EVALUATION OF THE EFFECTS OF LIVE YEAST ON RUMEN PARAMETERS AND IN SITU DIGESTIBILITIES IN BEEF CATTLE FED GROWING AND FINISHING DIETS

A Thesis

by

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Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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May 2019

Major Subject: Animal Science

ABSTRACT

Live yeast **(LY)** supplementation to ruminants has shown to increase nutrient digestibility and improve rumen environment by increasing pH in dairy cows. Few studies have determined the impact of LY in growing cattle receiving high-concentrate diets. Two studies were designed to evaluate effects of LY (*Saccharomyces cerevisiae*) on in vitro gas production (**IVGP**) fermentation dynamics, rumen parameters, and in-situ digestibility of dry matter **(DMD)** and neutral detergent fiber digestibility **(NDFD)** during three feeding phases [grower **(GRW),** transition **(TRANS)**, finisher **(FIN)**]. In the first study, eight ruminally-cannulated cattle were used in a randomized complete block design over 55 d to determine effects of top-dressed yeast at 0g/d (CON), 2.5 g/d (LY1), 5 g/d (LY2), 10 g/d (LY3). During the GRW diet, TRT altered DMD and NDFD ($P \leq 0.05$) and tended to affect rate of degradation **(kd)** and acetate-to-propionate ratio ($P \leq 0.10$). During the TRANS diet, TRT affected total gas production **(TGP)**, protozoa count **(PC)**, DMD, and NDFD ($P \leq 0.05$). Throughout the FIN diet, TRT affected kd, volatile fatty acid concentration, PC, DMD, NDFD ($P \leq 0.05$), and tended to impact CH_4 and pH ($P \leq 0.10$). We conclude that LY affected rumen parameters and digestibility, but dose-response varied by diet phase. The second study was similarly designed, instead with twenty ruminally-cannulated steers supplemented 45 d with LY at inclusions: CON (0g/d), 5 g/d (LY1), 10 g/d (LY2), 15 g/d (LY3). During GRW phase, TRT altered TGP of nonfiber carbohydrates **(NFC)** and kd of fiber carbohydrate (**FC**; $P \leq 0.05$). LY2 had the most TGP and fastest kd. TRT also influenced DMD and NDFD ($P \leq 0.05$) with LY2 providing greatest digestibility. For TRANS, TRT tended to affect NFC kd ($P = 0.078$) and

influenced pH and DMD ($P \leq 0.05$) where LY2 yielded highest pH, fastest kd, and greatest DMD. For FIN, TRT affected TGP and kd of the NFC pool, FC kd, CH_4, PC, DMD, and NDFD ($P \leq 0.05$). TRT response varied during the FIN phase. Overall, no constant dose-response pattern was observed; however, supplementation with LY affected IGVP, rumen parameters, and digestibility consistently with LY2 providing greatest benefit.

DEDICATION

This thesis is dedicated to every one of those who have helped me get this far in a never-ending journey of attaining more knowledge than I had the day before and who have had a hand in molding me into the person I am today. I still have a lot to learn and explore in this world, but I can't wait to spend the rest of my life doing just that.

To my parents, fiancé, brother, sister-in-law, beloved family, friends, and colleagues, I can't thank you all enough for being my constant source of inspiration and giving me the strength to push forward during the many times of self-doubt. Without you all, I do not know if I would have made it past the first semester. You all have and continue to provide moral, spiritual, emotional, and financial support. I appreciate your words of advice and encouragement more than you all could ever understand.

To my advisor: Dr. Tedeschi, it would take more than just one page of a limited dedication section to express all that I will endlessly be grateful for. I can't accurately express how much it means to me to have had you first believe in me, to take me on to work for you in your research program, and to constantly push me but never be too busy to take the time to teach me. I learned many invaluable things during my time with you, and I can only hope I get the chance to make a difference in the next generation of this industry as you have for me. Thank you for all the time and money you have invested into me during my tenure with you. I am honored to have you for a mentor.

Lastly, to my almighty God, whom I thank daily for the guidance, strength, fortitude, protection, and for giving me a healthy mind, body, and the ability to learn. For all of this, to Him I owe. I am so blessed.

ACKNOWLEDGEMENTS

I would like to acknowledge the partial financial support granted by the Texas A&M AgriLife Beef Initiative. I would also like to express my appreciation to Phileo Lesaffre Animal Care (Milwaukee, Wisconsin) for providing guidance on the design of the experiment and the selection of critical data to be measured, partial financial support to conduct this study, and feedback in interpreting the results.

Thank you to my committee chair, Dr. Tedeschi and to my committee members, Dr. Herring, and Dr. Anderson for their guidance and support throughout the course of this research. I also want to extend my deepest gratitude to the everyone in the business coordination department and all the men of the facility and feed staff for all their help throughout my tenure and during my every research trial at Texas A&M.

Thanks also go to my friends and colleagues and the department faculty and staff for making my time at Texas A&M University a great experience.

Finally, thanks to my mother, father, family and friends for their encouragement and support and to my fiancé for his patience and love. These past two and a half years haven't been the easiest, but we made it.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a thesis committee consisting of Dr. Luis O. Tedeschi, my advisor, Dr. Andy D. Herring of the Department of Animal Science, and Dr. Robin Anderson of the Department of Veterinary Physiology and Pharmacology.

All work conducted for the thesis was completed by the student, in collaboration with Dr. Luis O. Tedeschi of the Department of Animal Science.

Funding Sources

This graduate study was supported in partial by the Texas A&M AgriLife Beef Initiative and Phileo Lesaffre Animal Care.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION

OVERVIEW

The agriculture industry is a highly dynamic and evolving system in response to rapidly increasing demand for animal products. This is largely driven by the increasing population and urbanization which has begun to encroach upon agriculture land and resources (Herrero et at., 2009). This increase in demand, subsequent growth of the livestock industry, and waning of sources have made current production systems challenging.

For decades, research has been performed to find innovative techniques to keep up with the demands of the ever-growing population. The use of antimicrobial drugs to increase the growth and productivity of production animals has been implemented for that of 75 plus years worldwide. This was done to reduce low-level infections in animals, feed conversion efficiency, and promote growth in hopes of improving the health and thus the production efficiency of the animal (Van den Bogaard and Stobberingh, 2000). It is estimated that over one-half of the antibiotics produced and sold in the United States are used as feed additives in animal diets (Cromwell, 2002). Due to the ability of antibiotic residue to kill the beneficial microorganisms in the gastrointestinal tract, the accumulation in animal products is considered to be harmful for human consumption. In addition, the more an antibiotic is used, the more likely are resistant populations to develop (Vohra et al., 2016). As a result, the question of the appropriate use of such antimicrobials has risen, and the European Union and the USA have implemented bans on, or restricted the use of, certain antibiotics (Kesarcodi-Watson et al., 2008). Attributable to increased concern

regarding the use of antibiotics in animal production for consumption, there is much interest in exploring and discovering alternatives to antimicrobial feed additives.

In recent years, the industry has also witnessed an intensification in consumer's concern about the quality of animal products, the health and safety of production of animals for consumption, the health of people post-consumption of animal products, and the effects the livestock industry has on the environment. The purpose of using direct-fed microbial feed additives is to not only increase productivity but also to decrease the risk of transferring zoonotic diseases, reduce the antibiotic load that animals receive, sequentially decreasing the risk of antibiotic resistance, and to limit the excretion of pollutants (Chaucheyras-Durand et al., 2008). Active dry live yeasts such as *Saccharomyces cerevisiae* have been used as alternatives to antimicrobial feed additives for many years now (Lynch and Martin, 2002). This type of yeast is most commonly used in the making of bread and production of alcoholic beverages; however, supplementation with active dry yeast products in diets of ruminant animals has become a common practice for improving the efficiency of feed utilization performance of ruminants over 20 years ago (Moallem, 2009).

Yeast ferments carbohydrates to produce carbon dioxide. This process makes yeast useful when making bread. When yeast respires oxygen and produces carbon dioxide, it causes bread to rise. This specific property of yeast, the consumption of oxygen, is what makes it so beneficial to feed to ruminants. The rumen bacteria that degrade fiber require an anaerobic environment to function properly. The lower amount of oxygen that is in the rumen allows rumen bacteria to be more productive, multiply, and grow (Jouany, 2006). This sequentially increases the rumen's capacity to digest fiber. Several anaerobic

microbial communities like bacteria, fungi, and protozoa inhabiting the rumen are responsible for the digestion of the feed. Due to complex hydrolytic and fermentative processes, the rumen microorganisms provide the host animal with energetic and nitrogenous components that are essential to the animal's life. This high fermentative capacity has been the focus of research during the last century in order to help to develop more efficient ruminant production systems, as production level depends on the ability of the microbial ecosystem to convert organic matter into precursors of milk or meat. However, the nature of the feed given to ruminants to support productivity is one of several abiotic factors that can alter the balance of rumen microbial communities and their activities. This can lead to both a decrease in performance and an increased risk of health problems. Under these circumstances, live yeasts can be a useful tool to stabilize the rumen microflora and to limit these problems (Fonty and Chaucheyras-Durand, 2006).

Physiology of Yeast

Active dry live yeasts have become more commonly used in ruminant nutrition as direct-fed microbial feed additives to improve feed efficiency, performance, and at the same time, to prevent health disorders. They are particularly useful in high-producing ruminants whose rumen microflora are easily altered by high-energy dietary intake. Yeasts are single-celled organisms that are classified as fungi. In the rumen environment, live yeasts are considered to be allochthonous microorganisms, which are organisms that originate from a place other than that in which they are found. Nevertheless, when they are fed to ruminants daily, they can survive in the digestive tract and interact with autochthonous microbial populations, which are microbes that are native to the rumen environment (Fonty and Chaucheyras-Durand, 2008). Studies have revealed that yeast

concentration in the rumen significantly increases 30 minutes after ingestion of the daily dose, remains at constant levels for 7-8 hours then levels begin to decline, mainly due to rumen flow (Julien et al., 2016). Daily supplementation of live yeast is essential to maintain desired concentration in the rumen environment.

The drying process that results in a layer of dead cells around the outside of the yeast prill that protects the live yeast cells within is what makes active dry live yeasts so unique (Phileo Lesaffre Animal Care, Milwaukee, WI). This characteristic provides insurance that the live yeast cells within are retained in a stable environment. These products are generally characterized by a high concentration of viable cells, >10 billion cfu/g, and are most commonly being of the species *S. cerevisiae*. The biomass is dried to ensure cell viability and metabolic activity, and, in some products such as yeast culture, the cells are mixed with their fermentation medium (Chaucheyras-Durand et al., 2008).

There are two different products, culture or live cell product, in which yeast can be fed. Over the past few years, there has been increasing interest in comparing the effects of *S. cerevisiae* live cell products and *S. cerevisiae* culture products on ruminal fermentation. Yeast culture is generally produced by fermenting certain raw ingredients such as liquid and cereal grain with *S. cerevisiae* and drying the entire culture medium (Diamond V Mills, Inc., Cedar Rapids, IA). Live yeast cell supplements are reported to contain live *S. cerevisiae* cells that are fed alone or with a small amount of carrier. Unlike a yeast culture, live yeast does not include the entire culture medium. Live yeast is dried using special procedures that maintain a high live cell count, it is done to obtain an 100% active dry live yeast product that contains no cereal fillers (Saf Agri, Milwaukee, WI). While the yeast

culture supplements do contain some viable *S. cerevisiae*, the yeast live-cell supplements contain higher numbers of viable yeast (Lynch and Martin, 2002).

Yeast responses vary depending on whether you are feeding culture or active yeast, the strain of yeast used, the nature of the diet, and the physiological status of the animal (Chaucheyras-Durand et al., 2008). The nature of the interaction between the yeast cells and the rumen microorganisms is undoubtedly dependent upon the respective diet that is being fed, particularly its contents of readily fermentable carbohydrates, and by the microbial population of the rumen (Dawson, 1987). It is important to understand the underlying microbial mechanisms by which active dry live yeasts act in the rumen in order to optimize their utilization in ruminant nutrition going forward.

The Rumen Environment

In adult cattle, the rumen takes up a large proportion of the body cavity with a volume of 100-150 L. A healthy rumen's temperature resides around 39-40°C, and the mean pH, close to neutrality, has very low redox. This provides the diverse autochthonous microbial population a rich, strictly anaerobic environment (Hobson, 1997). The rumen is the main site of the very complex hydrolytic and fermentative processes that are carried out by facultative and anaerobic microbial communities such as bacteria, archaea, ciliate protozoa, flagellate protozoa, anaerobic fungi, and bacteriophage particles. These play a role in ruminant nutrition by fermenting and digesting the ingested complex plant polymers. These processes provide essential energy and protein components, detoxifies toxic compounds, stimulates the immune response, and inhibits the pathogenic microorganisms as well (Vohra et al., 2016). As described by Hobson and Stewart (1997), the microbial degradation does not break down the forage alone. During different intervals

after feeding, muscular contractions push a large fraction of herbage and saliva from the rumen into the mouth, where it is re-masticated. This chewing not only further mixes the forage with the saliva and microbes, but it also breaks it down further into smaller pieces before it is returned to the rumen.

The rumen microbes hydrolyze the plant celluloses, hemicelluloses, pectin, fructosans, starches and other polysaccharides to sugars which are fermented to produce various products. The initial products of the microbial actions are volatile fatty acids (acetate, propionate, and butyrate), methane, and carbon dioxide. The gases are eructated or excreted by the animal and are essentially waste products considered greenhouse gases that are harmful to the environment and are believed to contribute to global warming. The volatile fatty acids are absorbed through the rumen epithelium into the bloodstream and converted into sugars and lipids that are required by the animal for energy and tissue building (Aguiar and Wink, 2005). Proteins are hydrolyzed to amino acids and peptides; each amino acid is then deaminated to ammonia and a fatty acid. The latter may be further converted, while the bulk of the ammonia is absorbed through the rumen epithelium to be converted into urea (Hobson and Stewart, 1997).

Particular feeds like forage remain in the rumen for approximately two days; however, the exact time depends on the rate of degradation of the particles and the density of the rumen contents because they are not able to move from the rumen until the combined rumen fermentation and microbial action has reduced them to millimeter size. The actual size depends on the species of ruminant (Van Soest, 1994). The liquid and suspended matter pass on from the rumen in some 8-10 h; therefore, yeast supplementation must be administered daily.

In hopes of finding a means to increase production of cattle, feeding of high-energy based feed, mainly by the source of starchy grains and animal protein meals, has been implemented in feedlots and dairies worldwide. Due to rumen fill being a large control on feed intake, the animal can eat a much denser mass of concentrates than of forage. Since the microbial degradation of starch is much more rapid than that of plant fibers, large amounts of sugars and fermentation products can be produced at a rate that can surpass the buffering capacity of the rumen (Nocek, 1997). This causes gas production to exceed the capacity of the animal to get rid of it. Bloat, acidosis, and other problems can quickly become the result of concentrate feeding unless feeding patterns are carefully controlled.

When the rumen microflora undergoes stressful conditions, it commonly results in rumen dysfunction thereby reducing feed intake, digestion, health, performance, as well as increasing the contribution of environmental pollution, potentially leading to death (Vohra et al., 2016). One of the major problems in ruminants is ruminal acidosis. Ruminal acidosis is a serious condition in high producing dairy or beef cattle that results from ingestion of large amounts of feeds rich in ruminally fermentable carbohydrates that causes rumen disturbance (Nocek, 1997). It results from the accretion of acid or depletion of alkaline reserves in blood and body tissues and can not only cause a decrease in animal performance by facilitating erratic feed intake, but it can alter milk composition (Nocek, 1997) and cause health issues such as laminitis, bloat, metabolic acidosis, lameness, and can even lead to death (Enemark, 2008).

To overcome and help prevent these problematic issues, rumen microbiologists and ruminant nutritionists suggest the use of feed additives to manipulate the rumen microbial population and ruminal fermentation to take full advantage of the efficiency of feed

utilization in order to further increase ruminant productivity. Such feed additives to be used in a ruminant's diet should help a series of concerns as discussed below (Wallace and Newbold, 1995; Newbold, 1995; Nagaraja, 2012). They need to keep a more balanced ruminal pH by reducing the production and accumulation of lactate and increasing lactate fermentation. They should aid in the reduction of rumen pathogens, particularly those that can cause disease such as *Escherichia coli* O157, *Salmonella*, and *Campylobacter*. This will help reduce the risk of older livestock to develop ruminal acidosis or bloat and keep neonates from obtaining metabolic diseases like diarrhea. They also ought to improve ruminal energy utilization efficiency by lessening ruminal methanogenesis and decreasing the acetate to propionate ratio.

Furthermore, these feed additives need to improve the animal's ability to utilize ruminal nitrogen by reducing proteolysis, peptidolysis, and amino acid deamination in order to minimize production and excretion of ammonia that ends up in the environment. They should also facilitate the joining of ruminal energy and protein supply to enhance the synthesis of microbial protein. This not only allows for better efficiency of nitrogen metabolism, but it also decreases the overall nitrogen excretion by the animal. Additionally, these additives need to enhance the rumen microflora and increase microbial fiber digestion (Wallace and Newbold, 1995; Newbold, 1995; Nagaraja, 2012). These recommendations are why researchers have been focusing on natural feed additives, more specifically live yeast.

Implications of Yeast in the Rumen

Live yeasts have many of the properties as mentioned above and have been reported to efficiently limit the health problems of ruminants as well as increase

productivity (Lascano et al., 2009; Moallem, 2009; Newbold and Rode, 2006). Some results vary, but much research is still being executed. There have been studies that found supplementing yeast assisted with digestion and metabolism of feedstuffs in ruminants in multiple aspects such as increase nutrient digestibility, optimization of a proportion of volatile fatty acids, decrease in ruminal ammonia nitrogen, palliation of pH fluctuation, and stimulation of microorganism population (Chaucheyras-Durand et al., 2008). Additionally, it has been proven to provide multiple growth factors, pro-vitamins, and other stimulants to rumen microorganisms as well as balance the ruminal fluid redox potential to create ideal fermentation conditions for the rumen microbial populations (Jouany, 2006). The main purpose for using such direct-fed microbial feed additives in ruminant diets is to prevent rumen flora disturbances and disorders, especially those associated with high energy concentrates like those fed to finishing and high producing dairy cattle that make up our meat and milk production systems.

Effects on Ruminal pH and Acidosis

Ruminal pH plays a key role in in the fermentation of substrates by the microbes, so diets should be formulated, and supplements should be administered to maintain adequate and constant mean ruminal pH to ensure that does not significantly decrease and lead to ruminal acidosis (Tedeschi and Fox, 2018). Ruminal acidosis results from consumption of readily fermentable carbohydrates, causing a plummet in ruminal pH (Nocek, 1997). Lactic acid, an end product of ruminal microbial fermentation, is a major contender in acute cases of this nutritional disorder (Owens et al., 1998). Lactate becomes a major fermentative product at a pH <6. As the pH falls, lactate-producing bacterial species *Streptococcus bovis* outnumbers the lactate utilizing species *Megasphaera elsdenii*

and *Selenomonas ruminantium* (Nocek, 1997). If pH to falls too low in the rumen, *Lactobacilli* replaces *S. bovis*, initiating a spiraling effect with excessive lactate accumulation (Russell and Hino, 1985).

Effects of live yeasts have been extensively studied on lactate-metabolizing bacteria. In an in vitro study, Chaucheyras et al., (1996) discovered that one strain of *S. cerevisiae* was able to efficiently compete against *S. bovis* for the utilization of sugars. By reducing the availability of fermentable sugars available to the bacteria, the amount of lactate produced was in turn limited. This effect, however, was only observed with live yeast cells. Dead cells did not affect lactate production. Nisbet and Martin (1991) observed an increase of growth and metabolism of lactate-utilizing bacteria, such as *M. elsdenii* or *S. ruminantium* in vitro in the presence of different live yeasts due to an increase in the supply of different growth factors: amino acids, peptides, vitamins, and organic acids, all which are essential for the lactate-fermenting bacteria to perform their job efficiently. Moreover, other studies have reported that redox potential of the rumen fluid was lowered in the presence of live yeasts in lambs (Chaucheyras-Durand and Fonty, 2002) suggesting that live yeast cells could create a more favorable biological condition for growth and biological activities of the anaerobic microbes.

Effects on Methane Production

Hydrogen is produced by several hydrolytic and fermentative processes in the rumen and is mainly used to reduce carbon dioxide into methane by methanogens (Miller, 1995). This process where H2-producing and H2- utilizing microorganisms interact is called “interspecies hydrogen transfer” (Ianotti et al., 1973). The ability for hydrogen to be utilized by methanogens is beneficial to the degradation of plant cell wall carbohydrates in

the rumen (Wolin et al., 1997). However, as a result of this process, methane is eructated and/or excreted by ruminants at 400 to 500 liters per day in adult cattle and represents an 8–12% loss of carbon and available energy in the diet (Moss et al., 2000). Naturally, the amount of methane produced and expelled varies according to the type of diet (forage vs. concentrate) and the type of production system (intensive vs. extensive) (Sauvant et al.,1999) because methanogens are the most active in the pH range of 6.5-8.0 (Anderson et al., 2003), and each of these variables easily affect the pH.

Furthermore, methane is classified as a greenhouse gas, and emissions need to be decreased by any means possible as it contributes to the global warming effect (Moss et al., 2000). Various strategies have been investigated in order to alleviate ruminant methane production. Very little information regarding probiotic yeasts dealing with their potential effects on hydrogen transfer mechanisms and methanogenesis in vivo exist up to now. Future work investigation of such implications is crucial to determine the specific role of probiotic yeasts as an ecological tool to control methane emissions from the rumen.

Effects on Fiber Degradation

All ruminant diets contain some percentage of forage. The plant's cell wall primarily made up of cellulose, hemicellulose, and lignin, is insoluble, structurally complex, and not physically accessible (Nagaraja et al., 1992). Moreover, the enzymes in ruminant animals cannot digest them. The problem of all ruminant nutritionists to solve is to maximize nutrient intake and availability. In certain situations, yeasts have demonstrated their effectiveness on fiber-degrading microorganisms in the rumen. Most of these effects have been seen in vitro (Chaucheyras et al., 1995). However, in an in vivo study performed by Chaucheyras -Durand and Fonty (2001), it has been shown that cellulolytic

bacteria became established earlier in gnotobiotic lambs with only three species of bacteria *(Fibrobacter succinogenes, Ruminococcus albus, Ruminococcus flavefaciens*) as sole cellulolytic organisms that were supplemented with live yeast daily than those that were not supplemented. With the supplementation of yeast, the cellulolytic microflora remained stable at a high level. In a different study, the same three main cellulolytic bacterial species (*F. succinogenes, R. albus, R. flavefaciens*) were increased in the rumen of sheep receiving the yeast, confirming that yeast supplementation promoted growth and/or activity of these bacteria (Chaucheyras et al., 1997). One of the main factors that could explain the advantageous effect of live yeasts on fiber degrading bacteria relates back to the idea of the ability of yeast cells to scavenge oxygen (Newbold, 1995). Although the rumen is known to be considered anaerobic, traces of oxygen has been detected in situ, and it is recorded as high as 16 liters of oxygen can enter the ovine rumen daily during feed and water intake, rumination, or salivation (Newbold, 1995). This poses an issue as most of the ruminal microorganisms are highly sensitive to oxygen.

Conclusion

During the last decade, the mechanisms and attributions of live yeast on targeted rumen microbial communities, animal growth, health, and overall productivity have been extensively studied, at least for some strains of *S. cerevisiae.* Although research has certainly assisted in adding credibility on these probiotics for their use in ruminant nutrition, a lot remains to be studied to further explain the full effects of live yeasts in digestive processes. Live animal studies indicate that although positive effects on milk or meat production can occur, the animal response to such feed additives may be quite variable. These variabilities may result from a multitude of factors such as the nature of the

diet, animal physiological and genetic factors, production level of the animal, dosage rate, strain of yeast used, among many others. It will be of the utmost importance shortly to better understand the nature of interactions between the yeast probiotic, the autochthonous anaerobic microbial population, and the dietary components in order to accurately predict the impact of such a direct fed microbial in ruminant nutrition. This knowledge will also be essential to select a more targeted and reliable new generation of probiotics to capitalize on such a promising feed additive.

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CHAPTER II

EVALUATION OF THE EFFECTS OF LIVE YEAST ON RUMEN PARAMETERS AND IN SITU DIGESTIBILITY OF DRY MATTER AND NEUTRAL DETERGENT FIBER IN BEEF CATTLE FED GROWING AND FINISHING DIETS

SUMMARY

This study evaluated the effects of live yeast (LY; *Saccharomyces cerevisiae*) on rumen parameters and in situ dry matter digestibility (DMD) and neutral detergent fiber digestibility (NDFD) during three consecutive feeding phases: grower (GRW) for 27 d, transition (TRANS) for 14 d, and finisher (FIN) for 14 d. Eight ruminally-cannulated cattle (4 steers and 4 heifers) were blocked by sex into two pens containing Calan gate feeders and received a control (CON) diet (13.7% CP, 42.4% NDF, 88% DM) without LY for 10 d (d -10 to d -1). Animals were randomly assigned to treatments: CON or LY fed every morning (0800) at 2.5 g/d (LY1), 5 g/d (LY2), or 10 g/d (LY3) for 55 d. Digestibility was assessed on nine collection days using in situ nylon bags containing 5 g of GRW, TRANS, or FIN incubated for 48 h. In vitro gas production assays were conducted concurrently. Data were analyzed as a randomized complete block design with day as a repeated measure. During the GRW diet, treatment altered DMD and NDFD, and tended to affect the rate of degradation (kd) and the acetate-to-propionate ratio. During the TRANS diet, treatment affected total gas production, protozoa numbers, DMD, and NDFD. Throughout the FIN diet, there was an effect of treatment on kd, volatile fatty acid concentration, protozoa numbers, DMD, NDFD, and tended to impact methane and pH. We concluded that LY affected rumen parameters and digestibility, but the dose-response pattern varied depending on the type of diet.

INTRODUCTION

The beef production industry is a highly dynamic and evolving system that responds rapidly to increasing demand for animal-derived protein products that is driven by an increasing human population and a degree of urbanization. In previous years, the use of antimicrobial drugs to increase the growth and productivity of production animals has been used to promote feed conversion efficiency and growth and to reduce low-level infections in animals to improve the health and production efficiency of food animals (Van den Bogaard and Stobberingh, 2000); however, feeding antimicrobial feed additives for growth promotion is no longer an option.

Active dry live yeasts products such as *S. cerevisiae* have been used as alternatives to antimicrobial feed additives (Lynch and Martin, 2002). These products are most commonly used in the making of bread and production of alcoholic beverages; however, the use of probiotic yeast in diets of ruminant animals has become a common practice for improving the feed utilization efficiency of ruminants (Moallem, 2009). The establishment of more reducing condition in the rumen could assist in the growth of lactate-consuming and cellulolytic bacterial populations, sequentially aiding in the stabilization of the rumen and increase the rumen's capacity to digest fiber (Marden et al., 2008). Given the complex composition of ruminant feeds, live yeast can clearly alter the balance of rumen microbial communities and their activity (Fonty and Chaucheyras-Durand, 2006).

The supplementation of live yeast (**LY**) to ruminants has been shown to increase nutrient digestibility (Lascano et al., 2009), decrease methane production (Moallem, 2009),

and increase performance in dairy cows (Newbold and Rode, 2006), but few studies have determined their impact in the rumen of growing beef cattle receiving high concentrate feedlot type rations. Due to an increasing emphasis on efficiency, performance, and feed digestibility of growing cattle and the increasing public awareness of methane production in agriculture livestock, the present study was designed to evaluate the effects of supplementing LY on ruminal parameters and nutrient digestibility when fed to growing beef cattle during the three feeding phases: grower (**GRW**), transition (**TRANS**), and finisher (**FIN**).

MATERIALS AND METHODS

The effects of the inclusion of LY (*Saccharomyces cerevisiae* Sc47 CNCM I-4407, Actisaf ®, Phileo Lesaffre Animal Care, Milwaukee, WI, 1.10^{10} CFU/g) in diets of growing ruminants were examined in this study. Data in this study were collected from a 55-d in vitro and in situ trial and were analyzed to determine the total gas production as well as the fractional rate of degradation (**kd**) using the in vitro gas production technique (**IVGP**), total volatile fatty acid concentration (**VFA**), acetate to propionate ratio (**A:P**), lactate concentration, methane production (**CH_4**), rumen pH fluctuation, protozoa counts, dry matter digestibility (**DMD**), and neutral detergent fiber digestibility (**NDFD**).

All experimental procedures were executed, and animals were cared for according to the guidelines of the Texas A&M University Institutional Care and Use Committee (IACUC AUP protocol #2016-0362). Eight ruminally-cannulated mature cattle (n = 4 steers and n = 4 heifers at 36 and 24 months old, respectively) with body weight (**BW**) of 550 kg ± 75 kg from Texas A&M McGregor Research Center and Angelo State

University, respectively, were used in this experiment. Animals were blocked by sex and placed into two separate pens of four animals each. Each pen contained Calan gate feeders (American Calan, Northwood, NH), and water was available constantly. From d -10 to d -1, animals went through an adaptation period when they received a standard diet (13.7% CP, 42.4% NDF, 88% DM) without LY in the Calan gate feeders with the gates open so they would become acclimated to the bunks and Calan system. On d 0, they were fitted with the Calan sensor.

Treatments and Experimental Design

On d 0, treatments were randomly assigned to animals using a randomized complete block design (2 pens; 4 treatments; 4 animals per pen). Each animal within a block was assigned to a treatment. This allowed for two animals, one of each sex, per treatment. Treatments were as follows: control **(CON)**, **LY1** (2.5 g/d), **LY2** (5 g/d), and **LY3** (10 g/d) distributed by top-dressing. Weighed amounts of fresh feed were provided twice daily at 0800 and 1700 for 55 d. Each of the three-phase diets was fed sequentially as follows: grower (GRW) for 27 d (10 d for adaptation) fed during week one through five, transition (TRANS) for 14 d fed during weeks six and seven, and finishing (FIN) for 14 d during weeks eight and nine of the study (Table 1). Baseline ruminal contents were collected on d -1 and measurements were taken every 7-d following, for a total of nine collection days.

Rumen Sampling and Analyses

During the sample collection process, for each treatment, whole rumen contents were extracted from the cranial, middle, and caudal compartments of the rumen approximately 4 h after the morning feeding time for chemical analyses and to be used for in vitro gas

production (**IVGP**) and methane assays as described below. A combined rumen content (approximately 500 mL) were strained through eight layers of cheesecloth and placed into individual stainless-steel thermoses minimizing headspace to maintain both temperature and an anaerobic environment. Concurrently, in situ nylon bags were placed in each animal for a 48 h incubation. Rumen fluid was immediately transported to the laboratory and prepared for pH, CH_4, VFA, lactate analyses, protozoa counts, and the IVGP technique.

The pH of each rumen fluid sample was recorded using a VWR sympHony benchtop meter (VWR International, Radnor, PA). Then, subsamples were taken for volatile fatty acids (**VFA**) and lactate analyses. Approximately 8 mL of rumen fluid from each sample was transferred into individual falcon tubes containing 2 mL of metaphosphoric acid (2 Falcon tubes per animal) for both VFA and lactate analyses, and then frozen at -20ºC. VFA and lactate concentration was measured by gas chromatography (Hinton et al., 1990).

In Vitro Gas Production Measurements

Using a portion of the mixed rumen fluid, an in vitro anaerobic fermentation and gas production analysis (i.e., IVGP) was performed on a total of 288 samples (32 samples from each time point collection). Briefly, the IVGP technique utilizes an incubation chamber to mimic rumen temperature (39°C) with a multi-plate stirrer that houses thirty-two fermentation flasks (125 mL Wheaton bottles) (Tedeschi et al., 2009; Tedeschi and Fox, 2018; Ch. 9). Flasks were attached to pressure sensors that measure and record gas pressure every 5 minutes for 48h. Approximately 200 mg of ground diet (GRW, TRANS, and FIN depending on the period) was weighed and transferred into each 125 mL Wheaton bottles containing Teflon covered stir bars and dampened with 2.0 mL of distilled H_2O to prevent particle scattering during subsequent CO_2 flushing. Meanwhile, anoxic media

(Goering and Van Soest, 1970) was continuously flushed with O_2-free CO_2. Anoxic media was sealed with lightly greased butyl rubber stoppers and closed with aluminum crimps (Bellco Industries, Vineland, NJ). Bottles were placed in a 39 ºC incubator and connected to their respective pressure sensors via needle insertion. Ruminal fluid from treated cattle was then again filtered through 4 layers of cheesecloth and glass wool, into a flask continually flushed with CO_2, and 4 mL of rumen inoculum was injected anoxically into each fermentation bottle via a needle and syringe. The pressure inside the bottles was equalized to atmospheric pressure at time 0 by piercing rubber stoppers with a needle for approximately 5 seconds, prior to initiating recording. Once the pressure was equalized in all bottles, software recording was initialized, and atmospheric pressure was recorded. After 48 h of fermentation, software recording was terminated, and bottles were placed in the refrigerator to cease fermentation. Then, head space gas samples (1mL) were removed from each bottle and analyzed for methane concentration using the gas chromatography method (Allison et al., 1992). Final incubation pH was measured on the remaining rumen fluid, and 40 mL of neutral detergent solution (Van Soest et al., 1991) was added to each bottle which were then resealed and autoclaved for 15 min at 120ºC. Undegraded fiber was then filtered gravimetrically using Whatman 54 filter paper, oven dried at 60ºC for 48h, and weighed.

Protozoa Count

Protozoa counts were determined by methods described by Dehority (1984) without staining. About 1 mL of original rumen fluid samples were added to 10 mL formalin to achieve a 1:10 dilution of the original rumen contents. The counting technique was an adaptation of the procedure described by Purser and Moir (1959). A 1 ml aliquot of the

formalinized sample was pipetted with a 1 mL wide orifice (3 mm) into a Sedgewick Rafter counting chamber. Protozoa were counted at a 100x magnification with a counting grid 0.5 mm square in the eyepiece; 25 evenly spaced grids from the entire chamber surface were counted, and an average was computed for each rumen fluid sample (64 total samples).

In Situ Ruminal Incubations

There are many variants for the in situ incubation technique (Tedeschi and Fox, 2018; p. 148). Small nylon bags, 5 x 10 cm, 50 μg micron porosity (Ankom Technologies, Macedon, NY, USA) were weighed, filled with 5 g of ground sample (to pass a 2-mm screen), and sealed (Vanzant et al., 1998). Two sealed blank bags, three bags filled with the GRW diet, three bags filled with the TRANS diet, and five filled with the FIN diet (13 bags in total per animal) were incubated each week. The empty bags served as blanks to correct for feed particles and microorganisms that may adhere to the nylon. The small nylon bags were held together in a 32 x 42 cm polyester bag with a nylon zipper and weighted down with two sanitized, heavy bolts during the rumen fermentation period. After removal from the rumen, nylon bags were rinsed with distilled water to remove large particles of rumen contents off the bags and put through a series of washes until the water was colorless to remove rumen fluid contamination. Upon completion of the washing process, the bags were placed in a forced-air oven and dried at 60°C for 48 h in preparation for analyses.

Digestibility Analyses

Dry matter digestibility. After nylon bags were removed from the forced-air oven, they were placed in a desiccator and dry weights were obtained from the samples. The

residual weight of each sample was determined after drying to calculate in situ DMD by dividing the residue weight by the original sample weight before incubation.

Neutral detergent fiber digestibility. The NDFD was determined by methods described originally by Van Soest and Robertson (1980) using an Ankom 200 Fiber Analyzer (Ankom Technologies, Macedon, NY, USA). After dry weights were retrieved on the in situ bag samples, they went through an additional wash procedure in the ANKOM machine to determine the NDF residue. Bags were placed in the suspender (3 bags per level) the machine was filled with approximately 1900 to 2000 mL of NDF solution. After the temperature reached 100ºC, bags were placed into the solution, and the agitator was turned on for 70 minutes. Upon wash cessation, the bags went through a second and a third rinse with about 1900 mL of preheated distilled H_2O and were agitated for 10 minutes each time. The final rinse is approximately 1900 mL of room temperature distilled H_2O and agitated for 10 minutes. Bags were then removed from the suspender, excess water was manually removed, placed in a 100 mL beaker and covered with acetone for 3 to 5 minutes. Following this acetone bath, bags were removed, placed on a drying rack for 5 to 10 minutes then placed into a 55°C oven for 48 h. Once dry, they were immediately placed into a desiccator until final weights were able to be taken. The NDFD was calculated as follows:

$$\%\, NDFD = \left(1 - \frac{W_3 - (W_1 \text{x}\, C_1)}{W_2}\right) \text{x}\, 100$$

where W_1 is the bag tare weight, W_2 is the sample weight, W_3 is the dried weight of bag post incubation, and C_1 is the blank bag correction or the running average of the final oven dried weight divided by the original bag weight.

Statistical Analyses

The PROC MIXED of SAS (SAS Inst. Inc., Cary, NC) was used to analyze the data (IVGP-a, IVGP- b, methane, total volatile fatty acids (**VFA**) concentration, acetate:propionate ratio, lactate concentration, protozoa, DMD, and NDFD) as a complete randomized block design. Sex was the random effect, treatment was the fixed effect, and average dry matter intake (**DMI**) of each animal was used as a covariate. It was analyzed assuming a repeated measure design in which weeks of rumen fluid collection were the repeated variable, using the REPEATED statement of PROC MIXED of SAS (SAS Inst. Inc., Cary, NC), and animal within treatment was the subject. The effect of treatment was tested using the least square means and orthogonal contrasts. The PROC IML was used to obtain the orthogonal coefficients for linear, quadratic, and cubic contrasts because treatments were not equally spaced (0, 2.5, 5, and 10 g/d). The same statistical model was used for pH except that the initial pH of the animals was also used as a covariate in addition to average DMI. The interaction between treatment and the covariate was removed from the statistical model if not significant at $P < 0.05$.

RESULTS AND DISCUSSION

In vitro Fermentation and Rumen Parameters

Total gas production. The asymptote measurement or 48-h accumulated (total) gas production (mL) of IVGP is shown in Table 2 and Figure 1A. Though LY3 had the least total gas production, yeast treatments were not different while feeding the GRW diet ($P = 0.214$). Yeast treatment differed ($P = 0.035$) when cattle were fed the TRANS diet in which LY3 had the least gas production. For the FIN diet, yeast treatments tended to be different ($P = 0.065$), and an interaction between treatment and DMI was observed. The

LY1 had the least gas production when reported for the average DMI, but because of the interaction, the effect of yeast may differ depending on the DMI level. Figure 1A shows a distinct pattern throughout all feeding phases in which CON treatment produced the greatest amount of total gas. Mutsvangwa et al. (1992) stated that total gas production of a barley diet for beef cattle was on average less when supplemented with yeast culture (Yea-Sacc1026), but conversely, Tang et al. (2008) found that supplementation of a different type of yeast culture increased the total gas production of low-quality forages. The differences in the results of these two studies are likely attributed to the two products being of different strains and being supplemented to different diets. This shows relevance to the idea that both product type, strain, and diets all can influence the results seen from any type of yeast product supplementation. Both studies supplemented with yeast culture which is different from LY in the fact that LY contains high counts of viable active yeast cells and may be more proactive in the rumen. Wang et al. (2016) reported that when doses of LY (*S. cerevisiae*) were supplemented, total gas production was greater than the control. While this is different than what was found in the present study, Wang's et al. (2016) study also included supplementing different species of yeast, *Candida utilis*, *Candida tropicalis*, and *S. cerevisiae.* Supplementation of these different strains of yeast to cattle receiving two different low-quality forages, maize stover, and rice straw, resulted in *C. utilis* yielding lower total gas production than control and the other two species of yeast across both diets (Wang et at., 2016). Discrepancies between all these studies suggest again that the selection of yeast species, strain, product type (e.g., LY vs. culture), and ration composition should be taken into consideration when supplementing LY to cattle rations as it could affect the variables of interest.

Fractional rate of degradation. Yeast treatments tended to affect (P = 0.082) the fractional rate of degradation (Table 2, Figure 1B), but an interaction between treatment and DMI was observed for cattle consuming the GRW diet. There was not an effect of yeast treatment during the TRANS diet (P = 0.184), but an interaction between treatment and DMI was present. The LY1 had the fastest kd. When examining the FIN diet, yeast treatment impacted kd in a linear fashion (P =0.042) in which the control had the fastest kd after adjusting for an average DMI. Yeast treated animals had numerically higher rates of fermentation during the GRW and TRANS diets, but this was not significant, possibly due to a small sample size of rumen fluid donors. It is expected that increasing the sample size might result in significant effects of LY on kd.

Rumen Fluid Measurements

Volatile fatty acids. Total VFA concentration (m*M* of acetate + propionate + butyrate + isobutyrate + valeric + isovaleric) was not affected by yeast treatment while cattle were fed the GRW diet (P= 0.115) or the TRANS diet (P = 0.301). However, when cattle were fed the FIN diet, yeast treatment affected total VFA concentration in a quadratic fashion (P = 0.033) in which the LY2 rumen fluid had the greatest concentration of total VFA concentration (Table 2, Figure 2). Similar results were found by Bakr et al. (2015) when they witnessed total VFA concentration were significantly higher in the yeast-fed animals compared with the controls throughout the study. While our values did not reach significance for every diet, the common trend was present across treatments (Figure 3).

When examining the effects of LY on the A:P ratio (Table 2, Figure 3), DMI effect was significant, and a cubic pattern (P = 0.024) was observed for the GRW

diet. There was no significant interaction when cattle were fed the TRANS and FIN diets (P = 0.174 and P = 0.562; respectively, Table 2). The average A:P ratio decreased across all yeast treatments as well as the control as the diet shifted to a ration with a higher percent of concentrate. This follows in accordance with what Cho et al. (2014) reported when determining the effect of the energy level of the diet on the A:P ratio in the rumen of Hanwoo steers. As displayed in Table 2 and Figure 3, LY3 repetitively had the least A:P ratio throughout each diet. In a previous study performed by Uyeno et al. (2017), similar results were observed when supplementing different inclusions (0, 5, 10 g/d) of the same live yeast product to Holstein cows. While there was no significant effect of yeast treatment on the A:P ratio, 10 g/d of LY had a lower ratio during the study. This was attributed to the marginal decrease in the acetate concentration and an unchanged propionate concentration.

Lactic acid concentration, when represented as the average of each treatment per collection period, ranged from 0.76 to 10.0 μg/mL throughout the trial, which is within the acceptable range (Tedeschi and Fox, 2018; Russell, 2002). Nevertheless, there was no effect of yeast treatment on lactic acid concentration in the rumen during when cattle were fed the GRW diet (P = 0.996), TRANS diet (P =0.168), or the FIN diet (P =0.574), but the LY2 treatment had the least concentration consistently throughout all diets (Table 2).

Methane. Yeast treatment did not significantly impact methane production (P = 0.215), but as expected, DMI did in a linear fashion (P = 0.049) when cattle were fed the GRW diet. The LY3 had the least methane production (Table 2, Figure 4A). Treatment was not different (P = 0.265) for the TRANS diet but tended to be during the FIN diet (P = 0.052) where the CON treatment produced the least amount of methane (Table 2, Figure

4A). Although Carro et al. (1992) examined the effects of yeast culture, they reported the same patterns of methane production when cattle received three different levels of dietary concentrate in the ration. In our study, when cattle were fed low and medium concentrate rations, the treated animals produced less methane than did controls. When cattle were on the highest level of a concentrate ration, the control animals produced less methane than the treated animals. Similar responses of in vitro methane production have been observed when using a high-concentrate diet as a substrate by Dawson and Newman (1988) as well.

Protozoa count. No treatment or DMI effects or their interactions were observed on the protozoa numbers in the GRW diet ($P > 0.05$) (Table 2, Figure 4B); however, when cattle were fed the TRANS diet, yeast treatment ($P = 0.049$), DMI ($P = 0.045$), and their interaction ($P = 0.049$) were observed. At the average DMI, LY1 had the greatest count of protozoa ($P = 0.029$). During the FIN diet, yeast treatment affected protozoa numbers ($P < 0.05$) where LY2 had the greatest count of protozoa. When high-concentrate diets are fed, and ruminal pH decreases below 6.0, protozoa populations decrease (Franzolin and Dehority, 1996). Small amounts of roughage are often included in high-grain finishing diets to reduce digestive and metabolic problems and may have a positive effect on maintaining the ruminal protozoa populations (Kreikemeier et al., 1990). Newbold et al. (1996) reported no difference in protozoa when supplementing LY in the form of *S. cerevisiae*. Conversely, the present study suggests that supplementation of LY when transitioning to a grain-based diet may increase protozoa populations that can play a role in starch sequestration that reduces the rate of starch fermentation, thereby reducing the risk of cattle developing ruminal acidosis. As previously documented (Ushida and Jouany, 1996), the increased protozoa count during the FIN diet is consistent with the tendency of

increased methane production; thus, the inclusion of an ionophore might be beneficial to reduce methane when feeding LY. Further investigation is needed to fully understand the effects of LY on protozoa.

Ruminal pH. Yeast treatment tended to affect ruminal pH ($P = 0.104$) in a quadratic fashion ($P = 0.093$) when cattle were fed the GRW diet (Table 2). As shown in Figure 5, animals that received any inclusion of LY had greater pH than CON treatments after 21 d, suggesting an interaction between yeast treatment and time. The covariate DMI impacted ruminal pH ($P = 0.070$) when cattle received the TRANS diet while yeast treatment did not ($P = 0.308$). Treatments tended to behave in a quadratic pattern ($P = 0.089$) in which LY1 had the highest pH. In Figure 5, the increase in ruminal pH at week 5 for CON treatments was unexpected and does not follow the general trend for this treatment. All other treatments had a similar pattern of ruminal pH decreasing over time. When we removed this anomaly from the dataset, LY1 consistently produced a greater ruminal pH than did CON. For the FIN diet, treatment also tended to impact ruminal pH ($P = 0.061$) in linear and quadratic fashions ($P = 0.010$ and $P = 0.033$, respectively) as did DMI ($P = 0.002$), and there was an interaction between treatment and DMI ($P = 0.056$) (Table 2, Figure 5), suggesting that different levels of intake of DM might affect the LY effects in the ruminal pH. Overall, in our study, the ruminal pH in animals fed LY1 was higher than CON treatments, which is confirmed by previous studies (Thrune et al., 2009; Fiems et al., 1993, Erdman,1988). This finding is interesting considering the VFA concentration of LY1 throughout the study was higher than CON during the GRW and FIN diets, but according to Tedeschi and Fox (2018), VFA is not the only variable effecting ruminal pH. While pH is sensitive to the acid load in the rumen, it also depends on the

buffering capacity and the fractional rates of absorption of the fermentation acids through the rumen epithelium and their passage through the reticulum-omasum orifice. As long as passage rate, buffering capacity, and absorption rate are higher than the rate of VFA production, ruminal pH may still be high (Tedeschi and Fox, 2018). This could explain why we see values of ruminal pH of certain treatments higher than one would think when comparing them to their VFA concentrations during the same period.

In Situ Digestibility

Williams et al. (1991) found that the inclusion of *S. cerevisiae in* ruminant diets increased DMD of hay incubated in the rumen of steers fed a mixed ration of hay and rolled barley after 12 h; however, after 24 h, degradation was similar across all treatment groups. On the other hand, Newbold et al. (1996) did not observe that degradation was affected significantly by treatment; however, they did see a trend towards an increase in the population of cellulolytic bacteria in the rumen with yeast present, which is favorable for increased degradation, but as stated previously, the effect did not reach significance in our study. Carro et al. (1991) discovered that yeast culture has no significant effect on DM and NDF degradability with medium and low concentrate diets, but when supplementing the high- concentrate diet, LY resulted in significantly higher DM and NDF degradation. Collectively, these studies suggest an advantage to feeding LY to aid with degradation, though results still vary widely.

Dry matter digestibility. There was an effect of DMI ($P = 0.005$), yeast treatment ($P = 0.003$), and an interaction between treatment and DMI ($P < 0.003$) on DMD (Table 2, Figure 6A) in a linear ($P = 0.047$) and quadratic fashion ($P < 0.007$) for the GRW diet. For the TRANS diet, yeast treatment also differed ($P < 0.007$), and there was an interaction

observed between DMI and treatment ($P = 0.009$). When reporting treatment means for average DMI during the TRANS and GRW diets, LY2 resulted in higher DMD of the three yeast treatments ($P < 0.05$) but was not different from the control ($P > 0.05$). When cattle were fed the FIN diet, treatment affected the DMD ($P < 0.001$) in a cubic fashion ($P \leq 0.100$) in which LY3 had the highest DMD. Our results suggest that LY possibly increased the population of fiber-degrading bacteria or their activity, but they are contrary to the results of Carro et al. (1991) who found that LY resulted in greater DMD in low- to medium-concentrate diets but not with high concentrate diets. This difference could be because they were supplementing with a yeast culture, not live yeast as we did. As mentioned before, the biological differences between the two probiotic yeast products could influence what is observed, so additional data where LY products are the area of interest in determining the effects on DMD is needed to confirm our findings.

Neutral detergent fiber digestibility. For NDFD, as depicted in Table 2 and Figure 6B, TRT was different for all diets ($P < 0.05$), DMI affected the GRW diet ($P = 0.004$), and there was an interaction between DMI and yeast treatment ($P = 0.004$) during the TRANS diet. When the GRW diet was fed, LY1 and CON had the highest NDFD when treatment means were reported for the average of DMI. Throughout the TRANS diet, LY1 had the highest NDFD for average DMI as well, and while cattle were eating the FIN diet. The LY3 treatment and CON seemed to promote greater NDFD for unadjusted values. When examining values adjusted for DMI in low- and medium-concentrate diets, LY1 seems to have the greatest NDFD of the three yeast treatments, but in a high-concentrate diet comparing unadjusted values, LY3 provided the greatest NDFD. A study performed by Monnerat et al. (2013) found no significant changes in digestive parameters when

supplementation of two levels of yeast to beef cattle that were being fed diets containing different starch levels. The diverse results found throughout these studies solidifies the need for additional studies to determine the correlation between yeast treatment and diet composition on NDFD.

CONCLUSION

It is becoming critical to understand the nature of interactions between yeast probiotics, the ruminant gastrointestinal microbial population, and dietary components in order to predict the impact of probiotic supplementation on cattle nutrition. This acknowledgment is essential to select more targeted and reliable probiotics to capitalize on a promising antimicrobial alternative feed additive. The mechanisms and attributions of LY on targeted rumen microbial communities, animal growth, health, and overall productivity have been extensively studied, at least for some strains of *S. cerevisiae*. While there remains some perception of probiotics as "magical additives," research investigating the effects of probiotics has restored credibility to probiotic use in ruminant nutrition; however, much of the impact of LY supplementation remains unknown. Indeed, field studies indicate that positive effects on milk or meat production can be obtained, but the animal response to such feed additives may be quite variable depending upon various factors such as nature of the diet, level of productivity, animal physiological and genetic factors, dose, and strain of yeast used. Our results indicated that the daily supplementation of 2.5 g LY/d yielded a more rapid rate of fermentation in the TRANS diet, less total gas production in the FIN diet, higher protozoa counts, and greater DMD and NDFD in the GRW and TRANS diets, and a greater ruminal pH in all diets. The rumen fluid from cattle supplemented with 5.0 g LY/d had the least concentration of lactate throughout all diets

and had the greatest protozoa numbers when cattle were fed the FIN diet. The supplementation of 10 g LY/d provided the least A:P ratio and subsequently least methane production during the feedings of all diets, least total gas production during the GRW and TRANS diets, and the greatest DMD and NDFD while cattle were fed the FIN diet. Results regarding yeast treatment effects on total VFA varied across all diets with no statistically significant difference between treatments.

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Table 2.1. Diet formulation and chemical composition of growing period diets.

	Diets[1]		
Items	Grower	Transition	Finisher
Ingredients, % of the diet AF			
Cracked corn	40.0	52.5	65.0
Alfalfa pellets	28.0	21.75	15.5
Bermuda grass hay	8.00	9.00	10.0
Cottonseed hulls	15.0	7.50	0.00
Cow base mineral	1.50	1.25	1.00
Urea	1.00	1.00	1.00
Molasses	6.50	6.75	7.00
Limestone	0.00	0.25	0.50
Chemical Composition, % DM			
DM	88.0	87.8	87.4
CP	13.8	13.0	12.3
SP, % CP	56.4	54.0	47.1
ADIN	1.35	0.98	0.62
NDIN	1.66	1.48	1.48
ADF	25.5	19.1	13.4
NDF	36.9	31.7	27.3
Lignin	6.67	4.23	2.79
Sugar	6.90	6.40	6.20
Starch	29.6	37.3	45.5
Fat	2.88	2.85	2.77
Ash	6.09	5.35	4.49
Ca	0.84	0.80	0.74
P	0.37	0.37	0.35
Mg	0.21	0.19	0.16
K	1.57	1.34	1.10
S	0.24	0.23	0.23
Na	0.19	0.15	0.11
Fe	283	259	202
Mn	50.0	55.0	34.0
Zn	45.0	60.0	42.0
Cu	21.0	18.0	14.0
TDN	66.6	72.1	76.3
NEm (Mcal/kg)	1.50	1.68	1.81
NEg (Mcal/kg)	0.92	1.08	1.19

[1] 200 mg of monensin was supplemented to every animal during all feeding phases.

Table 2.2. Main effects of dry live yeast on rumen parameters of growing cattle for three types of diets.

	Dietary Treatment [1] (g/hd/d)				SEM	P- Values[2]			Contrasts			Covariate	
Variables	0	2.5	5	10		TRT	Time (T) [3]	TRT x T	L	Q	C	Initial pH	DMI
Grower													
IVGP- a [4], mL	10.10	9.23	8.31	8.21	1.12	NS	***	NS	NS	NS	NS	--	--
IVGP- b [4], %/h	0.192^{c}	0.429^{a}	0.223^{bc}	0.548^{ab}	0.083	†	*	NS	†	*	NS	--	[5]
Total VFA	52.8^{b}	62.5^{a}	59.1^{ab}	54.7^{b}	2.99	NS	NS	†	NS	NS	NS	--	0.044
A:P Ratio	3.06	2.82	2.83	2.81	0.183	†	*	NS	NS	NS	*	--	0.021[5]
Lactate	2.51	2.67	2.50	2.54	0.100	NS	NS	NS	NS	NS	NS	--	--
Methane	8.71^{a}	7.59^{ab}	8.30^{a}	5.58^{b}	0.689	NS	***	***	*	NS	NS	--	0.048
pH	5.71^{ab}	5.76^{a}	5.65^{b}	5.75^{a}	0.030	†	†	†	NS	†	NS	0.108	--
Protozoa	13.6	15.3	13.9	9.71	3.21	NS	*	*	NS	NS	NS	--	--
DMD, %	78.1^{a}	78.1^{a}	70.2^{b}	72.6^{b}	1.40	*	***	***	*	*	***	--	0.005[5]
NDFD, %	82.7^{a}	82.7^{a}	77.3^{b}	78.5^{b}	1.00	*	***	***	NS	NS	NS	--	0.004[5]
Transition													
IVGP- a[4], mL	15.9^{a}	7.31^{b}	9.00^{b}	6.55^{b}	2.73	*	*	NS	NS	NS	NS	--	--
IVGP- b[4], %/h	0.199^{b}	0.696^{a}	0.305^{b}	0.593^{ab}	0.110	NS	NS	NS	NS	NS	NS	--	5
Total VFA	60.9	55.0	49.2	59.0	5.13	NS	*	NS	NS	NS	†	--	--
A:P Ratio	2.78	2.15	2.77	2.11	0.253	NS	*	NS	NS	NS	NS	--	--
Lactate	2.62	1.40	1.22	1.54	0.100	NS	NS	NS	NS	†	†	--	--
Methane	14.2	15.2	13.2	12.5	1.12	NS	NS	*	NS	NS	NS	--	--
pH	5.79	5.85	5.73	5.65	0.088	NS	***	†	NS	†	NS	0.050	0.070
Protozoa	10.7^{ab}	13.7^{a}	6.30^{bc}	1.87^{c}	2.10	*	†	†	NS	NS	NS	--	0.045[5]
DMD, %	75.5	76.0	72.2	72.0	1.90	*	NS	NS	NS	NS	NS	--	[5]
NDFD, %	78.7	79.2	77.8	76.4	1.90	*	***	*	NS	NS	NS	--	[5]
Finisher													
IVGP- a [4], mL	12.4^{a}	7.07^{b}	12.2^{ab}	16.4^{ab}	2.51	†	NS	NS	NS	NS	NS	--	0.027[5]
IVGP- b [4], %/h	0.278	0.239	0.187	0.047	0.111	*	NS	†	*	NS	NS	--	0.007[5]
Total VFA	55.7^{b}	60.3^{ab}	73.1^{ab}	64.9^{a}	4.42	*	NS	NS	NS	*	NS	--	--
A:P Ratio	2.17	2.04	2.34	1.80	0.293	NS	NS	†	NS	†	†	--	--
Lactate	2.64	4.93	1.55	2.87	0.240	NS	†	NS	NS	NS	NS	--	--
Methane	11.9^{b}	16.3^{ab}	18.9^{a}	15.3^{ab}	2.28	†	NS	NS	NS	NS	NS	--	--
pH	5.24	5.43	5.36	5.19	0.094	†	*	NS	*	*	NS	0.638	0.002[5]
Protozoa	5.67^{b}	10.2^{ab}	18.5^{a}	16.9^{a}	3.00	*	*	NS	NS	NS	NS	--	--
DMD, %	71.8^{a}	69.3^{a}	64.3^{b}	72.0^{a}	2.00	***	NS	NS	NS	NS	†	--	--
NDFD, %	75.7^{a}	72.3^{bc}	70.2^{c}	73.7^{ab}	1.60	***	***	*	NS	NS	NS	--	--

[1] Treatment values are given as least squares means.

[2] NS = $P \geq 0.10$; † = $P \leq 0.10$; * = $P < 0.05$; *** = $P < 0.001$

[3] All animals were fed for 9 weeks (1 week adaptation, 8 weeks observation). DMD and NDFD samples were collected for 7 weeks, protozoa and lactate for 8 weeks, and all other variables were collected for 9 weeks.

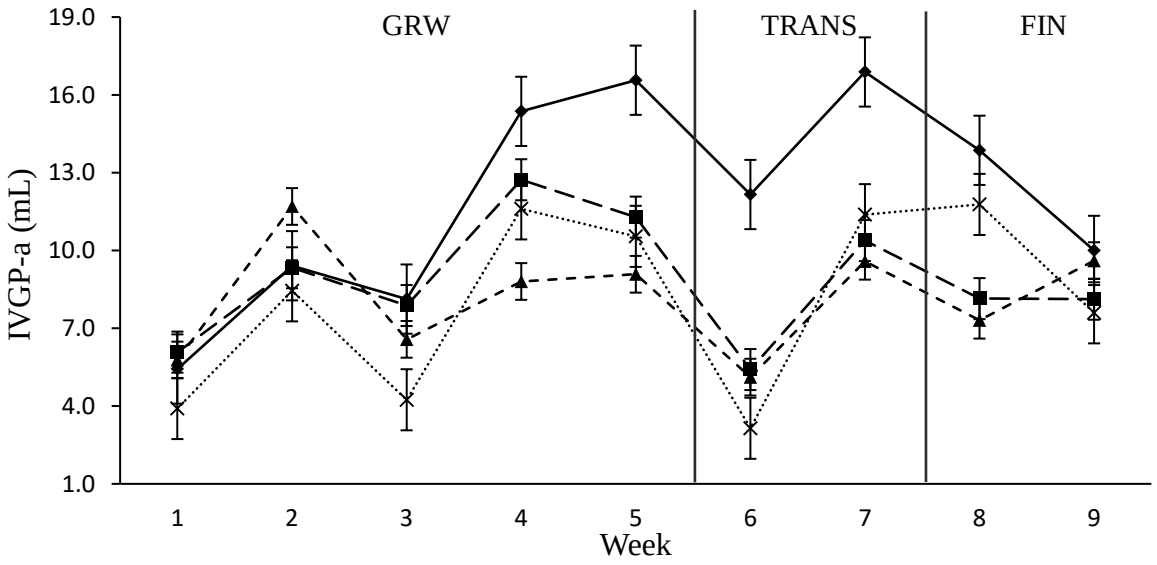
[4] A = the asymptote measurement of the exponential model (total gas production). B = the fraction rate of gas production of the exponential model.

[5] There was a significant ($P < 0.01$) interaction between dietary treatment and DMI, so the dietary treatment means are reported for the average of DMI.

[a,b,c] Means with different superscripts differ by $P \leq 0.05$

Figure 2.1. Effects of dry live yeast on the in vitro (A) total gas production and (B) fractional rate of fermentation for ♦ = CON, ■ = LY1 (2.5g/d), ▲= LY2 (5g/d), and ×= LY3 (10g/d). GRW diet was fed weeks 1-5, TRANS was fed weeks 6 and 7, and FIN diet was fed weeks 8 and 9.

(A)



(B)

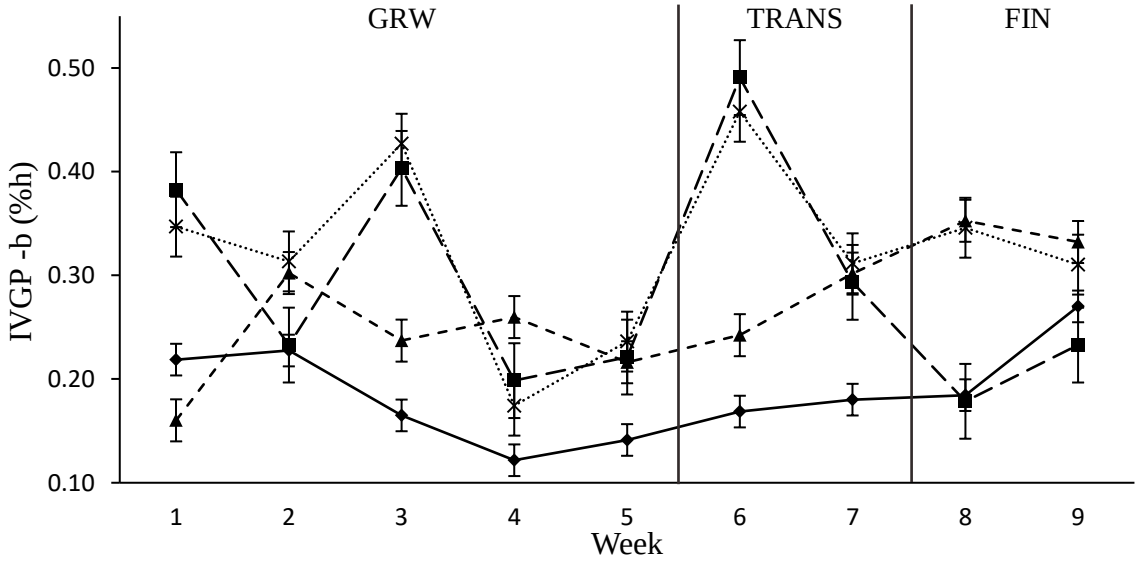


Figure 2.2. Effects of dry live yeast on the total volatile fatty acids concentration in the rumen (white = CON (0g/d), black = LY1 (2.5g/d), grey = LY2 (5g/d), diagonal stripes = LY3 (10g/d)). **GRW** diet was fed weeks 1-5, **TRANS** was fed weeks 6 and 7, and **FIN** diet was fed weeks 8 and 9.

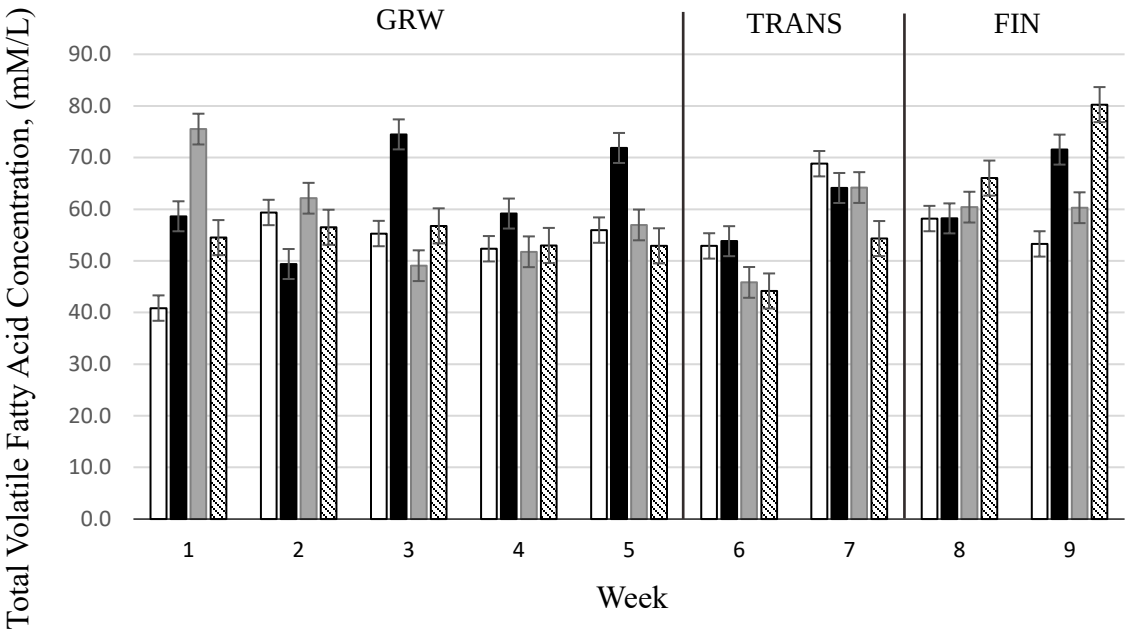


Figure 2.3. Effects of dry live yeast (Actisaf hr+) on the the acetate:propionate ratio in the rumen (white = CON (0g/d), black = LY1 (2.5g/d), grey = LY2 (5g/d), diagonal stripes = LY3 (10g/d)). **GRW** diet was fed weeks 1-5, **TRANS** was fed weeks 6 and 7, and **FIN** diet was fed weeks 8 and 9.

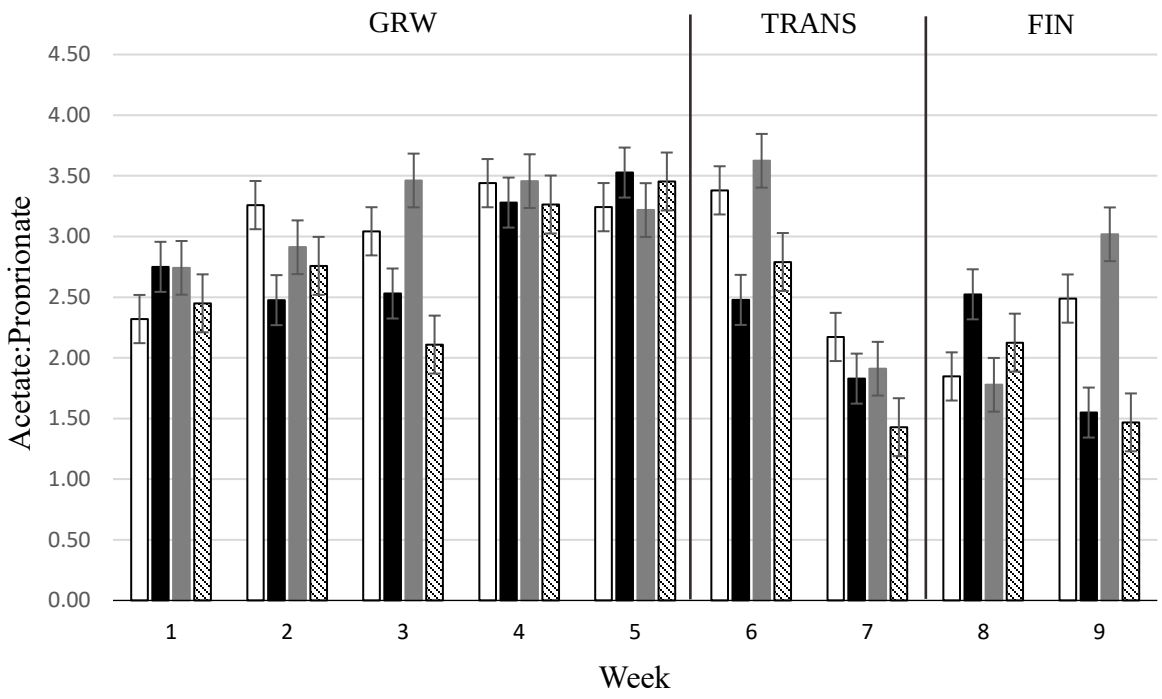


Figure 2.4. Effects of dry live yeast on (A) methane production and (B) protozoa (♦ = CON (0g/d), ■ = LY1 (2.5g/d), ▲= LY2 (5g/d), ×= LY3 (10g/d)). **GRW** diet was fed weeks 1-5, **TRANS** was fed weeks 6 and 7, and **FIN** diet was fed weeks 8 and 9.

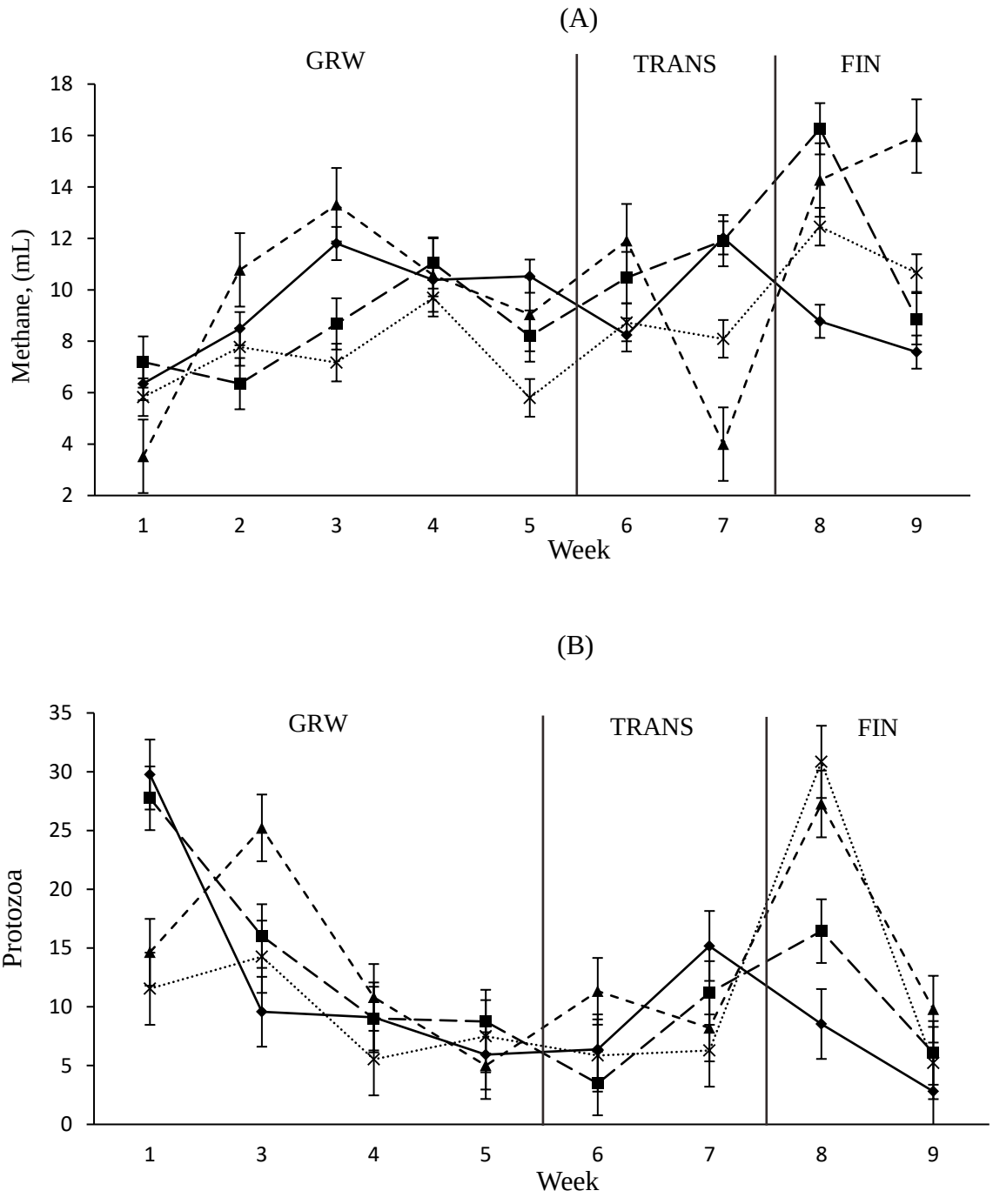


Figure 2.5. Effects of dry live yeast on rumen pH (♦ = CON (0g/d), ■ = LY1 (2.5g/d), ▲= LY2 (5g/d), ×= LY3 (10g/d)). **GRW** diet was fed weeks 1-5, **TRANS** was fed weeks 6 and 7, and **FIN** diet was fed weeks 8 and 9.

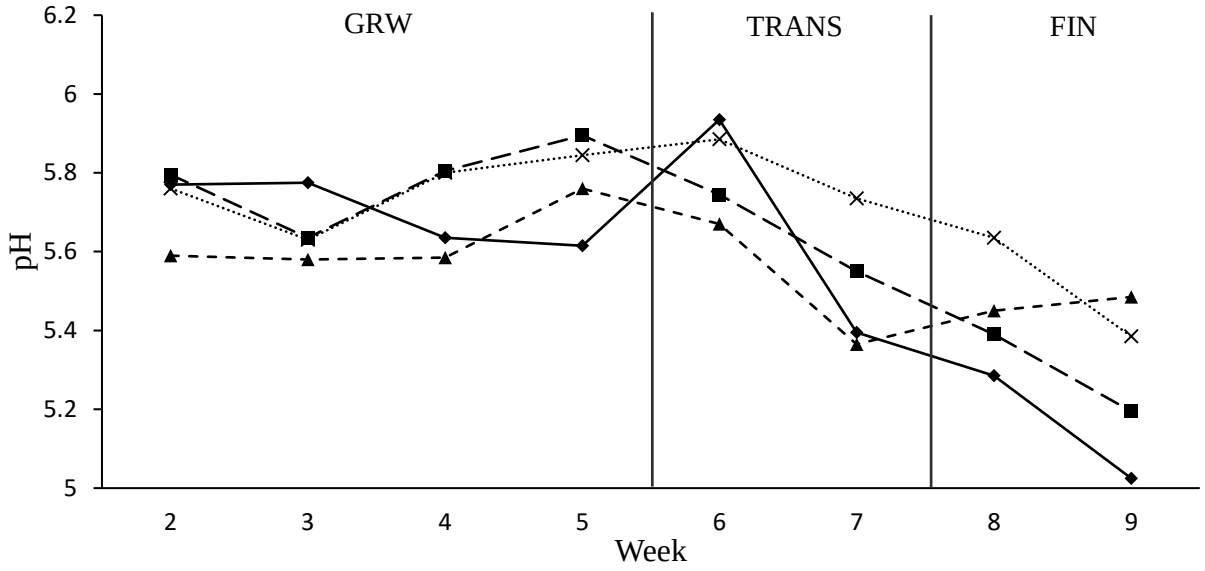
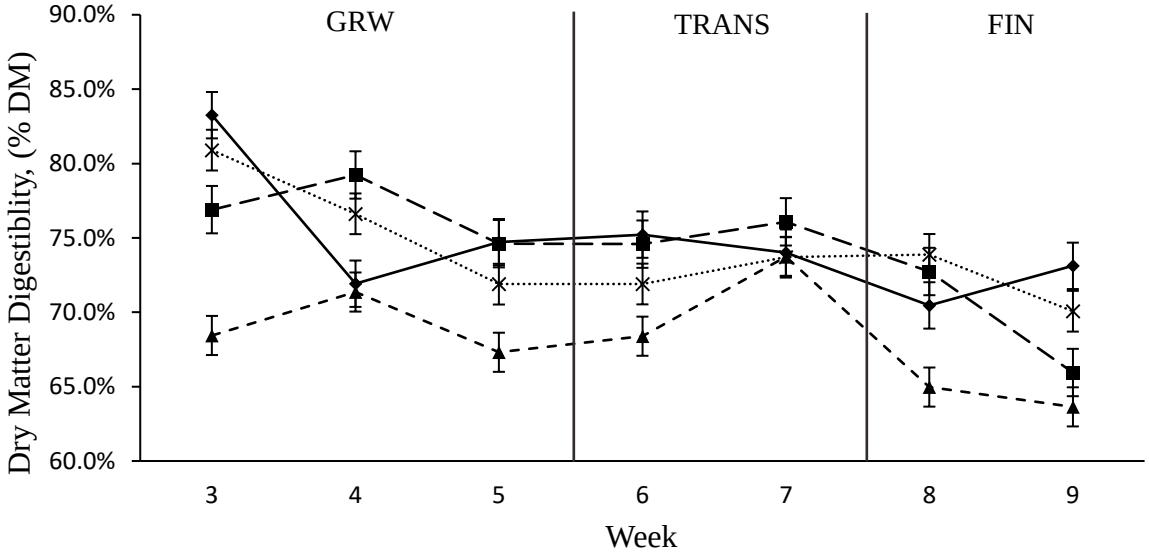
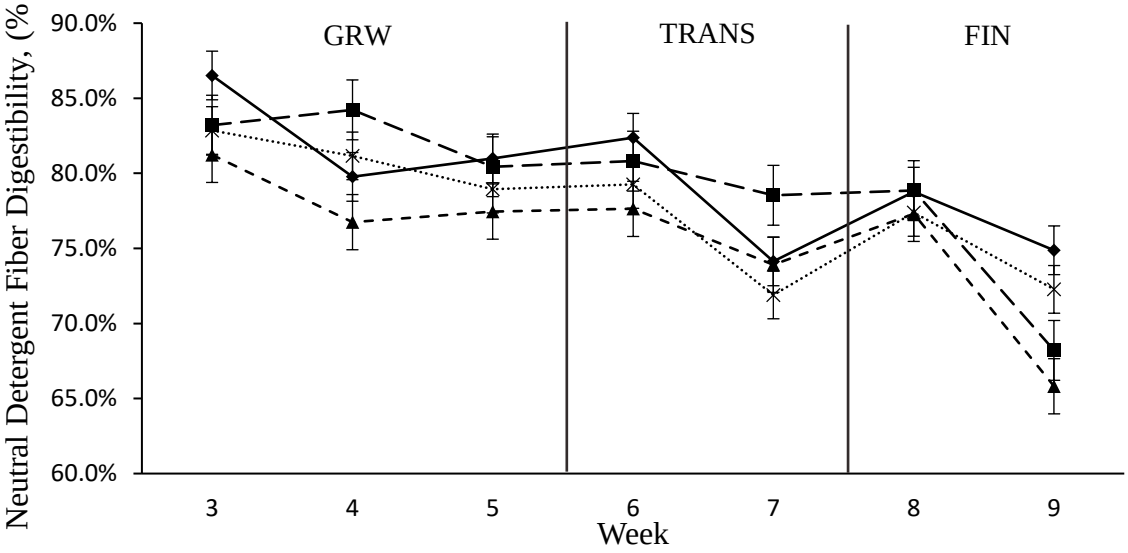


Figure 2.6. Effects of dry live yeast on (A) dry matter digestibility and (B) neutral detergent fiber digestibility for ♦ = CON, ■ = LY1 (2.5g/d), ▲= LY2 (5g/d), and ×= LY3 (10g/d). **GRW** diet was fed weeks 3-5, **TRANS** was fed weeks 6 and 7, and **FIN** diet was fed weeks 8 and 9.



(A)

(B)



CHAPTER III

EVALUATION OF DIFFERENT INCLUSION LEVELS OF DRY LIVE YEAST IMPACTS ON VARIOUS RUMEN PARAMETERS AND IN SITU DIGESTIBILITY OF DRY MATTER AND NEUTRAL DETERGENT FIBER IN GROWING AND FINISHING BEEF CATTLE

SUMMARY

The objective of this trial was to determine the effects of supplementing dry live yeast (**LY**; *Saccharomyces cerevisiae*) on in vitro gas production (**IVGP**) fermentation dynamics, pH, and CH_4 concentration at 48 h, and in situ rumen parameters and digestibility of dry matter (**DMD**) and neutral detergent fiber (**NDFD**) of growing cattle during three feeding phases: grower (**GRW**) for 17 d (38% steamed-flaked corn (**SFC**)), transition (**TRANS**) for 15 d (55.5% SFC: 1.2 Mcal/kg NEg), and finisher (**FIN**) for 13 d (73% SFC: 1.23 Mcal/kg NEg). Twenty British-crossbred, ruminally-cannulated steers (183 kg ± 44 kg) six months of age were blocked by weight into five pens containing Calan gate feeders and received a control (**CON**) diet (17.2% CP, 35.8% NDF, 86.7% DM) without LY on d -12 to d 0. Animals were randomly assigned to treatments (five animals per treatment): CON or LY at inclusion rates of 5 g/d (**LY1**), 10 g/d (**LY2**), or 15 g/d (**LY3**) top dressed every morning at 0800 for 45 d. The DMD and NDFD were assessed during seven separate collection days using in situ nylon bags containing 5 g of GRW, TRANS, or FIN diets, incubated at 1200 for 48 h. Protozoa counts (**PC**) were determined during five collection periods. Data were analyzed as a repeated measure within a randomized complete block design (random effect of pen). For GRW, TRT altered the total gas production of the nonfiber carbohydrate (**NFC**) (P = 0.045) and the fractional

rate of degradation (**kd**) of the fiber carbohydrate (**FC**) pool ($P = 0.001$) in a cubic pattern ($P \leq 0.05$): LY2 had the most gas production and fastest kd. TRT also influenced DMD ($P = 0.035$) and NDFD ($P = 0.012$) with LY2 providing the greatest digestibility. For TRANS, TRT tended to affect the NFC kd ($P = 0.078$) and influenced pH ($P = 0.04$) and DMD ($P < 0.001$) in which LY2 yielded the fastest kd, highest pH, and greatest DMD. For FIN, there was an effect of TRT on total gas production ($P < 0.001$) and kd ($P = 0.004$) of the NFC pool, FC kd ($P = 0.012$), CH_4 concentration ($P < 0.001$), PC ($P < 0.001$), DMD ($P = 0.039$), and NDFD ($P = 0.008$). LY1 had the highest PC and provided the greatest DMD and NDFD. LY2 had the fastest kd of both the NFC and FC pools and had the least CH_4 concentration. LY3 had the greatest NFC gas production. No specific dose-response pattern was observed, but supplementation with LY affected IGVP, rumen parameters, and digestibility consistently. LY2 (10 g/d) provided the most beneficial result for all diets.

INTRODUCTION

The continued escalation of livestock production will likely continue due to an increasing worldwide demand for livestock products. Researchers are continually investigating ways that beef cattle production can become a more efficient and economical process. Meat yields have been improved by supplementing livestock with different sources of feedstuffs and feed additives that provide not only appropriate levels of protein, vitamins, minerals, and energy, but also adequate animal health. Due to current trends in consumer preferences and government regulation through directives such as the Veterinary Feed Directive, interest has been sparked in finding additional means where we can still receive similar results as the current medicated feed additives. This has increased the use of alternative additives in animal nutrition. One of these alternative sources, direct fed

microbial (**DFM**) have been consistently investigated because they have been shown to improve animal performance due to their ability to modify the rumen environment and overall function (Tedeschi et al., 2011). Of these DFM, live yeast (**LY**) is one of the most studied, specifically *S. cerevisiae*. The effect of LY has been extensively studied on dairy cattle (Desnoyers et al.,2009). The role of LY in ruminants is not clearly defined, but it is suggested to improve dry matter digestibility (**DMD**), stabilize ruminal pH, thus increasing performance in intensive feeding systems. This is thought to occur because of the alteration of fermentative pathways from lactate to propionate by increasing the lactate-utilizing and cellulolytic bacterial populations (Chauchryras et al., 1996, Lila et al., 2004). It has been found that supplementing yeast assisted with digestion and metabolism of feedstuffs in ruminants in multiple aspects such as increase nutrient digestibility, help reach the optimal proportion of volatile fatty acids, decrease ruminal ammonia nitrogen, palliation of pH fluctuation, and stimulation of microorganism population (Chaucheyras-Durand et al., 2008).

Additionally, it has been proven to provide various growth factors, pro-vitamins, and other stimulants to rumen microorganisms (Jouany, 2006). Moreover, *S. cerevisiae* is said to have the ability to decrease the redox potential of the rumen (Marden et al., 2008) and promotes a more favorable environment for the development of microorganisms, mainly cellulose consumers, which maximize the fiber degradation rates as well (McAllister et al., 2011).

The effects of such DFM on beef cattle under feedlot conditions are not as investigated as well compared to dairy cattle. Therefore, the objective of this study was to determine the benefits of supplementing LY to growing beef cattle receiving three

consecutive feedlot diets, grower (**GRW**), transition (**TRANS**), and finisher (**FIN**) phases, when examining multiple rumen parameters and in situ DMD and neutral detergent fiber digestibility (**NDFD**).

MATERIALS AND METHODS

All experimental procedures were executed, and animals were cared for according to the guidelines of the Texas A&M University Institutional Care and Use Committee (IACUC protocol #2018-0039). The effects of the inclusion of LY (*Saccharomyces cerevisiae* Sc47 CNCM I-4407, Actisaf HR+, Phileo Lesaffre Animal Care, Milwaukee, WI, 1.10^{10} CFU/g) in diets of growing beef cattle were examined in this study. Data were collected from a 45-d in vitro and in situ trials and were analyzed to determine the total gas production as well as the fractional rate of degradation (**kd**) using the in vitro gas production technique (**IVGP**), methane concentration (**CH_4**), rumen pH, protozoa counts, **DMD**, and **NDFD**.

Animals, Equipment, and Feeding Regimen

Twenty British-crossbred, ruminally-cannulated steers (183 kg ± 44 kg) 6 months of age from Texas A&M AgriLife Research Center in McGregor, TX, were used in this experiment. Cattle were blocked by weight resulting in two pens of heavyweight steers, one pen of medium weight steers, and two pens of lightweight steers that were housed in five separate pens of four animals each. Each pen contained Calan gate feeders (American Calan, Northwood, NH), and water was always accessible. On d -12, cattle were fitted with the Calan sensor. The Large Ruminant Nutrition System (**LRNS**; http://www.nutritionmodels.com/lrns.html; Accessed on June 20, 2018; Tedeschi and Fox,

2018) was used to formulate all diets using the following ingredients: medium chopped alfalfa hay, bermudagrass hay, dried distiller's grain, steamed flaked corn, and a mineral supplement (Table 1). From d -12 to d -1, animals were stepped up to the grower diet (17.21% CP, 35.8% NDF, 86.7% DM) without LY supplement in the Calan gate feeders so they could become acclimated to their individual bunks in the Calan system and adjust to a total mixed ration. Beginning on d 0, each of the three-phase diets was fed sequentially as follows: grower (**GRW**) for 17 d fed during weeks one and two, transition (**TRANS**) for 14 d fed during weeks three and four, and finishing (**FIN**) for 14 d during weeks five and six of the study (Table 1).

Treatments and Experimental Design

Additionally, on d 0, treatments were randomly assigned to animals using a randomized complete block design (5 pens; 4 treatments; 4 animals per pen). Each animal within a block was assigned to a treatment. This allowed for five animals per treatment. Treatments were as follows: control **(CON)**, **LY1** (5 g/d), **LY2** (10 g/d), and **LY3** (15 g/d). Each animal was offered its weighted amount of ration twice daily at 0800 and 1700 in its corresponding bunk. Treatments were top dressed and thoroughly handed mixed during the morning feeding. Baseline ruminal contents were collected on d -1 and weekly collections were taken every 7 d following except for only 5 d between each of the 3 collections during the TRANS phase. There was a total of eight collection days.

Rumen Sampling and Analyses

During the collection process for each treatment, whole rumen contents were extracted from the cranial, middle, and caudal compartments of the rumen and split into two

portions: one portion was frozen for future chemical assays and the second portion was used in the **IVGP** assay as described below. Through the rumen cannula, a combined rumen content (approximately 1 L) was suctioned with a rumen fluid extractor that contained a plastic tube with a strainer cap at the end to prevent a large mass of rumen particles being retrieved. Rumen fluid samples were strained through eight layers of cheesecloth and immediately placed into individual stainless-steel thermoses minimizing headspace to maintain both temperature and an anaerobic environment. Rumen fluid was immediately transported to the Ruminant Nutrition Laboratory with members of the collection team to be prepared for the IVGP technique, pH measurements, and for subsamples to be taken and stored for protozoa counts to be executed at a later time. Meanwhile, two members stayed behind and placed in situ pre-prepared nylon bags into each animal and took rumen pH measurements of individual animals at three separate locations approximately 16 inches from the outside of the cannula opening at each location: the reticulum, the dorsal portion of the rumen, and the caudal portion of the rumen. The pH of each rumen fluid sample was immediately recorded using a Thermo Scientific Orion A221 portable pH meter (Thermo Fisher Scientific, Waltham, MA).

In Vitro Gas Production Measurements

About 6 mL of rumen fluid subsamples from each like-treated animal were homogenized to make a representative sample of each treatment (30 mL). Using a portion of the treatment specific homogenous samples as the inoculum, an in vitro anaerobic fermentation and gas production analysis (i.e., IVGP) was performed on a total of 384 samples (48 samples from each time point collection performed in two separate fermentation chambers). The IVGP technique has been previously described in detail

(Tedeschi et al., 2009; Tedeschi and Fox, 2018; Ch. 9), but briefly, it utilizes an incubation chamber to mimic rumen temperature (39°C) with a multi-plate stirrer housing 24 or 36 Wheaton bottles in each chamber. Approximately 200 mg of each of the phase-specific the diets (GRW, TRANS, and FIN depending on the feeding phase), ground to 2 mm, was weighed and transferred into 125 mL Wheaton bottles containing equal sized magnetic stir bars. Samples were dampened with 2.0 mL of distilled H_2O to prevent particle scattering during subsequent CO_2 flushing to maintain an oxygen-reduced atmosphere. Meanwhile, an anoxic media (Goering and Van Soest, 1970) was continuously flushed with O_2-free CO_2, and then14 mL was added to each bottle always under constant CO_2 flushing. Each bottle was then sealed with lightly greased butyl rubber stoppers and closed with aluminum crimps (Bellco Industries, Vineland, NJ). Bottles were instantly placed in the 39 ºC incubator and connected to their respective pressure sensors via needle insertion. Representative rumen fluid samples from treated cattle were then again filtered through 4 layers of cheesecloth and glass wool, into a flask continually flushed with CO_2, and 4 mL of previously prepared rumen inoculum was injected anoxically into each Wheaton fermentation bottle via a needle and syringe which contained either a blank, alfalfa has as the laboratory standard, or phase-specific diet in quadruplicates, respectively. Internal pressure was equilibrated to atmospheric pressure at time 0 by piercing rubber stoppers with a needle for approximately 5 seconds, before initiating recording. Once the pressure was equalized in all bottles, software recording was initialized, and atmospheric pressure was recorded at 5-minute intervals for 48 h. Real-Time plotting of the fermentation profile over time for each bottle was monitored for abnormalities. After 48 h of fermentation, software recording was terminated, and bottles were placed in a refrigerator (-8ºC) to cease

fermentation. The headspace gas was sampled (1 mL) from each bottle and analyzed for methane concentration using the gas chromatography method (Allison et al., 1992). The Final incubation pH was measured on the remaining rumen fluid. Then, 40 mL of neutral detergent solution (Van Soest et al., 1991) was added to each bottle, resealed, and autoclaved for 15 min at 120ºC. The undegraded fiber was then filtered gravimetrically using Whatman 54 filter paper, oven dried at 60ºC for 48h, and the residue was weighed to calculate IVGP dry matter digestibility. All steps of the IVGP process was completed for two separate chambers which allowed for 48 samples per collection (24 in each chamber).

Protozoa Counts

In accordance with methods described by Dehority (1984), protozoa counts were performed without staining. The counting technique was an adaptation of the procedure described by Purser and Moir (1959). In summary, about 1-mL subsample of the original rumen fluid from each animal was added to 10 mL formalin to achieve a 1:10 dilution of the original rumen contents. A 1 ml aliquot of the formalinized sample was pipetted into a Sedgewick Rafter counting chamber using a 1 mL pipet with a 3 mm wide orifice. Protozoa were counted at a 100x magnification with a counting grid 0.5 mm square in the eyepiece. Twenty-five evenly spaced grids from the entire chamber surface were counted for each rumen fluid sample (120 total samples). Protozoa per mL of rumen fluid were then calculated as follows: the sum of protozoa counted in all twenty-five grids was multiplied by the dilution factor which was then multiplied by the multiple of the volume of a square and the total number of squares counted (25).

In Situ Ruminal Incubations

There are many variants for the in situ incubation technique (Tedeschi and Fox, 2018; p. 148). In our study, small nylon bags, 5 x 10 cm, 50 μg micron porosity (ANKOM Technologies, Macedon, NY, USA) were weighed, filled with 5 g of ground sample (to pass through a 2-mm screen), and sealed (Vanzant et al., 1998). Two sealed blank bags and five bags filled with the phase-specific diet (GRW, TRANS, FIN) were incubated into every animal for a 48 h period each week. The empty bags were used as blanks to correct for feed particles and microorganisms that may have adhered to the nylon bags after incubation. The small nylon bags were held together in a 32 x 42 cm polyester bag with a nylon zipper during the 48 h incubation period. After removal from the rumen, nylon bags were rinsed with distilled water to remove large particles of rumen contents off the bags and were washed through a series of ten three-minute washes cycles in a washing machine consisting of a 2-minute wash and a 1-minute spin (Vanzant et al., 1998). Upon completion of the washing process, the bags were placed in a forced-air oven and dried at 55°C for 48 h in preparation for further analyses.

Digestibility Analyses

Dry matter digestibility. After nylon bags were removed from the forced-air oven, they were placed in a desiccator, and individual dry weights were obtained from all samples. The residual weight of each sample was determined after drying to calculate in situ DMD by dividing the residue weight by the original sample weight before incubation.

Neutral detergent fiber digestibility. After dry weights were retrieved on each in situ bag sample, they went through additional wash cycles in the ANKOM machine to determine the NDF residue. The NDFD was determined by methods described originally

by Van Soest and Robertson (1980) using an Ankom 200 Fiber Analyzer (Ankom Technologies, Macedon, NY, USA). In summary, individual bags were placed in the suspender (3 bags per level), and the machine was filled with approximately 1900 to 2000 mL of NDF solution. After the temperature reached 100ºC, bags were placed into the solution, and the agitator was turned on for 70 minutes. Upon wash cessation, the bags went through a second and a third rinse with about 1900 mL of preheated distilled H_2O and were agitated for 10 minutes each time. The final rinse is approximately 1900 mL of room temperature distilled H_2O and agitated for 10 minutes. Bags were then removed from the suspender, the excess of water was manually removed, placed in a 100 mL beaker and covered with acetone for 3 to 5 minutes. Following this acetone bath, bags were removed, placed on a drying rack for 5 to 10 minutes then placed into a 55°C oven for 48 h. Once dry, they were immediately placed into a desiccator until final weights were able to be taken. The NDFD was calculated as follows:

$$\%\, NDFD = \left(1 - \frac{W_3 - (W_1 \text{x}\, C_1)}{W_2}\right) \text{x}\, 100$$

where W_1 is the bag tare weight, W_2 is the sample weight, W_3 is the dried weight of bag post incubation, and C_1 is the blank bag correction or the running average of the final oven dried weight divided by the original bag weight.

Statistical Analyses

The effect of treatment was tested using the least square means and orthogonal contrasts. The PROC IML was used to obtain the orthogonal coefficients for linear, quadratic, and cubic contrasts. The interaction between treatment, week, DMI, or the

covariate was removed from the statistical model if not significant at $P < 0.05$. Data were considered significant at alpha level $P \leq 0.05$, and tendencies were discussed at $P \leq 0.10$.

In vitro analysis. The PROC MIXED of SAS (SAS Inst. Inc., Cary, NC) was used to analyze the data from representative rumen fluid from like-treated animals (IVGP-a, IVGP- b, IVGP- d, IVGP- e, and methane) as a complete randomized block design. Incubation box was the random effect, treatment was the fixed effect, and incubation bottle within the box was the subject.

In situ analysis. The PROC GLIMMIX of SAS (SAS Inst. Inc., Cary, NC) was also used to analyze the data collected from animals (pH, protozoa, DMD, and NDFD) as a complete randomized block design. The pens were the random effect, treatment was the fixed effect, and animal within pen was the subject. The average dry matter intake (**DMI**) of each animal was used as a covariate for all animals variables. Similarly, the initial pH and initial protozoa concentration of the animals were also used as covariates. The weeks, or time (T), of rumen fluid collections were analyzed as repeated measures design using the REPEATED statement.

RESULTS AND DISCUSSION

In vitro Fermentation

S. cerevisiae live cells have been shown to stimulate fermentation of mixed ruminal microorganisms (Lila et al., 2004). The IVGP technique allowed for insight into the fermentative capacity of each of the adapted, representative, treated rumen fluid samples. The total gas production (Table 2, Figure 1A, 1B), and the fractional rate of degradation (Table 2, Figure 2A, 2B) of each treated, representative sample was computed for two separate pools (non-fiber carbohydrate pool (NFC) and fiber concentrate (FC)

pool) during the 48 h fermentation period (Schofield et al., 1994; Tedeschi and Fox, 2018). Specific variables within these two pools are IVGP-a (total gas production of the NFC pool) (Figure 1A), IVGP- b (factional rate of degradation of the NFC pool) (Figure 2A), IVGP- d (total gas production of the FC pool) (Figure 1B), and IVGP- e (factional rate of degradation of the FC pool) (Figure 2B). Adjustments to the parameters of the IVGP were done as proposed by Tedeschi and Fox (2018; Ch 9).

Total gas production. A TRT by T interaction was observed ($P < 0.05$) during the fermentation of the NFC (IVGP- a) when cattle were fed the GRW and FIN diet, and there was an effect of T ($P < 0.001$) during the TRANS diet. Overall, TRT did not significantly affect IVGP-a during the feeding of the GRW or TRANS diet; however, TRT tended to respond in a cubic pattern during the GRW ($P = 0.081$) with LY1 producing the most total gas. When looking at the weeks when cattle were fed the FIN diet, there was an effect of TRT ($P < 0.001$) and T ($P < 0.001$) in addition of the interaction of the two, as mentioned above. The TRT responded in a quadratic ($P < 0.001$) and cubic ($P < 0.051$) pattern where LY3 produced the greatest amount of total gas overall. When comparing individual TRT within the same run, differences were able to be detected in week 1, 2, 4, 5, 6 (Figure 1A). This illustrates why we see a TRT by T interaction when comparing TRT effects across all weeks. This interaction is most likely due to the different diets being fed during each of the feeding phases (GRW: week 1, 2, TRANS: week 3, 4 5, and FIN: week 6, 7). These interactions suggest that TRT responses may have different outcomes depending on the diet being fed and how long animals have been fed the specific diet (Table 2, Figure 1A).

During the fermentation of the FC pool (IVGP- d) (Figure 1B), TRT ($P = 0.045$) and T ($P = 0.021$) affected total gas production in a cubic fashion ($P = 0.014$) when the

cattle received the GRW diet in which LY2 had the greatest total gas production. No differences were observed overall when cattle went through TRANS ($P \geq 0.05$), but TRT responded in a linear fashion ($P = 0.045$) in which LY1 produced the greatest amount of gas averaged over the whole period. When comparing TRT within each T period of the TRANS phase, TRT influenced total gas production in T period 4 which LY3 had the least total gas production ($P \geq 0.05$). There was a TRT by T interaction detected ($P = 0.003$) as well as an effect of T ($P < 0.001$) during the FIN phase, but not TRT effect was observed ($P = 0.470$).

The total gas production is assumed to represent the digestibility of the substrate being fermented (Tedeschi and Fox, 2018). TRT responses varied from diet to diet, but the inclusion of live yeast increased total gas production suggesting greater digestibility of the substrate incubated. These findings are in accordance with Tang et al. (2008) and Elghandour et al. (2014) who reported that supplementation of a yeast culture increased the total gas production when incubating different types of diets. Although they supplemented with a yeast culture product which does not contain a high count of live yeast cells like a complete live yeast product does, they observed similar results possibly indicating yeast alone, no matter the product (culture or live), may interact with the rumen environment in some manner. The intensity of this interaction may vary depending on the particular yeast product. The results we found are likely due to increased production of propionate acid caused by an improvement in rumen fermentation which in turn increased carbon dioxide via the succinate-propionate pathway (Wolin and Miller, 1988). The inclusion of direct fed microbials like yeast-based products can not only improve total gas production but can also make qualitative changes in the gases produced through increasing animals and rumen

efficiency to help contribute fewer negative effects on the environment (Hristov et al., 2013).

Fractional rate of degradation. There was an interaction of TRT by T ($P = 0.010$) on the fractional rate of degradation (kd) of the NFC pool (IVGP- b; Table 2, Figure 2A) when cattle were fed the GRW diet. There was no overall effect of TRT or T independently ($P = 0.477$, $P = 0.679$, respectively), but as shown in Figure 2A, there were some differences in TRT between week 1 and the interaction can clearly be identified by TRT responding invertedly between week 1 and 2. This indicates that the effects of TRT may be dependent upon how long animals receive LY and how adapted they are to a particular diet. During the TRANS and FIN phase, there was an interaction of TRT and T ($P = 0.002$, $P = 0.003$, respectively) as well as an independent effect of and T ($P < 0.001$) in both feeding phases. TRT also tended to affect the kd ($P = 0.078$) in the TRANS phase in a quadratic pattern ($P = 0.052$) and had a significant effect ($P = 0.004$) in the FIN phase with a cubic response ($P = 0.002$), where LY2 numerically had the fastest kd overall during both feeding phases. Figure 2A reflects the differences of the TRTs within each run and displays a clear image of how TRT responses may be dependent upon how long animals are on the diet in each feeding phase.

Similar interactions were observed for the kd of the FC pool (IVGP- e; Table 2, Figure 2B) throughout the feeding phases. An effect of TRT ($P = 0.001$), T ($P < 0.001$), and a tendency for the interaction of the two ($P = 0.095$), was observed during the feeding of the GRW diet as well as a cubic pattern for TRT response ($P = 0.006$). LY2 had the quickest kd averaged over the entire GRW phase. When cattle went through the TRANS phase an influence of T was detected ($P = 0.020$), but there was no effect of TRT, contrast

pattern, or a TRT by T interaction detected ($P = 0.509$, $P \geq 0.05$, $P = 0.197$, respectively). However, numerically, CON had the slowest kd throughout the TRANS phase. A TRT by T ($P < 0.001$) interaction, effect of TRT ($P = 0.012$), as well as significant cubic and linear pattern responses ($P = 0.024$, $P = 0.045$, respectively) occurred within the FIN diet. Coinciding with results in the GRW phase, LY2 resulted in the numerically fastest kd in the FIN phase as well.

The fractional rate of degradation indicates the proportion of the substrate that disappears per unit of time. Our findings support Dawson et al. (1990) conclusion that active dry live yeast remains able to stimulate rumen microbes. Ando et al., (2004) experienced the same results of increase kd with the inclusion of dried brewers' yeast which are commonly cultures of *S. cerevisiae* species. While brewer's yeast does not contain live yeast organisms like that of the live yeast product used in the current study, it is still adding credibility to yeast's ability to alter the rumen environment.

Rumen Fluid Measurements

Methane. Decreasing methane emissions from ruminants without sacrificing animal production is a constant objective of ruminant nutritionists. Using live yeast as a means of mitigation have been studied, but most results are inconclusive (Martin et al., 2010). Some studies performed have posed the idea that various yeast products might stimulate the acetogens to compete or to co-metabolize hydrogen with methanogens, thereby reducing methane emissions (Mwenya et al., 2004; Elghandour et al., 2014). There was quite a variability in TRT response on methane production overall (Table 2, Figure3), as expected by a variable that is affected by many factors simultaneously (Van Soest, 1994; Tedeschi and Fox, 2018). During the GRW phase, there was only an effect of T

observed ($P < 0.001$) in which more methane was produced during week 2 than in week 1 (26.30 vs. 40.30 mL, respectively; $P < 0.001$). This was likely initiated by an increase in DMD experienced from week 1 to 2 in the GRW phase. There was an interaction detected between TRT and T ($P < 0.001$) as well as an effect of T ($P < 0.001$) in the TRANS phase. Methane increased significantly from week 3 to 4 to 5 (10.56 mL, 11.98 mL, 12.59 mL, respectively*;* $P < 0.001$). This could be expected since there was an increase in the percentage of starch in the diet from one run to another. With an increasing percentage in starch, the rumen microbes could have adapted, and an increase in protozoa populations could have been present to aid in the digestion of the starch. Protozoa are known methanogens, so they could have contributed to the increase in methane from period to period. There was no recorded effect of TRT overall, but as illustrated in Figure 5, the response varied depending upon what week was observed. There was a TRT by T interaction ($P < 0.001$) during the FIN phase as well. There was also an effect of TRT ($P < 0.001$) on methane production in a liner, quadratic, and cubic fashion ($P < 0.001$, $P = 0.004$, $P = 0.027$, respectively) in which CON had the highest methane production.

Protozoa. In this trial subsamples of rumen fluid were taken from each animal to perform protozoa counts on. Hence, it is very likely there was variation between samples attributable to the different DMI and initial protozoa per animal. We expected an interaction between TRT and DMI and TRT and initial protozoa. In fact, each interaction of each was observed during all phases (Table 3, Figure 4). Thus, depending on the DMI and initial protozoa count (IPC), treatments may have different outcomes. On account of these outside effects and interactions, values are reported for the average of DMI and IPC.

Figure 4 represents the interaction between IPC on the protozoa counts over T with IPC being in the 25% quantile (Figure 4A), 50% quantile (Figure 4B), and 75% quantile (Figure 4C). When cattle were fed the GRW diet, IPC ($P < 0.001$) and DMI ($P = 0.002$) affected protozoa count and a TRT by DMI interaction ($P < 0.001$) was observed. There was a significant quadratic effect of TRT ($P = 0.047$) in which LY2 and LY3 had the lowest count of protozoa/mL of rumen fluid. Rumen ciliate protozoa are the most numerous protozoa species in the rumen, and they readily digest starch (Michalowski, 2005; Williams, 1989). Our finding may be desirable in a high forage, GRW type diet because there is little dietary starch that needs to be slowly degraded. Because of the relationship of the number of protozoa and methane production, fewer protozoa in the rumen could be advantageous, but the reduction in methane emissions vary by diet (Hegarty, 1999). When cattle went through the TRANS period, there tended to be an interaction of TRT and IPC ($P = 0.108$), and TRT tended to respond again in a quadratic fashion ($P = 0.063$), with LY2 having the lowest count of protozoa. Animals were offered a 50% forage 50% concentrate step up ration when these samples were collected during week 4. This is still a high forage content diet, so a lower count of protozoa could still be advantageous when considering a subsequent lower production of methane. During the FIN phase, there was a significant effect of TRT ($P < 0.001$), DMI ($P = 0.017$), and IPC ($P = 0.001$), as well as an interaction between TRT and DMI ($P < 0.001$) and TRT and IPC ($P < 0.001$). The TRT responded most significantly in a quadratic pattern ($P < 0.001$) in which the CON diet had the lowest count of protozoa. These findings are in agreeance with Shen et al., (2018) who discovered that total protozoa counts were significantly greater in the rumen fluid of cattle that were supplemented with an *S. cerevisiae* fermentation product

top dressed while receiving high starch diets. With the FIN diet of our trial being high in starch, a higher concentration of protozoa may be desirable due to the protozoa's ability to digest more slowly than other microbes. This may aid in keeping the animal's ruminal pH more stable and more favorable pH, reduce the likelihood of experiencing acidosis when receiving concentrate diets rich in available starch like those fed in confined feeding programs and decrease the redox potential (Slyter, 1976). Because cellulolytic bacteria are susceptible to these parameters, protozoa indirectly stimulate the bacterial cellulolytic activity and supply their activity to the rumen microbial ecosystem (Jouany and Ushida, 1999).

Ruminal pH. The digestive health of cattle and the ability for ruminants to digest feed efficiently in order to perform relies heavily on the pH of the rumen (Shabat et al., 2016), and a good understanding is a necessary prerequisite in order to manipulate the microbiota in order to optimize rumen function and productivity (Jami and Mizrahi, 2012). If a bovine's ruminal pH drops below 5.6 for longer than 180 min, they can begin to experience subacute ruminal acidosis (Plaizier et al., 2008), which can begin to kill the rumen microflora, damage the papilla that is responsible for nutrient absorption, reduce feed efficiency, and can even become as serious as death (Owens et al., 1998). Low pH commonly occurs after an animal is fed a diet with a high percentage of starch. These high starch diets are beneficial when it comes to putting weight on cattle, and they are commonly fed in confinement feeding programs; however, it is important that cattle keep a high ruminal pH in these types of settings, so they do not experience issues as mentioned before. Due to their confinement feeding regimens, it is important that these feeding programs find sources that help maintain a healthy ruminal pH and minimize the

occurrence of liver abscesses (Tedeschi and Gorocica, 2018). Several studies have shown that certain strains of active live yeast may be particularly effective at raising and stabilizing ruminal pH throughout diets that differ in their acidotic potential (Bach et al., 2007; Guedes et al., 2008; Marden et al., 2008; Crossland et al., 2019) and under thermoneutral conditions (Crossland et al., 2018). Effects of dry live yeast on pH are illustrated in Table 3 and Figure 5. Although TRT only had a significant effect during the TRANS phase ($P = 0.041$), LY2 had consistent higher pH throughout the feeding phases. There were no effects observed when cattle were fed the GRW diet other than TRTs responding in a linear fashion ($P = 0.042$) with LY2 providing the highest ruminal pH. In addition to the effect of TRT, there was also an effect of T ($P = 0.041$), initial pH ($P = 0.022$), and TRT by DMI interaction ($P = 0.044$) as well as a tendency of an interaction between TRT and T ($P = 0.091$), and a three-way interaction between TRT, T, DMI ($P = 0.104$) when cattle went through the TRANS phase (Table 2, Figure 6). There was a significant effect of T ($P = 0.036$), TRT by T ($P = 0.046$), T by DMI ($P = 0.034$), and a TRT by T by DMI ($P = 0.035$) interaction during the FIN phase.

In Situ Digestibility

Ruminal microbes play a crucial role in the degradation of forage. Specifically, the numbers of rumen microbes and their activity have a direct effect on the efficiency of forage degradation (Hungate, 1966). Increased concentrations of ruminal fibrolytic bacteria have been observed to result from yeast supplementation (Wiedmeier et al., 1987; Harrison et al., 1988; Dawson et al., 1991; Crossland et al., 2018). In the present study, in situ digestibility of both DMD and NDFD was increased by the addition of the low to medium inclusion level of yeast throughout all feeding phases (Table 3 Figure 6). Thus, as in

agreement with the above reports, it can be concluded that the addition of yeast activates rumen microbes.

Dry matter digestibility. TRT influenced DMD in a cubic pattern ($P = 0.035$) more significantly than others where LY2 presented the greatest DMD when cattle were fed the GRW diet. T tended to effect DMD ($P = 0.062$), and there were TRT by T ($P < 0.001$), TRT by DMI ($P = 0.005$), and TRT by T by DMI ($P < 0.001$) interactions as well. There was more variation, in TRT response during the TRANS phase than there were in the GRW or FIN (Figure 7, Table 2), and this could be related to the way the cattle were transitioned from the GRW to the FIN diet (3 different GRW: FIN step ups consisting of 75:25, 50:50, 25:75). An influence of TRT ($P < 0.001$), DMI ($P < 0.001$), TRT by T ($P < 0.001$), TRT by DMI ($P < 0.001$), and TRT by T by DMI ($P < 0.001$) on DMD was observed during the TRANS phase. TRT responded linearly ($P = 0.001$), quadratically ($P = 0.003$), and cubically ($P = 0.011$) in which LY2 yielded the greatest DMD. The FIN phase resulted in effect of TRT ($P = 0.039$) in a quadratic response ($P = 0.022$) with influence of TRT by T ($P = 0.009$), TRT by DMI ($P = 0.053$), and TRT by T by DMI ($P = 0.030$) interactions in which LY1 had the greatest DMD. Although LY1 resulted in the greatest DMD when cattle were fed the FIN diet, LY2 and LY3 were not far behind, but as can be seen in Figure 7, LY2 seemed to stay very constant DMD throughout all feeding phases and had the least variation (Figure 6A). This could be due to the pH of the rumen being higher throughout the feeding phases which allowed for a more favorable fermentation environment, and a more adaptive, productive microbial population (Chaucheyras-Durand et al., 2008). A less acidic and more anaerobic ruminal environment would help stimulate the growth of fiber-degrading microorganisms (Callaway and Martin,

1997) and could improve fiber degradation in the rumen (Williams et al., 1991). Although their study was done in vivo, our results are consistent with Crossland et al., (2018) in the sense that LY supplementation resulted in great DMD.

Neutral detergent fiber digestibility. Almost identical results were observed during each feeding phase on NDFD (Table 3, Figure 6B), and the same TRT response pattern with LY2 having the greatest numerical NDFD with the least variation throughout each phase minus the FIN was observed as well. This is likely to be the result of the higher pH and more productive rumen microbial population in LY2 treated cattle as previously discussed. Specifically, TRT influenced NDFD during all feeding phases ($P \leq 0.012$). Additionally, a TRT by T, TRT by DMI, and TRT by T by DMI interactions were observed throughout all feeding phases ($P < 0.05$). An effect of DMI ($P < 0.001$) was only seen during the TRANS phase. While not reaching significance, Crossland et al., (2018) observed an increase in NDFD in live yeast treated inoculum over the control. This NFDF was measured on in vitro fermentation batches, and they resonate that the variation between fermentation batched was the reason for it not being significant.

CONCLUSION

Our results indicated that the daily supplementation of 5.0 g LY/d could yield less methane production when high-forage diets are fed, but higher protozoa counts, greater DMD, and greater NDFD might be observed when high-concentrate diets are fed. The rumen fluid from cattle supplemented with 10 g LY/d provided the greatest amount of in vitro gas production for nonfiber and fiber-carbohydrate pools as well as the fastest fractional rate of fermentation for high-forage diets (i.e., GRW and TRANS phases). The supplementation of 10 g LY/d also provided the greatest DMD and NDFD during the same

feeding time as well, and it provided the highest ruminal pH throughout all phases. The administration of 15 g LY/d provided no additional measurable benefits over the other inclusions during the high-forage diet (GRW phase), but it showed to have a higher total gas production and kd of the fiber carbohydrate pool as well as the least amount of methane production during the transition phase. Additionally, the 15 g LY/d presented the greatest total gas production of the NFC pool, fastest kd of both pools, and least methane production during the finisher phase (high-concentrate diets). Many studies have claimed that some type of probiotic yeast supplementation can be beneficial for ruminal health and subsequent ruminal productivity, but hardly any specific conclusions are given about the optimal inclusion of live yeast throughout entire feeding phases like those of confinement feeding in feed yards. More titration-type studies are needed to narrow down to the optimum concentration of dietary live yeast supplementation. While there is some slight variation within some variables, overall, our results indicated that daily supplementation of live yeast at the inclusion of 10 g LY/d may be the most optimal dosage for growing cattle being fed in confinement when considering the health and subsequent productivity of the rumen. The conclusion is based upon the specific inclusion rate's ability yield a higher pH which commonly leads to an increase in microbial growth and an improvement in feed digestibility.

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Table 3.1. Ingredient and chemical composition of diets fed to steers during each growing period.

	Diets[1]		
Items	Gower	Transition	Finisher
Ingredients, % of the diet AF			
Alfalfa hay, medium chopped	25	16.15	7.3
Bermudagrass hay	8.0	7.6	7.2
Dried distiller's grains	22	15.5	9.0
Steam Flaked Corn	38	55.5	73
Mineral	1.0	1.0	1.0
Limestone	1.0	1.0	1.0
Urea	0.5	1.0	1.5
Molasses	4.5	2.25	0.0
Chemical Composition, % DM			
DM	86.7	90.7	87.3
CP	17.2	18.0	16.1
SP, % CP	31.7	31.0	35.4
ADIN	1.9	1.7	1.2
NDIN	2.4	2.1	1.8
ADF	24.8	13.9	10.7
NDF	35.8	23.8	21.9
Lignin	6.0	3.7	2.7
Sugar	6.5	3.7	2.1
Starch	24.7	37.3	55.2
Fat	3.7	4.3	2.9
Ash	6.5	6.1	4.5
Ca	1.2	1.0	0.70
P	0.44	0.46	0.38
Mg	0.29	0.23	0.16
K	1.1	0.95	0.71
S	0.36	0.29	0.19
Na	0.23	0.18	0.13
Fe	221.0	329.7	256.0
Mn	65.0	63.3	47.0
Zn	63.0	58.7	40.0
Cu	26.0	18.0	13.0
Cl	0.44	0.32	0.30
TDN	68.8	76.5	78.4
NEm (Mcal/kg)	1.6	1.8	1.9
NEg (Mcal/kg)	1.0	1.18	1.23

[1] 200 mg of monensin was supplemented to every animal during the grower and transition feeding phases, and 360 mg was supplemented to every animal during the finisher feeding phase.

Table 3.2. Effect of dry live yeast on in vitro gas production (IVGP) parameters and methane production of representative rumen fluid samples from like-treated growing steers fed three types of diets.

	Dietary Treatment[2] (g/hd/d)				*SEM*	*P- Values*			*Contrasts*		
Items[1]	CON (0g)	LY1 (5g)	LY2 (10g)	LY3 (15g)		TRT	Time (T)[3]	TRT x T	L	Q	C
Grower											
IVGP- a[4], mL	5.98	7.2	6.42	7.18	0.528	0.195	0.053	0.041	0.191	0.625	0.081
IVGP- d[4], mL	7.75[ab]	7.19[b]	8.27[a]	7.93[ab]	0.309	0.045	0.021	0.247	0.246	0.710	0.014
IVGP- b[4], 1/h	14.3	12.1	14	13.7	1.16	0.479	0.679	0.010	0.978	0.391	0.199
IVGP- e[4], 1/h	3.10[a]	2.78[b]	3.35[b]	3.21[b]	0.173	0.001	<0.001	0.095	0.063	0.371	0.001
Methane, mL	33.3	31.4	34.2	34.2	2.61	0.508	<0.001	0.615	0.409	0.540	0.264
Transition											
IVGP- a[4], mL	13.29	13.76	12.966	14.35	0.528	0.278	<0.001	0.127	0.313	0.387	0.151
IVGP- d[4], mL	10.2[a]	10.5[a]	9.71[a]	9.44[b]	0.333	0.124	0.757	0.507	0.045	0.389	0.284
IVGP- b[4], 1/h	15.3[ab]	15.4[ab]	16.2[a]	14.1[b]	0.560	0.078	<0.001	0.002	0.263	0.052	0.164
IVGP- e[4], 1/h	3.31	3.51	3.5	3.55	0.121	0.509	0.020	0.703	0.197	0.542	0.637
Methane, mL	13.1	12.7	13.2	12.6	0.493	0.487	<0.001	<0.001	0.486	0.645	0.193
Finisher											
IVGP- a[4], mL	10.5[b]	9.68[bc]	8.39[c]	12.6[a]	0.701	<0.001	<0.001	0.005	0.113	0.001	0.051
IVGP- d[4], mL	10.5	9.94	9.21	9.98	0.573	0.470	<0.001	0.003	0.381	0.252	0.509
IVGP- b[4], 1/h	19.2[ab]	16.6[b]	21.2[a]	14.5[b]	1.32	0.004	<0.001	0.003	0.123	0.125	0.002
IVGP- e[4], 1/h	4.20[a]	4.08[ab]	4.29[a]	3.81[ab]	0.104	0.012	0.961	<0.001	0.045	0.090	0.024
Methane, mL	9.78[a]	8.70[b]	7.14[c]	7.57[c]	0.248	<0.001	0.220	<0.001	<0.001	0.004	0.027

[a,b,c] Means with different superscripts differ by $P \leq 0.05$

[1] Items are variables analyzed during each feeding phase using representative rumen fluid sampled from like treated animals.

[2] Dietary treatment values are given as least squares means.

[3] All animals were fed for 8 weeks (12 d adaptation and 7 observation periods consisting of 17 d GRW, 15 d TRANS, 13 d FIN)

[4] a = the asymptote measurement of the nonfiber carbohydrate pool (total gas production). d = the asymptote measurement of the fiber concentrate pool (total gas production). b = the fractional rate of degradation of the nonfiber carbohydrate pool. e = the fractional rate of degradation of the fiber concentrate pool.

Table 3.3. Effects of dry live yeast on rumen parameters and in situ digestibility of dry matter and neutral detergent fiber in growing steers fed. three types of diets.

	Dietary Treatment[2] (g/hd/d)				***SEM***	***P- Values[3]***							***Contrasts***			***Covariate***		
Items[1]	CON (0g)	LY1 (5g)	LY2 (10g)	LY3 (15g)		TRT	Time (T) [4]	DMI	TRT x T	T x DMI	TRT x DMI	TRT x T x DMI	L	Q	C	IpH[5]	IPC[5]	IPC x TRT[6]
Grower																		
Protozoa, Log10/ mL	10.4[a]	104[a]	10.1[b]	10.1[b]	0.061	0.175	0.164	0.002	0.884	--	<0.001	--	0.300	0.047	0.167	--	<0.001	0.369
pH	6.31[a]	6.33[a]	6.45[a]	6.26[b]	0.086	0.145	0.525	0.182	0.751	0.883	0.157	0.823	0.042	0.753	0.229	0.398	--	--
DMD, %	75.6	75.2	77.6	73.8	1.76	0.035	0.062	0.797	<0.001	0.005	0.042	<0.001	0.058	0.556	0.035	--	--	--
NDFD, %	81.1	80.7	82.2	79.7	1.23	0.012	0.177	0.796	<0.001	0.021	0.014	<0.001	0.018	0.520	0.029	--	--	--
Transition																		
Protozoa, Log10/ mL	10.4	10.5	10.2	10.4	0.085	0.118	--	0.335	--	--	0.314	--	0.232	0.063	0.842	--	0.233	0.108
pH	6.26[b]	6.31[a]	6.44[a]	6.37[a]	0.079	0.041	0.014	0.943	0.091	0.184	0.044	0.104	0.178	0.069	0.027	0.022	--	--
DMD, %	78.4	78.5	81.3	77.0	1.89	<0.001	0.364	<0.001	<0.001	0.685	<0.001	<0.001	0.001	0.003	0.011	--	--	--
NDFD, %	84.4	84.9	86.6	84.2	1.06	<0.001	0.438	<0.001	<0.001	0.242	0.001	<0.001	0.002	0.032	0.031	--	--	--
Finisher																		
Protozoa, Log10/ mL	10.2[c]	10.6[a]	10.3[b]	10.5[a]	0.048	<0.001	0.100	0.017	0.134	--	<0.001	--	0.020	<0.001	0.014	--	0.001	<0.001
pH	5.68	5.66	5.75	5.47	0.108	0.956	0.036	0.512	0.046	0.034	0.924	0.035	0.719	0.769	0.781	0.178	--	--
DMD, %	78.0	81.5	79.9	80.0	1.76	0.039	0.135	0.277	0.009	0.045	0.053	0.030	0.145	0.022	0.620	--	--	--
NDFD, %	85.3	86.6	86.3	86.2	0.672	0.008	0.096	0.454	0.000	0.021	0.012	0.001	0.551	0.001	0.539	--	--	--

[a,b,c] Means with different superscripts differ by $P \leq 0.05$

[1] Items are variables collected during each feeding phase on individually treated animals. DMD= dry matter digestibility. NDFD= neutral detergent dry matter digestibility.

[2] Dietary treatment values are given as least squares means.

[3] There was a significant ($P \leq 0.05$) interactions between dietary treatment, T, and DMI, so the dietary treatment means are reported for the average of DMI and average overall runs in that feeding phase.

[4] All animals were fed for 8 weeks (12 d adaptation and 7 observation periods consisting of 17 d GRW, 15 d TRANS, 13 d FIN). Protozoa were only collected during 5 of the periods, and all other variables were collected for 7 periods.

[5] IpH= initial pH. IPC=initial protozoa concentration

[6]There were a significant ($P \leq 0.05$) interactions between dietary treatment and DMI and dietary treatment and initial protozoa, so the dietary treatment means are reported for the average of DMI and the average concentration of initial protozoa count.

Figure 3.1. Effects of dry live yeast on the in vitro (A) total gas production of the nonfiber concentrate pool and (B) total gas production of the fiber concentrate pool (♦, solid line = CON, ■, long dashes = LY1 (5g/d), ▲, short dashes = LY2 (10g/d), and ×, dotted line = LY3 (15g/d)). **GRW** diet was fed during weeks 1 and 2, **TRANS** was fed during weeks 3-5, and **FIN** diet was fed during weeks 6 and 7.

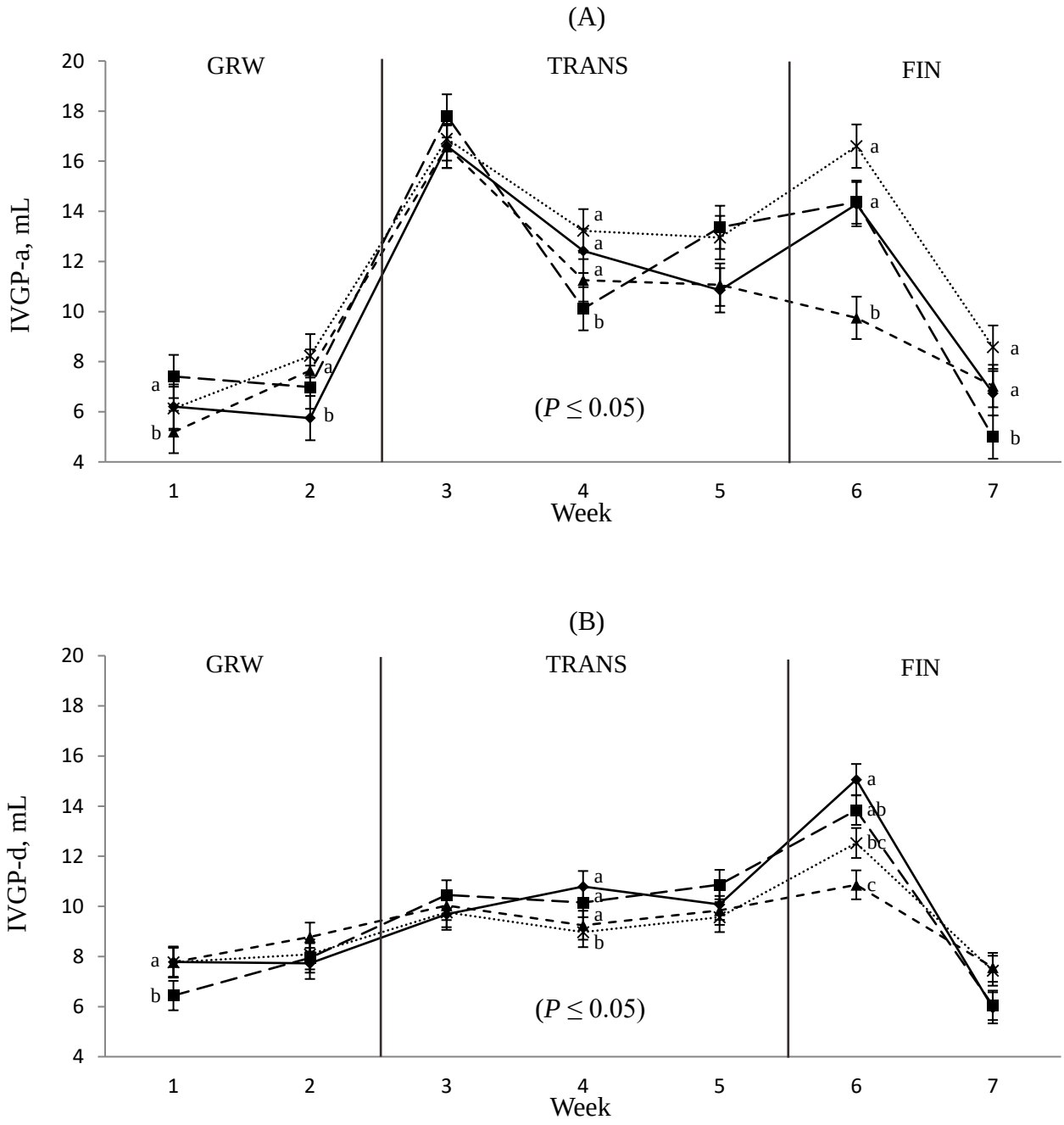
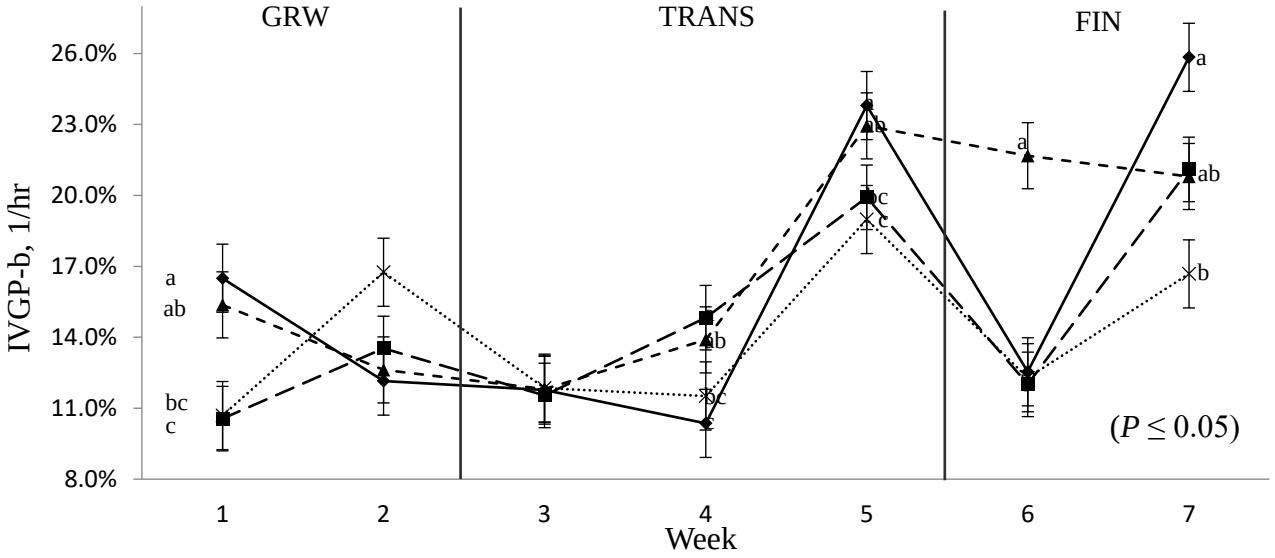


Figure 3.2. Effects of dry live yeast on the in vitro (A) fractional rate of degradation of the nonfiber concentrate pool and (B) fractional rate of degradation of the fiber concentrate pool (♦, solid line = CON, ■, long dashes = LY1 (5g/d), ▲, short dashes = LY2 (10g/d), and ×, dotted line = LY3 (15g/d)). **GRW** diet was fed during weeks 1 and 2, **TRANS** was fed during weeks 3-5, and **FIN** diet was fed during weeks 6 and 7.

(A)



(B)

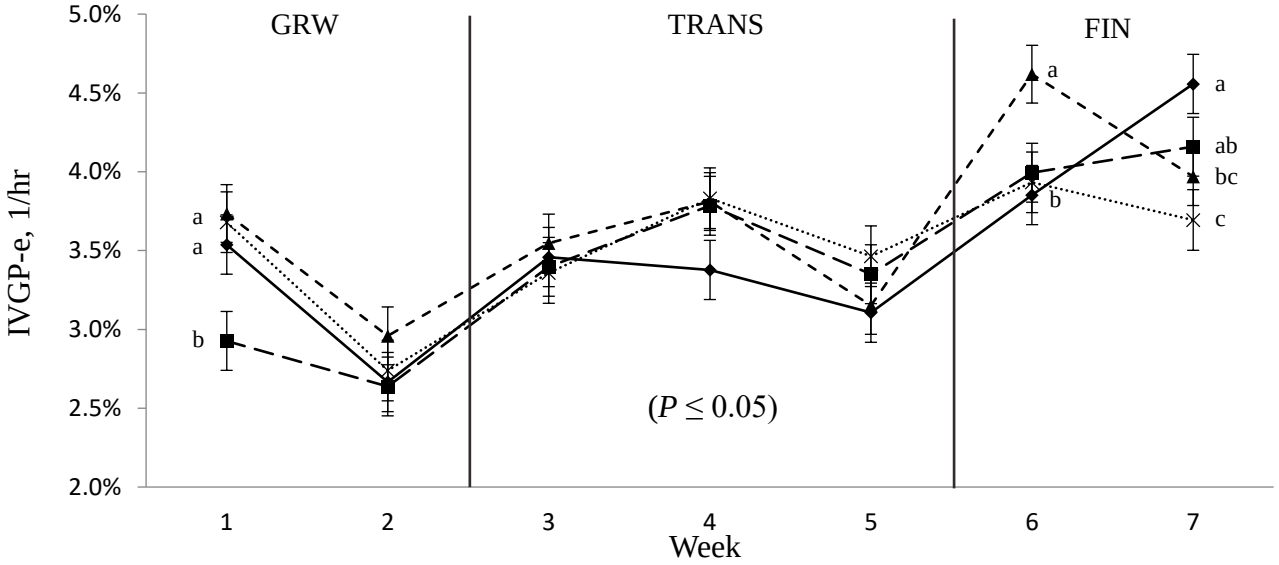


Figure 3.3. Effects of dry live yeast on the methane production of growing cattle (♦, solid line = CON, ■, long dashes = LY1 (5g/d), ▲, short dashes = LY2 (10g/d), and ×, dotted line = LY3 (15g/d)). **GRW** diet was fed during week 1 and 2, **TRANS** was fed during week 3-5, and **FIN** diet was fed during week 6 and 7.

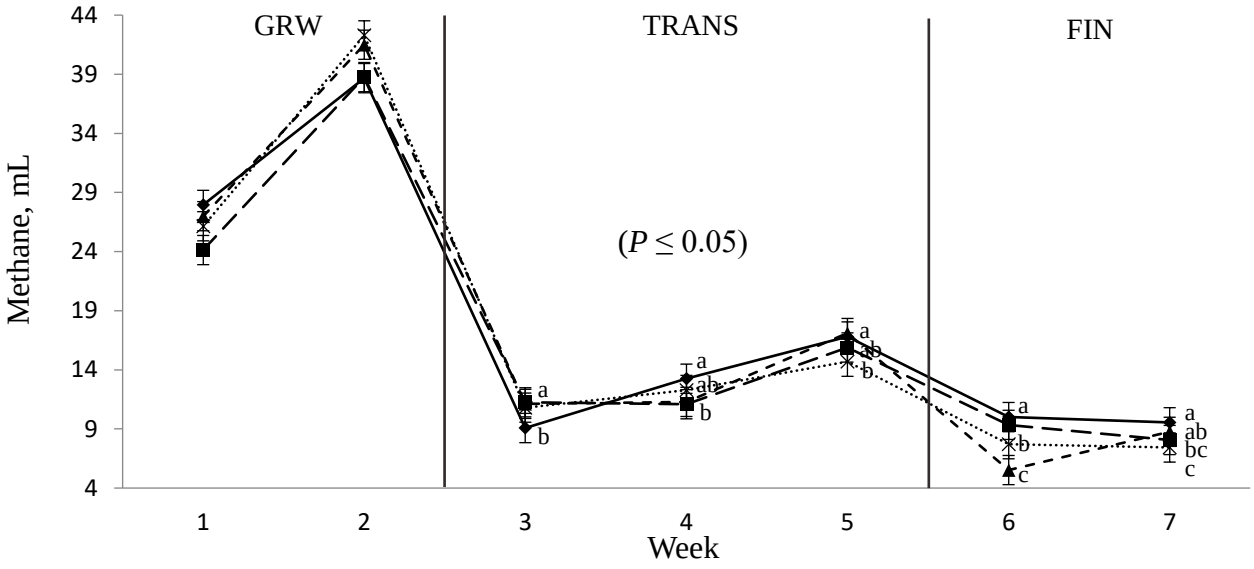


Figure 3.4. Effects of dry live yeast on the protozoa counts in growing cattle at different levels of initial protozoa counts (A- 25%, B- 50%, and C- 75% of the distribution) (♦, solid line = CON, ■, long dashes = LY1 (5g/d), ▲, short dashes = LY2 (10g/d), and ×, dotted line = LY3 (15g/d)). **GRW** diet was fed during weeks 1 and 2, **TRANS** was fed during week 4, and **FIN** diet was fed during weeks 6 and 7.

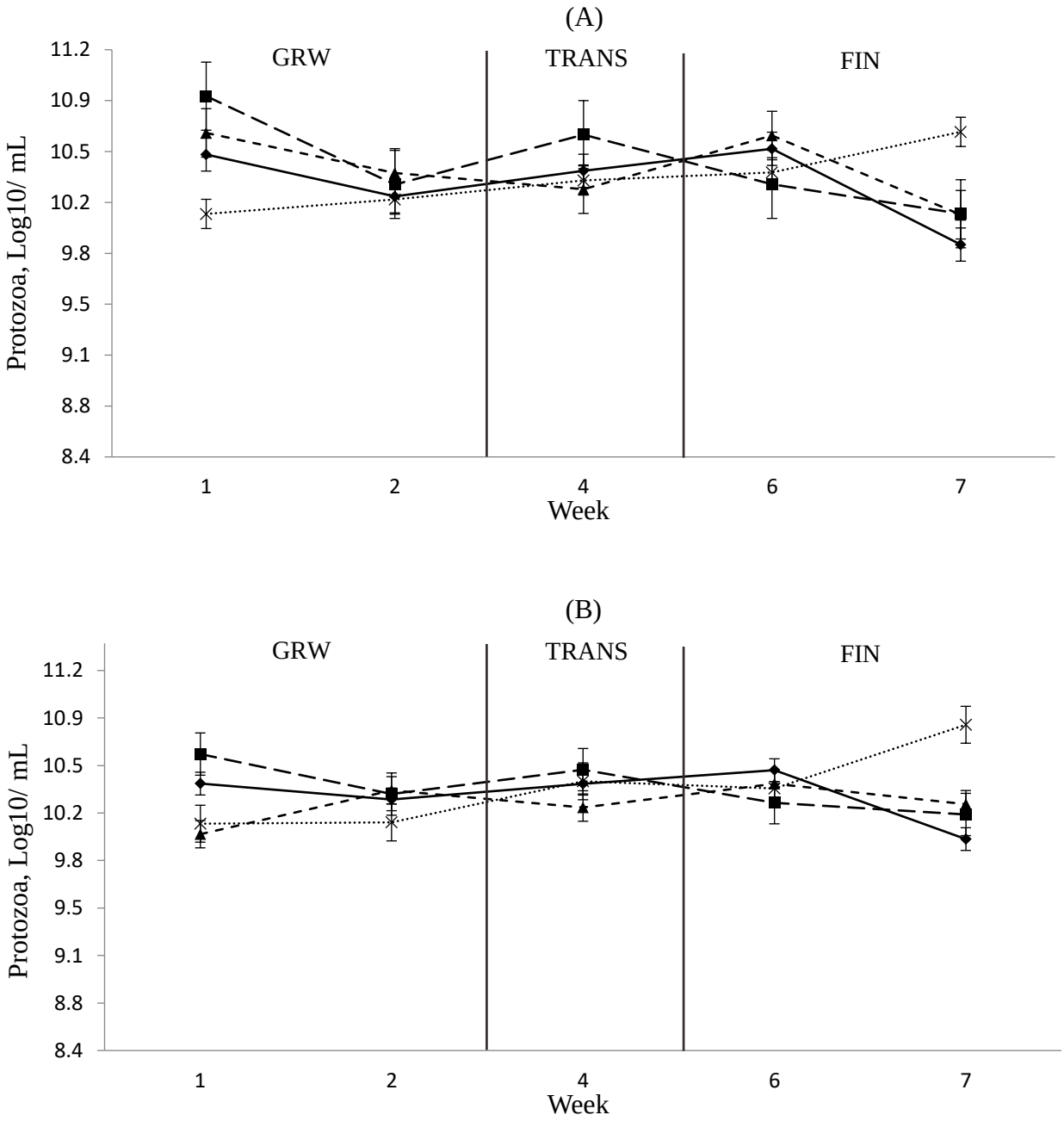


Figure 3.4. Continued

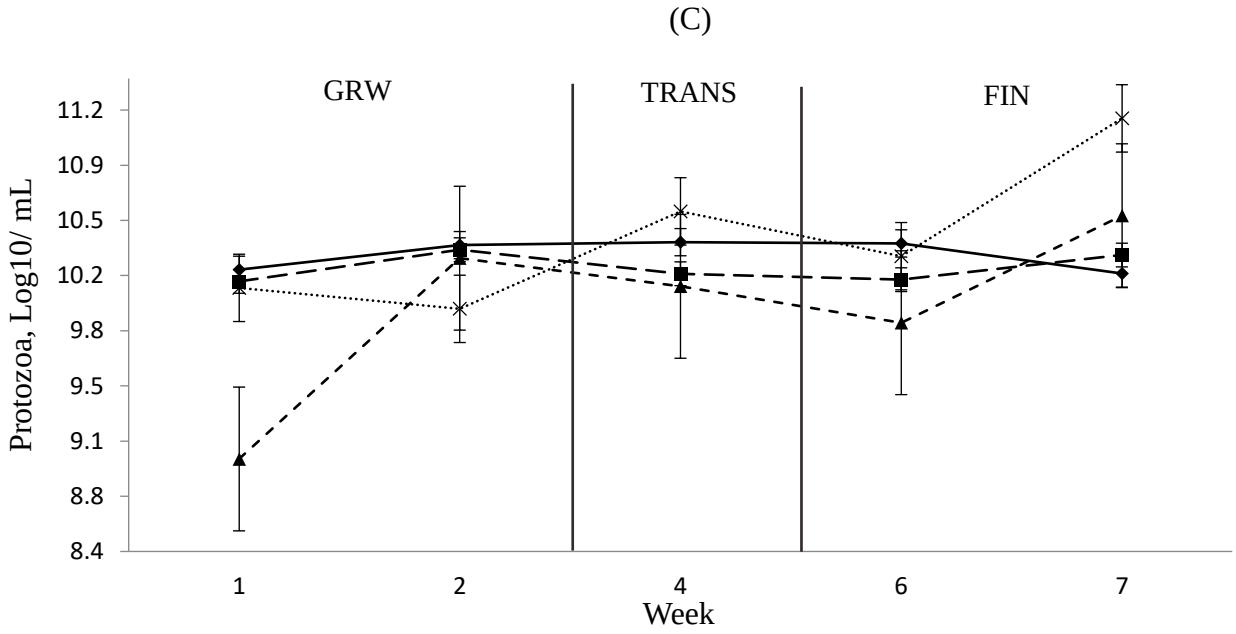


Figure 3.5. Effects of dry live yeast on the ruminal pH of growing cattle (♦, solid line = CON, ■, long dashes = LY1 (5g/d), ▲, short dashes = LY2 (10g/d), and ×, dotted line = LY3 (15g/d)). **GRW** diet was fed during weeks 1 and 2, **TRANS** was fed during weeks 3-5, and **FIN** diet was fed during weeks 6 and 7.

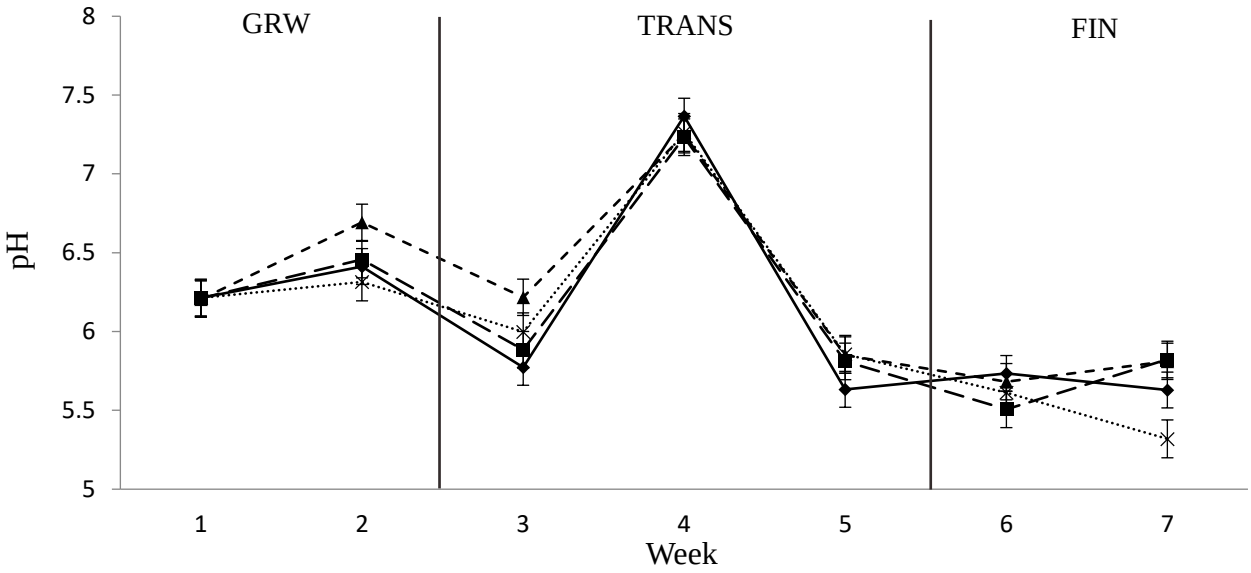
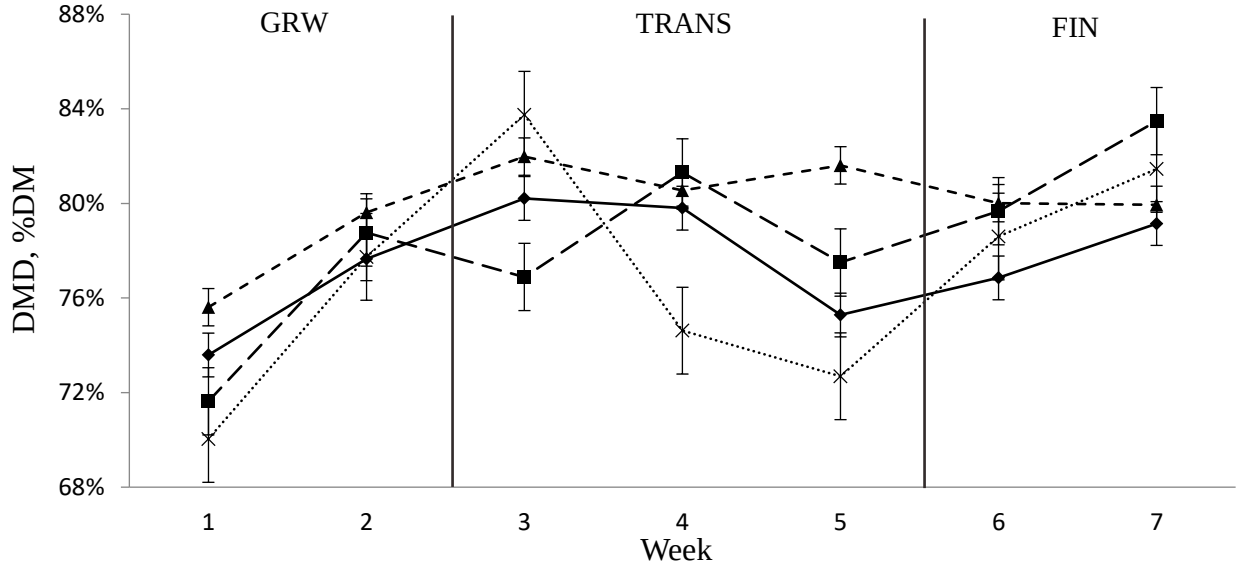
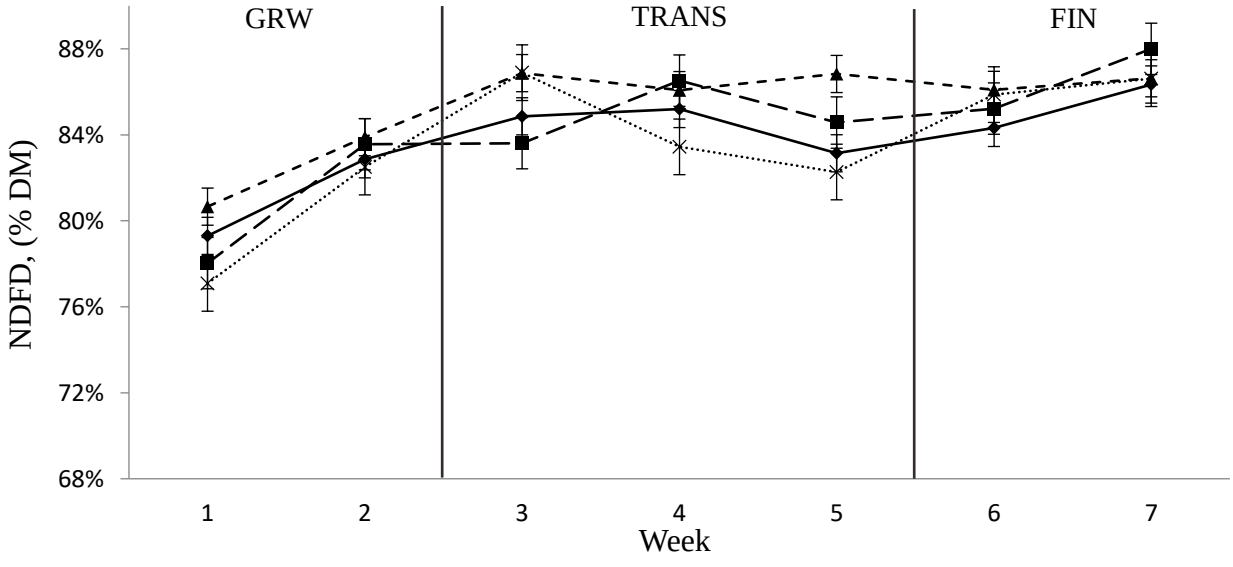


Figure 3.6. Effects of dry live yeast on (A) dry matter digestibility (DMD) and (B) neutral detergent fiber digestibility (NDFD) in growing cattle (♦, solid line = CON, ■, long dashes = LY1 (5g/d), ▲, short dashes = LY2 (10g/d), and ×, dotted line = LY3 (15g/d)). **GRW** diet was fed during weeks 1 and 2, **TRANS** was fed during weeks 3-5, and **FIN** diet was fed during weeks 6 and 7.

(A)



(B)



CHAPTER IV

CONCLUSION

During the last decade, the mechanisms and attributions of LY on targeted rumen microbial communities, animal growth, health, and overall productivity have been extensively studied for some strains of *S. cerevisiae.* Although research has certainly assisted in adding credibility on these probiotics for their use in ruminant nutrition, a lot remains to be studied to further explain the full effects of live yeasts in digestive processes. Field studies indicate that although positive effects on milk or meat production can be obtained, the animal response to such feed additives may be quite variable depending upon factors such as nature of the diet, level of productivity, animal physiological and genetic factors, dose, and strain of yeast used, etc. It will be of great importance in the near future to better understand the nature of interactions between the yeast probiotic, the autochthonous anaerobic microbial population, and the dietary components in order to further predict the impact of such a probiotic in ruminant nutrition. Evaluating and characterizing the effects of LY on growing beef cattle may help to improve our understanding and help us determine how it may be implicated in the health and nutrition sector in a feedlot setting. In these two studies, we evaluated the effects of feeding a LY product in a feedlot setting. While we did not detect a specific TRT response during all growing periods, we did observe that supplementing LY consistently seemed to have beneficial effects on IGVP patterns, rumen parameters, and digestibility consistently with LY2 (10 g/d) providing the most beneficial result for all diets.