen pH.

Feeding lactate utilizing bacteria to ruminants is a common practice to increase lactate utilization in the rumen. *Megasphaera elsdenii* is a lactate utilizing bacteria that was shown to modify rumen fermentation and prevent accumulation of lactate throughout the transition from low to high concentrate diets (Kung and Hession, 1995). When feeding readily degradable and soluble carbohydrates, *M. elsdenii* seems to be the main rumen lactate utilizing bacteria (Counotte *et al.*, 1981) as it uses lactate, glucose and maltose and converts them to VFA (Russell and Baldwin, 1978), thereby competing with lactate-producing organisms for substrate. Kung and Hession (1995), reported that the pH of cultures treated with *M. elsdenii* was below 5.5 at 4 h, and remained at approximately 5.3, whereas the control decreased to 4.8. Total VFA concentrations of cultures treated with *M. elsdenii* were more than twice those of controls, but acetate concentration did not differ after 2 h, while concentrations of butyrate, isobutyrate, isovalerate, propionate and valerate for control and *M. elsdenii* inoculation differed. Greening *et al.* (1991) reported that inoculation with *M. elsdenii* decreased minimal pH and lactate concentration in acidotic beef cattle. Robinson *et al.* (1992) reported effects of inoculation with *M. elsdenii* on DM intake, rumen pH, osmolarity, lactate, as well as VFA concentration in acute acidosis-induced steers fed a 90% concentrate diet. In this study, the interaction between inoculation and the day of diet change moderated pH, lactate, VFA and DM intake, but steers inoculated with *M. elsdenii* consumed 24% more DM. A link between *M. elsdenii* and milk fat depression has recently been identified (Palmonari *et al.*, 2010), but the main limitation of *M. elsdenii* is that it is a strict anaerobe and must be maintained in an anaerobic environment to maintain a viable culture (DiLorenzo, 2010).

Propionibacterium is a lactate utilizing bacteria that mainly produces propionate rather than ferment lactate (Krehbiel *et al.*, 2003). Propionate is quantitatively the most vital single precursor for glucose synthesis among the main rumen VFA, and consequently has a major impact on hormonal release and tissue distribution of nutrients (Nagaraja *et al.*, 1997). In growing ruminants, as well as lactating cows, propionate has been estimated to represent 61 to 67% of glucose release (Huntington, 2000). Propionate spares glucogenic AA in gluconeogenesis, and consequently reduces the energetic cost of metabolizing protein and,

possibly, the heat increment of fermentation (Van Soest, 1994). Nutrient intake is typically much lower than nutrient demand during early lactation in dairy cows, and rumen supply of propionate will likely be inadequate to supply the energy required to support milk synthesis (Overton *et al.*, 1999). Also, a decrease in the acetate: propionate ratio is linked to a reduction in enteric methane production agreeing to the stoichiometric laws of chemical balance (Van Soest, 1994). When the acetate: propionate ratio decreases, CH₄ production declines, and energy retention by cattle should theoretically increase (Wolin, 1960). The VFA proportions in the rumen depend on the species of microorganisms and their culture conditions. For example, propionate production by *Propionibacterium* is usually accompanied by formation of acetate and CO₂ and this occurs for stoichiometric reasons, and to maintain the hydrogen and redox balance (Krehbiel *et al.*, 2003). *Propionibacterium* accounted for 40 to 50% of the lactate utilizing bacteria on occasion, but the population of *Propionibacterium* usually seems very low (Krehbiel *et al.*, 2003). Thus the concept of daily or periodic supplementation of *Propionibacterium* may be on the basis of the increment of propionate production when cattle are fed a high concentrate diet (Krehbiel *et al.*, 2003).

2.4.2.2 Competitive Attachment

Jones and Rutter (1972) suggested that attachment of bacteria to the intestinal wall is important for enterotoxin producing strains like *E. coli* to induce diarrhea. Therefore it seems likely that bacterial DFM could compete with pathogens for sites of adherence on the intestinal surface. Attachment is believed to support proliferation and reduce peristaltic removal of bacteria (Salimen *et al.*, 1996). In support, Muralidhara *et al.* (1977) found that homogenates of washed intestinal tissue collected from piglets dosed with *L. lactis* had significantly higher numbers of attached *Lactobacilli*, and lower *E. coli* counts than scouring or normal (control) pigs. Similarly, in the study of Abu-Tarboush *et al.* (1996), the adherence of *L. acidophilus* to the GIT was confirmed in young calves where the organisms used were comparable with the GIT. Adhesion of bacteria to the GIT is believed to be mediated nonspecifically by physicochemical factors, or specifically by adhesive bacterial surface molecules and epithelial receptor molecules (Holzapfel *et al.*, 1998). Nonspecifically, the ability of bacteria to adhere to epithelial cells appears to depend on interactions between an acidic muco-polysaccharide forming the outer layer of the bacterial cell wall and a similar muco-polysaccharide layer on the intestinal cells (Fuller and Brooker, 1974). Fibrils (extracellular attachments of bacteria) are often found on the adhering bacteria and might reinforce attachment to the gut wall (Fuller and Brooker, 1980).

Competitive attachment as a mode of action of DFM has recently gained a lot of interest among researchers because of the concept of competitive exclusion where the focus is to inhibit food-borne pathogens in the GIT, and results have shown a decrease in *E. coli* O157:H7, a food borne pathogen in the GIT (Krehbiel *et al.*, 2003).

2.4.2.3 Antibacterial effects

Bacterial species such as *Lactobacilli* have shown inhibitory action against pathogens. For example *Lactobacillus acidophilus* has been shown to be antagonistic toward enteropathogenic *E. coli*, *Clostridium perfringens*, *Salmonella typhimurium*, *Staphylococcus*

aureus (Gilliland and Speck, 1977). A study by Mann *et al.* (1980) indicated that a strain of *E. coli*, which causes illness and death when it is the sole microbial species in the GIT of young lambs, could be tolerated in the presence of *Lactobacilli*. Lactic acid has been shown to be effective in decreasing counts of coliforms throughout the GIT of piglets (Ratcliffe *et al.*, 1986), perhaps due to a reduced pH due to increased acid production, which can prevent growth of many pathogens (Fuller, 1977).

Lactobacilli produce hydrogen peroxide that appears to be responsible for the antagonistic interaction between bacteria (Gilliland and Speck, 1977). Hydrogen peroxide demonstrated to have bactericidal activity *in vitro* (Reiter *et al.*, 1980), but might have little participation in the gut since oxygen is required for its formation by *Lactobacilli*. Some reports suggest that antimicrobial proteins and/or bacteriocins either facilitate or mediate antagonism by *L. acidophilus* (Gilliland and Speck, 1977) but, because of the presence of proteolytic enzymes, their importance in the GIT might be limited.

In general, most yeast and lactic acid producing bacteria are destroyed by heat during feed pelleting (Kung, 2001). Spore forming bacteria (*Bacillus*) have advantages as probiotics for humans and animals (Ripamonti *et al.*, 2009), who also suggested that the inability to form spores provides DFM with higher resistance to stressors during their production and storage (Hyronimus *et al.*, 2000), and a higher resistance to gastric and intestinal environmental conditions (Hong *et al.*, 2005). Several recent studies demonstrated DFM effects of *Bacillus* (spore forming bacteria) on ruminant performance and *Bacillus* species are now known to inhibit gastrointestinal infection by pathogens, and/or by producing antimicrobials (Seo *et al.*, 2010).

Kritas *et al.* (2006) studied effects of both *B. licheniformis* and *B. subtilis* on young lambs and milking ewes where dietary inclusion of the DFM tended to reduce mortality of young lambs and increase the daily milk yield of the ewes. Another experiment with *Bacillus* (Qiao *et al.*, 2009) increased yields of 4% fat corrected milk and DM intake, while milk protein percentages were increased after *B. licheniformis* supplementation. In the latter study, total rumen VFA and acetate concentrations were higher with *B. licheniformis* treatment than in the other two groups of *B. subtilis* treatment, or in animals that received the control diet.

2.4.2.4 Immune Response

Modulation of the host animals immunity may represent a mechanism of action by which DFM promote intestinal health, as well as the overall well-being of the host animal (Isolauri *et al.*, 2001). The animal host immune system is capable of mounting both adaptive and innate immune responses against a wide diversity of pathogens. In addition to its part in digestion and absorption of nutrients, the GIT provides its host with a protective defense against a continuous presence of antigens from food and microorganisms in the gut lumen (Krehbiel *et al.*, 2003). Besides epithelial cells, immune cells in the GIT consist of dendritic cells, macrophages, natural killer cells, neutrophils, and B and T lymphocytes which are aggregated in Peyer's patches, lamina propria and intra-epithelial regions (Krehbiel *et al.*, 2003). This protective effect of the gut flora stems from the observation that shows germ-free

animals are more prone to disease than conventional animals with a broad intestinal flora population. For example Collins and Carter (1978) showed that germ-free mice were killed with 10 cells of *Salmonella enteritidis*, while it required 10^6 cells to kill a conventional mouse. The presence of a gut flora is the key factor in this difference because the LD₅₀ for germ-free and conventional mice is the same if they are challenged *in vivo* (Fuller, 1989). Upon infection by an antigen, immune cells are rapidly activated aiding to enhance phagocytosis as well as producing a vast selection of humoral mediators (Zhang and Ghosh, 2001). Antimicrobial peptides, Interleukin (IL)-1, IL-6, interferons (IFN), reactive oxygen/nitrogen intermediates and tumor necrosis factor- α (TNF- α) are among the first humoral mediators produced in reaction to pathogenic bacteria, and they collectively provide either direct protection for the host or help with development of specific immune responses (Krehbiel *et al.*, 2003). Cytokines formed later during microbial infection direct responses towards either cell-mediated T-helper type-1 (Th1) or humeral Th type-2 (Th2) immunity (Krehbiel *et al.*, 2003). Interleukin- 2 and IL-12 promote development of Th1 cells from naïve T cells, whereas IL-4, IL-10, and transforming growth factor- β (TGF- β) inhibit the production of TNF- α , IL-1, IL-6, IL-12, and IFN- γ , and thus enhance Th2 immune responses (Infante-Duarte and Kamradt, 1999).

2.5 Yeast based DFM

2.5.1 Introduction

Yeasts are single celled fungi which ferment carbohydrates and reproduce by budding (Chaucheyras-Durand *et al.*, 2008). Live yeasts and yeast cultures based on *Saccharomyces cerevisiae* are commonly used in diets of ruminants in commercial production systems (Adesogan, 2009). The strain of *S. cerevisiae*, as well as the number and viability of cells, vary widely among commercial products (Chaucheyras-Durand *et al.*, 2008), but a blend of live and dead *S. cerevisiae* cells are found in many commercial products (Adesogan, 2009). Products containing more live cells are classified as ‘live yeasts’ whereas those containing more dead cells together with the growth medium are classified as ‘yeast cultures’ (Adesogan, 2009). Not all strains of yeast are capable of stimulating digestion in the rumen (Newbold and Rode, 2006), which is not related to the number of living cells, but rather to their metabolic activity (Newbold and Rode, 2006).

2.5.2 Strain specific effects

Only 7 of over 50 yeast strains tested had the ability to stimulate growth of fiber digesting bacteria in the rumen (Dawson and Hopkins, 1991). Some studies suggest that very few strains of yeast have the ability to stimulate both bacteria associated with lactate utilization and the beneficial fiber digesting bacteria. Newbold *et al.* (1996) demonstrated that baker’s yeast and brewer’s yeast strains differ in their abilities to stimulate these critical groups of rumen microbes. The baker’s yeast strains had a limited ability to bring about microbial stimulation. Overall these studies suggest that care must be taken in selecting yeast strains for use as ruminant feed additives, and may explain some of the variability in production responses in the literature.

2.5.3 Modes of action of yeast DFM

2.5.3.1 Microbial stimulation

Ruminants fed live *S. cerevisiae* based DFM can have a significant increase in desirable live rumen bacteria (Fonty and Chaucheyras-Durand, 2006) and up to a 50% increase in viable rumen bacteria (Wallace and Newbold, 1993). The rumen fungus *Neocallimastix frontalis* is stimulated by adding live yeasts and yeast cultures because they supply thiamine, a B-vitamin required by rumen fungi for zoosporogenesis (Fonty and Chaucheyras-Durand, 2006), and live yeasts can also cause enhanced plant cell wall colonization by fungi (Chaucheyras-Durand *et al.*, 2008). Fiber digesting bacteria such as *Fibrobacter succinogens*, *Butyrivibrio fibrosolvans* and *Ruminococcus species* can also be stimulated by feeding yeast products, associated with increased fiber digestion and DM intake (Chaucheyras-Durand *et al.*, 2008).

Some studies have suggested that a more basic mechanism may be involved in the overall stimulation of the growth of beneficial rumen bacteria (Girard and Dawson, 1995). These studies lead to the isolation of a small protein-like N containing compounds that stimulate bacteria to enter logarithmic growth and thus stimulate microbial activity. The basic chemical characteristics of this stimulatory compound are consistent with those of small biologically active peptides (Girard 1996). Stimulatory activities of these small peptides can be demonstrated in studies with pure cultures of rumen bacteria (Girard, 1996). Synthetic tryptophan containing peptides have shown to bring about comparable stimulatory effects and to stimulate growth of representative fiber digesting bacteria from the rumen (Denev *et al.*, 2007). These stimulatory activities were not associated with individual AA, and occurred at concentrations well below those that would suggest that these compounds were limiting nutrients (Denev *et al.*, 2007). These small protein-like N containing compounds appear to serve as metabolic triggers that stimulate beneficial rumen bacteria to enter an exponential growth phase (Denev *et al.*, 2007). This stimulatory activity towards specific strains of rumen bacteria may explain many of the observed effects of live yeasts in the rumen that are otherwise difficult to understand. However the stimulatory peptides seem to be rumen unstable (Denev *et al.*, 2007) and thus difficult to isolate. The activity of proteolytic enzymes, and the rapid uptake of the peptides by microorganisms, may quickly eliminate these compounds from the rumen. This observation is consistent with requirements for metabolically active yeast (live yeasts) preparations that have been observed by a number of investigators (Dawson *et al.*, 1990; El Hassan *et al.*, 1993). It seems that the metabolically active yeast cells (live yeasts) can produce a continuous source of these peptides, and thus can continually provide low levels of stimulation that benefits many strains of rumen bacteria (Denev *et al.*, 2007).

2.5.3.2 Oxygen scavenging

Although the rumen environment is anaerobic, some dissolved oxygen is always present (Fonty and Chaucheyras-Durand, 2006). Oxygen enters the rumen through salivation, rumination and water intake (Fonty and Chaucheyras-Durand, 2006). Most rumen microorganisms, such as *Fibrobacter succinogens*, are anaerobic and highly sensitive to

oxygen (Chaucheyras-Durand *et al.*, 2008). One of the main possible benefits of live yeasts on fiber-degrading bacteria is the ability of the yeast cells to scavenge oxygen (Chaucheyras-Durand *et al.*, 2008). The redox potential of the rumen under *in vitro* and *in vivo* conditions was reduced with inclusion of live yeasts (Chaucheyras-Durand and Fonty, 2002), which implies that the live yeast cells created a more favourable condition for growth and activity of anaerobic microorganisms (Chaucheyras-Durand and Fonty, 2002). Live yeasts can release vitamins and other growth factors that are closely associated bacterial cells, and their impact on redox potential can also be microorganism mediated and not just a direct effect on oxygen consumption (Jouany, 2006).

2.5.3.3 pH Modulation

Live yeasts can stabilise rumen pH by stimulating entodiniomorphid protozoa (Chaucheyras-Durand *et al.*, 2008). Protozoa compete with the amylolytic bacteria in the rumen for starch as they engulf starch granules and thereby protect it from being fermented to lactate by bacteria (Mendoza *et al.*, 1993). Protozoa ferment starch at a much slower rate than amylolytic bacteria and the main fermentation end products are VFA rather than lactate (Mendoza *et al.*, 1993). By delaying fermentation and producing fermentation end products which don't cause such a prominent decline in pH, protozoa have a stabilizing effect on rumen pH (Fonty and Chaucheyras-Durand, 2006). Entodiniomorphid protozoa also take up some lactate and thereby inhibit it from accumulating in the rumen (Adesogan, 2009). Thus live yeasts can indirectly reduce the risk of rumen acidosis (Adesogan, 2009), and live yeast has been found to stimulate the activity of *Selenomonas ruminantium* which utilizes lactic acid (Nisbet and Martin, 1990).

2.5.3.4 Effect on rumen maturity and digestion

Although the newborn rumen is germ-free at birth, it is very quickly colonised by an abundant and complex microbial population (Chaucheyras-Durand *et al.*, 2008). The animal's mother, as well as other animals, produces saliva and faeces which comes into contact with the new-born animal (Chaucheyras-Durand *et al.*, 2008), and this, together with consumed vegetation contaminated with microbes, provides a continuous supply of rumen microorganisms which colonize the developing rumen as the conditions in the rumen become more favourable (Hobson and Stewart, 1997). The mother ruminant and offspring usually have prolonged contact with one other in extensive farming systems (Chaucheyras-Durand *et al.*, 2008), but in more intensive farming systems the separation of the young from its mother occurs soon after birth (Fonty *et al.*, 1987). This can lead to the transition from liquid milk to solid feed occurring before complete microbial colonisation of the developing rumen has occurred (Fonty *et al.*, 1987). An imbalance in the rumen microorganism composition may often be caused by this practice, and this can lead to digestive disorders and a higher risk of microbial infections which have been reported to be one of the main causes of mortality and economic losses in livestock (Collado and Sanz, 2007). The development of rumen functions such as digestion efficiency and absorption ability rely on the establishment of a complex microbial ecosystem in the rumen (Hooper *et al.*, 2001). Development of the immune system, as well as gut health, also depends on rumen microbial establishment (Hooper *et al.*, 2001).

Chaucheyras-Durand and Fonty (2001) observed that lambs fed live *S. cerevisiae* yeasts daily had a higher rate of establishment of the microbial population, and this population was more stable than those in the lambs receiving no supplementation. Bacterial communities have to be previously colonised in the rumen for ciliate protozoa to establish (Fonty *et al.*, 1988). However Chaucheyras-Durand and Fonty (2002) found that ciliate protozoa appeared more rapidly in the rumen of lambs fed live yeast products, suggesting that maturation of the rumen microbial ecosystem is accelerated by their supplementation (Chaucheyras-Durand and Fonty, 2002). Galvao *et al.* (2005) found that live yeast cultures resulted in a high efficiency of growth performance in young calves that did not receive colostrum, especially before weaning increasing average daily gain (ADG) and blood glucose levels. They also found that live yeast culture supplementation decreased the occurrence of diarrhoea in these animals (Galvao *et al.*, 2005). In a calf study by Lesmeister *et al.* (2004) where a yeast culture was supplemented, there was a positive calf performance response on DM intake, and ADG increased. These positive responses could be correlated to improved rumen development of characteristics such as papillae length and width, as well as rumen wall thickness (Lesmeister *et al.*, 2004).

Most research agrees that yeast and yeast culture supplementation strategies can have measurable effects on rumen fermentation, and some advantageous changes in rumen digestion have been reported. Studies in several laboratories have shown that live yeast supplementation can affect digestive processes in the rumen (Williams and Newbold, 1990; Newbold *et al.*, 1996; Wallace, 1996). Naturally, the extent of DM digestion was not significantly changed although the initial rate of digestion was influenced by addition of live yeasts to diets of ruminants (Kumar *et al.*, 1997). This is a characteristic of yeast supplementation that has been measured *in vitro* (Dawson and Hopkins, 1991) and *in vivo* (Williams and Newbold, 1990; Kumar *et al.*, 1997). Dry matter intake is often considered to be a function of initial rates of fiber digestion, and early stimulation of rumen activity can be expected to have a positive impact on DM consumption, which may be the driving force to improved animal performance.

2.5.3.5 Effects on rumen Nitrogen and Energy usage

In vitro addition of yeasts and yeast cultures has led to decreases in methane production in some studies (Lynch and Martin, 2002), while in others it showed only small differences (McGinn *et al.*, 2004). This inconsistency might be due to strain specific effects, stage of lactation of the cows or too short study duration in *in vitro* which allow insufficient time for yeasts to stimulate the growth of other rumen microbes (Newbold and Rode, 2006).

Live yeasts and yeast cultures fed to ruminants can increase rumen bacterial numbers, which leads to a higher rate of fermentation and microbial protein synthesis (Chaucheyras-Durand *et al.*, 2008). This should lead to increased fermentation which increases NH₃ uptake by microbes (Chaucheyras-Durand *et al.*, 2008), but published data have not been consistent in this regard. For example in some instances a decrease in rumen NH₃ concentrations occurred (Chaucheyras-Durand *et al.*, 2005) but in others there were no decreases in rumen NH₃ concentrations (Erasmus *et al.*, 1992).

Energy is required to support microbial protein synthesis and not all ammonia is incorporated into microbial protein (Chaucheyras-Durand *et al.*, 2008). It is therefore unfavourable to the host animal when excessive amounts of AA and peptides are rapidly converted to ammonia by rumen microbes (Chaucheyras-Durand *et al.*, 2008). High rumen ammonia levels consequently cause a large amount of N to be excreted in the urine as urea after absorption from the rumen as the ammonium ion (Chaucheyras-Durand *et al.*, 2008). Excreted urea is rapidly mineralised to ammonia and can be converted to nitrous oxide which has a global warming potential 12 times that of methane and 296 times that of carbon dioxide (Chaucheyras-Durand *et al.*, 2005). Nutritional strategies that aim to reduce N losses in the rumen are becoming more important because the role of livestock in global warming has been a concern for some time (Moss *et al.*, 2000). The ammonia concentration is the rumen N related parameter which is normally considered when determining the impact of yeasts *in vivo* (Newbold *et al.*, 1995). Rumen ammonia levels vary widely and depend to a large extent on factors including the nature of the diet as well as animal and microbial characteristics (Newbold *et al.*, 1995). Chaucheyras-Durand and Fonty (2001) found that in lambs raised in microbially controlled environments containing a very basic rumen microbial population, that the presence of live yeast in their diet reduced rumen ammonia concentrations. This also occurred in the rumen of newborn lambs (Chaucheyras-Durand and Fonty, 2001). In a ruminant study by Kumar *et al.* (1994), daily live yeast feeding caused similar effects on ammonia concentrations. These studies show that the presence of live yeasts can cause a change in the N metabolism of rumen microbes. Many *in vitro* studies have indicated that the growth and activity of proteolytic rumen bacteria can be influenced by yeast strains that inhibit their action on protein and peptides (Chaucheyras-Durand *et al.*, 2008). Chaucheyras-Durand *et al.* (2005) found competition between bacteria and live *S. cerevisiae* cells for energy supply and a direct inhibitory effect of yeast peptides on bacterial peptidases which may be a mechanism of yeast action that inhibits bacterial action. Chaucheyras-Durand *et al.* (2008) suggested that a production response to a probiotic yeast depended on the dietary level of soluble N, which included ammonia, amino acids and peptides. Microbial growth can be enhanced, and N loss can be reduced, by live yeast feeding when the optimal dietary balance between carbohydrate supply and soluble N are achieved (Chaucheyras-Durand *et al.*, 2008). The risk of acidosis can be decreased by not wasting digested carbohydrates as excessive production of VFA, but by incorporating them into the microbial mass by means of increased fermentative coupling (Chaucheyras-Durand *et al.*, 2008). However Erasmus *et al.* (1992) found that their live yeast product did not increase the amount and composition of microbial N reaching the duodenum in dairy cows, and Putnam *et al.* (1997) reported similar results.

2.5.3.6 Effects on animal performance

Animal performance can be improved by live yeast and yeast culture feeding (Adesogan, 2009). The performance enhancement varies depending on stage of lactation, diet composition and management (Adesogan, 2009). Dry matter intake, milk yield and milk solids have all been increased by addition of live yeasts and yeast cultures to ruminant diets (Robinson and Erasmus, 2008), and feeding a yeast culture led to a slight increase in feed efficiency in lactating dairy cows (Robinson and Erasmus, 2008).

2.6 Fungal DFM

2.6.1 Introduction

Fungi used as DFM include *Aspergillus niger* and *Aspergillus oryzae* fermentation extracts consisting of fungal spores and mycelium commonly dried onto a wheat bran base (Wallace and Newbold, 1995). *A. oryzae* is the most commonly fed fungi to ruminants and is known to contain high levels of amylase activity (Tricarico *et al.*, 2005).

2.6.2 Modes of action of fungal DFM

2.6.2.1 Rumen environment

Like yeasts, treatments with *A. oryzae* have increased total and cellulolytic rumen bacterial populations (Wiedemeier *et al.*, 1987) and this can be partly attributed to the dicarboxylic acids in *A. oryzae* extracts (Hobson and Stewart, 1997), and increases in numbers of cellulolytic bacteria also contribute to improved fiber digestion. Like live yeast and yeast cultures, *A. oryzae* extracts are used in the food industry as flavor enhancers, suggesting that an increase in palatability may be a mode of action (Wallace and Newbold, 1995) but for this theory to be affective, dietary inclusion levels of the extracts should be much higher than has been the case in *A. oryzae* studies.

Addition of *A. Oryzae* has resulted in variable effects on rumen pH (Wiedemeier *et al.*, 1987), methane production (Nisbet and Martin, 1990), microbial yield (Gomez-Alarcon *et al.*, 1990), amino acid deamination (Frumholtz *et al.*, 1989), and microbial N flow (Gomez-Alarcon *et al.*, 1990). Effects on DM intake and digestibility are also inconsistent.

Cellulase, esterase, and xylanase enzymes in the extract possibly account for the fiber hydrolysis that is often reported when *A. oryzae* is included to substrates *in vitro* or to ruminant diets (Varel *et al.*, 1993). Some research showed improved fibrolysis can be diet and forage species specific (Gomez-Alarcon *et al.*, 1990, Wallace and Newbold, 1995).

2.6.2.2 Effects on animal performance

Dairy calves fed *A. Oryzae* were weaned 1 week earlier (Adesogan, 2009) which is associated with increased numbers of rumen bacteria as well as rumen VFA concentrations. Increases in DM intake, DM digestibility and milk yield have been observed when *A. Oryzae* was fed to ruminants. Thus *A. Oryzae* treatment affected digestibility about two thirds of the time and improved milk yield about half of the times it was fed (Adesogan, 2009). Fungal feed additives tend to have small effects on rumen NH₃ and VFA concentrations (Newbold *et al.*, 1995). Wallace and Newbold (1995) also reported that, across eight studies, *A. Oryzae* feeding resulted in 4.3% more milk (Adesogan, 2009). Like yeasts, effects of *A. Oryzae* also tend to be dependent on diet composition and lactation stage.

2.7 Summary

Lack of organism specificity, appropriate dose, survival in the rumen and difficulty in defining when ruminants are actually stressed are some of the reasons for a lack of a production response when DFM are fed. Direct fed microbial products are often fed to high producing dairy cows in early lactation due to their negative energy balance, and young calves during rumen development and weaning because of metabolic stress. In this literature review, the different DFM feed additives (*e.g.*, bacterial, yeast, fungal), their modes of action and their effects on performance of ruminant animals were discussed. Although positive results have been obtained with all these additives, results are not consistent and thus why specific products was tested in this trial.

The fact that a DFM product is ruminally bioactive and increases animal performance does not prove that it was the rumen bioactivity which caused the increased animal performance. Indeed, all the proposed ruminal modes of DFM action are theoretically sound, but the feeding levels in animal studies are virtually always too low to support them. While studies should be completed in specific farming systems in order to ensure that DFM are efficacious, more research is needed on the proposed modes of actions of DFM in order to better understand the metabolic reasons for their efficacy and to better predict the animal responses when they are fed.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study location, duration and experimental design

The study was conducted on a commercial dairy farm near Hanford (CA, USA) and encompassed 12 weeks starting on the 23rd of January 2014 and ending on the 17th of April 2014. The experimental period was divided into 3 periods of 4 weeks each, using a 3 × 3 Latin square experimental design.

In every experimental period there was a 3 week adaption period followed by a 4th week for sample collection. Samples were collected of individual feedstuffs as well as the total mixed ration (TMR), the DFM yeasts, urine, blood, milk and faeces. Body condition scoring (BCS) was completed at the start of the study and at the end of each experimental period.

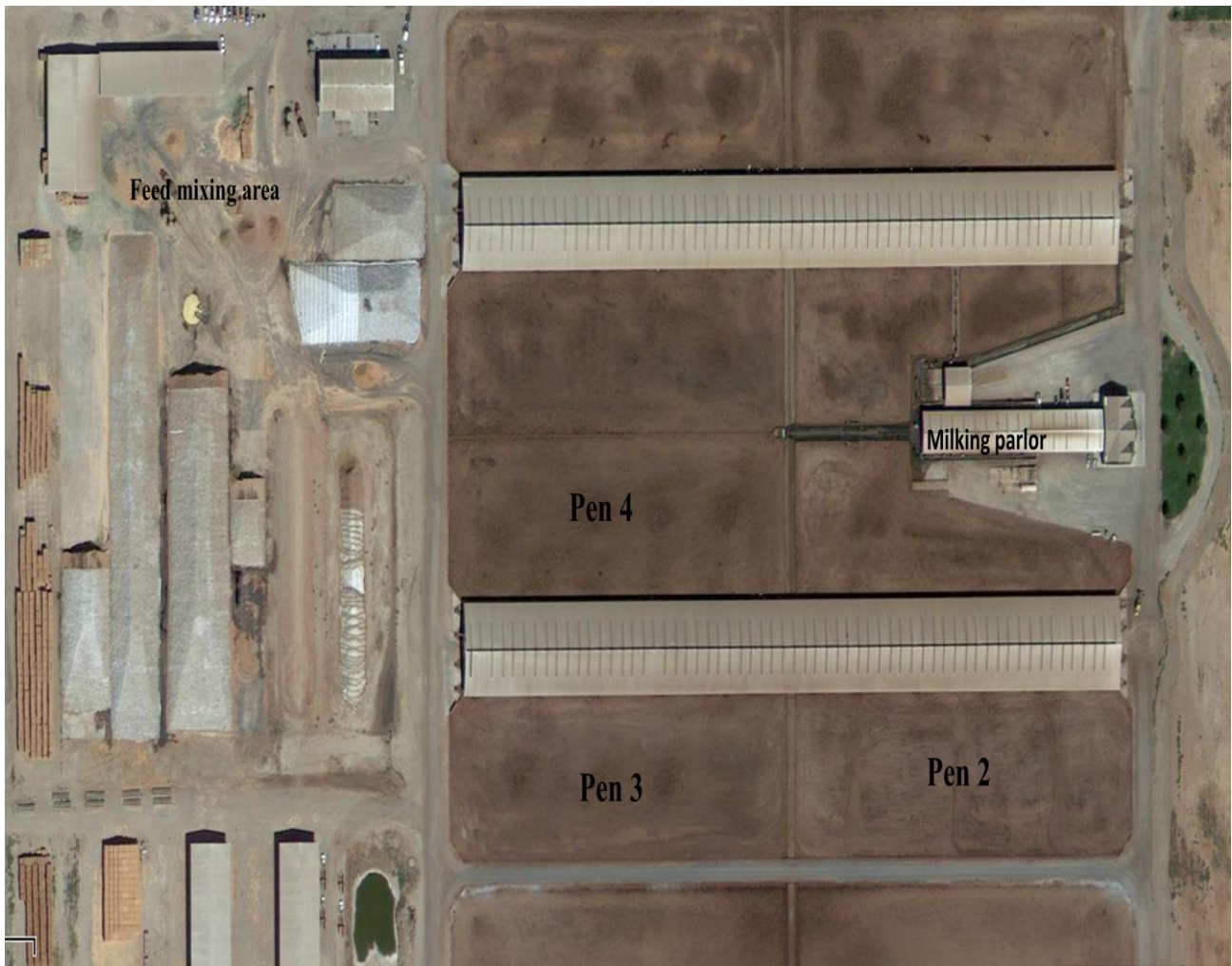


Fig.3.1 Aerial view of the commercial dairy farm near Hanford (CA, USA)

The experiment was designed to ensure that all samples from the cows could be collected while they were in ‘lock up’ (*e.g.*, the 45 minute period directly after the first milking of the day when cows come back to fresh feed and when the farm breeding staff

assessed the health of the cows, did pregnancy diagnoses and artificial insemination of the cows).

The experimental design was a 3×3 Latin-square with 3 pens, 3 dietary treatments and 3 experimental periods. According to Dr. Paul Weimer, a rumen microbiologist at the University of Wisconsin (pjweimer@wisc.edu) a 3 week adaptation period is sufficient to prevent any carryover effects from previous diets or feed additives on rumen fermentation. Each pen was subjected to a different dietary treatment during each experimental period. The dietary DFM yeast based additive changed with each period between pens so that at the end of the study each pen had been fed each of the experimental treatments. The treatment TMR's differed only in that they contained, or did not contain, the different DFM yeast based feed additives. Each pen received one of the following treatments during the experimental periods:

- 1) Basal TMR (Control).
- 2) Control supplemented with the 'DV XPC' yeast culture (14 g/cow/d).
[Diamond V Mills, Cedar Rapids, IA, USA]
- 3) Control supplemented with the Yeasture DFM (10 g/cow/d).
[Cenzone Tech, Inc., San Marcos, CA, USA]

Table 3.1 Assignment of treatments to each pen during the experiment.

Period	Pen 2	Pen 3	Pen 4
1	Diamond V XPC	Cenzone Yeasture	Control
2	Cenzone Yeasture	Control	Diamond V XPC
3	Control	Diamond V XPC	Cenzone Yeasture

3.2 Experimental animals

Three pens containing an average of 315 multiparous high producing Holstein cows not yet confirmed in calf and milked three times daily in a double 35 herringbone parlor were used. Cows were moved out of these 'high' pens to a common 'mid' pen after being confirmed pregnant at about 200 days in milk (DIM). Thus cows were selected to participate in the study relative to animal based parameters based on their DIM being below 115 at the beginning of the study in order maximize the likelihood that they would remain in their initially assigned pens for the entire experiment.

3.3 Pens

The pens were similar with a single feed bunk under a roof structure equipped with 297 free stalls with dried manure solids as bedding which was restored weekly, as well as 295 functional head gates/pen (used to examine cows in morning 'lock up') with water misters above the headlocks. There were also rubber mats in the walkway between the free stalls and the milking parlor as well as along the feed bunk in the pen in order to minimize foot and leg injuries. Cows were also allowed access to an enclosed dirt lot outside of the roofed area daily, except during morning 'lock up'. Clean drinking water was available at all times.

3.4 Weather data

Weather data was collected using a HOBO Temperatures/Relative humidity logger (HOBO U23 Pro v2, Onset, Cape Cod, Mass, USA) that recorded temperature (temp) and humidity every 30 min for the duration of the study. The weather logger was placed in a pen area, protected by a Hobo shield, at a sufficient height to eliminate animal interference. Weather data is shown in Appendix on Fig A1 and Table A1.

3.5 Diets

The TMR were fed twice a day, but the yeast additives were only included in the first load that was fed in the morning when the cows were being milked. The second feeding, to ensure *ad libitum* feed was available all day, was fed between 11:30 and 12:30 h and did not contain the DFM yeast as it was not fed pen specific. The TMR's were pushed up several times during the day to encourage consumption.

Diet ingredients included lucerne hay of high, medium and low quality, whole crop wheat silage, whole crop maize silage, rolled maize grain, wet citrus pulp, fresh chop lucerne and a high cow premix that included almond hulls, cottonseed (whole upland), mineral premix, canola meal (solvent extracted), mold binder, sodium bicarbonate, tallow, distillers dried grain with soluble (DDGS) and liquid molasses.

3.6 The DFM yeast additives

Diamond V XPC (DV XPC) is a yeast culture manufactured by Diamond V mills (Cedar Rapids, IA, USA) containing 15% CP (minimum), 1.5% crude fat (minimum), 25% crude fiber (maximum) and 9% ash (maximum)/kilogram of additive as defined by the manufacturer. Product ingredients are *Saccharomyces cerevisiae* yeast and some of the media on which it was grown, many being processed grain by-products and cane molasses. The DV XPC was fed at 14 g/cow/d according to manufacturer recommendations.

Cenzone Yeasture (Yst) is a yeast based DFM manufactured by Cenzone Tech, Inc. (San Marcos, CA, USA). Yeasture contains 18% CP (minimum), 1.6% crude fat (minimum) and 9% crude fiber (maximum), *Bacillus subtilis* of minimum 1.8×10^{11} CFU and *Saccharomyces cerevisiae* of minimum 4.5×10^{12} cells/kg of additive as defined by the manufacturer. The ingredients include *Saccharomyces cerevisiae* yeast and its cell wall extract, *Aspergillus oryzae* fermentation extract, *Bacillus subtilis* and fermentation extract. Yeasture was fed at 10 g/cow/d according to manufacturer recommendations.

3.7 Feeding of the yeast additives, feed mixing and feed delivery

The DFM yeast additives were weighed on a weekly basis into bags for daily feeding that were pre-marked by pen and stored within easy access to the feeding staff.

A premix was prepared prior to mixing the TMR consisting of almond hulls, cottonseed (whole upland), mineral premix, canola meal (solvent extracted), mold binder, low quality lucerne hay, sodium bicarbonate, tallow, DDGS and liquid molasses.

The TMR's were mixed by loading all feed ingredients into a two screw vertical mixing wagon (Model 1200 T Supreme Feed Processor, Duport TMR Equipment Co., Inc., Visalia, CA, USA) that was on an electronic scale while electronically recording the actual weight of all feeds added. The DFM yeast additives were added to the TMR with the premix. The "TMR tracker" system (Digi-Star LLC, Fort Atkinson, WI, USA) kept an electronic record of the actual ingredient levels in each batch of TMR.

3.8 Sampling and data collection

3.8.1 Sampling TMR's and feedstuffs

During each collection period TMR and feedstuff samples were collected on day 21 and day 26. A 3.7 liter plastic bag was used for bulky and wet ingredients such as silages and citrus pulp, while 1 liter plastic bags were used for less bulky ingredients such as canola meal and DDGS.

The TMR samples were collected according to guidelines of Robinson and Meyer (2010), by walking down the bunk line and taking 10 handfuls of the TMR (about 1.1 kg total) from predetermined locations in the middle of the TMR bunk line directly after it was fed. No cows were in the pens during feeding and sampling. Larger particles were cut into smaller pieces and the sample was mixed by turning it upside down and then quartering it on a clean surface before packing half of it into a 3.7 L plastic bag.

Hays and straw was sampled with a golf club style hay probe (Seifert Analytical, Lodi, CA, USA) by taking 8 to 15 samples. Other samples were collected with a gloved hand by taking 4 to 8 handfuls of the sample and placing it in either in a 3.7 L plastic bag or 1 L plastic bag depending on its bulkiness. All the samples were stored immediately at -20°C.



Fig3.2 Quartering of TMR samples

3.8.2 Dry matter intake

Each morning orts were pushed out of the bunklines to clear the feed bunks prior to fresh TMR being fed. Orts were also weighed each morning and the amount of orts was recorded for each pen. The amount of TMR fed/pen was also recorded electronically by the Digi – Star electronic system.

3.8.3 Urine collection

On day 26 of each experimental period a group of 130 to 160 cows/pen were marked (selecting the cows with DIM < 115) with 3M Nexcare waterproof plaster (3M Consumer Health Care, St. Paul, MN, USA) and Super 77 multipurpose adhesive glue (3M Stationary Products Division, St, Paul, MN, USA) by sticking the plaster with the corresponding cow number on the rump of the cow to ensure that cow numbers could be accessed from behind the cows during urine collection (Figure 3.3).



Fig3.3 Example of how the cows were marked for urine collection

Urine collection occurred on day 27 of each experimental period while cows were in morning lockup. Urine was collected from the free urine flow of voluntarily urinating cows which had been marked as described above. Once the sample was collected into a 500 ml plastic cup, a lid was placed on top and the urine collector wrote the cow number from the plaster next to the cup number in a booklet and then placed the cup on ice in a cooler box.

Pre-marked urine tubes were set out and marked for the period and pen for urine sub-sampling. The specific gravity (SG) was measured using a pen refractometer (Digital Hand-held "PEN" Refractometer PEN-RI, ATAGO, Tokyo, Japan). Into each urine tube 1.4 ml of a

0.5 molar sulphuric acid dilution was added to prevent microbial degradation of allantoin (Al) and then 7 ml of the urine was added to the tube with the corresponding sample number. The pH of these samples was measured with a pH meter (Oyster 10 series, EXTECH instruments, Nashua, NH, USA) to ensure that the pH was below 3. Urine samples were then diluted with 27 ml of deionized water and samples placed in a freezer at -20°C .

3.8.4 Blood sampling

Blood collection occurred on day 28 of each experimental period by sampling tail vein blood from the same pre-selected group of 16 cows/pen in each period. Cows were selected from those from which urine had been collected in period 1 and the first cows encountered in the pens were sampled. Blood collection was from the coccygeal vein into a green top evacuated tube containing K_2 EDTA (Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA). After the sample had been collected, blood samples were placed on ice.

Blood sub-sampling was completed by centrifuging (SorvallTM ST 16 R Centrifuge series, Thermo Scientific Inc, Waltham, MA, USA) them at 4°C at $2100\times g$ for 15 min. Plasma was transferred into Eppendorf tubes and then placed in a freezer at -20°C .

3.8.5 Fecal sampling

Fecal samples were collected on day 28 of each period from the same pre-selected group of 18 cows/pen in each of the periods. Cows were selected from those from which urine had been collected in period 1 and the first cows encountered in the pens were sampled. If spontaneous defecation did not occur, samples were obtained by inserting one hand into the cow's rectum to stimulate defecation. After collection, sample containers for each cow were wiped and frozen at -20°C .

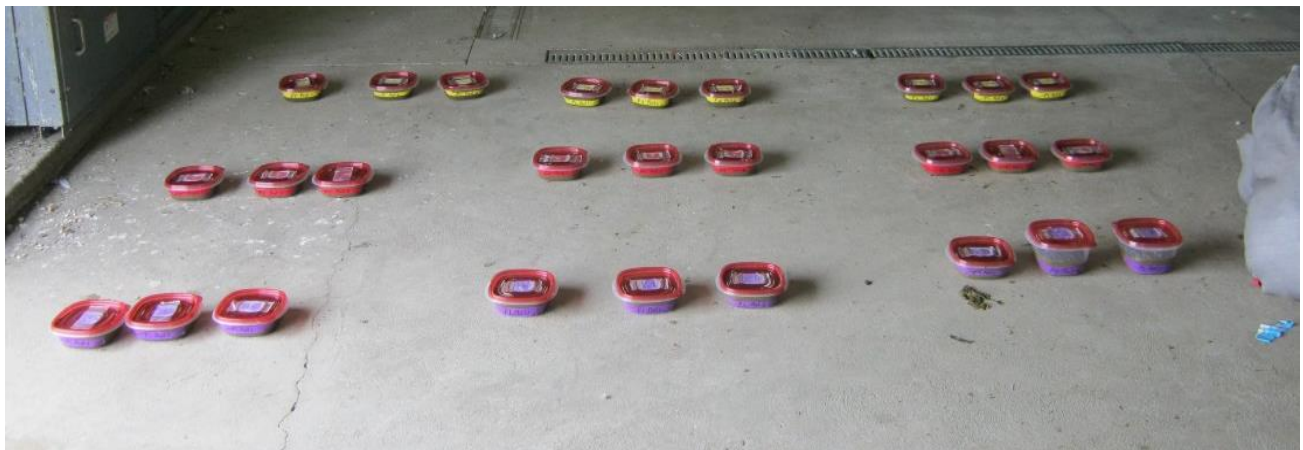


Fig.3.4 Pooled fecal samples

At the end of the last fecal sampling period, fecal samples were pooled by mixing three groups of 6 cow's fecal samples/pen to create 3 representative fecal samples/pen/period. This was completed to support the assumption that the TMR consumed by these groups of 6 cows/pen groups were representative of all cows in the pen in order to support digestibility calculations. Cow groups were created by sequential cow number.

3.8.6 Milk sampling

The Dairy Herd Improvement Association (DHIA) in Hanford (CA, USA) collected milk samples at the end of each experimental period on day 28 using WB Auto Samplers (Tru-Test Incorporated, Mineral Wells, TX, USA) to create composite milk sample for each cow and to record total milk yield. Cows used for production data were all cows that were in their same pens for all 3 periods.

3.8.7 Body condition scoring

A BCS was assigned to cows at the beginning of the study and at the end of each experimental period in order to calculate change in BCS. The 5 point scoring system was used where a score of 1 indicates severe under condition and a score of 5 indicates severe obesity (Wildman *et al.*, 1982; Ferguson *et al.*, 1994). Body condition scoring was completed to the nearest 1/8 point after the morning milking while the cows were in lockup.

3.9 Sample preparation and assays

3.9.1 Feed

Feed and TMR samples was analysed for DM, ash, CP, ash free neutral detergent fiber (aNDFom), acid detergent fiber (ADF), lignin(sa), free sugars (soluble carbohydrates), acid detergent insoluble CP (ADICP), macro and micro minerals and fat (EE). Three TMR samples/period (1 pooled sample/pen) and 1 pooled sample of each feed/period were analysed at the UC Davis Service Lab.

The DM content of the TMR and wet ingredients (*e.g.*, lucerne fresh chop, silages, citrus pulp) was calculated by measuring gravimetric weight losses in a forced air oven for 48 h at 55°C, leaving it to air equilibrate for 24 h, with analytical DM determined as the gravimetric weight loss by heating the air equilibrated sample to 105°C for 3 h (NFTA, 2001). The final DM value was then calculated by multiplying the air equilibrated DM value by the analytical DM value. All samples were ground to pass a 1 mm screen on a model 4 Wiley Mill (Arthur H. Thomas, Philadelphia, PA, USA). Total N and ADIN were determined by the Leco method with an N gas analyzer using an induction furnace to ignite samples to 900°C and a thermal conductivity detector to determine the N content (Method 942.05, AOAC, 2005). The CP was calculated from the N content as $N \times 6.25$. Lignin(sa) was determined by the reflux method using sulfuric acid and heat to dissolve solubles, leaving a residue of lignin(sa). The ADF was determined gravimetrically as the residue remaining after AD extraction (Method 973.18, AOAC, 1997). The NDF was determined by the reflux method using sodium sulfite and heat (Van Soest *et al.*, 1991). Heat stable amylase was added to all samples and NDF is reported ash free (*e.g.*, aNDFom). Total ash determination was based on gravimetric loss by heating samples to 550°C for 8 h. Soluble carbohydrates were determined by high-performance liquid chromatography (HPLC) using a Phenomenex Luna NH₂ (250 mm × 4.6 mm) HPLC column at a flow rate of 2.75 ml/min, acetonitrile: water (78:22) (Johansen *et al.*, 1996). Soluble N was determined by the borate phosphate buffer procedure (Krishnamoorthy *et al.*, 1982). EE was quantified using a standard Soxhlet

extraction during which fat was dissolved in ethyl ether and residues determined gravimetrically after drying (Method 2003.05, AOAC, 2006).

Most minerals (*e.g.*, P, S, Ca, Mg, Na, Zn, Mn, Fe, Cu, Co, Mo) were determined using a nitric acid/hydrogen peroxide microwave digestion/dissolution of samples and quantitative determination by atomic absorption spectrometry (AAS) and inductively coupled plasma by atomic emission spectrometry (ICP-AES) (Meyer and Keliher, 1992). Total K was determined by atomic emission spectrometry (AES) and Cl by chloridometer after both minerals were extracted by 20 g/l acetic acid (Johnson and Ulrich, 1959). Total Se was extracted by nitric/perchloric acid digestion/dissolution and determined by vapor generation using inductively coupled plasma atomic emission spectroscopy (Tracy and Moeller, 1990).

3.9.2 *In vitro* gas production

In vitro gas production used the method described by Blümmel and Ørskov (1993) with calibrated 100 ml syringes of 31 mm internal diameter (Model Fortuna, Häberle Labortechnik, Lonsee – Ettlenschieb, Germany). Each sample of 200 mg was incubated in 30 ml buffered rumen liquor in a water bath at 39°C. Rumen fluid was collected from 2 dry cows fed an all hay diet, and the rumen liquor was filtered through 3 layers of cheese cloth. Gas recordings were at 0, 4, 24, 30 and 48 h where the 4 h reading is an indicator of the rapidly fermentable fraction of the ration (Groot *et al.*, 1996), the 24 h value is indicative of the metabolizable energy (ME) value of the diet at maintenance (Menke and Steingass, 1988), the 30 h value is indicative of the energy available to high producing cows (Robinson *et al.*, 2004), and the 48 h cumulative gas production is used as an indication of the diets practical extent of *in vitro* digestibility (Robinson *et al.*, 2004). A known reference lucerne hay was used as the internal standard with a 200 mg sample weighed to duplicate syringes. A mixture containing 472.5 ml distilled water, 236.25 ml main element solution, 236.25 ml buffer solution, 1.20 ml resazurin solution and 0.1185 ml trace element solution per liter was prepared in an Erlenmeyer flask. After heating the solution to 39°C under continuous flushing with CO₂, a reduction solution consisting of 45.0 ml distilled water, 1.875 ml 1N NaOH, and 296.25mg Na₂S•9H₂O was added. The ratio of rumen liquor to buffer was 1:2. Syringes were stirred gently by hand before each measurement. Total gas values were adjusted for blank incubations, but not for the lucerne hay standard as its values were within range for all *in vitro* runs.

All 19 samples (*e.g.*, 3 TMR/period, 1 control TMR with DV XPC added/period, 1 control TMR with Yeasture added/period as well as 2 standards and 2 blanks) were assayed in one run. The control TMR with directly added DV XPC and Yeasture were prepared to ensure viable cell cultures which could have been impacted by the 105°C drying of the TMR samples that contained them and was sampled from the bunklines. To do this the DFM yeasts were added to the control diets and mixed in a tumble drier for 12 min.

3.9.3 Urine

Only cows with repeated urine samples (*e.g.*, collected in 2 or 3 periods) were used for allantoin (AL) analysis. Urine samples were analysed by a colorimetric method according to

Chen and Gomes (1992). Standards was prepared to create working concentrations of 20, 40, 60, 80 and 100 mg/L AL. Urine samples were thawed and then centrifuged (IEC Centra CL3, Thermo Scientific Inc, Waltham, MA, USA) at 1200×g for 15 min at 20 to 22°C to remove precipitate which can influence the colorimetric reading. Samples were diluted 60 times to fit the standard curve. A duplicate standard curve was included at the start and end of each run. Two inter-run standard samples were used in each run to assess variation among runs but, because all inter-run standards were within 0.1 of the average over all runs, runs were accepted without inter-run correction. Each urine sample was analysed in duplicate with the average used as the final concentration.

3.9.4 Blood

Blood samples were sent to the Molecular Structure Facility (University of California, Davis, CA, USA) for physiological AA (*e.g.*, free plasma AA) analysis. Four cows/pen/period, selected to be part of the fecal cows were assayed. Samples were acidified with sulfosalicylic acid to precipitate intact proteins and then AA were quantified using a Hitachi 8800 AA analyzer (Beckman Coulter, Inc., Tokyo, Japan) utilizing a lithium citrate buffer system and ion-exchange chromatography to separate AA followed by a “post-column” ninhydrin reaction detection system.

3.9.5 Faeces

Fecal samples were analysed for DM, ash, ash free neutral detergent fiber (aNDFom), acid detergent fiber (ADF), lignin(sa), starch and CP. All analysis was as described earlier for feed and TMR samples.

3.9.6 Milk

Milk fat, true protein and lactose concentrations as well as somatic cell counts (SCC) were determined using infrared (IR) spectroscopy at the Dairy Herd Improvement Association (DHIA) laboratory in Hanford (CA, USA).

3.10 Calculations

DM intake

Dry matter intake was calculated by recording the amount of TMR offered/pen/d for each day during the 7 day collection week and then subtracting allorts values to allow calculation of intake/pen/wk during the collection week. The ‘as is weight’ was then multiplied with the DM proportion of the TMR to obtain the DM intake/pen. The DM intake/pen/wk was then divided by the sum of the cows/pen/d for the collection week to yield DM intake/cow/d.

Urine volume

Urine volume (L/d) was estimated using an equation derived from data published by Burgos *et al.* (2005) as:

$$\text{Urine volume} = 332.66 \times ((\text{SG}-1) \times 1000)^{-0.884}$$

Daily PD excretion and rumen Microbial CP flow

Calculations were according to Chen and Gomes (1992). The AL values were first converted from mg/l AL to mmol/l AL and then multiplied by the daily urine volume to determine daily AL excretion/cow followed by calculation of total urine PD (mmol/d) excretion as:

$$\text{Milk PD (mmol/d)} = \text{Total urine PD (mmol/d)} \times 0.05$$

Where: AL and uric acid in milk is estimated to be 5% of that excreted in urine.

$$\text{Total excretion of PD was: (mmol/d)} = \text{Milk PD (mmol/d)} + \text{Total urine PD (mmol/d)}.$$

Microbial purines absorbed (mmol/d) was then calculated as:

$$(\text{Excretion of PD in urine (mmol/d)} - 0.385 \times W^{0.75}) / 0.85.$$

Where $(0.385 \times W^{0.75})$ is Endogenous PD contribution, $W^{0.75}$ is the metabolic body weight of the cow and 0.85 is the recovery of absorbed PD.

Finally intestinal flow of microbial N (gN/d) was calculated as:

$$\text{Microbial purines absorbed (mmol/d)} \times 70/0.116 \times 0.83 \times 1000$$

Where: 70 is the N content of purines is 70mg N/mmol. Digestibility of purines is 0.83. The ratio of purine-N: total N in mixed rumen microbes is taken as 11.6:100 and microbial CP was calculated as microbial N \times 6.25.

Production, body condition score and total energetics:

Milk energy (MJ/kg) was calculated as:

$$(((41.63 \times \text{g/kg fat}) + (24.13 \times \text{g/kg true protein}/0.934) + (21.6 \times \text{g/kg lactose}) - 11.72) \times 4.185) \times 2.2046$$

According to Tyrrell and Reid (1965) where 0.934 is the conversion factor from true to crude protein. 4.185 converts Mcal to MJ 2.2046 convert Mcal/lb to Mcal/kg.

Milk energy output (MJ/d) was calculated as:

$$\text{Milk energy (MJ/kg)} \times \text{milk yield (kg/d)}$$

Differences in BCS were calculated by subtracting the initial BCS from the final BCS for each cow in each period to obtain change in BCS. The energetic value of the BCS change was calculated as:

$$(\text{BCS} \times 300/28) \times 4.184$$

Where 1 unit BCS change over 28 days 300Mcal net energy for lactation (NE_L) (Chilliard *et al.*, 1991) with the factor of 4.184 converting Mcal/day to MJ/day.

NE maintenance was calculated as:

$$((\text{BW})^{0.75} \times 0.08) + \text{NEmact (NRC, 2001)}$$

Where: NEmact = variable to calculate NE activity requirements

$$= ((\text{walking distance}/1000 \times \text{trips}) \times (0.00045 \times \text{BW})) + (0.0012 \times \text{BW})$$

Walking distance to milking parlor is 0.5 km, trips is the number of trips to the milking parlor/day, body weight (BW) is assumed to be 675 kg.

NE_L density (MJ/kg DM) of TMR's was estimated using the biological response of the cows, as expressed in the partial NE output, and measured DM intake as:

NE output (MJ/d) / DM intake (kg/d)

Whole tract nutrient digestibility

Whole tract nutrient digestibility was calculated as:

$$1000 - (1000 \times ((\text{g/kg lignin(sa)}_{\text{TMR}} \times 0.95 / \text{g/kg lignin(sa)}_{\text{Faeces}}) \times (\text{g/kg nutrient}_{\text{Faeces}} / \text{g/kg nutrient}_{\text{TMR}})))$$

Assuming that lignin(sa) in the TMR is 950 g/kg indigestible and will be recovered in faeces (Stensig and Robinson, 1997).

In vitro gas production

Gas production (ml/g OM) was calculated as:

$$((\text{gas production/h since last recording}) - (\text{Blank piston hour since last recording})) / (\text{TMR analytical DM, g/kg}) / (\text{TMR OM, g/kg})$$

ME (MJ/KG DM) was calculated as:

$$\text{ME (MJ/kg DM)} = 1.25 + (0.0292 \times 24\text{h gas (ml)}) + (0.0246 \times \text{g/kg fat}) + (0.0143 \times (\text{g/kg CP} - \text{g/kg ADICP}))$$

According to Robinson *et al.* (2004).

3.11 Statistical analysis

For all statistical analysis only cows that were in their originally assigned pens for each of the 26 consecutive Dairy Comp 305 (Valley Ag Software, Tulare, CA, USA) herd data backups during the 12 week study were used. Any cow that moved from their originally assigned pen for any reason was not used. This resulted in an initial list group of 386 cows for milk production, 329 cows for BCS and 64 cows for urine based response parameters. Outlier analysis completed blind to treatments identified 20 cows which were removed: 11 cows from the milk data set, 8 cows from the BCS dataset, and 1 cow from the urine dataset. This resulted in a total number of cows used for statistical analysis of 375 for milk production, 321 for BCS and 63 cows for urine parameter analysis. As previously described, all these cows were in their initially assigned pens for the entire study.

Animal production data, BCS, SG, urine volume, urine AL, microbial CP flow and plasma AA were analysed using the MIXED Model of the Statistical Analysis Software (SAS, 2000) for a 3 × 3 Latin Square design with cow within pen as the random effect and period, pen and treatment as class variables.

For DM intake ($n = 3$ pens, calculated on a pen basis with 3 pens/period), TMR components and composition, TMR nutrient profile, whole tract digestibility and *in vitro* gas

production at 4, 24, 30 and 48 h the General Linear Model (GLM) of SAS (2000) was used with period, pen and treatment as fixed effects.

Significance of differences between each DFM yeast treatment and the control was determined using the PDIFF function in SAS (2000), with $0.05 < P < 0.10$ accepted as a tendency to differ and $P < 0.05$ as indicator of a significant difference. The 2 products was not tested against one another because it was 2 different products as described earlier.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Ration evaluation

The nutrient profile of the feeds fed are shown in Tables 4.1 and 4.2 while the ingredient composition of the experimental diets is in Table 4.3 and the nutrient profile of experimental diets is in Table 4.4.

Table 4.1 Chemical analysis (\pm standard deviation ^b) of forage and wet byproduct ingredients used in the total mixed rations fed to dairy cows (g/kg DM). ^a

	Lucerne hay (HQ) ^c	Lucerne hay (MQ) ^d	Wheat silage	Maize Silage	Lucerne fresh chop	Citrus Pulp
Dry matter	933 (3.4)	938 (7.9)	318 (13.2)	311 (3.5)	266 (16.1)	152 (30.0)
Organic matter	876.0 (10.29)	890.5 (11.37)	880.0 (3.66)	932.1 (1.26)	894.8 (39.42)	956.3 (5.79)
Crude protein.	214.6 (8.32)	210.8 (27.88)	104.0 (3.44)	76.0 (1.44)	216.1 (1.99)	82.1 (5.60)
aNDFom ^e	320.7 (16.01)	362.3 (30.99)	503.0 (33.29)	435.7 (5.86)	332.8 (42.07)	197.5 (45.65)

^a Average of a total of 6 samples, 2 samples collected during the last week of each of the 3 periods.

^b Standard deviation of the mean.

^c High quality lucerne hay as classified by the dairy.

^d Medium quality lucerne hay as classified by the dairy.

^e Neutral detergent fiber assayed with heat stable amylase expressed exclusive of residual ash.

The nutrient profile of feeds in Tables 4.1 and 4.2 are generally similar to California feeds as reported by Swanepoel *et al.* (2010) and Rauch *et al.* (2012) and similar to feeds listed in NRC (2001). The aNDFom of the canola pellets were slightly lower than values reported by Swanepoel *et al.* (2010), but within the range of NRC values. The low quality lucerne hay had a higher concentration of aNDFom compared to results presented by Swanepoel *et al.* (2010) but it was within the ranges reported by Rauch *et al.* (2012). Wheat straw was much lower in CP compared to Swanepoel *et al.* (2010) and the aNDFom was slightly higher compared to Swanepoel *et al.* (2010), but it was within the range reported by Rauch *et al.* (2012).

The TMR of the three treatment diets were similar in ingredient (Table 4.3) and nutrient (Table 4.4) composition and there were no differences among them. The only substantial difference between diets was addition of the DFM yeast additives. The ingredient composition and nutrient profile of the diets are very similar to those reported by Swanepoel *et al.* (2014) and Rauch *et al.* (2012). The diet was composed of 16 ingredients which may explain the low variation in inclusion rates of ingredients when compared to other studies, as

only 10 to 14 ingredients are commonly used to formulate a TMR in typical California dairy rations (Swanepoel et al., 2010). It may also explain the low levels of variation when comparing the diet nutrient profile to the diets of Swanepoel et al. (2014) and Rauch et al. (2012). Overall, the basal experimental TMR was judged to be representative of typical California dairy rations and the TMR complies with the nutritional requirements of large breed dairy cows at a similar level of production, with a small oversupply of most micro minerals according to NRC (2001) recommendations.

Table 4.2 Chemical analysis (\pm standard deviation ^b) of concentrate and premix ingredients used in the total mixed rations fed to dairy cows (g/kg DM).^a

	Maize (rolled grain)	Almond hulls	Cottonseed, (whole upland)	Canola (pellets)	Lucerne, hay (LQ) ^c	Wheat straw	DDGS ^d (meal)
Dry matter	848 (4.6)	944 (1.5)	889 (10.0)	919 (3.0)	938 (2.1)	955 (8.4)	907 (1.2)
Organic matter	986.8 (0.12)	904.1 (42.88)	953.3 (1.16)	924.4 (2.68)	888.3 (3.00)	880.7 (0.29)	944.5 (1.34)
Crude protein	81.7 (1.93)	51.3 (2.25)	295.5 (2.56)	406.7 (5.81)	166.7 (6.59)	40.0 (0.40)	314.5 (3.98)
aNDFom ^e	90.0 (8.04)	285.0 (34.00)	462.0 (10.23)	215.3 (3.06)	441.0 (33.88)	722.0 (3.01)	288.0 (9.54)

^a Average of a total of 6 samples, 2 samples collected during the last week of each of the 3 periods.

^b Standard deviation of the mean.

^c Low quality lucerne hay as classified by the dairy.

^d Dried distillers grains with solubles (maize grain).

^e Neutral detergent fiber assayed with heat stable amylase expressed exclusive of residual ash.

Table 4.3 Ingredient composition (g/kg DM) of the TMR fed to high producing dairy cows: control (C), DV XPC (XPC) and Yeasture (Yst) treatments. ^a

	Treatments			SEM ^c
	C	XPC	Yst	
Lucerne, Hay (HQ) ^d	44.3	45.1	44.5	0.34
Lucerne, Hay (MQ) ^e	48.4	47.3	46.6	0.92
Wheat, silage (Whole crop)	134.0	133.8	134.8	0.27
Maize, silage (Whole crop)	128.1	128.7	128.5	0.33
Maize, grain (rolled)	190.6	190.2	190.9	0.03
Citrus, Pulp (with orange and lemon)	29.5	29.4	29.3	0.08
Lucerne (fresh chop)	32.5	33.2	32.1	4.68
Almond, hulls ^b	100.7	100.7	100.8	0.32
Cottonseed, whole upland ^b	44.9	44.9	44.9	0.14
Mineral, premix ^b	14.8	14.8	14.8	0.05
Canola meal (solvent extracted) ^b	135.6	135.6	135.7	0.43
Lucerne hay (LQ) or Wheat straw ^b	16.4	16.4	16.4	0.05
Sodium Bicarbonate ^b	4.7	4.7	4.7	0.01
Fat, rumen inert ^{bf}	10.4	10.4	10.4	0.03
DDGS ^{bg}	68.6	68.5	68.6	0.22
Molasses (liquid) ^b	9.0	9.0	10.0	0.03

^a Based on two TMR samples collected/period/diet (*i.e.*, 6 samples per diet). Additions of the DFM yeast products were too low to provide meaningful additions to the diet on a DM basis.

^b Ingredients used to create premix.

^c Standard error of the mean.

^d High quality lucerne hay as classified by the dairy.

^e Medium quality lucerne hay as classified by the dairy.

^f Energy 2. Virtus Nutrition, LLC. 520 Industrial Way, Corcoran, CA, USA.

^g Dried distillers grains with solubles (maize grain).

No significant differences occurred among diets.

Table 4.4 Nutrient profile of the TMR fed to high producing dairy cows fed the control (C), DV XPC (XPC) and Yeasture (Yst) treatments. ^a

	Treatments			SEM ^b
	C	XPC	Yst	
Dry matter (g/kg as fed)	523	521	525	12.1
g/kg DM				
Crude protein	166.3	166.8	163.0	1.80
ADICP ^c	78.9	78.1	77.3	1.40
aNDFom ^d	308	312	315	3.5
ADF	229	233	235	1.3
Lignin(sa) ^e	50	51	52	1.2
Crude fat	43.8	45.4	44.6	0.92
Starch	179	172	169	11.1
Free sugars	35	36	37	2.9
Ash	91.3	89.7	89.9	0.94
Ca	9.0	9.4	9.2	0.22
Mg	3.01	2.94	2.95	0.030
K	16.3	16.2	16.4	0.22
P	4.16	4.13	4.09	0.071
S	2.96	2.94	2.93	0.019
Na	3.69	3.81	3.71	0.035
Cl	5.3	5.4	5.3	0.16
mg/kg DM				
Zn	85	87	85	0.7
Mn	50.4	50.7	51.1	0.47
Cu	16.6	16.6	16.8	0.31
Co	0.50	0.48	0.53	0.010
Mo	1.37	1.38	1.34	0.020
Se	0.48	0.49	0.50	0.009
Fe	395	421	459	0.8

^a Based on 2 TMR samples collected/period/diet (i.e., 6 samples per diet).

^b Standard error of the mean.

^c Acid detergent insoluble crude protein expressed as g/kg CP.

^d aNDF expressed exclusive of residual ash.

^e Lignin assayed with sulfuric acid.

No significant differences occurred among diets.

4.2 Milk yield, milk composition and BCS

There was a milk yield response ($P = 0.01$) when the Yeasture was fed compared to the control (Table 4.5). This included increases in yields of true protein ($P = 0.01$), lactose ($P = 0.01$) and energy ($P = 0.02$), as well as a tendency for the milk fat yield ($P = 0.07$) to increase. The overall composition of the milk (g/kg), as well as the SCC, was not affected by Yeasture. In contrast, there was no impact on any of those response parameters when DV XPC was fed and compared to cows fed the control diet. In addition there was no difference in average BCS, or BCS change, *versus* Control for either of the DFM yeasts supplemented to the diets.

Table 4.5 Productive performance and BCS of high producing dairy cows fed the control (C), DV XPC (XPC) and Yeasture (Yst) treatments.

	Treatments			SEM ^a	<i>P</i>	
	C	XPC	Yst		C vs. XPC	C vs. Yst
Yield (kg/d) ^b						
Milk	47.77	47.92	48.93	0.412	0.75	0.01
Fat	1.56	1.56	1.59	0.016	0.94	0.07
True protein	1.35	1.36	1.39	0.011	0.39	0.01
Lactose	2.27	2.28	2.33	0.020	0.62	0.01
Energy (MJ/d)	132.3	132.7	135.5	1.14	0.76	0.02
Composition (g/kg) ^b						
Fat	3.28	3.26	3.27	0.028	0.53	0.84
True protein	2.85	2.86	2.85	0.011	0.29	0.75
Fat : Protein ratio	1.15	1.14	1.15	0.008	0.27	0.85
Lactose	4.76	4.76	4.75	0.008	0.52	0.43
Energy (MJ/kg)	2.78	2.78	2.78	0.013	0.75	0.75
SCC ($\times 1000$ cells/ml)	65.2	68.8	79.1	9.18	0.71	0.16
Body Condition ^c						
BCS (units)	2.48	2.46	2.48	0.023	0.24	0.93
Change in BCS (units/28 d)	0.08	0.10	0.07	0.014	0.15	0.85

^a Standard error of the mean.

^b $n = 375$ cows.

^c $n = 321$ cows.

4.2.1 Effect of DFM yeasts on milk production and composition

There is no published research utilizing DV XPC which makes direct comparison of our results to those of others not possible since the general area of DFM yeast products is virtually exclusively product based. Nevertheless our results can be compared to Diamond V Mills XP as there is research available on effects of DV XP on dairy cow production, and both products are *S. cerevisiae* yeast culture based products. The difference between DV XPC and XP, as stated by the manufacturer on their website, is that DV XPC is a concentrated low inclusion form of DV XP (*i.e.*, a 4 times higher recommended dietary inclusion rate for XP

versus XPC). According to Poppy *et al.* (2012), DV XP and XPC are equivalent products except for their concentrations of some active metabolites. Erasmus *et al.* (2005) reported a small numerical increase in milk yield by feeding DV XP, but found no difference in milk composition except for increased milk CP concentrations. Robinson and Garrett (1999) also found no significant effect of DV XP on milk production in spite of a small numerical increase. Robinson (1997) also found no significant effect on milk production when XP was fed. However when Robinson and Erasmus (2008) summarized results of 7 DV XP lactation studies and reported increases in milk (3.6%), milk fat (4.9%) and CP (2.4%) yields, as well as 3% higher daily milk energy output; this is in general agreement with a meta-analysis by Poppy *et al.* (2012). The overall lack of a performance response increase of the DV XPC supplemented cows in our study suggest that it is not equivalent to DV XP, at least at the feeding level which we applied. Zaworski *et al.* (2014) reported a decrease in the SCC of cows treated with DV XP which is in contrast to our study where no effect of yeast supplementation on SCC occurred.

Because there is only one published study on the Yeasture product, and since it is a unique product containing *S.cerevisiae*, *B. subtilis* and *A. oryzae* fermentation extracts, it makes comparisons to other products difficult (Stretenovic *et al.*, 2008). Nevertheless, our results can be compared to other live yeast (*S.cerevisiae*) products as Yeasture is marketed as a live yeast based product, as well as bacterial cocktails that were composed out of similar bacterial species. The only published study which utilized Yeasture as a supplement for lactating dairy cows (Stretenovic *et al.*, 2008) reported a 2.6 kg increase in 4% fat corrected milk response when it was fed to early lactation cows. This results supports the 1.2 kg/d increase in milk yield found in our study. However in contrast to Stretenovic *et al.* (2008), milk fat and lactose concentrations were not affected in our study and this is in agreement with Bitencourt *et al.* (2011) who fed a live yeast culture. In contrast to our results, Kristensen *et al.* (2014) reported no difference in milk production when a live yeast product was fed to dairy cows in a commercial system, but found that its addition decreased milk protein concentrations, which is also in agreement with a literature review of Robinson (2013) which showed that addition of live yeast cultures decreases milk CP %. Erasmus *et al.* (1992), Higginbotham *et al.* (1999) and Soder and Holden (1999) found no effects on milk production or milk components when live yeast products were fed to dairy cows, but Higginbotham *et al.* (1999) found that, due to a numerical increase in milk yield and some milk components, that there was an increase in milk fat, protein and solids nonfat yield when a live yeast product is supplemented. Unlike in our study, Stretenovic *et al.* (2008) fed Yeasture and found that milk SCC were lower. This is in agreement with Higginbotham *et al.* (1999) who showed a tendency for the SCC to decrease when live yeast was fed. However, overall, the SCC values were very low in our study suggesting mastitis in the herd was low, and perhaps making it unlikely that there was an opportunity to further decrease SCC.

4.2.2 Effect of DFM yeast on BCS

Data on BCS in our study is difficult to compare to those of others who fed live yeasts or yeast cultures due to the low numbers of cows, short experimental periods and individual scoring differences in those other studies.

While the effects of live yeasts and yeast culture supplementation on BCS is not always clear from the published literature, Robinson (1997) showed that cows supplemented with DV XP lost less BCS compared to the control, Erasmus *et al.* (2005) however, showed no difference in BCS between Control and the DV XP supplemented diets.

Higginbotham *et al.* (1999) also reported no effect on BCS when a live yeast product was fed, which is in agreement with Soder and Holden (1999) as well as our results. In a literature review on the effect of yeast products on the performance of lactating and growing animals, Robinson (2013) reported a slight increase in body weight gain when live yeast products were fed.

4.3 Energy balance

Yeasture supplementation increased ($P = 0.02$) milk energy output compared to the control, but there was no effect when supplementing DV XPC (Table 4.6.) In both the Yeasture ($P < 0.01$) and XPC ($P = 0.01$) treatments there was an increase in total NE output/d, although the diet NE_L density for both treatments vs. Control did not differ ($P > 0.05$). These NE_L values are according to standards for diets fed to this class of cows and within ranges found by Swanepoel *et al.* (2014) and suggested by the NRC (2001). While it is clear that both the Yeasture and XPC had an effect on total energy output, their modes of action differed with Yeasture primarily impacting milk energy output whereas DV XPC fed cows had a higher BCS energy output, leading to similar diet NE_L densities of the two yeast treatments.

Table 4.6 Energy balance of high producing dairy cows fed the control (C), DV XPC (XPC) and Yeasture (Yst) treatments.

	Treatments				<i>P</i>	
	C	XPC	Yst	SEM ^a	C vs. XPC	C vs. Yst
Milk energy output (MJ/d)	132.3	132.7	135.5	1.14	0.76	0.02
BCS energy (MJ/d)	3.39	4.67	3.22	0.626	0.15	0.85
Total net energy (MJ/d)	185.5	187.1	188.4	0.13	0.01	< 0.01
NE _L (MJ/Kg DM)	6.80	6.94	6.91	0.164	0.59	0.66

^a Standard error of the mean.

^b Maintenance energy assumed to be 49.69 for all treatments

n = 3 pens.

Erasmus *et al.* (2005) showed that total NE output differed when a yeast culture was fed to dairy cows, and this is in agreement with Robinson (1997) and Robinson and Garrett (1999) who demonstrated that yeast culture supplementation was associated with an increase in NE_L density and showed that NE_L density tended to be higher for multiparous vs. primiparous cows, which is in agreement with the results of our study.

Kristensen *et al.* (2014) in contrast to results from our study, showed no effect on energy efficiency when a live yeast product was fed, and this is in agreement with Soder and

Holden (1999) who reported no effect on energetics when a live yeast product was fed. In contrast, a review article by Robinson (2013) showed an increase in energy efficiency when live yeast cultures were fed.

4.4 Dry matter intake and digestibility

It is clear that there was no DM intake response to either treatment and no difference in fecal DM proportion (Table 4.7), but there was a reduction in apparent total tract digestibility of OM for the Yeasture treatment ($P = 0.02$), a tendency for OM digestibility to decrease with XPC ($P = 0.08$), a decrease in apparent CP digestibility for the Yeasture treatment ($P < 0.01$) and a tendency to a decreased apparent CP digestibility for the XPC treatment ($P = 0.05$). In contrast, there was no difference in starch and aNDFom digestibility. Dry matter intake is within the normal NRC recommendations for large breed dairy cows at the same level of production (NRC, 2001).

Table 4.7 Dry matter intake, fecal dry matter and total tract apparent digestibility of TMR's for dairy cows fed the control (C), DV XPC (XPC) and Yeasture (Yst) treatments.

	Treatments			SEM ^c	<i>P</i>	
	C	XPC	Yst		C vs. XPC	C vs. Yst
Dry matter intake, kg/d	27.3	27.0	27.3	0.64	0.75	0.98
Fecal dry matter g/kg DM	132	119	132	0.73	0.21	0.97
Apparent Digestibility ^a						
aNDFom ^b	441	431	436	11.5	0.57	0.77
Organic matter	698	678	669	7.7	0.08	0.02
Starch	980	988	990	5.3	0.32	0.22
Crude protein	650	619	593	10.7	0.05	< 0.01

^a Based on two TMR samples collected/period/diet (*i.e.*, 6 samples/diet).

^b aNDF expressed exclusive of residual ash.

^c Standard error of the mean.

Robinson and Erasmus (2008) found a 1.8 kg/d increase in DM intake in a summary of 7 DV XP studies, which differs from our results with XPC, although those diets contained lower overall fiber concentrations. In contrast Schingoethe *et al.* (2004) reported a decrease in DM intake when DV XP was supplemented. Erasmus *et al.* (2005) and Robinson (1997) found that there was no effect on DM intake when DV XP was fed to cows in early lactation and Robinson and Garret (1999) showed a numerical increase in DM intake when DV XP was fed, which is in agreement with a meta-analysis by Poppy *et al.* (2012). Arambel and Kent (1990) and Hristov *et al.* (2010) found no effect on apparent total tract digestion when DV XP was fed and this is in contrast to our results where apparent total tract digestibility of OM ($P = 0.08$) and CP ($P = 0.05$) for DV XPC supplemented cows tended to be lower compared to the control treatment.

Both Soder and Holden (1999) and Piva (1993) reported no effect on DM intake when a live yeast was fed, which is in agreement with our results but not with data of Stretenovic *et al.* (2008), who found an increase in DM intake. Kristensen *et al.* (2014) reported no effect on NDF and OM digestibility in dairy cows fed live yeasts, which is in agreement with Erasmus *et al.* (1992), but contrasts to their data which shows an increase in apparent CP and ADF digestion when a live yeast culture was fed. Chiquette (1995) supplemented a combination of *A.oryzae* and live *S.cerevisiae* yeast and found no effect on digestibility of OM, ADF or NDF in dairy cows, but there was a tendency for lower apparent CP digestibility in steers and no effect on apparent OM and CP digestibility in dairy cows (Chiquette 1995).

4.5 *In vitro* gas production

No differences occurred between the Control diet gas production and that of the treatment diets, or when comparing the different treatments to each other (Table 4.8). When comparing these diets to results of Rauch *et al.* (2012), our diets were more fermentable, at least as judged by higher gas production (*i.e.*, Control at 24 h is 212 ml/g OM).

Table 4.8 *In Vitro* gas production and predicted ME of the diets for dairy cows fed the control (C), DV XPC (XPC) and Yeasture (Yst) treatments.

	Treatments ^a					SEM ^b
	C	XPC	Yst	C + XPC	C + Yst	
Gas production at (ml/g OM)						
4 h	89	92	91	92	93	2.4
24 h	296	304	304	305	306	6.1
30 h	319	326	329	326	330	6.8
48 h	350	348	360	360	363	8.3
ME ^c , 1×M	9.9	10.1	10.2	10.2	10.2	0.18

^a 'C', 'XPC' and 'YST' is samples of the diets fed to the cows which were dried and ground. 'C + XPC' and 'C+ YST' are samples of the control diet which was dried and ground and then had XPC and YST added to recreate the XPC and YST diets, but without the XPC and YST having been oven dried.

^b Standard error of the mean.

^c UC Davis approach to estimate ME (MJ/kg DM) of a feed (Robinson *et al.*, 2004). 1× M, ME requirements for maintenance.

No significant difference was observed between treatments.

Doto and Liu (2011) showed that *Bacillus licheniformis* and *Clostridium butyricum* did not have an effect on *in vitro* gas production when added separately but, once DV XP was added, gas production increased. The lower concentrations of DV XP inclusion in the study by Doto and Liu (2011) tend to agree with the data of our study where no increase in gas production was observed.

Sosa *et al.* (2011) studied *A. oryzae* addition to forage *in vitro* and found a tendency towards increased gas production with high concentrations but no effects were with lower concentrations, which is in agreement with our gas production data. They also found an increased *in vitro* Organic Matter Digestibility (IVOMD). Lila *et al.* (2004) found a linear increase in gas production as the live yeast supplementation level increased, but it did not

seem to have an effect at lower levels of addition. Nevertheless they did demonstrate that the change was not due to methane and H₂ production because these gas concentrations were similar among treatments. Sosa *et al.* (2011) used *A. oryzae* and demonstrated that the *in vitro* digestibility of NDF and DM was not altered, which is in agreement with a review by Robinson (2013) who showed a slight decrease *in vitro* NDF digestion with addition of live yeasts.

4.6 Urine SG, volume, measured allantoin and calculated CP flow

There was no differences in any of the urine data (*i.e.*, SG, urine volume, measured allantoin concentrations and calculated microbial CP flow) due to addition of either DFM yeast product (Table 4.9).

Table 4.9 Urine SG, volume, measured allantoin and calculated microbial CP flow from the rumen in dairy cows fed the control (C), DV XPC (XPC) and Yeasture (Yst) treatments.

	Treatments			SEM ^a	<i>P</i>	
	C	XPC	Yst		C vs. XPC	C vs. Yst
Specific gravity (SG)	1.027	1.028	1.027	0.0006	0.35	0.55
Urine volume (L/d)	18.4	18.1	18.9	0.45	0.50	0.46
Measured AI concentration (mg/L)	59.1	62.1	59.1	1.94	0.17	0.99
Microbial CP flow	2239	2309	2286	47.1	0.16	0.37

^a Standard error of the mean.

n = 63 cows.

In contrast to our results where no increase in allantoin concentrations were found by supplementing XPC, Hristov *et al.* (2010) reported a tendency for allantoin concentrations to increase when DV XP was supplemented to diets of multiparous Holstein cows.

Soder and Holden (1999) and Bitencourt *et al.* (2011) found no effect on the creatinine to allantoin ratio when a live yeast product was fed, which is in agreement with our data where microbial CP flow from the rumen did not change, suggesting that rumen microbial growth and/or numbers were not altered by treatment. In contrast, Erasmus *et al.* (1992) showed a tendency to an increase of non-ammonia N (NAN) flow from the rumen when diets were supplemented with a live yeast due to higher microbial CP flow. Dawson *et al.* (1990) also showed an increase in cellulolytic bacteria numbers in rumen contents of steers that were fed a forage diet supplemented with a live yeast product, and this is in agreement with Robinson (2013) who, in a review article, reported that rumen bacterial counts of both cellulolytic and non-cellulolytic bacteria increased with live yeast supplementation.

4.7 Blood plasma amino acid concentrations

Total essential AA (EAA) tended to be higher (*P* = 0.07) when cows were fed the Yeasture. This higher level of EAA was driven mainly by an increase in threonine (*P* = 0.03) tryptophan (*P* = 0.02), valine (*P* = 0.08) and histidine (*P* = 0.06). Although total non-essential AA (NEAA) concentrations did not differ when the Yeasture was fed, there was an increase

in concentrations of glycine ($P = 0.04$), asparagine ($P = 0.03$), tyrosine ($P = 0.05$), serine ($P = 0.07$), proline ($P = 0.06$) and taurine ($P = 0.07$). In contrast, when cows were fed DV XPC, there were no differences in blood AA concentrations (Table 4.10). All results are within the normal range of plasma AA for early lactation multiparity dairy cows as reported by Swanepoel *et al.* (2014).

Table 4.10 Free amino acid concentrations ($\mu\text{g/ml}$) in plasma of dairy cows fed the control (C), DV XPC (XPC) and Yeasture (Yst) treatments.

	Treatments			SEM ^a	<i>P</i>	
	C	XPC	Yst		C vs. XPC	C vs. Yst
<i>n</i> = 12 cows ^b						
Essential amino acids						
Threonine	10.9	11.3	13.2	0.71	0.69	0.03
Valine	28.2	27.2	31.6	1.83	0.55	0.08
Methionine	3.53	3.43	4.02	0.202	0.73	0.11
Isoleucine	13.8	12.9	15.3	0.92	0.33	0.16
Leucine	21.7	20.2	23.8	1.25	0.31	0.17
Phenylalanine	8.5	7.6	9.0	0.40	0.10	0.41
Tryptophan	12.2	13.5	14.2	0.68	0.14	0.02
Lysine	10.9	10.1	12.2	0.68	0.37	0.14
Histidine	6.6	7.4	7.7	0.44	0.17	0.06
Arginine	12.2	11.8	13.6	0.71	0.73	0.13
Total essential amino acids	129	125	145	6.5	0.69	0.07
Lys: Met ratio	3.1	3.0	3.1	0.14	0.28	0.92
Non-essential amino acids						
Homocystine	0.67	0.77	0.62	0.061	0.13	0.45
Aspartic acid	1.1	1.2	1.2	0.07	0.59	0.70
Tyrosine	9.02	8.75	10.7	0.61	0.75	0.05
Serine	7.5	8.0	8.8	0.50	0.46	0.07
Glutamic acid	6.0	5.8	6.4	0.27	0.63	0.18
Glutamine	39.3	42.2	40.8	3.09	0.51	0.73
Glycine	22.2	25.3	27.2	2.16	0.18	0.04
Alanine	20.3	19.5	22.5	1.20	0.65	0.19
Proline	11	11.1	12.6	0.62	0.85	0.06
Asparagine	4.5	4.8	5.4	0.30	0.43	0.03
Taurine	5.7	6.6	6.9	0.49	0.19	0.07
Total non-essential amino acids	127	134	143	7.2	0.51	0.13

^a Standard error of the mean.

^b A group of 4 cows/pen/period randomly selected from the group of eligible blood cows and sent for AA analysis as this was determined to be sufficient to identify significant differences between treatments and the control.

Piva (1993) found only a numerical increase in total blood proteins when cows were fed a live yeast product, and this is in agreement with Ayad *et al.* (2013) who also found a

numerical increase in the total plasma protein content of cows fed a live yeast product. No published results were found on effects of feeding live yeast or yeast culture products on AA concentrations of dairy cows.

4.8 General discussion

4.8.1 Introduction

When supplementing DFM products to diets of ruminants there are many factors that affect their impact on animal production. For example the animal's response to dietary yeast supplementation depends on the dosage, type of microorganism, basal diet and general feed management practices (Newbold *et al.*, 1995). Commercially available yeast products vary according to species, strain, amount of live cells and the microorganism's growth medium (Erasmus *et al.*, 1992). Not all of these products will have the same effect on animal performance, and the mode of action may well differ between products (Desnoyers *et al.* 2009).

4.8.2 DFM yeasts and diet interactions

Desnoyers *et al.* (2009), in a review article on the modes of action of live yeast and yeast culture products effects on rumen parameters and milk production, discussed differences that occur in experimental conditions that might affect the treatment response. For example, a positive effect on OM digestibility due to yeast supplementation was decreased by an increased proportion of concentrate in the diet, but increased by an increased proportion of dietary NDF. They also found that the positive effect of live yeast and yeast culture supplementation on DM intake increased as the proportion of concentrate in the diet increased. Factors that affected the milk yield response were DM intake level as well as the diet concentrate, NDF and CP levels. The diet fed in our study was low in concentrate, and relatively low in CP, which may explain the lack of a DM intake response. When comparing the diet in our study to other studies where a DM intake response occurred, it is clear that those were generally diets with lower fiber levels compared to the diet in our study with its relatively high fiber level. Robinson and Erasmus (2008) found that increased dietary fiber levels have a strong effect on suppressing the DM intake and production response when live yeast and yeast culture products are fed to dairy cows. Williams and Newbold (1990) suggested that live yeasts appear to be more beneficial when high starch diets are fed during early lactation and suggested that this benefit was based on the ability of yeast cultures to moderate rumen lactate concentrations. In contrast, our treatments had a positive effect on animal performance when both the treatments was supplemented to a low starch diet indicating the importance to consider a product specific response and to emphasize that there are many factors affecting the production response.

4.8.3 Effects of Yeasture supplementation

Erasmus *et al.* (1992) showed that AA's in digesta flow from the rumen was higher when cows were fed a live yeast. The AA that drove this increase was threonine, serine, glutamic acid and cystine. They concluded that this change in AA flow was mainly due to an increase in measured microbial CP flow from the rumen. Dawson *et al.* (1990) stated that

yeast cultures might influence the AA profile flowing from the rumen by selectively stimulating some species of anaerobic bacteria. Purser and Buechler (1966) investigated the AA composition of rumen bacteria and determined that the AA composition of a mixture of bacteria in the rumen is relatively constant, but noted that when individual species of rumen bacteria were compared to each other, differences did occur. A comparison of the AA compositions of the four abundant rumen bacteria *Selenomonas ruminantium*, *Butyrivibrio fibrisolvens*, *Bacteroides amylophilus*, and *Bacteroides rumenicola* shows that large differences in concentrations of some AA do occur. Ranges of AA in these bacteria are (g/100 g of total AA) threonine, 4.7 to 5.7; valine, 6.4 to 11.4; methionine, 2.2 to 3.3; isoleucine, 6.3 to 7.4; leucine, 7.7 to 8.6; lysine, 8.3 to 14.9; and phenylalanine, 4.7 to 5.6. This suggests that any feed supplement with a selective stimulatory effect on growth of bacterial species in the rumen could cause a population shift that can lead to an alteration in the AA profile of the rumen bacterial fraction (Erasmus *et al.*, 1992). Furthermore, Bergen (1967) suggested differences in AA composition exist among bacterial species that digest fiber and those that digest nonstructural carbohydrates.

Erasmus *et al.* (1992) in a study where live yeast was supplemented, stated that the AA that are most limiting to lactating dairy cows are lysine, methionine, phenylalanine, threonine and histidine. They found all those AA levels to increase in the digesta flowing out of the rumen, although only the threonine increase was statistically supported. Erasmus *et al.* (1992) thus suggest that live yeasts may alter the duodenal AA profile, and can provide animal nutritionists with a tool to manipulate duodenal AA profile. However data from our study do not show any influence on microbial CP flow (g/d) from the rumen with the Yeasture supplemented product. Although DFM yeasts impacts on rumen bacteria species profile have been shown in other studies, it seems unlikely to have occurred in our study if total rumen outflow was not impaired. The *in vitro* gas production of the treatment diets through 48 h show that there was no immediate effect of DFM yeast on rumen fermentation, and total tract apparent digestibility data show no difference in the digestion of aNDFom that further supports the conclusion that there was no increase in fermentation in the rumen, while discounting the possibility of a bacterial species shift being responsible for the increased concentration of some AA in blood plasma.

Williams and Newbold (1990) suggested that live yeasts may alter the site of nutrient digestion and this might affect apparent total tract digestibility and lead to an inaccurate representation of the effect of yeast cultures on digestion. This can be seen in our study where whole tract OM and CP apparent digestibility decreased with the Yeasture treatment. As there was no reason to believe that the true digestibility of OM and CP would differ due to treatment, since the basal diets were all the same and since aNDFom and starch digestion was not impaired, it is possible that the decreased apparent OM and CP digestion was because more OM and CP entered the small intestine in the form of digestive enzymes. This suggests that intestinal health might have been improved due to supplementing the Yeasture product, which could have led to increased true digestion which is supported by the higher plasma AA levels of cows fed the Yeasture diet.

Effects of DFM products on intestinal health have been studied extensively in monogastric animals, especially their effects on GI health in poultry (Giannenas *et al.*, 2012). However very little research has been done on effects of DFM products on post ruminal GIT health in ruminants. The increased plasma AA levels in the Yeasture treated cows and the increased milk production in the Yeasture treated cows suggests that an increase in post ruminal absorption efficiency might have caused the higher plasma AA levels which drove higher milk yield. Indeed one would expect to see a decrease in the AA concentrations in the blood plasma as milk yields increase, such as in the case of the DV XPC treatment and as commonly observed in literature (Trottier, 1997; Xu *et al.*, 1998) but, as the opposite occurred with the Yeasture treatment, these higher plasma AA levels in the Yeasture cows suggests an increase in intestinal AA absorption efficiency.

Live yeasts are naturally found in the rumen, but the rumen temperature does not promote their growth since optimal yeast growth occurs at about 25°C (Lund, 1974), and live yeast has very little ability to remain viable in the rumen more than 24h and thus has a limited ability to multiply (Kung *et al.*, 1997). Chaucheyras-Durand *et al.*, (2012) stated that yeast probiotics passing through the rumen might affect intestinal homeostasis thereby influencing the animal health and affecting the immune system. Gut micro-flora have important effects on host animal health, nutrition and performance by affecting utilization of nutrients and development of the GI system of the host (Barrow, 1992). This interaction is complex and, depending on the activity and composition of the gut micro-flora, it can have either negative or positive effects on animal health (Giannenas *et al.*, 2012).

Probiotics are defined as live microorganisms which, when supplemented in adequate amounts, demonstrate a health benefit to the host animal (Gaggia *et al.*, 2010). Prebiotics are defined as food components (growth factors, micro nutrients and pro-vitamins) that will beneficially affect the host animal by improving growth of intestinal bacteria (Gibson and Roberfroid, 1995). Symbiotics are defined as a mixture of both probiotics and prebiotics that positively affects the host by improving implantation and survival of live microbial dietary supplements in the GIT (Gibson and Roberfroid, 1995). Probiotic products influence the intestinal microbial system of monogastric animals in multiple ways. Some of this include augmenting toll-like receptor signaling, competitive exclusion of pathogenic bacteria (*E. coli*), enhancing dendritic cell-induced T cell hyporesponsiveness, improving T cell homing to mesenteric lymph nodes, increasing antibody production, promoting epithelial barrier integrity, reducing epithelial cell apoptosis and regulation of the local mucosal cell-mediated immune responses (Ng *et al.*, 2009).

The microbial population within the GI tract of mammals can be considered to be a metabolically active organ with a wide biodiversity in terms of species and the high number of cells (Gaggia *et al.*, 2010). A normal bacteria biome in the rumen and post ruminal GIT is an important health asset with a nutritional purpose and a protective impact on the intestinal structure and homeostasis (Gaggia *et al.*, 2010). In the GIT of mammals the mucosal barrier separates the internal environment from the luminal environment (Gaggia *et al.*, 2010) and the mucus layer is formed by the interaction of numerous mucosal secretions such as mucin

glycoproteins, surfactant phospholipids and trefoil peptides (Guarner and Malagelada 2003). The intestinal epithelium and mucus provide the first physical line of defense that mediates the active resident bacteria, pathogens and antigens and the three main types of immune cells involved are surface enterocytes, M cells and intestinal dendritic cells (Gaggia *et al.*, 2010). Resident bacteria may exert a dual purpose by stimulation of mucosal mechanism defense while having an effect on maintenance of homeostasis of the immune response (Gaggia *et al.*, 2010). Stressors that lead to malfunction of the intestinal barrier, and an increase in intestinal permeability may have a negative impact on gut microbial composition and increase susceptibility to enteric pathogens (Gareau *et al.*, 2009). An example of animals exposed to such a stressor is high producing dairy cows; especially in early lactation induced negative energy balance.

Lee *et al.* (2009), Giannenas *et al.* (2012) and Salim *et al.* (2013) demonstrated altered intestinal morphometric measurements when *B. subtilis* bacterial strains were fed to broilers, and showed an increase in villus height and crypt depth in the small intestine of poultry. This effect might also occur in the rumen of mature cows, as well as in the post ruminal GIT, leading to increased villus height and width thereby increasing the surface area available for absorption thereby leading to increased absorption efficiency. It is also possible that this might explain the decreased apparent digestibility of OM and CP in our study. For example an increase in post ruminal health and an increased surface area available for absorption means that there is also an increased surface area for attachment of microbes to the gut wall and thus a larger microbial biome and, due to the increased GI health, endogenous secretion levels would be higher, and so this increased gut health would lead to an increase in absorption efficiency and production as observed with the Yeasture treatment.

Our results show that use of Yeasture effected the AA concentrations in the blood plasma of cows. This might be due to an increase in concentrations of AA's in the digesta (less likely as previously discussed) and/or increased absorption efficiency (more likely as discussed). Indeed many EAA concentrations increased with Yeasture supplementation, as did some of the NEAA. The AA's that did increase also support an increase in GIT health in the Yeasture treated group, as these AA were threonine, tryptophan, glycine and asparagine. Threonine has a direct influence on synthesis of the mucin proteins required to maintain the intestinal immune function, but also has a direct influence on lymphocyte proliferation, enhancement of anti-body production and inhibition of apoptosis (Li *et al.*, 2007). Li *et al.* (1999) showed that the animal's immune system is sensitive to changes in dietary threonine intake and high levels of dietary threonine did increase mucosal concentrations of IgG and IgA in pigs challenged with *E. coli* (Wang *et al.*, 2006). Tryptophan catabolism renders serotonin that inhibits production of inflammatory cytokines and superoxide as well as other products such as N-acetylserotonin (NAS) that is an antioxidant and inhibits production of inflammatory cytokines and superoxides, melatonin that is an antioxidant and inhibits production of inflammatory cytokines and superoxide and anthanilic acid (ANS) that enhances immune system and inhibits production of pro-inflammatory T-helper-1 cytokines (Li *et al.*, 2007). A tryptophan deficiency in poultry diets impaired the immune response of the birds (Konashi *et al.*, 2000) and furthermore Esteban *et al.* (2004), reported that 300 mg

of tryptophan administered to rats enhanced phagocytosis by macrophages as well as the innate immune response. Glycine can degrade to nucleic acids that play a role in lymphocyte proliferation (Li *et al.*, 2007), but it is also a potent antioxidant (Fang *et al.*, 2002). Tsune *et al.* (2003) concluded that glycine is a novel anti-inflammatory, immune modulator and cytoprotective nutrient. Finally asparagine degrades into nucleic acids that play a role in lymphocyte proliferation (Li *et al.*, 2007). Improved nutrient and energy utilization, as well as increased absorption of nutrients in cows fed Yeasture may have had an effect on increasing the innate immune response.

This inconsistent responses and sometimes unexplained positive response with yeast products might have been due to a post ruminal effect that has not been exploited in most studies with yeast supplementation. There is an urgent need for more research in this field.

4.8.4 Effects of DV XPC supplementation

Supplementation of XPC did result in an increased metabolic efficiency, similar to that described by Robinson (1997), and occurred due to a slightly lower DM intake, slightly higher milk production and a slightly higher BCS gain which, when combined in energetic terms, resulted in a higher ($P < 0.05$) NE output when compared to the control diet, but was essentially the same as that of the Yeasture fed cows. The small numerical increase in NE density of the XPC diet seems to be a consistent response with DV XP, as also reported by Robinson (1997), Robinson and Garrett (1999) and Erasmus *et al.* (2005).

Yeast cultures have been shown to modify rumen fermentation and stimulate bacterial growth (Robinson and Garrett, 1999, Erasmus *et al.* 2005) and, in our study the XPC treatment did show that numerically there was a higher microbial CP flow, although it was not statistically supported. This slight microbial stimulation that occurred might have occurred because more thiamine, a B-vitamin needed for bacterial growth was available in the XPC. We did not examine any other rumen parameters and thus cannot comment on whether any other ruminal changes did occur, but the small increase in microbial CP flow might indicate that protein and energy coupling in the rumen was improved. This however cannot be shown, as no effect was seen in apparent total tract aNDFom digestion and because *in vitro* data showed no immediate effect of the XPC supplementation on gas production. The above mentioned discussion, therefore, is mere speculation.

Ingvartsen and Moyes (2013) proposed that improved nutrient and energy utilization, as well as increased absorption of nutrients in cows fed yeast cultures may have an effect on increasing the innate immune response and this may play a role in improving animal production, but this did not occur in our study when XPC was supplemented. The plasma EAA concentration were slightly lower and does not support an increased absorption efficiency, as shown with Yeasture supplementation, but the contrasting results between Yeasture, and XPC cows does show that the mode of action differs between the products.

CHAPTER 5

CONCLUSION

Effects of yeast based DFM products on rumen fermentation have been extensively studied in ruminant animals. However our results provide no evidence to suggest that there was a rumen effect with either DFM yeast product because the apparent total tract fiber digestion and microbial CP flow were not affected by either of the DFM yeasts. However, results show an increase in energetic output with both DFM products, suggesting an increase in efficiency of nutrient use due to feeding both yeast products, but the mechanisms by which production was influenced differed between products.

The Yeasture DFM product affected total energy output primarily by increasing milk and milk component production that may have occurred due to an increase in post ruminal GIT health leading to an increase in absorption efficiency from the small intestines. It can be speculated that the DV XPC product effect on total energy output may have been due to a small increase in microbial CP flow and a possible improved coupling of the protein and energy utilization in the rumen. These differences in animal response between Yeasture and XPC suggest that dietary nutrients were utilized and partitioned differently between treatments, indicative of a different mechanism of action.

Overall, results provide a basis for future studies to investigate DFM yeast products, especially relative to and their post ruminal GIT affects. We propose that when yeast based DFM products (Yeasture) are supplemented to the diet that a post rumen response may occur to improve gut health in the small intestine thereby leading to increased nutrient absorption efficiency.

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APPENDIX

Fig A1 Temperatures during the three collection weeks.

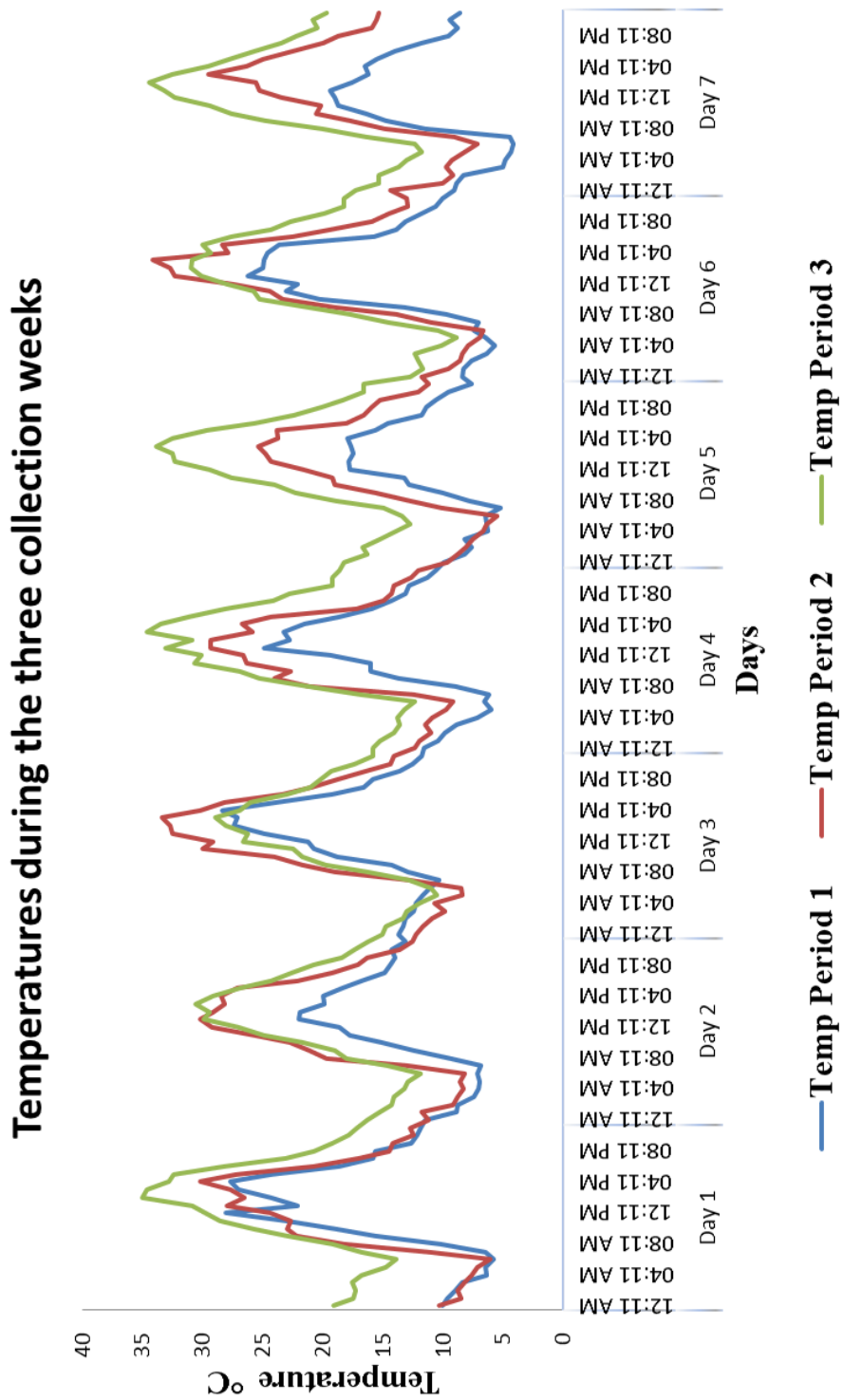


Table A1. Weekly measured rainfall during experimental period.

Week	Rain measured (mm)
Week 1	2
Week 2	5
Week 3	Tr ^a
Week 4	Tr ^a
Week 5	2
Week 6	14
Week 7	0
Week 8	0
Week 9	4
Week 10	10
Week 11	0
Week 12	0

^a Tr = Trace of water (not measurable).

Table A2. Common yeast products that are commercially available.

Product	LY	YC	YDFM	YBP
ADM Citristim	●			
Altech Yea-Sacc	●			
Biosaf Actisaf SC 47	●			
Biosaf procreatin 7	●			
Biotech Yea-Sacc	●			
Cenzone Yeasture			●	
Diamand V Mills XP		●		
Diamond V Mills XPC		●		
Dox-al Thepax Dry	●			
Hansen Biomate Yeast Plus	●			
Lallemand Levucell SC	●			
Lallemand Pro Ternative	●			
Pharmaqua BIOTECH	●			
Saf Agri PMX70SBK	●			
SAF Products BioYeast	●			
Vi-Cor Celmanac				●
Western yeast culture MOS-CON				●

LY = Live yeast DFM.

YC = Yeast Culture DFM.

YDFM = Yeast based DFM including bacterial culture.

YBP = Yeast based product DFM.

Table A3. Research articles and product types used to compare to results of DV XPC and Cenzone Yeasture.

References	LY	YC	YDFM	DFM
Arambel <i>et al.</i> (1990)		•		
Ayad <i>et al.</i> (2013)	•			
Bitencourt <i>et al.</i> (2011)	•			
Chiquette (1995)			•	
Dawson <i>et al.</i> (1990)			•	
Doto and Liu (2011)		•		
Erasmus <i>et al.</i> (1992)	•			
Erasmus <i>et al.</i> (2005)		•		
Higginbotham <i>et al.</i> (1999)	•			
Hristov <i>et al.</i> (2010)		•		
Kristensen <i>et al.</i> (2014)	•			
Lila <i>et al.</i> (2004)	•			
Piva (1993)	•			
Poppy <i>et al.</i> (2012)		•		
Robinson (1997)		•		
Robinson (2013)	•			
Robinson and Erasmus (2008)		•		
Robinson and Garrett (1999)		•		
Schingoethe <i>et al.</i> (2004)		•		
Soder and Holden (1999)	•			
Sosa <i>et al.</i> (2011)				•
Stretenovic <i>et al.</i> (2008)			•	
Zaworski <i>et al.</i> (2014)		•		

LY = Live yeast DFM.

YC = Yeast Culture DFM.

YDFM = Yeast based DFM including a bacterial culture.

DFM = Direct fed microbial product (*e.g.*, *A. Oryzae*).