EVALUATION OF THE EFFECTS OF *SACCHAROMYCES CEREVISIAE* (LEVUCELL) ON PERFORMANCE, PHYSIOLOGICAL, AND BEHAVIORAL RESPONSES DURING SUBACUTE ACIDOSIS IN BEEF STEERS

A Thesis

by

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ABSTRACT

The objective of this study was to evaluate the behavioral and physiological responses to live-yeast (LY) supplementation during subacute ruminal acidosis (SARA) in beef steers fed a high-concentrate diet. In the 1st study, 48 crossbred beef steers were assigned to LY or control dietary treatments, with individual-animal dry matter intake (DMI) and feeding behavior data collected daily for 70 d via an electronic feed intake measurement system. Although LY supplementation did not affect DMI or F:G, duration of BV and meal events were longer causing BV and meal eating rate to be slower in LY-fed steers compared to control-fed steers. These results suggest the potential for LY supplementation to mitigate SARA in cattle fed a high-concentrate diet. In the 2nd study, 48 steers were randomly assigned to 1 of 4 treatments (n = 12): (1) control diet and non-SARA, (2) control diet and SARA, (3) LY diet and non-SARA, and (4) LY diet and SARA. Steers in the SARA treatments were subjected to SARA challenge protocols by disrupting daily DMI from 60 to 140% of baseline DMI during 7-d periods. Steers were placed in pens equipped with the GrowSafe System and weighed weekly to evaluate the effects of LY supplementation during imposed SARA challenges on DMI, feeding behavior patterns, and performance. Six steers per treatment were fitted with reticulo-rumen boluses to measure rumen pH and temperature. In general, the SARA treatment caused reductions in mean, maximum and minimum ruminal pH and increased daily variance of pH and duration and AUC of pH < 5.8, confirming that the experimental SARA challenge protocols disrupted the rumen environment. Live-yeast supplementation consistently reduced DMI and F:G throughout the trial and increased

mean and minimum ruminal pH, while decreasing daily variance of ruminal pH and duration and AUC of pH < 5.8. Significant diet x SARA interactions illustrated the benefit of supplementing LY during SARA, such that LY supplementation increased mean and maximum ruminal pH in SARA-treated steers, but not in non-SARA steers. Additional, LY supplementation decreased duration and AUC of pH < 5.8 to a greater extent in SARA-treated steers than in non-SARA steers (diet x SARA interactions; $P < 0.01$). Results from this study demonstrate the efficacy of the experimental SARA challenge protocols, and that LY supplementation favorably altered the rumen environment, especially during SARA challenges.

DEDICATION

This thesis is dedicated to my mother and father. Thank you for your unconditional encouragement, support, and love over the last 25 years. You have shaped me into who I am today and this achievement is just as much because of you both.

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

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NOMENCLATURE

LY	Live yeast
SARA	Subacute ruminal acidosis
BV	Bunk visit
TTB	Time to bunk
NFI	Non-feeding interval
BW	Body weight
ADG	Average daily gain

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Digestive disorders are one of the leading causes of morbidity and mortality in US feedlots, second only to respiratory disease (Nagaraja and Lechtenberg, 2007). Over the last 20 years, feedlot diets have shifted to lower roughage inclusion rates in order to optimize production efficiency (Stock, 2000). However, roughages are an important component of feedlot diets that contribute to animal health by providing a scratch factor to stimulate rumination and increase saliva production. Rumination is positively correlated with buffering capacity, which results in a more basic rumen environment to support optimal ruminal fermentation. Without adequate amounts of roughage, buffering capacity will decrease and cause digestive disorders. The cost per unit of net energy of roughage feeds is also typically higher than that of concentrate feeds (Nagaraja and Lechtenberg, 2007) and roughage feeds are more difficult to handle and often times do not mix consistently into diets. Thus, typically feedlot diets are comprised of high amounts of readily available, non-structural carbohydrates in the form of grain and grain by-products leaving only 8-12% DM of the diet for roughage inclusion (Samuelson et al., 2007). Consequently, rumination patterns have been disrupted leading to a decreased buffering capacity and undesirable metabolic disorders. Amongst those digestive disturbances, acidosis is one of the most well known because of its prevalence and impact on animal and economic performance.

Simultaneously, the livestock industry has been plagued with a multitude of issues that have renewed focus on strategies to improve feed efficiency. By the year 2050, the world population will increase to 9.8 billion (US Census Bureau, 2008), which will nearly double the demand for animal-protein products (FAO, 2009). Furthermore, an increasing demand for urban development will reduce land resources available for livestock production, and the production of biofuels from grains will continue to increase feed costs. In addition, public concerns for antibiotic resistance and production methods have put pressure on the industry to adopt management practices that may not always be the most cost effective.

In an attempt to mitigate the effects of digestive disturbances and increase feed efficiency, the use of feed additives and management strategies have been adopted to enhance rumen fermentation. Ionophores are commonly used to mitigate digestive disturbances, but their use is being questioned as the concern for antibiotic resistance increases. Other methods such as supplementing dietary fat or buffers, or substituting non-starch feed by-products like DDGs have also been evaluated. More recently, direct-fed microbials (DFM) have been examined as a potential alternative to antimicrobial use because of their ability to alter microbial populations of the gastrointestinal tract (NASEM, 2016). Live yeasts (LY), in particular *Saccharomyces cerevisiae*, have been evaluated extensively in the dairy industry, but fewer studies have examined the effects of LY to mitigate digestive disorders, like acidosis.

Ruminal acidosis

Overview

Acidosis is a digestive disorder that exists as a continuum of degrees of ruminal acidity (Britton and Stock, 1989), and negatively impacts animal health and performance. In feedlot cattle, the common cause of acidosis is a large consumption of rapidly fermentable carbohydrates resulting in a decrease in ruminal pH levels due to an increase in the concentration of organic acids. If low ruminal pH levels are sustained, this will alter microbial populations, physiological function of the rumen, and fermentation products (Nagaraja and Lenchtenberg, 2007). Bouts of acidosis can be categorized into subacute (SARA) and acute ruminal acidosis based on ruminal pH levels and evidence of clinical signs (Nagarja and Lechtenberg, 2007). Ruminal pH thresholds used to characterize acidosis in beef cattle vary in literature; pH values below 5.6 (Brown et al., 2000; Vyas et al., 2014; Nagaraja and Lechtenberg, 2007) or 5.8 (Beauchemin et al., 2003; Ghorbani et al., 2002; Schwartzkopf et al., 2004) have both been used to defined SARA, whereas, acute ruminal acidosis can further be defined by much lower sustained ruminal pH levels (typically < 5.0). Furthermore, acidosis can be classified as SARA or acute ruminal acidosis by evidence of clinical signs. Acute acidosis will cause lethargy, incoordination, anorexia, and watery, and/or foamy stools that can lead to significant dehydration (Nagaraja and Lechtenberg, 2007). Ruminal motility can cease due to a buildup of organic acids like butyrate and lactate (Crichlow and Chaplin, 1985), and in acute cases cattle death can occur. Conversely, during SARA, cattle may not exhibit observed clinical signs other than reduced feed intake.

Schwartzkopf-Genswein and colleagues (2003) estimated the economic losses due to SARA to be 15 to 20 dollars per animal. In cases where cattle recover from sustained acidotic bouts (either acute or SARA), animal performance may be impaired due to ruminitis, laminitis, or liver abscesses (Nagaraja and Lechtenberg, 2007). Thus, there is a need to further develop strategies to mitigate the incidence of SARA and reduce its economic impact within the feedlot industry.

Causes of acidosis

The incidence of SARA is more prevalent in feedlots than acute ruminal acidosis (Britton and Stock, 1986; Owens et al., 1998), however it is difficult to identify and treat SARA cases due to the lack of visual clinical signs. In order to prevent acidosis, it is vital to understand its causes. In beef feedlots, the dietary cause of acidosis is a large consumption of rapidly fermentable carbohydrates and too little effective fiber. Current feedlot finishing diets consist of a large amount of non-structural carbohydrates that rapidly ferment and produce a significant volume of organic acids and consequently favor a reduction in ruminal pH. Organic acids are normal by-product of anaerobic fermentation and when ruminal absorption matches production, ruminal pH will range from 5.6 to 6.5 throughout the day and the rumen will remain stable (Nagaraja and Lechtenberg, 2007). When the rates of acid production are greater than absorption, organic acid levels will accumulate and cause ruminal pH to shift to acidotic range. Reductions in roughage inclusion in feedlot diets will increase organic acid production rates due to availability of more non-structural carbohydrates and decreased buffering capacity due to reduced rumination. Along with dietary causes, management or

environmental factors that affect intake patterns can affect the incidence of acidosis. Diets that have large proportions of rapidly fermentable carbohydrates leave little margin for feeding pattern inconsistencies. Acidotic bouts usually occur during the transition period when cattle are adapting to high grain diets, but can continue into the feeding period if normal feed-intake patterns are interrupted (Nagaraja and Lechtenberg, 2007; Owens et al., 1998). Any disturbances that cause an animal to consume a large amount of non-structural carbohydrates have the potential to create an acidotic bout. If the disturbance is coupled with a period of unintentional fasting— i.e. if a feeding is skipped, time between feedings is prolonged, or a pen of cattle is not fed enough— then the likelihood of acidosis occurring increases. External environment can also impact the incidence of acidosis. Periods of volatile weather can change feed intake patterns and disrupt homeostasis within the rumen. High temperatures that cause heat stress will increase panting, which decreases saliva production and inhibits a ruminant's buffering capacity.

Methods of detection

Rumen pH has traditionally been used to diagnose acidosis, however methods to measure rumen pH either poorly reflect the whole rumen environment (rumen fluid sampling) or are expensive (reticulo-rumen boluses). Rumen temperature has been shown to negatively correlate with rumen pH (Kimura et al., 2012), however patterns follow microbial and fermentation activity. Reduced intake may be the first visible sign of SARA within a pen of feedlot cattle (Nagaraja and Lechtenberg, 2007). If roughage levels in the diet are increased and feed intake and gain both increase, cattle were most

likely being influenced by SARA; conversely, if there is not a problem, then feed intake will increase, but gain will remain the same (Nagaraja and Lechtenberg, 2007), causing a decrease in feed efficiency. Commonly, feedlots measure feed intake on a pen basis, thereby making individual detection even more difficult. Cattle experiencing SARA will have loose or watery stools, or exhibit tenderness while walking which is characteristic of laminitis, but these are better indicators for acute cases. AlZahal and colleagues (2008) reported that ruminal temperature may have the potential to predict ruminal pH and aid in diagnosing SARA, however the study found dairy cows that spent more time with acidic rumens had greater rumen temperatures, contrary to the current studies findings where steers with higher duration under the pH threshold 5.8 had lower rumen temperatures. Additionally, AlZahal found a strong negative correlation ($R2 = 0.77$) between nadir rumen pH and corresponding ruminal temperature (2008).

Current strategies to mitigate SARA

Current strategies to mitigate SARA include the use of feed additives (i.e. ionophores, dietary buffers, or added fats), the use of low-starch by-products, and implementation of feeding management protocols to promote more constant feeding patterns. Ionophores have been found to improve feed efficiency (Richardson et al., 1976; Goodrich et al., 1984), reduce daily feed intake variation (Burrin et al., 1988; Stock et al., 1995) and increase ruminal pH when fed to finishing cattle (Nagaraja et al., 1981; Burrin and Britton 1986). Dietary buffers have been used to control SARA in feedlot cattle (Horn et al., 1979). Additionally, adding dietary fat has been shown to slow the rate of starch degradation, thereby decreasing the incidence of acidosis

(Huffman et al., 1992). However, Krehbiel and colleagues (1995) suggested that the potential of fat supplementation to mitigate SARA depends on the type and amount of fat, the type of grain, and corresponding roughage inclusion rates. Grain by-products can serve to reduce the incidence of SARA by serving as a substitute for a portion of the cereal grains in the diet (Nagaraja and Lechtenberg, 2007). Various strategies to transition growing calves from high-roughage to high-concentrate diets have been evaluated to minimize SARA.

Current SARA challenge models in literature

Various SARA challenge models have been evaluated to induce bouts of acidosis in beef cattle fed high-concentrate diets. Most studies have used ruminally-cannulated animals with relatively low numbers of animals. In addition, some studies only collected serial measurements of pH with a pH meter, whether by inter-ruminally or by collecting rumen fluid via an esophageal tube. These methods often fail to capture true rumen pH conditions due to daily and diurnal fluctuations in both feed intake and rumen pH. Denwood and colleagues (2018) proposed a drift analysis in which an individual animal's previous pH average was used as a comparison to future values. Crossland et al. (2019) also found this method useful in evaluating pH changes during the transition period because it decreased differences due to inter-animal variation. The use of reticulo-rumen boluses to continuously measure rumen pH is ideal, however they are expensive to implement.

In dairy cattle, Krause and Oetzel (2005) developed a SARA challenge model by restricting feed intake for 1 day by 50%, and then feeding a 1-h size-restricted meal of a

ground barley/wheat mixture was offered before TMR. Rumen pH was measured in ruminally-cannulated animals using indwelling electrodes to provide continuous measurements. Using this SARA challenge model, both Krause and Oetzel (2007) and DeVries et al. (2008, 2009) successfully reduced ruminal pH. Other studies have used other SARA challenge models, including: (1) the addition of supplementing intraruminal doses of glucose (Krehbiel et al., 1995), (2) the substitution of processed wheat, barley, or steam flaked corn (Beauchemin et al., 2003; Brown et al., 2000; Ghorbani et al., 2002; Gozho et al., 2005; Gozho et al., 2007; Keunen et al., 2002; Khafipour et al., 2009; Malekkahi et al., 2015; Villot et al., 2017; Vyas et al., 2014), (3) alterations in roughage to concentrate ratios (Penner et al., 2007), (4) feed intake disruptions (Cooper et al., 1997) including periods of (4a) feed restriction (Owens et al., 1998) and (4b) subsequent overfeeding (Horn et al., 1979; Schwartzkopf et al., 2004), or (5) a combination of multiple methods (Nagata et al., 2018; Vyas et al., 2014) in order to induce acidotic bouts and study the effects.

Feed efficiency

Overview

Feed input expenses are one of the largest variable costs to beef producers (Archer et al., 1999); any changes that can be made in feed efficiency could dramatically alter the profitability for individuals within the beef production chain. Consequently, feed efficiency research has been a critical topic of interest within the cattle industry. Whether it is amount of beef produced per feed consumed or similarly, milk produced, any change in feed efficiency that can occur which reduces feed input will decrease

production cost losses. Feed efficiency has been defined with numerous traits involving the ratio of inputs and outputs (Carstens and Tedeschi, 2006; Crews, 2005) including the ratio of feed to gain (F:G), as well as residual feed intake (RFI).

Feed:gain ratio has traditionally been used to assess efficiency of performance in growing animals, however observing F:G alone as a selection method will lead to an increase in the size of the breeding herd (Archer et al., 1999; Crews, 2005; Moore et al., 2009; Nkrumah et al., 2004) because it's association with feed requirements. An ideal feed efficiency trait would account for genetic variation in feed efficiency, without depending on genetic variation in output traits (Carstens and Tedeschi, 2006) like RFI. Individual animal performance varies because of factors such as age, gender, environmental conditions, and breed composition. However, even when comparing the animals of the same type under similar environmental conditions, feed intake differences can still be observed resulting in similar average daily gains (ADG). Residual feed intake was proposed by Koch et al. (1963) to address this issue and can be defined as a measure of the difference in actual individual animal dry matter intake (DMI) compared to expected DMI, where expected feed intake is calculated by regressing actual intake by gain and body size. Residual feed intake measures the variations in intake that occur for animals of the same type (breed, age, sex) consuming the same diet by calculating a feed efficiency trait by regressing feed intake against gain and metabolic body size. This measure is moderately heritable and genetically independent of growth traits (Arthur et al., 2001; Lancaster et al., 2009) making it a genetic selection trait that is growing in popularity. Multiple biological processes attribute to the inter-animal variation

quantified by RFI including digestibility, heat production, methane production and composition of gain (Carstens and Kerley, 2009). This review will focus on changes in feed efficiency due to the rumen environment.

Variations in feed efficiency due to rumen environment

The Beef NRC (2016) summarized 5 studies by Verge et al. (2008), Beauchemin et al. (2010), Pelletier et al. (2010), Lupo et al. (2013), and Stackhouse et al. (2012) that estimated the carbon footprint associated with beef cattle production in North America. The studies concluded that 55 to 63% of total greenhouse gas emissions from beef cattle production are produced via enteric CH_4. Nagaraja (2012) concluded that CH_4 emissions account for 2 to 12% of GE intake. Studies conducted in cattle fed high-concentrate diets found that CH_4 production contributed to 2.4 to 3.8% of GE intake (Todd et al., 2014; Harper et al., 2013). Consequently, selecting animals with lower levels of methane and adopting technologies that reduce methane emission would improve feed efficiency and reduce the carbon footprint of beef production system.

Variations in the energetic cost associated with CH_4 production can arise due to multiple factors such as differences in DMI and rumen microbial populations. Dry matter intake in particular can be mitigated by selection for RFI. Multiple studies have shown positive correlations between RFI and CH_4 production, such that as RFI increases so does CH_4 production (Nkrumah et al., 2006; Hegarty et al., 2007; Waghorn and Hegarty, 2011; Fitzsimons et al., 2013; McDonnell et al., 2016). Inefficient animals are characterized as having higher CH_4 production, however when CH_4 production was observed as a function of DMI (L CH_4/d/kg DMI) in these studies, significant

differences between high and low RFI groups disappeared. This suggests that differences in CH_4 production between RFI groups are mostly associated with differences in DMI.

Differences in rumen microbial populations and its relation with methane production have been proposed in more recent studies as a cause of variation in RFI amongst animals of a given population. Methanogens are a type of microorganism that produce CH_4 as a by-product of fermentation. Methanogens have various functions within the rumen, but most importantly act as a hydrogen sink in the process of regenerating oxidized co-factors (NAD+) in the rumen (Alende et al., 2017). Unfortunately, this path to deter excess hydrogen within the rumen is inefficient because of the production of CH_4. While differences in total methanogen populations have not been reported (Craberry et al., 2014), differences in the composition of these microbes have been detected (Zhou et al., 2009; Li and Guan, 2017). Steers with high RFI had higher amounts of *Methanobrevibacter ruminatium,* whereas low RFI steers had a higher population of *Methanomassillicoccales. M. ruminatium* produce CH_4 by utilizing CO_2, formate, and hydrogen as substrates (Russell and Rychlik, 2001 and Miller et al., 1986; reported by Li and Guan, 2017), whereas *Methanomassillicoccales* utilizes methanol and methylamines as a major energy and carbon source to produce NH_4^+ and CH_4 as a byproduct (Poulsen et al., 2013 and Sollinger et al., 2016; reported by Li and Guan, 2017). Consequently, low RFI steers could have provided more NH_4^+ for nitrogen recycling within the rumen, suggesting a potential for feed efficiency variation. More research needs to be done on the microbial population and its effect on ruminant animal efficiency.

Direct-fed microbials

Overview

Consumer perceptions of the livestock industry has changed in the past 20 years, such that many European countries now ban the use of growth promoting antibiotics in animal feeds due to potential risks of spreading antibiotic resistant genes (Hong et al., 2005) or concerns of antibiotic residues in animal products (Seo et al., 2010). The industry has adapted by evaluating the use of direct fed microbials (DFM) on animal performance. Direct-fed microbials, as defined by the US Food and Drug Administration (FDA) are "a source of live (viable) naturally occurring microorganisms" (FDA). Frequently used DFM supplements for ruminant animals include fungal cultures *Aspergillus oryzae* and *Saccharomyces cerevisiae* and lactic acid bacteria such as *Lactobacillus* or *Streptococcus* (Yoon and Stern, 1995), amongst others. Commonly, fungal cultures such as live yeasts have been utilized because of their ability to be processed and easily supplemented daily while still remaining viable. Other DFM like lactic acid utilizing or producing bacteria are limited in their application because of their anaerobic nature that reduces their viability through preparation, delivery, and inside the gastrointestinal tract; these DFM need to be orally drenched as a mean of supplementation which is labor intensive and may be cost-prohibitive (Seo et al., 2010). This has led to increased use of fungal cultures and active dried live yeasts due to their ability to be supplemented daily through premix inclusion without requiring substantial extra labor. Three primary methods have been utilized to enhance ruminant-animal production (Yoon and Stern, 1995): (1) use of additives or preservatives for silage, (2)

replacement or reduction in the use of antibiotics, and (3) improvement in milk production or feed efficiency. This review of literature will focus on how LY supplementation alters feed efficiency in growing and finishing beef cattle.

Effects on rumen environment and animal performance

The beef and dairy industries have evaluated the effects of LY usage, however, contradictory reports exist, potentially due to variability in dosages, feeding times and frequencies, strains, and effects on animals in different environments (Seo et al., 2010). In diets where live yeast has been supplemented, bacterial populations have been repeatedly shown to increase, most likely due to its ability to scavenge oxygen in the rumen (Rose, 1987) and decrease redox potential (Jouany et al., 1999), which would be beneficial for anaerobic cellulolytic bacteria (Seo et al., 2010). These changes would be beneficial in diets with a high proportion of roughage. Live yeast strain *S. cerevisiae* has also been observed to be beneficial to cattle high-concentrate diets where they compete with starch utilizing bacteria (Lynch and Martin, 2002) and prevent the accumulation of lactate in the rumen (Chaucheyras et al., 1995; Seo et al., 2010). Chaucheyras-Durand et al. (2007) reviewed the modes of action and effects of LY supplementation on ruminant animals and concluded that beneficial effects of LY were due to: (1) improvement in rumen maturity by favoring microbial establishment, (2) stabilization of ruminal pH by interacting with lactate-metabolizing bacteria, and (3) increase of fiber degradation by interacting with plant-cell wall degrading microorganisms (Chaucheyras-Durand et al., 2007). The ability of LY to stabilize ruminal pH and alter organic acid production is beneficial for metabolic disorders such as acidosis. Higher rumen pH and lower lactate

concentrations have been repeatedly observed in studies where cannulated animals were supplemented with live yeast (Chaucheyras-Durand and Fonty, 2006; Williams et al., 1991). Live yeast strain *S. cerevisiae* was also shown to compete with *S. bovis* and encourage growth of lactate-utilizing bacteria in *in vitro* studies, which would decrease the amount of lactate produced and limit accumulation within the rumen (Chaucheyras-Durand et al., 2007).

In a review (Jeyanathan et al., 2014) summarizing the effects of live yeast supplementation on methane production, varying results were observed in both *in vivo* and *in vitro* studies, most likely due to differences in conditions and live yeast products between studies. If LY supplementation can effectively reduce CH_4 emissions, it has the potential to increase animal efficiency by accounting for 2% to 12% of dietary gross energy losses (Nagaraja, 2012). In addition to stabilizing rumen pH and modifying methane production, a meta-analysis over 18 trials for animals fed growing and finishing diets, LY has been observed to modify performance and carcass characteristics (Wagner et al., 2016). Live-yeast supplemented cattle had increased ADG (6.5%), DMI (1%), G:F ratios (2.6%), and final BW (2.9 kg), as well as a greater percentage of animals grading choice or higher and a tendency to reduce liver abscesses compared to control cattle. Additionally, in dairy cattle, common effects of LY supplementation include an increase in dry matter intake and milk production (El-Ghani, 2004; Sniffen et al., 2004; Jouany, 2006; Stella et al., 2007).

Summary and conclusion

Digestive disorders like acidosis are the second leading cause of morbidity and mortality in US beef feedlots due to low roughage to concentrate ratios. As the feedlot industry continues to feed low-roughage diets to improve feed efficiency and reduce the cost of gain digestive disorders, like acidosis, have unintentionally resulted. Acidosis negatively effects animal and economic performance by impacting rumen function and animal health. Negative impacts on feed efficiency during a time when the demand for beef is steadily increasing need to be addressed; however, common methods of mitigating acidosis will begin to be scrutinized as consumer perception of antibiotic usage shifts. Using LY supplementation as a method to reduce the incidence of acidosis when feeding high concentrate diets has the potential to be a viable strategy. Inclusion of LY in high concentrate diets has been shown to stabilize ruminal pH and mitigate lactic acid production, giving it the potential to mitigate acidosis and increase feed efficiency.

CHAPTER 2

THE EFFECTS OF *SACCHAROMYCES CEREVISIAE* ON GROWTH EFFICIENCY, PHYSIOLOGY, FEEDING BEHAVIOR, AND CARCASS QUALITY IN YEARLING STEERS FED A HIGH-GRAIN DIET

Introduction

By the year 2050, the increase in the demand for animal-protein products will nearly double due to an increasing world population that is expected to reach 9.8 billion (FAO, 2009; US Census Bureau, 2008). Also, an increase in the demand of land for urban development will decrease land resources available for livestock production while a push for more "environmentally friendly" bio-energy production will increase feed costs. Traditional means of increasing feed efficiency will begin to be questioned as consumer concerns of antibiotic residues in animal products (Seo et al., 2010) and antibiotic resistant genes (Hong et al., 2005) grow. As a result, DFM have been proposed as a potential alternative to traditional antibiotic use because of their ability to alter microbial populations of the gastrointestinal tract (NASEM, 2016). Live-yeast strain *Saccharomyces cerevisiae* has also been observed to be beneficial for cattle fed a high concentrate diet, where they compete with starch utilizing bacteria (Lynch and Martin, 2002) and prevent the accumulation of lactate in the rumen (Chaucheyras et al., 1995; Seo et al., 2010). Furthermore, LY supplementation has been shown to optimize rumen function for more efficient feed utilization and animal health,. Therefore, the objectives of this study were to evaluate the effects of Levucell SC (*Saccharomyces cerevisiae*

CNCM I-1077) supplementation on performance, feeding and physical behaviors, rumen temperature, and carcass-quality traits in yearling steers fed a high-grain diet.

Materials and Methods

Animals, diets and experimental procedure

All animal care and use procedures were in accordance with guidelines for use of Animals in Agricultural Teaching and Research and as approved by animal use protocol (AACUC: 2014-019A).

Forty-eight crossbred beef steers (25% Bos indicus; 435 kg; 14.5 months of age) born and raised at the McGregor AgriLife Research Center (McGregor, TX) or the Beef Cattle Systems Research Center (College Station, TX) were used for this trial. Steers were blocked by source (McGregor vs. College Station) and randomly allocated into 1 of 2 dietary treatments: (1) control and (2) live yeast (LY; *Saccharomyces cerevisiae CNCM* I-1077; Levucell SC; Lallemand Animal Nutrition), with 2 pen replicates per treatment (n = 12 steers per pen; 24 steers per treatment).

During the 70-d study, steers were fed a high grain (dry-rolled corn) based diet (Table 2.1). The control diet included the experimental diet plus a treatment premix containing a carrier product (2% DM basis; provided by Lallemand Animal Nutrition), whereas LY diets included premixes (2% DM basis) containing Levucell SC (10×10^9 cfu/hd/d). The experimental diets did not contain tylosin or monensin. Upon arrival at McGregor AgriLife Research Center, steers were stepped up onto the experimental diet and acclimated to eat from GrowSafe feed bunks (3 bunks per pen). Following the 21-d adaptation period, dietary treatments were introduced (day 0). The actual inclusion rates

of the treatment premixes were adjusted throughout the trial to maintain approximate target consumptions rates of 2 oz per animal per day. Diet samples were collected every 14 d, and stored at 20°C. At the end of the study, samples will be composited by weight and sent to Cumberland Valley Analytical Services (Waynesboro, PA) to determine nutritional profiles. During this trial, shade was not provided.

Data collections

During the 70-d period, steers were weighed at 14-d intervals. On day 0 of the trial, boluses (Bella Ag™) were placed in the rumens of 10 steers per treatment (5 steers per pen replicate). The boluses were programmed to record rumen temperature at 15-min intervals, and data transmitted wirelessly to a base station located next to the data acquisition computer for the GrowSafe system. Additionally, HOBO accelerometer devices were placed on the left hind leg of the steers with rumen boluses to record physical activity (lying vs. standing, total step counts).

During the 70-d trial, diets were fed once daily, and feed bunks cleaned once weekly with ort samples collected and stored at 4°C as deemed necessary. Feed intake and feeding behavior traits were measured daily using the GrowSafe feed intake measurement system. Throughout the trial, the system was monitored at least twice daily to ensure accuracy of the feed intake data. To ensure data quality of the feed intake and feeding behavior data, the average assigned feed disappearance (AFD) rate was computed daily, and data for all animals in a pen deleted if AFD for a given day is less than 90%.

During the trial, steers were monitored twice daily for clinical symptoms of illness, and rectal temperature measured in steers with high clinical scores. Steers with rectal temperatures of ≥ 40.0°C were administered antimicrobial therapy and subsequently returned to their pen. Treatment premixes and treatment total mixed ration samples were collected weekly and stored at 4°C. The treatment premix samples were composited by weight at 3-wk intervals, and samples analyzed for yeast counts. Upon completion of the trial, weekly samples were composited by weight and analyzed.

At the end of the 70-d individual intake measurement trial, all steers were moved to group pens and maintained on their respective treatments until harvest at an approximate low-choice quality grade endpoint. At an approximate low-choice quality grade endpoint, steers were transported to Sam Kane Beef Processors Inc. (Corpus Christi, TX) for harvest and carcass data collection. Yield grade and USDA quality grade factors were measured at 48 h post-harvest, and lean color and pH measured. Liver and lung weights were recorded, and subjectively evaluated for signs of abscesses.

Calculations and statistical analysis

For analytical purposes, the trial was divided up into 2 periods: (Period 1) days 0 to 28, (Period 2) days 0 to 70. Data collected during this experiment was analyzed according to randomized complete block design (RCBD) where animal served as the experimental unit. Performance, DMI, feeding behavior, rumen temperature, physical activity and carcass response variables were summarized on an individual basis for the period of interest and analyzed using a mixed-linear model using PROC MIXED procedures of SAS (SAS Version 9.4, SAS Institute Inc., Cary, NC), with fixed (dietary

treatment) and random effects (pen) included in the model. Correlation coefficients among dependent variables will be generated using the PROC CORR procedure of SAS. Daily diurnal patterns were analyzed for DMI and BV eating rate by first apportioning the trait for every trial-hour from day 0-70, then summarizing values by h of day (HOD; h 0 through 24) for each steer. A time-series analysis using a mixed-linear model of PROC MIXED (SAS, 9.4) that included fixed effects of dietary treatment and h of day, and the REPEATED option where animal served as the subject repeated throughout HOD.

Individual growth rates were calculated using a linear regression model of PROC GLM (SAS, 9.4) in which body weight measurements were fitted against relative study day. Regression coefficients of the model were then used to symbolize ADG throughout the period of interest. Feed efficiency was computed as both feed:gain ratio (F:G), and as residual feed intake (RFI) defined as observed DM intake minus expected DM intake, with expected DM intake derived from multiple linear regression DM intake on mid-test $BW^{0.75}$ and ADG.

In addition to bunk visit (BV) event data (frequency and duration), a 2-pool Gaussian-Weibull distribution model was fitted to log-transformed NFI data, and the intercept of the 2 distributions (NFI within and between meals) used to define meal criterion. Individual-animal meal criterion was then be used to compute frequency and duration of meal events, meal length and size, and meal eating rate.

Variation of feeding behavior and meal traits were also measured for this trial, post hoc. Day-to-day variance were computed using the standard deviation of the

residuals from a linear regression model of PROC GLM (SAS, 9.4) in which feeding behavior traits were fitted against relative study day for individuals.

Results

Only 4 steers were treated for clinical symptoms of BRD during the trial, and all responded to the first antimicrobial therapy. Thus, LY treatment did not affect the health status of steers during the trial.

Days 0 to 28

Analyses of response variables for the first 28 d of the trial are summarized in Table 2.2. Live-yeast treatment did not affected performance, DMI or feed efficiency during the first 28 d of the trial. The frequency of BV events was 11% less ($P < 0.05$) in LY-fed steers compared to control-fed steers. Bunk visit duration tended ($P = 0.08$) to be higher in LY- versus control-fed steers, which resulted in LY-fed steers having 18% slower ($P < 0.05$) BV eating rates than the control-fed steers. Meal criterion tended ($P = 0.08$) to be longer, and meal frequency tended ($P = 0.12$) to be reduced in LY- compared to control-fed steers. Although meal duration was not affected by dietary treatment, meal-eating rate tended ($P = 0.12$) to be slower in LY-fed steers compared to control-fed steers. Additionally, head-down (HD) duration tended ($P = 0.08$) to be higher, and the ratio of HD to MD duration 29% greater ($P < 0.05$) in LY-fed compared to control-fed steers. During the first 28 d of the trial, the LY-fed steers approached the feed bunks 29 min sooner ($P < 0.05$) each day upon feed-truck delivery compared to control steers. There were no differences in the day-to-day variation of DMI, BV frequency, or meal frequency between treatments, although there was a tendency ($P = 0.07$) for BV duration

to be 15.4% more variable in LY-fed cattle. Daily variations in meal duration, max NFI, and HD duration were greater ($P < 0.05$) for LY-fed compared to control-fed steers. In contrast, the LY-fed steers had less ($P < 0.05$) daily variation in TTB than control-fed steers.

Days 0 to 70

Analyses of performance, feed efficiency, DMI, and feeding behavior response variables for the first 70 d of the trial are summarized in Table 2.3. During the 70-d trial, performance, DMI and feed efficiency traits were not affected by LY treatment. There was a tendency ($P = 0.07$) for LY-fed steers to have 9% lower BV frequency, and BV and HD durations were 22 and 40% longer ($P < 0.05$), respectively, compared to control-fed steers. The increase in BV duration due to LY supplementation resulted in 18% slower BV eating rate compared to control steers.

The LY-fed steers displayed substantially different meal behavior patterns than control steers. The LY-fed steers had 58% longer ($P < 0.05$) meal criterion, which led to a tendency ($P = 0.07$) for LY-fed steers to have fewer meals compared to control steers. The LY-fed steers had 13% greater ($P < 0.05$) meal duration and 27% longer ($P < 0.05$) meal lengths compared to the control steers. Consequently, since there were no differences in overall DMI, meal-eating rates of LY-fed steers were 10% slower ($P < 0.05$) than control-fed steers. Similar to results found during the first 28 d of the trial, the dietary treatment did not affect the BV per meal ratio, but did increase ($P < 0.05$) HD:MD ratio by 28% during the 70-d trial. Also similar to the first 28 d of the trial, the LY-fed steers approached the feed bunks 25 min sooner ($P < 0.05$) each day after feed-

truck delivery compared to control-fed steers. Similar to the first 28 d, daily variation in meal duration, max NFI, and BV and HD duration were greater ($P < 0.05$) for LY-fed steers in the 70-d period compared to control steers. In contrast, daily variation in TTB was 31% lower ($P < 0.05$) than control steers. Live yeast-fed steers had 19% lower day-to-day variation in meal frequency, a trait that was not affected by dietary treatment during the first 28 d of the trial.

Figures 2.1 and 2.2 illustrate the diurnal patterns for DMI and BV eating rate between the 2 treatments. The *P*-values at the top of each figure illustrate results from the time-series analysis for diurnal patterns. Live-yeast treatment did not affect ($P = 0.11$) diurnal DMI patterns during the 70-d trial. The control-fed steers had a faster BV eating rates throughout the day then LY-fed steers. Although the dietary treatment x hour interaction was not significant, the hour-to-hour variation in BV eating rates was numerically greater in control-fed then LY-fed steers.

Rumen temperature and physical activity

Results for rumen temperature and physical activity for the 70-day trial are summarized in Table 2.4. Live-yeast treatment did not affect average rumen temperature during the 70-d trial. Physical activity data were collected during 3 14-d periods from days 0-14, days 28-42, and days 56-70. Physical activity was not affected by period, or live-yeast treatment x period interaction ($P > 0.25$). When averaged across the 3 14-d periods, live-yeast treatment did not affect frequency or length of standing bouts, or daily duration of standing. A difference between the control- and LY-fed steers in standing frequency during the first 14-d period was detected such that LY-fed steers had

15% fewer ($P < 0.05$) standing bouts that tended to be 25% longer in duration compared to the control-fed steers.

Carcass and liver-lung scores

Analyses of carcass characteristics as well as subjective liver and lung scores are presented in Table 2.5 and 2.6. Following the 70-d trial, steers were maintained on their respective dietary treatments for an additional 26 d until harvest. Live-yeast treatment did not affect hot-carcass weight, LM area, KPH or marbling scores in this trial. However, backfat depth and yield grade were 17% and 13% greater ($P < 0.05$), respectively, in LY-fed steers than control-fed steers. Warner Bratlzer shear force measurements of longissimus dorsi muscle steaks at 1- and 14-d post mortem aging were not affected by LY treatment. Additionally, carcass color and pH were not affected by dietary treatment. Thirty-three percent of the all steers presented with liver abscesses (Table 2.6; scores of 2 or 3) postharvest. Additionally, 25% of all steers had lung scores of 2 or 3. Dietary treatment did not affect the incidence of liver abscesses or lung lesions during this trial ($P \geq 0.37$).

Phenotypic correlations

Phenotypic correlations are presented in Table 2.7. In agreement with previous studies, ADG was positively correlated ($P < 0.05$) with DMI (0.64), and negatively correlated with F:G ratio (-0.64). As expected, RFI was not correlated with initial BW or ADG in this trial. Dry matter intake was moderate to highly correlated with most of the meal traits, likely also impacting the BV per meal ratio. Additionally, most of the meal traits and the BV per meal ratio were significantly correlated with ADG, which is

surprising, but could be due to the strong relationship between ADG and DMI. Bunk visit and HD duration were also significantly positively correlated with ADG.

Discussion

The results of this study address the impact of LY supplementation on performance, feeding and physical behaviors, rumen temperature, and carcass-quality characteristics in yearling steers fed a high-grain diet.

Performance

In the current study, performance of steers during the first 28 d was as expected for the animal type and diet fed during this trial. Both F:G and G:F data are shown to illustrate the challenges of analyzing ratio-type feed efficiency traits during relatively short trial periods. During the entire 70-d trial, no differences were detected on performance due to LY supplementation. Contrary to the current study, in a review of yeast products by Shurson (2017), 8 studies were reported to yield positive results for growth performance and milk production due to yeast supplementation. Variable results of the effects of LY on performance exist within literature, most likely due to variability in trial design (i.e. animal type, diet, yeast product, and yeast concentration).

Feeding behavior and DMI

In the current study, there was no difference in DMI due to LY supplementation, similar to the results of a similar study conducted by DeVries and Chevaux, 2014. The current study is in agreement with previous dairy literature that suggests LY has the potential to modify feeding behavior (Bach et al., 2007; DeVries and Chevaux, 2008). Bach and colleagues (2007) reported active dry yeast to decrease the non-feeding

interval between meals in dairy cows, implying greater frequency of meal consumption. Additionally, DeVries and Chevaux (2008) reported greater meal frequency associated with LY supplementation in dairy cow diets. In the current study, LY supplementation tended to decrease BV and meal frequency. In a study with dairy cows, DeVries and Chevaux (2014) found that LY supplementation decreased meal criterion and increased meal frequency and size, contrary to the findings of the current study. As mentioned, DMI was not affected by LY supplementation; concurrently, BV duration was longer leading to slower eating rates for LY supplemented cattle. These results propose positive effects on the rumen environment due to LY supplementation. In beef feedlots, the dietary cause of metabolic disorders is a large consumption of rapidly fermentable carbohydrates and too little effective fiber. In to the current study, eating rate was slower for LY-fed steers. Steers supplemented with LY spent more time eating, ate less often and at a reduced rate compared to control steers. Together these feeding behavior patterns point to LY supplementation potentially aiding in mitigating metabolic stress in steers fed high-concentrate diets. Interestingly, time to bunk was faster in LY-fed steers compared to control-fed steers, suggesting LY supplementation influenced appetite sustenance.

Day-to-day variance was evaluated post-hoc for this study and did not yield results as expected. Duration response variables (BV, meal, and HD) yielded higher daily variance, whereas other metrics like Max NFI, meal frequency, and time to bunk exhibited lower values. It was hypothesized that LY would decrease day-to-day variance, given that it showed positive implications on feeding behavior. The nature of

day-to-day variation calculation implies a variable must be linearly distributed throughout time, therefore deviations from linearity could skew the results. Phenotypic correlations between day-to-day variance traits and performance metrics suggest a relationship with ADG. Steers that were had higher daily variation in meal and HD duration, yet lower day-to-day variation in meal frequency and TTB had higher ADG.

Rumen temperature and physical activity

Rumen temperature was not affected by LY supplementation in the current study. Rumen temperature has been proposed as a potential predictor of acidotic bouts, where nadir rumen pH has been found to have a strong negative correlation to corresponding ruminal temperature (AlZahal et al., 2008); therefore, lower rumen temperatures would be a positive response indicating a more favorable rumen environment for performance. In a study by DeVries and Chevaux (2014), rumen temperature was lower in LY supplemented compared to control treatments, contrary to the results of the current study. Live yeast has been shown to yield favorable results in high roughage diets because it stimulates the growth of cellulolytic microorganisms and increasing fiber digestibility. The current study had much lower roughage concentrations than the study by DeVries and Chevaux (2014) –10% versus 57.7%, so potentially the effects of LY were amplified in the higher roughage diet explaining the differing results in rumen temperature changes. Additionally, study location (i.e. weather; Ontario, Canada vs. Texas, USA) and animal type (lactating dairy cows vs. growing beef steers) could have also played a role in varying results.

Live-yeast supplementation decreased standing bouts and tended to decrease lying duration in the current study during the first 14 d, implying decrease restlessness. Gonzalez et al. (2010) observed lying times decreased in recently band-castrated bulls compared with steers, implying the results from this study characterize discomfort. Cattle behavior may be altered by contact with people and exposure to processing procedures (Ishiwata et al., 2007), indicating the LY group of cattle could have responded differently to new stressors implemented during the beginning of the study. Previous exposures to handling equipment or prior temperament scores (chute scores or exit velocity) were not obtained on the steers, therefore only speculations can be made to the true value of the observed result.

Carcass and liver-lung scores

In the current study, LY supplementation yielded greater backfat depth and higher yields grades, although HCW, LMA, Warner Bratlzer sheer force measurements, or carcass color and pH were not affected by dietary treatment. Crossland et al (2018) reported no difference in HCW or LMA consistent with the current study. In some studies, Saccharomyces cerveisiae has been found to be beneficial (Ovinge et al., 2018; Geng et al., 2015) in improving carcass quality, whereas not in others (Mir and Mir, 1994; Maggioni et al., 2009) like in the current study. Live-yeast treatment did not affect the incidence of liver abscesses or lung lesions during this trial, but previous studies have yielded a tendency to reduce liver abscesses compared to control cattle (Wagner et al., 2016). Liver scores prevalence likely reflects the fact that tylosin was not included in

the experimental diets during this trial. Additionally, lung scores incidence indicates some degree of lung consolidation associated with bovine respiratory disease.

Conclusion

This study evaluated the effects of LY supplementation on growth efficiency, feeding and physical behaviors, rumen temperature, and carcass-quality characteristics in finishing cattle fed a high-grain diet. There was no affect of LY supplementation on performance, intake, or feed efficiency, but feeding behavior patterns were substantially altered. Bunk-visit and HD duration were greater in LY supplemented steers and subsequently BV eating rates were slower. Meal criterion was greater due to LY supplementation leading to reduced meal frequency, greater meal duration, and subsequently slower meal eating rates for LY-fed steers compared to control steers. Altogether, the response of feeding behavior patterns to LY supplementation may have had positive effects on the rumen environment. Time to bunk was also faster for LY-fed steers suggesting that LY supplementation influenced appetite sustainability. Additionally, day-to-day variation traits were varied such that LY supplementation increased variation in duration traits (BV, HD, and meal) and max NFI, but decreased daily variation in meal frequency and time to bunk.

CHAPTER 3

THE EFFECTS OF *SACCHAROMYCES CEREVISIAE* ON PERFORMANCE, PHYSIOLOGICAL, AND BEHAVIORAL RESPONSES DURING SUBACUTE ACIDOSIS CHALLENGES IN BEEF STEERS

Introduction

Current feedlot diets are composed of non-structural carbohydrates in the form of grain and grain by-products leaving only 8-12% DM of the diet for roughage inclusion (Samuelson et al., 2007). Unintentionally, this change lead to were an increase in undesirable digestive disorders. Currently, the feedlot industry in the United States is challenged with digestive disorders as one of the leading causes of morbidity and mortality, second only to respiratory disease (Nagaraja and Lechtenberg, 2007) due to the characteristics of finishing diets and management techniques.. Amongst those digestive disturbances, acidosis is one of the most well known and researched because of its prevalence and impact on animal and economic performance. Traditionally, ionophores have been utilized to mitigate the causes of acidosis, however growing public concern about antibiotic usage and resistance within the livestock industry has lead to exploring other non-antibiotic methods of reducing or mitigating the incidence of acidosis. Direct-fed microbials have been proposed as a potential alternative to antimicrobial use because of their ability to alter microbial populations of the gastrointestinal tract (NASEM, 2016). Live yeasts (LY), in particular *Saccharomyces cerevisiae*, have been evaluated extensively in the dairy industry, but remain to be well defined for feedlot scenarios although it poses the potential to mitigate digestive

disorders, like acidosis. In the previous chapter, LY supplementation was proposed as a potential mitigating tactic for acidosis because it reduced BV eating rates in steers consuming high-concentrate diets. Therefore, the objective of this study was to evaluate the potential of LY (*Saccharomyces cerevisiae* strain I-1077; Levucell SC; Lallemand Animal Nutrition) to mitigate the effects of an experimentally induced SARA challenge in growing beef steers consuming a high-concentrate diet.

Materials and Methods

Animals, diets and experimental procedure

All animal care and use procedures were in accordance with guidelines for use of Animals in Agricultural Teaching and Research and as approved animal use protocol (AACUC: 2018-018A).

Upon arrival at the Texas A&M Beef Cattle Systems facility (College Station, TX), 48 Angus crossbred steers (initial BW = 343 ± 3) originating from McGregor research herd were placed on wheat pasture until the beginning of the trial. On day -21, steers were weighed and assigned an EID tag (Allflex USA, Inc., Dallas, TX), placed in pens equipped with 3 electronic feed bunks (GrowSafe Systems) and fed a grower diet (Table 3.8) without live yeast in order to adapt to the bunks and diet. Thereafter, steers were weighed weekly and pen rotations occurred on weigh days to account for any potential random effects of pen. On day -7, steers were stratified by BW, previous ADG, and exit velocity, and randomly allocated to a 2 x 2 factorial treatment arrangement with Factor 1 being a diet with added live yeast (LY; *Saccharomyces cerevisiae* strain I-1077 at 2 x 10^{10} cfu per d; Levucell SC; Lallemand Animal Nutrition) and without (Control), and

Factor 2 being induction of subacute ruminal acidosis (SARA) or negative control (non-SARA). Treatments (n = 12) were as follows: (1) control SARA, (2) control non-SARA, (3) LY SARA and (4) LY non-SARA. Live yeast treatments began on day 0 and lasted for the entirety of the study (day 105). For the entirety of the study, steers were fed steers were fed by hand 3x per day at 0800, 1100, and 1600 h with 30%, 30% and 40% of daily feed allowance allocated at each feeding, respectively. Feed calls were evaluated daily each morning with a goal of maintaining empty bunk time for approximately 4 to 6 h per day. On day 21, steers were transitioned to finisher diets (Table 3.8), with and without LY, during a 14-d period using a 2-ration method. Once fully on the finisher diet, SARA treatment groups underwent 2 SARA challenge periods. The experimental timeline for the trial is illustrated in Figure 2.1.

Acidosis challenge models

After uninterrupted adaptation to the finisher diet, steers in the SARA treatment groups were exposed to 2 subsequent SARA challenge models (Figure 2.1; day 56 to 97) while steers of non-SARA treatments were fed as normal. Both challenge models were designed to mimic disruptive feed delivery, which can lead to a ruminal acidosis bout. Each SARA challenge was comprised of a 3 wk with the 1st wk to determine baseline feed intake, the 2nd to impose disruptive feed delivery, and the 3rd wk to return to a normal feeding regimens. During week 2 of the first SARA challenge (SARA #1), the amount of feed intake was altered on a daily basis from 60 to 140% of average feed intake measured during week 1; steers were fed 1x per day at 0800 h on the first day of imposed challenge. The second SARA challenge (SARA #2) protocol was executed

similar to SARA #1, with the exception that finely ground wheat was substituted for 20% of the steam-flaked corn in the diet in order to further disrupt the rumen environment (Finisher-GW; Diet composition presented in Table 3.8). During the 3rd wk, after each SARA challenge, steers were return to 100% feed delivery and fed 3X per day in a manner similar to the non-SARA steers.

Rumen boluses

To facilitate continuous collection of rumen pH and temperature measurements, indwelling reticulo-rumen boluses (Smaxtec) were inserted orally into half (n = 6 per treatment group) of the steers with a balling gun on day -7. Before insertion, boluses were calibrated using a buffer solution of pH 7. Throughout the trial, data was continually recorded by boluses and radio-transmitted to a base station, with data stored in the cloud. The Smaxtec monitoring system averaged data at 10-min intervals. Data was serially downloaded at the end of the trial and aggregated on a daily basis to include average, maximum, minimum, and standard deviation of pH, average temperature, and average activity. Duration of time spent with pH < 5.8 was also computed on a daily basis for individuals by the summation of measurements < 5.8 multiplied by 10. Area under the curve of pH < 5.8 was computed using trapezoidal summation between consecutive pH readings. Ruminal pH threshold < 5.8 were used to characterize SARA during this trial in accordance with previous literature (Beauchemin et al., 2003; Ghorbani et al., 2002; Schwartzkopf et al., 2004).

Feed sampling and analyses

Diet samples were collected weekly, and stored at 20°C. At the end of the study, samples were composited by weight and sent to Cumberland Valley Analytical Services (Waynesboro, PA) to determine nutritional profiles. Diet premix samples were also collected weekly for live yeast counts and stored at 20°C until being sent to a designated lab for LY analysis.

Arterial blood samples and analyses

Arterial blood samples were collected the morning of the start of each SARA challenge protocol, and the 7th day of feed-intake disruption (days 63 and 70 for SARA #1 and days 84 and 91 for SARA #2). Samples were collected from steers that received reticulo-rumen boluses (n = 6 per treatment) via the intermediate branch of the caudal auricular artery using lyophilized heparin 1 mL syringes (Cat.# 9025TRU, Agri*Life* Arterial Blood Sampler, CareFusion, Yorba Linda, CA) designed for blood gas analysis. Analysis was performed chute side with a VetScan iStat®1 Analyzer (Abaxis North America, Union City, CA) using i-STAT CG4+ cartridges to obtain blood pH, HCO_{3}-, base excess (BE), saO_2, and lactate measurements. Data for samples that had saO_2 values of < 90% were deleted from analysis, due to likelihood that venous rather than arterial blood was sampled.

Carcass and liver characteristics and analyses

Steers were maintained on the same dietary treatments throughout the study until they reached an approximate backfat depth of 1.27 cm. Steers were harvested at Cargill Meat Solutions (Friona, TX). Instrument grading was used to collect carcass

characteristics 1 d after slaughter and evaluations for liver scores were collected by a trained team of individuals from West Texas A&M University.

Feeding behavior and DMI

Steers were housed in 4 pens (n = 12), each equipped with 3 electronic feed bunks (GrowSafe Systems Ltd., Airdrie, AB, Canada) to facilitate collection of feed intake and feeding behavior data on an individual-animal basis. The GrowSafe system continually collects feeding behavior and intake data based on its ability to detect and record EID tag presence within a load bar mounted feed bunk equipped with an antenna. Individual animal feed intake was calculated by measuring feed disappearance associated with EID detection within a bunk. Each EID detection also signifies the start of a bunk visit (BV) event which continues until: (1) time since consecutive EID recordings exceeds 100 seconds, (2) EID was detected within another feed bunk, (3) another animals EID was detected at the same feed bunk (Mendes et al., 2011). The daily number of independent bunk visit events and total time spent at the bunk, represent BV frequency and BV duration, respectively. Total feed disappearance associated with an individual within a 24-h period represents daily feed intake. From these traits, additional feeding behavior traits were computed including BV eating rate and time to bunk (TTB). Bunk visit eating rate (g/min) was calculated as the ratio of daily DMI to daily BV duration and TTB (min) was also computed by taking the difference in time between the first feeding event for an individual and the first feed supply event of the day. Throughout the trial, assigned feed disappearance (AFD) rates were computed and monitored daily to assess data quality. Days where the average AFD for any given pen

(computed as the average of the AFD's from the 3 used bunks) was < 90% were considered failed days and not included in data analysis. Post hoc, average daily empty bunk time and total daily feed supply amounts were determined to provide further insight into behavioral data.

Calculations and statistical analysis

The experimental timeline used for data analysis within this study ranges from day -14 to 105. Data collected during this experiment were analyzed according to randomized complete block design (RCBD) with a 2 x 2 factorial treatment arrangement where animal served as the experimental unit. Individual growth rates were calculated using a linear regression model of PROC GLM (SAS, 9.4, SAS Institute Inc., Cary, NC) in which body weight measurements were fitted against relative study day. Regression coefficients of the model were used to symbolize ADG throughout the period of interest. Feed efficiency was computed as F:G and G:F ratios. In addition to rumen bolus parameters, feeding behaviors were also summarized on an individual-animal basis for the period of interest and analyzed using the MIXED procedure (SAS 9.4). For response variables with significant ($P \leq 0.05$) diet x SARA interactions, mean separation tests were performed using Student's t-test.

To further evaluate the effects of imposed SARA challenges (days 56 to 97), time-series analysis was performed using MIXED procedure of SAS with a model that included fixed effects of diet, SARA, and day and all possible interactions. To account for random variation within individuals and throughout time, the REPEATED option of SAS was utilized.

Results

For data analysis, the trial was divided into 4 periods: (1) days -14 to 0, (2) days 0 to 35, (3) days 35 to 105, and (4) days 56 to 97.

Days -14 to 0

Prior to LY supplementation, steers were adapted to a grower diet for a period of 14 d. Main effect and subclass means for this analysis are presented in Tables 3.9 and 3.10, respectively. These results are presented to illustrate that treatment did not affect DMI, feeding behavior, or rumen bolus response variables during this 14-d adaption period, with one exception of BV eating rate. Bunk visit eating rate was 15.5 g per min greater ($P < 0.05$) in control-fed steers compared to LY-fed steers. For this study, daily feed calls were made to target approximately 4 to 6 h of empty bunk time. Pen means for feed supply level (kg/d) and mean empty-bunk time (EBT; min/d) are presented in Tables 3.9 and 3.10 to assess treatment differences in feed delivery.

Days 0 to 35

The dietary treatment was initiated following the 14-d adaptation period, and all steers were maintained on the grower diet for 21-d prior to being transitioned to the finisher diet over a period of 14-d. The dietary treatment, but not SARA treatment was implemented during this 35-d period. The main effect and subclass means for DMI, feeding behavior, and rumen bolus response variables are presented in Tables 3.11 and 3.12, respectively, for this 35-d period (21-d grower and 14-d transition).

During this 35-d period, LY supplemented steers consumed 9% less ($P < 0.05$) DMI than control steers even though feeding behavior patterns were not affected by

dietary treatment. Furthermore, BV eating rate was slower ($P < 0.05$) for LY-fed steers compared to control steers, although there was a diet x SARA treatment interaction ($P < 0.01$) for BV eating rate. This interaction was due to the fact that in the steers fed the control diet, non-SARA steers consumed feed at a much faster ($P < 0.05$) rate than SARA steers (191 vs. 111 g/min), whereas, there was no difference in BV eating rate between the non-SARA and SARA steers fed the LY diet (120 vs. 133 g/min). There were substantial SARA and diet x SARA treatment interactions for feeding behavior traits despite the fact that the SARA treatment had not yet been imposed. Although DMI was not affected by SARA treatment or the diet x SARA treatment interaction, BV frequency and duration, and head-down (HD) duration were significant. The interaction for BV and HD duration was due to the fact that for non-SARA treatment, LY-fed steers spent more ($P < 0.05$) time eating than control steers, but the same trend did not follow for the SARA treatment steers. Additionally, LY supplementation decreased BV frequency in non-SARA treated steers, but increased BV frequency it SARA steers, compared to the control diet.

The only rumen response variable that was affected by diet or SARA treatments was variance of rumen pH (diet x SARA interaction; $P < 0.05$). The variance of rumen pH was greater ($P < 0.05$) in the non-SARA control-diet steers than the other 3 treatments,

Days 35 to 105

The main effect and subclass means for DMI, feeding behavior, and rumen bolus response variables for the 70-d period are presented in Tables 3.13 and 3.14. During this

70-d period, the LY steers consumed 12% less ($P < 0.01$) DMI than control steers. There was no effect of the SARA treatment or diet x SARA interaction on DMI. There was a diet effect and diet x SARA interaction ($P < 0.05$) for BV frequency. Live yeast treatment increased ($P < 0.05$) BV frequency by 33% in SARA steers, but had no effect on BV frequency in non-SARA steers. There was a tendency ($P = 0.09$) for a diet effect for BV duration, such that LY supplementation numerically reduced BV duration by 13%. Head down duration was not affected by dietary or SARA treatments. The diet x SARA interaction for TTB was due to the fact that in SARA steers, LY supplementation caused the steers to approach the feed bunks 15 min sooner ($P < 0.05$) each day compared to steers fed the control diet, whereas LY treatment did not affect time to bunk in the non-SARA steers.

During the 70-d period, there were no differences detected for rumen bolus response variables due to dietary or SARA treatment, with the exceptions for mean rumen temperature and maximal pH. Mean rumen temperature tended ($P = 0.09$) be 0.19% lower in SARA compared to non-SARA steers. The tendency ($P = 0.08$) for the diet x SARA interaction for maximal pH was due to the fact that LY decreased ($P < 0.05$) maximum pH by 3.4% in non-SARA steers, but increased ($P < 0.05$) maximum pH by 2.1% in SARA steers. Collectively, these results indicate that the 2 weekly periods of disruptions in feed delivery had minimal effects when considering the entire 70-d period.

Days 56 to 97

The time-series analyses for each of the SARA challenge periods (SARA #1 – days 56 to 76 and SARA #2 – days 77 to 97) are shown in Tables 3.15 through 3.18. The

results from the time-series analyses of the DMI and feeding behavior data are presented in Tables 3.15 (main-effect means) and 3.16 (subclass means). Rumen pH and temperature data are presented in Tables 3.17 (main-effect means) and 3.18 (subclass means). Summaries of the time series analysis of the DMI and feeding behavior data (Tables 3.15 and 3.16), and rumen bolus data (Tables 3.17 and 3.18) are presented in Tables 3.19 and 3.20. For these summary tables: (1) in the presence of significant diet x SARA interactions, the percentage differences in LY versus control diet subclass means are compared within SARA treatment and (2) in the absence of diet x SARA interactions, the percentage change in diet and SARA main-effect means are presented. Additionally, Figures 3.4 through 3.10 are presented for response variables with significant diet x SARA interactions, with DMI and feeding behavior data shown in Figures 3.4 to 3.6, rumen pH parameters in Figures 3.7 to 3.9, and rumen temperature in Figure 3.10.

During SARA #1, there was a diet x SARA interaction for DMI (Table 3.15 and 3.19). Live-yeast supplementation reduced ($P < 0.05$) DMI 17% in the non-SARA treatment, whereas the reduction ($P < 0.05$) in DMI due to LY supplementation was only 9% in the SARA treatment. Conversely, the diet x SARA interaction for BV frequency was due to LY supplementation having no affect on non-SARA steers, but increasing ($P < 0.05$) 23% in the SARA steers. Diet x SARA interactions were not detected for the other feeding behavior traits. However, BV and HD durations were 11 and 16% less ($P < 0.5$), respectively, and the HD:BV duration ratio 4% less ($P < 0.05$) for steers fed the LY diet compared to steers fed the control diet. Additionally, HD duration and the HD:BV

duration ratio was 14% longer ($P < 0.05$) and 8% greater ($P < 0.05$), respectively, for SARA compared to non-SARA steers.

Despite the fact that the GrowSafe system failed during days 84 through 87 of SARA #2, the effects of diet and SARA treatments on DMI and feeding behavior patterns were remarkably similar to SARA #1. The diet x SARA interaction ($P < 0.05$) for DMI was due to LY supplementation reducing DMI to a greater extent in the non-SARA steers (18%) compared to SARA steers (7%). Additionally, the diet x SARA interaction for BV frequency was due to LY supplementation increasing with a greater magnitude for SARA steers (52%) than non-SARA steers (NS). As during SARA #1, there was no diet x SARA interactions for the other feeding behavior traits. In all steers, the reductions ($P < 0.01$) in BV (14%) and HD duration (21%), and HD:BV (8%) due to LY supplementation during SARA #2 were similar to the reductions observed during SARA #1.

During SARA #1, diet x SARA interactions were detected for mean pH, maximum pH, pH variance, and duration and AUC of pH < 5.8, but not for minimum pH, rumen temperature, or mean activity. In non-SARA steers, LY supplementation reduced ($P < 0.05$) mean pH by 1.4% and maximum pH by 4.6%, and reduced pH variance by 26%. Conversely, in SARA steers, LY supplementation increased ($P < 0.05$) mean pH 1.4% and maximum pH by 2.1%, and caused pH variance to 6.9% higher ($P < 0.05$). Furthermore, duration of pH < 5.8 was numerically reduced 44% (NS) in non-SARA steers, but was reduced ($P < 0.05$) 61% in the SARA steers, while AUC of pH < 5.8 was reduced by a greater magnitude in the SARA steers fed LY (73% reduction; $P <$

0.05) compared to the non-SARA steers fed LY (67% reduction ($P < 0.05$). In all steers (main-effect means), minimum pH and rumen temperature were 2.2% and 0.38% lower ($P < 0.05$) in SARA compared to non-SARA steers. There was no effect of SARA treatment on mean activity level. Further, all steers (main-effect means) fed the LY diet had 1.2% higher minimum pH, 0.29% lower rumen temperature, and 10% greater mean activity levels than control-fed steers.

As during SARA #1, diet x SARA interactions ($P < 0.05$) were detected for mean pH, maximum pH, pH variance, and duration of pH < 5.8, but not for minimum pH in SARA #2. Contrary to SARA #1, there was no diet x SARA interaction for AUC of pH < 5.8 in SARA #2. In the non-SARA treatment steers, LY supplementation numerically reduced mean pH by 0.5% (NS), and reduced ($P < 0.05$) maximum pH by 2.6% and pH variance by 20%. Conversely, in SARA steers, LY supplementation increased ($P < 0.05$) mean pH by 2.6%, maximum pH by 3.0% and caused pH variance to be numerically higher (NS) by 4.5%. Similar to SARA #1, duration of pH < 5.8 was reduced ($P < 0.05$) in non-SARA and SARA steers fed the LY diet, but the magnitude of the reduction was greater in SARA steers (76%) than in non-SARA steers (49%). Unlike during SARA #1, a diet x SARA interaction was not detected for AUC of pH < 5.8. Steers fed the LY diet (main-effect means) had 65% reduced AUC of pH < 5.8 compared to steers fed the control diet. The AUC of pH < 5.8 was not affected by SARA treatment during SARA #2. In all steers (main-effect means), minimum pH was 2.2 % lower ($P < 0.05$) in SARA compared to non-SARA steers and 1.2% greater ($P < 0.05$) for LY-fed steers compared to control-fed steers. In contrast to SARA #1, there was also a diet x SARA interaction

for rumen temperature. Live-yeast supplementation caused rumen temperature to be 0.19% higher ($P < 0.05$) in non-SARA, but LY supplementation did not affect rumen temperature in SARA steers. Thus, the treatment effects on rumen pH were remarkably similar during the 1st and 2nd SARA challenge protocols (Table 3.20) despite the fact that the 2nd SARA challenge had less of an apparent disruptive effect based on fewer SARA x day interactions.

Additional analysis were conducted using the same time-series model with DMI for the 2 weeks following the transition period (days 35 to 49) considered as a covariate (results not presented). Results from the covariate model conveyed the same treatment differences as the model without the covariate, with the exception of a diet x SARA interactions for the ratio of HD to BV duration in SARA #1 and BV duration in SARA #2.

Figures 3.11 through 3.18 illustrate diet x day and SARA x day means for both SARA challenge protocols, with diet x day means in Figure 3.11 and SARA x day means in Figures 3.12 through 3.18. Furthermore for SARA x day means, DMI and feeding behavior are presented in Figures 3.12 through 3.14, rumen pH data in Figures 3.15 through 3.17, and rumen temperature data in Figure 3.18. In these figures, the ↓ indicates the day that the feed-intake disruption protocol was initiated.

For the most part, there was no diet x day interactions detected for rumen parameters, DMI, or feeding behavior data, except for on BV duration and eating rate during SARA #2 (Figure 3.11). Seeing a difference in the effects on feeding behavior traits between SARA #1 and #2 was not surprising given: (1) system failure for 4/7 d

during challenge week, and (2) the adaptive nature witnessed on response variables with successive SARA challenges.

Figures 3.12 through 3.14 (SARA #1 graphs, left) illustrate that the feed-intake disruption protocol impacted daily variance in DMI and BV frequency, duration, eating rate, and TTB, but not for HD duration, or the ratio of HD:BV duration for the 1st SARA challenge. As anticipated, the first SARA challenge varied (SARA x day; $P < 0.01$) DMI daily where DMI ranged between approximately 5 and 14 kg per day from days 63 through 69 that encompassed feed fluctuations. More so, DMI for SARA steers was significantly less ($P < 0.05$) on days 63, 65, 67, and 69 and greater ($P < 0.05$) on day 64 than control steers. Day 63 was the first day of feed fluctuations in SARA #1 where feed availability was restricted to 60% of baseline intake and steers were fed only 1x per day at 0800 h. Furthermore, days 65, 67, and 69 were also feed restricted to 60% baseline intake, but feedings occurred 3x per day. Bunk visit frequency was also significant ($P < 0.05$) for the SARA x day interaction during SARA #1 such that SARA steers exhibited less ($P < 0.05$) bunk visits for days 63 and 65 compared to Non-SARA steers. The difference in BV frequency is not detected on days 67 and 69. A different trend seemed to be detected for BV duration. During SARA #1, there was no difference in BV duration on feed restriction days (days 63, 65, 67, and 69), with the exception of a tendency on day 63 for SARA steers to have less time spent eating. However, when steers were offered 140% of baseline intake (days 64, 66, and 68), BV duration appeared to be greater on days 64 ($P < 0.05$) and 68 ($P < 0.10$), and numerically greater ($P > 0.10$) on day 66. Bunk visit duration was not different in SARA compared to non-SARA steers

on feed restriction days. Consequently, BV eating rate was lower on days 63 and 65 ($P < 0.05$), and numerically lower on days 67 and 69. The difference in BV eating rate (g/day) is due to the decrease in DMI coupled with similar BV duration on feed restriction days. Therefore SARA steers ate and visited the bunk less and had slower BV eating rates on feed restriction days and had higher BV duration on excess feed delivery days compared to non-SARA steers. There was no SARA x day interaction for HD duration or the ratio of HD:BV duration during SARA #1. Collectively, these results follow expectations for daily feed delivery fluctuations. Unfortunately, we cannot evaluate similar metrics for the 2nd SARA challenge due to the GrowSafe system crashing during a 4-d period.

For SARA #1, there were SARA x day interactions for mean rumen pH and maximal pH, hour-to-hour variance of rumen pH, and a tendency for minimal pH (Figures 3.15 and 3.16; left graphs); however, SARA x day interactions were not detected for duration and AUC of pH < 5.8 (Figure 3.17), or mean activity levels. The SARA x day interaction also affected rumen temperature (Figure 2.9), such that temperature was lower in SARA treatments than controls most day of challenge. Interestingly, mean and maximum rumen pH were lowest on feed restriction days (days 63, 65, 67, and 69), contrary to expected. Mean and maximum pH values for SARA steers on feed restriction days were significantly ($P < 0.05$) lower than control steers, with the exception of day 63 when values were approximately the same. Mean and maximum pH were not different in SARA steers on excess feed days (days 64, 66, and 68) compared to control, with the exception of maximum pH being higher ($P < 0.05$) in

SARA treatments on day 66. Minimum pH was significantly lower ($P < 0.05$) during both feed excess and restriction days, with the exception of the first day: day 63). Consequently, the variance of pH from hour-to-hour was highest and statistically different in SARA steers compared to control on feed excess days. The variance of pH was highest in SARA treatments on day 64, which was the first day excess feed was offered. Therefore, SARA treatment induced lowest mean and maximum pH values on feed restriction days, higher pH variance on feed excess days, and lower minimum pH throughout the whole challenge week. Collectively these results illustrate that the 1st SARA challenge disrupted the rumen environment. These trends are repeated in SARA #2, but with less severity. For SARA #2, there were SARA x day interactions for maximal pH and hour-to-hour variance of rumen pH, but not for mean and minimal pH, duration and AUC of pH < 5.8, rumen temperature, or mean activity levels. The SARA treatment still induced lowest mean and maximum pH values on feed restriction days, higher hour-to-hour variance of pH on feed excess days, and tended to yield lower minimum pH throughout the whole challenge week. These results suggest that SARA #1 disrupted rumen environment to a greater extent than SARA #2.

Arterial blood analysis

The main-effect means (3.21) for arterial blood measurements are presented for the 1st and the 7th day of the disruptive feed delivery phases for both SARA challenges. There were no differences detected due to diet, SARA, or the diet x SARA interaction in blood pH, HCO_{3-}, base excess (BE), lactate or saO_2 levels before either SARA challenge throughout the study. Live-yeast supplemented steers tended ($P = 0.12$) to have lower

HCO_{3-} and BE levels following SARA #1 however, this trend was not observed following the 2nd SARA challenge. Following SARA #1, there were no observed differences due to SARA except for a tendency (P = 0.08) for SARA cattle to have higher saO_2 levels. Following SARA #2, there was a tendency for blood pH and base excess to increase by 5.3 and 54%, respectively. Additionally, lactate was 46% lower (P < 0.05) for SARA steers following SARA #2. Collectively, these results indicate the SARA challenges had minimal affects on arterial blood gas measurements.

Performance and feed efficiency

Performance and feed efficiency data for this study are presented for 2 periods: days 0 to 35, and days 35 to 105. During the first 35-d period (Table 3.22), there were no differences due to dietary or SARA treatments for initial or final BW, ADG, or F:G. Although the LY-fed steers consumed 9.2% less (P < 0.01) DMI compared to the control-fed steers, there was no affect of dietary treatment on F:G. Likewise, during the 70-d period (Table 3.23), there were no effects of diet or SARA treatments on initial or final BW, or ADG. However, steers fed the LY diet consumed 12.4% less (P < 0.01) DMI, and consequently had 20.7% more favorable (P < 0.05) F:G than steers fed the control diet. During days 35 to 105 of the study, there were no differences in ADG, DMI or F:G due to the SARA treatment.

Carcass and liver scores

Carcass data and liver scores analysis are presented in Table 3.26. Steers fed the LY diet tended (P = 0.08) to have 5.7% larger LMA than steers fed the control diet, but there was no difference in HCW, YG, marbling scores, QG distributions, or liver abscess

scores. Additionally, the SARA treatment did not affect HCW, backfat depth, LMA, YG, or liver abscess scores. However, SARA steers tended (P = 0.08) to have lower marbling scores, which resulted in numerically fewer prime-grade and more select-grade carcasses. This tendency can be explained by the diet x SARA interaction (P = 0.06), where LY supplementation reduced marbling scores 12% in non-SARA steers, but increased marbling scores 6% in SARA steers.

Discussion

Feeding behavior and DMI

Feeding behavior patterns were altered due to LY supplementation, consistent with results from studies conducted with dairy cattle (Bach et al., 2007; DeVries and Chevaux, 2008). During the SARA challenge periods, LY decreased both BV and HD durations, although the significant diet x SARA indicated that LY-fed steers had greater reduction in DMI and increase in BV frequency in non-SARA treatments than SARA treatments. Changes in DMI and feeding behavior patterns due to LY supplementation may have positively impacted the rumen environment and fermentation during SARA challenges. Live-yeast strain *Saccharomyces cerevisiae* has also been observed to be valuable in high concentrate diets as they compete with starch utilizing bacteria (Lynch and Martin, 2002) and prevent the accumulation of lactate in the rumen (Chaucheyras et al., 1995; Seo et al., 2010), and improve ruminant efficiency. Although dietary treatment did not affect ADG, LY-fed steers had reduced DMI making them more efficient.

When prior DMI was used as a covariate in the time series analysis for days 56 to 97, no changes in the effects of diet, SARA, or the diet x SARA interaction in

comparison to the previous model were observed. This illustrates that the results of the original model were not due to differences in DMI.

Substantial SARA x day interactions for feeding behavior traits confirms that the SARA challenge protocols effectively altered feeding behavior patterns. During this study, steers were fed to target 4 to 6 h of empty bunk time. During days 0 to 35, differences due to SARA treatment were observed despite the fact that the SARA treatment had not yet been initiated. The mean pen feed supply and empty bunk time metrics from the GrowSafe system were evaluated to understand these SARA treatment effects. During this 35-d period, feed supply levels were fairly similar between pens and ranged from 107 to 121 kg/d. However, empty bunk time was substantially greater for the non-SARA control-fed steers than the other 3 treatments (427 min/d vs. 133 $\pm$ 28 min/d). These differences in empty bunk time likely contributed to the SARA treatment effects on feeding behavior during this period. During the 70-d period that encompassed the 2 SARA challenge protocols, empty bunk time was more consistent amongst treatments (242 $\pm$ 48 min/d). Most of the difference in feeding supply came from greater levels being supplied to the control treatment (124 kg/d) compared to the LY treatment (107 kg/d). The 17 kg difference between dietary treatments could have contributed to the effect of diet on DMI, BV frequency, and BV duration, and thus caution needs to be taken when interpreting this data.

Rumen pH and temperature

The results of this trial demonstrate that the experimental SARA challenge protocols successfully induced reductions in ruminal pH, a measurement commonly used to evaluate acidotic status in ruminants.

Focusing on the first14-d period prior to the beginning of LY supplementation, no differences in rumen bolus parameters were observed due dietary or SARA treatments, or their interaction, as expected. During the first 35-d of LY supplementation, the variance of pH was effected by SARA treatment and the diet x SARA interaction, which may be due to the higher mean empty bunk time of the non-SARA control steers compared to the other 3 treatments (427 min/d vs. 133 $\pm$ 28 min/d).

The true impact of the SARA challenge protocols becomes evident when looking at the results from the time series analysis from days 56 through 97. In general, the SARA challenge protocols reduced mean, maximum, and minimum pH and increased variance of pH and AUC and duration of pH < 5.8. Schwartzkopf-Genswein et al. (2004) evaluated the effects of intake disruptions on rumen parameters and has similarly found that mean pH tended to be lower and pH remained below 5.8 longer in the feed disruption treatment compared to the control cattle. Furthermore, LY supplementation increased mean and minimum pH and decreased variance of pH and AUC and duration of pH < 5.8 suggesting positive benefits of LY on the rumen environment. Additionally, significant diet x SARA interactions were observed, such that LY supplementation mean and maximum ruminal pH were increased in SARA steers, but decreased in non-SARA steers. The decrease in mean and maximum pH for LY-fed non-SARA steers compared

to control-fed non-SARA steers could be indicative of more efficient ruminal environment and greater fermentation. Furthermore, the diet x SARA interactions for duration and AUC of pH < 5.8 are due to stated variables decreasing when LY was supplemented in SARA treated steers to a greater extent than when supplemented to non-SARA steers. Together these results demonstrate the benefits of LY supplementation on the rumen environment, especially during SARA. Live-yeast supplementation has been reported to stabilize ruminal pH by interacting competing with *S. bovis* and encouraging the growth of lactate-utilizing bacteria in *in vitro* studies lactate-metabolizing bacteria (Chaucheyras-Durand et al., 2007). Additionally, higher rumen pH has been repeatedly observed in studies where cannulated animals were supplemented with live yeast (Chaucheyras-Durand and Fonty, 2006; Williams et al., 1991), similar to that of the current trial.

In general, similar results were found during both SARA challenge protocols, however SARA #2 did not respond as drastically as SARA #1 to feed intake fluctuations. In general, mean pH was higher, and variance of pH and duration and AUC of pH < 5.8 were lower in SARA #2 compared to SARA #1. During SARA #1, exposure to SARA challenge affected ($P \leq 0.01$) all rumen bolus parameters, whereas during SARA #2, duration and AUC of pH < 5.8 were not affected. These findings align with a study by Nagata and colleagues (2018) where the effects of repeated SARA challenges were evaluated in dairy cows. The study consisted of 4 SARA challenge periods characterized by feeding a high forage diet for 7 d followed by a high grain diet for 7 d (Nagata et al., 2018). The effects of challenge were more evident in the 1st and 2nd

challenges, but were mitigated during the 3rd and 4th challenges. Mean and minimum pH were higher in challenges 3 and 4 compared to 1st and 2nd challenges and duration and AUC of pH < 5.6 values were lower (Nagata et al., 2018). Dohme and colleagues (2008) also exposed dairy cows of differing acidotic risk to repeated SARA challenges, however results were inconsistent with the current study. The study consisted of 3 challenge models starting with 3 pre-challenge days of ad libitum intake, followed by 1 feed restriction day, then 1 challenge day in which a ground barley and wheat mixture was fed for 1 h followed by TMR ad libitum (Dohme et al., 2008; similar to model used by Krause and Oetzel, 2005). Contrary to the current study and the study by Nagata et al. (2018), Dohme et al. (2008) observed the most drastic response during the 3rd challenge. Interestingly, during the 3rd challenge some cows avoided the grain allocation on the challenge day, yet SARA was still induced for the individuals. Potentially, the feed restriction day destabilized the rumen microbial population by starving some bacteria (Van Kessell and Russel; 1997) so that when the TMR was reintroduced, rapid intake combined with destabilization reduced rumen pH (Dohme et al., 2008). Variations in SARA challenge protocols amongst studies make it difficult to conclude why differences in changes occur, although adaptations in bacterial composition and densities in response to ruminal pH changes have been found (Hook et al., 2011; Petri et al., 2013). The study by Nagata et al. (2018) found greater bacterial diversity of the rumen population during the 4th challenge compared to the 1st challenge, however the current study did not evaluate rumen microbial populations.

Challenge day also affected rumen response variables as evident by significant SARA x day interactions. The 2nd wk of the SARA challenge protocols were characterized by alternating feed delivery daily from 60 (feed-restriction) to 140% (feed-excess) of previously determined baseline intake. Surprisingly, the mean and maximum ruminal pH values of the SARA steers were lowest on feed-restriction days and similar to non-SARA steers during the feed-excess days. This difference could have been due to differences in water intake, keeping in mind the pH of water is approximately 7 and its consumption will cause increases in ruminal pH if < 7. Differences in DMI during feed-restriction and -excess days most likely caused differences in water intake, as DMI and water intake have been reported to be positively related (Murphy et al., 1983; Hicks et al., 1988; Loneragan et al., 2001). Dry-matter intake was greater on feed-excess days compared to -restriction days, thus water consumption was also greater and ruminal pH would have increased to a greater extent.

Mean ruminal temperature was influenced in SARA #1 and SARA #2, such that SARA treated steers had lower temperatures than non-SARA steers. Ruminal temperature has been reported as a potential predictor of ruminal pH and diagnostic tool for SARA (AlZahal et al., 2008). AlZahal and colleagues (2008) found dairy cows that spent more time with acidic ruminal pH had greater ruminal temperatures, contrary to the current studies where SARA-steers had greater duration of pH < 5.8 and lower rumen temperatures. Additionally, AlZahal et al. (2008) found a strong negative correlation between nadir rumen pH and corresponding ruminal temperature, although nadir rumen pH was not analyzed during the current study.

Overall for this study, mean ruminal pH was higher compared to previous studies. Crossland et al. (2018) conducted a study using reticulo-rumen boluses to evaluate the effects of LY during the transition period of growing beef steers. The transition period serves as a comparable SARA challenge model to the current study. In the study conducted by Crossland et al. (2018), steers had an average mean ruminal pH of approximately 6.18 during the transition period. In the current study, the average mean pH of SARA-steers was 6.28 from days 56 to 97. Steam flaked corn was used as the majority of the diet during this study, whereas in the Crossland study (2018), cracked corn was primarily utilized. The steam flaked corn used for this trial were produced at a feed mill approximately 88.5 km from the cattle feeding facility. The relative high rumen pH and the performance of the cattle indicates that the quality of the steam flaked corn used in this study was lower quality.

Previous challenge models have evaluated the implications of variable feed delivery on the rumen environment, however these approaches typically last 3 days or less. In dairy cattle literature, Krause and Oetzel (2005) developed a SARA challenge model that has been repeated (DeVries et al., 2008 and 2009); feed intake was restricted for 1 day by 50%, then a 1-h size-restricted meal of a ground barley/wheat mixture was offered before TMR delivery. Rumen pH was measured on ruminally-cannulated animals using indwelling electrodes, which provided continuous measurements. Both Krause and Oetzel (2007) and DeVries et al. (2008 and 2009) successfully decreased ruminal pH using this method. Other studies use different methods including: (1) the addition of intraruminal doses of glucose (Krehbiel et al., 1995), (2) the substitution of

processed wheat, barley, or steam flaked corn substitutions (Beauchemin et al., 2003; Brown et al., 2000; Ghorbani et al., 2002; Gozho et al., 2005; Gozho et al., 2007; Keunen et al., 2002; Khafipour et al., 2009; Malekkahi et al., 2015; Villot et al., 2017; Vyas et al., 2014), (3) alterations in the roughage to concentrate ratios (Penner et al., 2007), (4) feed intake disruptions (Cooper et al., 1997) including periods of (4a) feed restriction (Owens et al., 1998) and (4b) subsequent overfeeding (Horn et al., 1979; Schwartzkopf et al., 2004), or (5) a combination of multiple methods (Nagata et al., 2018; Vyas et al., 2014) in order to induce acidotic bouts. The current model serves as a novel SARA challenge protocol in that it attempts to cause acidotic bouts over 7 days. The current study continuously measured rumen pH using indwelling reticulo-rumen boluses, whereas other studies have utilized pH meters or indwelling electrodes on cannulated animals. Results of this study illustrate the effectiveness of the SARA challenge models at reducing rumen pH.

Arterial blood analysis

In the current study, no differences were found on arterial blood parameters due to dietary treatment. Similarly, Ghorbani and colleagues (2002) measured blood variables in response to consuming high concentrate diets and likewise found no difference in blood pH due to LY supplementation. Arterial blood sampling after each SARA challenge period (SARA #1 and #2) yielded results contrary to expected after a week of feed intake disruptions. After SARA #1, no differences due to SARA treatment were observed. After SARA #2, blood pH and base excess increased ($P < 0.10$) and lactate decreased ($P < 0.05$) due to SARA treatment. Brown and colleagues (2000)

evaluated the effects of two types of SARA challenge protocols on blood parameters in cannulated beef steers. In a SARA challenge model that offered 50% concentrate diet ad libitum to concentrate- or forage-adapted steers, blood pH, bicarbonate, and base excess were greater 7 days following treatment. On the contrary, after 7 days of intraruminal dosing processed grain, blood pH, bicarbonate, and base excess were decreased. Blood pH is rather resistant to fluctuations because it acid-base balance is highly regulated (Owens et al., 1998), therefore decreased blood pH following intraruminal dosing of process grain was more systemically disrupted compared to alternating diets, especially since animals often decreased DMI in response to increased ruminal acidity (Fulton et al., 1979). In the current study and the study by Brown et al. (2000), caution should also be taken in interpreting these results given that these were time point measurements. Krehbiel and colleagues (1995) performed a time series study in lambs that evaluated blood parameters at serial time points following an intraruminal dose of glucose and blood pH declined sharply 8 to 12 h after treatment. Therefore, future studies should consider arterial blood measurements at serial time points during SARA challenge to more accurately evaluate the effects.

Performance and feed efficiency

Performance and feed efficiency data were analyzed separately for 2 study periods: days 0 to 35 and days 35 to 105. During the first 35 days, there were no diet or SARA treatment effects on any response variables except DMI, which was 9.2% less in LY supplemented steers compared to control steers. In studies with dairy cows, common effects of LY supplementation include an increase in DMI and milk production (El-

Ghani, 2004; Sniffen et al., 2004; Jouany, 2006; Stella et al., 2007). From days 35 to 105, LY supplementation reduced DMI and F:G compared to control-fed steers. Live yeast strain *S. cerevisiae* has been observed to be beneficial for cattle fed high-concentrate diets where they compete with starch utilizing bacteria (Lynch and Martin, 2002) and prevent the accumulation of lactate in the rumen (Chaucheyras et al., 1995; Seo et al., 2010), which may be the mechanism for increasing efficiency in the current study. In a meta-analysis of 18 trials for cattle fed growing and finishing diets, LY has been shown to reduce in F:G ratios (2.6%), consistent with the current study. Wagner et al. (2016) also found that LY supplementation increased ADG (6.5%) and DMI (1%), whereas the current study observed no differences in ADG, and DMI was reduced.

Carcass and liver scores

In the current study, LY supplementation yielded heavier carcasses with larger LMA and lower YG values. In a meta-analysis of over 18 trials for cattle fed growing and finishing diets, LY supplementation resulted in a greater percentage of animals grading choice or higher (Wagner et al., 2016), but in the current study marbling score and quality grade were not affected. Lower marbling scores were observed for SARA challenged steers, although LY-fed steers exposed to challenge had 6% higher marbling scores than control. Contrary to expected, liver scores were not affected by SARA challenge in the current study, although LY supplementation has previously yielded a tendency to reduce liver abscesses compared to control cattle (Wagner et al., 2016). The relatively high incidence of liver abscesses in the current study likely reflects the fact that tylosin was not included in the experimental diets.

Conclusion

This study evaluated the effects LY supplementation during 2 experimental SARA challenge models on DMI and feeding behavior, rumen parameters, physical activity, arterial blood responses, performance, carcass characteristics and liver scores. Two experimental SARA challenge models were designed to mimic disruptive feed delivery and induce SARA by alternating feed delivery for 7-d periods. Disruption in the rumen environment due to the experimental SARA challenge protocols was evident by decreases in mean, maximum, and minimum rumen pH, and increases in AUC and duration of pH < 5.8 in SARA-treated steers compared to non-SARA steers. Both SARA challenge models effectively changed feeding behavior patterns as evident by substantial SARA x day interactions for DMI, BV frequency and duration, TTB, and BV eating rate. Supplementation with LY consistently reduced DMI and improved F:G, as ADG was not affected during the study. Additionally, LY supplementation caused mean and minimum pH to increase and variance of pH and duration and AUC of pH < 5.8 to decrease. Live-yeast supplementation was beneficial to the rumen environment, especially during SARA challenges as evident by diet x SARA interactions for DMI, mean and maximum ruminal pH, and duration and AUC of pH < 5.8. During SARA challenges, DMI was lowered in both SARA treatment groups, but to a greater extent in non-SARA steers compared to SARA-treated steers. In SARA treated steers, LY supplementation caused mean and maximum rumen pH to increase, whereas it did not in non-SARA steers. Additionally, duration and AUC of pH < 5.8 were lowered to a greater extent when LY was supplemented to SARA steers compared to non-SARA

steers. Overall, SARA #1 and SARA #2 responded similarly, with the exception of duration and AUC of pH < 5.8, which were not affected during SARA #2. Together these results demonstrate the efficacy of a novel experimental SARA challenge model and illustrate LY supplementation as beneficial to the rumen environment during SARA.

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APPENDIX FIGURES AND TABLES

Chapter 2 Figures and Tables

Table 2.1. Ingredient and chemical composition of the experimental diet.

Item	Value
Ingredient (As-fed basis)	
Dry-rolled corn, %	56.0
Dried distillers' grain, %	24.0
Chopped alfalfa, %	10.0
Molasses, %	5.5
Vitamin-mineral premix, %[1]	2.5
Dietary treatment premix, %[2]	2.0
Chemical Composition (Dry-matter basis)	
Dry matter, %	89.3
CP, %	12.7
NDF, %	24.9
ME, Mcal/kg	2.84

[1]Vitamin-mineral premix contained minimum 15.5% Ca, 2,800 ppm Zn, 1,200 ppm Mn, 12 ppm Se, 14 ppm Co, 30 ppm I, 45.4 KIU/kg Vit-A, 2.3 KIU/kg Vit-D, 726 IU/kg Vit-E.

[2]Dietary treatment premixes contained limestone, dried distillers grain (as carrier), and carrier or LY product.

Table 2.2. Effects of LY treatment on performance, feed efficiency, and feeding behavior traits in finishing steers during the first 28 days of the trial.

	Dietary treatment			
Item	**Control**	**LY**	**SE**	**P-value**
No. of steers	24	24	---	---
Performance and feed efficiency				
Initial BW, kg	440.6	445.5	6.5	0.59
BW (day 28), kg	489.7	491.9	7.7	0.84
ADG, kg/d	1.75	1.66	0.09	0.47
DMI, kg/d	12.05	11.81	0.43	0.61
DMI, % BW	2.59	2.51	0.04	0.33
F:G ratio	7.26	7.63	0.32	0.56
G:F ratio	0.145	0.140	0.006	0.61
Bunk visit (BV) traits				
BV frequency, events/d	46.6	41.4	2.9	**0.04**
BV duration, min/d	84.0	97.9	6.8	*0.08*
BV eating rate, g/min	157.2	128.6	9.7	**0.03**
Meal traits				
Meal criterion, min	7.25	9.51	1.28	*0.08*
Meal frequency, events/d	10.38	9.20	0.37	0.12
Meal duration (MD), min/d	149.8	157.4	6.5	0.47
Meal length, min/event	15.8	18.1	1.1	0.25
Meal size, kg/event	1.26	1.35	0.08	0.44
Meal eating rate, g/min	84.3	76.8	3.30	0.12
Max non-feeding interval, min	446.6	470.0	8.4	0.17
Intensity traits				
Time to bunk, min	69.7	40.8	3.3	**<0.01**
HD duration, min/d	37.2	49.1	6.3	*0.08*
BV per meal, events/meal	4.86	4.68	0.36	0.69
HD per MD ratio	0.237	0.306	0.033	**0.03**
Day-to-day variance traits				
DMI SD[1], kg/d	1.95	2.13	0.20	0.15
BV frequency SD[1], events/d	7.68	8.03	0.57	0.54
BV duration SD[1], min/d	14.9	17.2	0.7	*0.07*
Max NFI SD[1], min	121.4	151.6	12.9	**0.01**
Meal frequency SD[1], events/d	2.00	1.70	0.28	0.17
Meal duration SD[1], min/d	21.1	25.0	0.9	**0.02**
HD duration SD[1], min/d	7.31	10.1	0.7	**0.02**
Time to bunk SD[1], min/d	103.7	82.6	4.7	**0.03**

[1]SD = Day-to-day variation

Table 2.3. Effects of LY treatment on performance, feed efficiency, and feeding behavior traits in finishing steers during the 70-d trial.

	Dietary treatment			
Item	**Control**	**LY**	**SE**	**P-value**
No. of steers	24	24	---	---
Performance and feed efficiency				
Initial BW, kg	438.5	443.7	4.4	0.56
BW (day 70), kg	547.3	555.2	5.8	0.50
ADG, kg/d	1.56	1.59	0.03	0.59
DMI, kg/d	12.91	13.31	0.22	0.36
DMI, % BW	2.62	2.66	0.03	0.48
F:G ratio	8.38	8.47	0.20	0.75
G:F ratio	0.121	0.120	0.002	0.87
RFI, kg/d	-0.079	0.091	0.146	0.56
Bunk visit (BV) traits				
BV frequency, events/d	40.4	36.8	1.2	*0.07*
BV duration, min/d	74.7	91.0	5.4	**0.02**
BV eating rate, g/min	187.8	153.5	7.1	**0.02**
Meal traits				
Meal criterion, min	6.85	10.08	0.60	**0.01**
Meal frequency, events/d	9.50	8.33	0.40	*0.07*
Meal duration (MD), min/d	129.2	146.2	6.3	**0.04**
Meal length, min/event	14.6	18.6	0.8	**0.02**
Meal size, kg/event	1.45	1.68	0.06	*0.09*
Meal eating rate, g/min	103.2	92.9	2.50	**0.05**
Max non-feeding interval, min	475.9	490.2	7.3	0.33
Intensity traits				
Time to bunk, min	61.3	36.6	3.5	**0.01**
HD duration, min/d	32.5	45.6	6.5	**0.03**
BV per meal, events/meal	4.55	4.62	0.20	0.87
HD per MD ratio	0.240	0.308	0.016	**0.04**
Day-to-day variance traits				
DMI SD[1], kg/d	2.13	2.26	0.04	0.15
BV frequency SD[1], events/d	8.90	8.44	0.39	0.26
BV duration SD[1], min/d	16.1	18.4	0.5	**0.02**
Max NFI SD[1], min	118.8	142.4	4.0	**0.01**
Meal frequency SD[1], events/d	2.16	1.76	0.13	**0.03**
Meal duration SD[1], min/d	25.0	27.9	0.7	**0.04**
HD duration SD[1], min/d	8.18	10.9	1.1	**0.01**
Time to bunk SD[1], min/d	99.1	68.5	5.3	**0.01**

[1]SD = Day-to-day variation

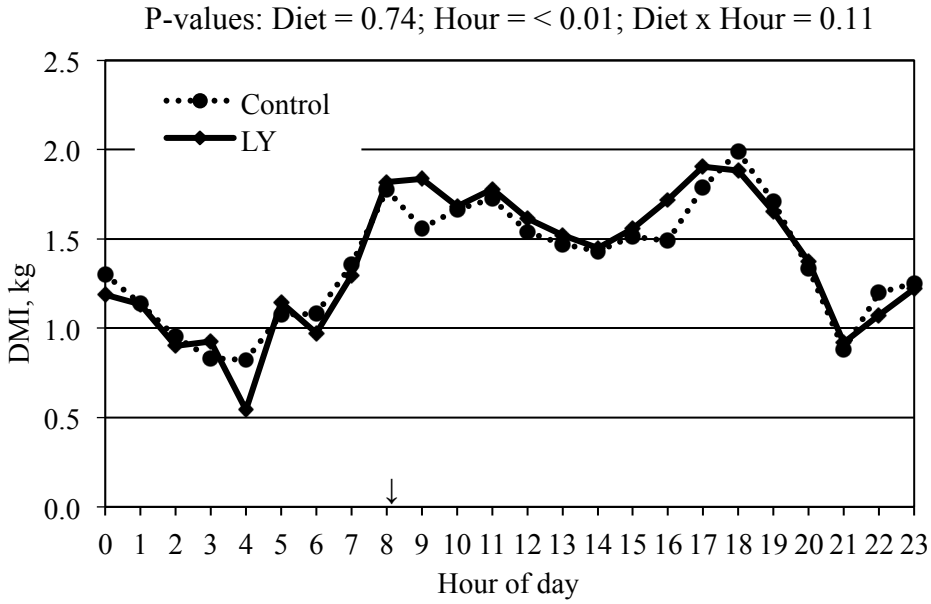


Figure 2.1. Effects of LY treatment on diurnal patterns for DMI during the entire 70-d trial.
Steers were fed at approximately 0800 h each day (↓).

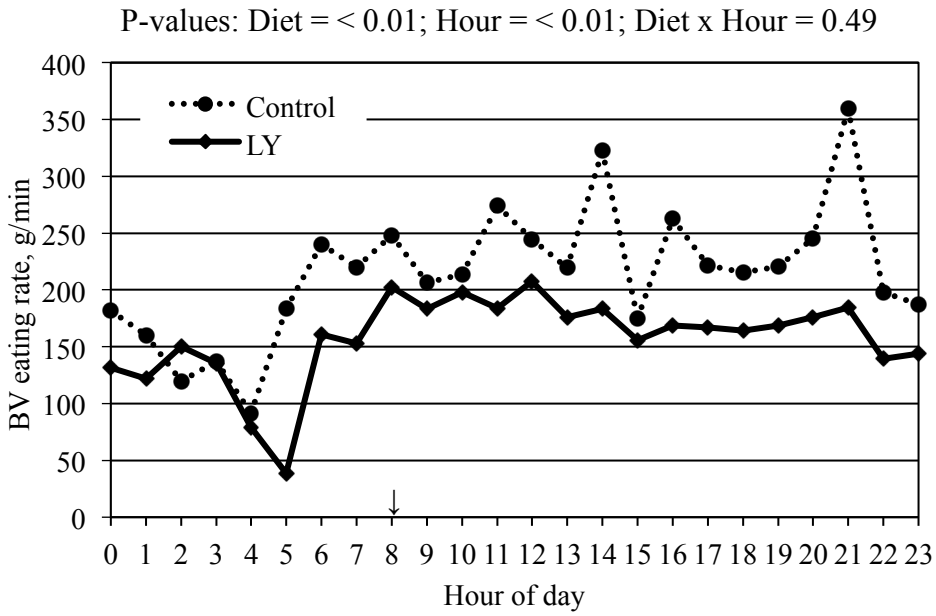


Figure 2.2. Effects of LY treatment on diurnal patterns for BV eating rate during the entire 70-d trial.
Steers were fed at approximately 0800 h each day (↓).

Table 2.4. Effects of LY treatment on rumen temperature and physical activity during the trial**.**

	Dietary treatment			
Item	**Control**	**LY**	**SE**	**P-value**
No. of steers	10	10	---	---
Rumen Temperature[1]				
Average, °C	39.67	39.6	0.1	0.72
Physical Activity (Trial				
Standing duration, min/d	709.0	714	16	0.83
Standing length, min/event	53.5	61.0	4.8	0.28
Standing frequency, event/d	15.4	13.3	1.4	0.30
Physical Activity (D 0 to 14)[2]				
Standing duration, min/d	678.6	701	18	0.39
Standing length, min/event	47.8	59.7	6.2	0.08
Standing frequency, event/d	15.1	12.8	1.2	**0.03**
Physical Activity (D 28 to 42)[2]				
Standing duration, min/d	754.3	737	14	0.39
Standing length, min/event	63.0	64.0	6.1	0.90
Standing frequency, event/d	13.0	13.2	1.2	0.90
Physical Activity (D 56 to 70)[2]				
Standing duration, min/d	695.9	695	31.5	1.00
Standing length, min/event	50.6	55.1	5.6	0.55
Standing frequency, event/d	18.0	14.5	3.4	0.47

[1]Data ± 3 SD from mean rectal temperature within animal were deleted for this analysis.

[2]Treatment x period interactions were non-significant (P > 0.25).

Table 2.5. Effects of LY on carcass traits for finishing steers.

	Dietary treatment			
Item	**Control**	**LY**	**SE**	**P-value**
No. of steers	24	24	---	---
Hot carcass weight, kg	360	365	6	0.57
Backfat depth, cm	1.55	1.81	0.14	**0.05**
LMA, cm^2	86.7	84.5	1.4	0.28
Kidney, pelvic and heart fat,	2.0	2.0	0.0	1.00
Yield grade	3.20	3.61	0.23	**0.02**
Marbling score	467	452	14	0.36
Quality grade[1]	Low CH	Low CH		
Carcass Color and pH				
L* color	44.4	44.5	0.4	0.73
a* color	17.3	17.3	0.4	1.00
b* color	8.98	8.95	0.29	0.93
pH	5.15	5.61	0.23	0.16
WBSF (1-d post mortem	2.94	3.03	0.17	0.71
WBSF (14-d post mortem	1.93	1.95	0.14	0.88

[1]The numeric quality grade data ranged from 200-799, with Choice ranging from 400-699.

Table 2.6. Effects of LY treatments on subjective lung and liver scores for finishing steers.

	Dietary treatment		
Item	**Control**	**LY**	**P-value**
No. of steers	24	24	---
Liver scores (1 to 3)[1]			
1	67%	58%	0.56
2	29%	42%	0.37
3	4%	0%	0.98
SEM	9.6%	10.1%	---
Lung scores (1 to 5)[2]			
1	75%	67%	0.76
2	21%	33%	0.53
3	4%	0%	0.98
SEM	0.10%	0.10%	---

[1]Liver scores: (**1**) no abscesses, (**2**) 1 or 2 small, unorganized abscesses, or 2 to 4 well-organized abscesses or abscess scares, and (**3**) 1 or more large, or multiple small, active abscesses.
[2]Lung scores: 1 to 5, with 1 being normal and 5 being severe (>50%) consolidation of any lung lobe with lesions associated with BRD.

Table 2.7. Pearson correlations[1] between performance, feed efficiency, and feeding behavior for finishing steers during the 70-d trial.

Trait	Initial BW	ADG	DMI	F:G ratio	RFI
Performance traits					
Initial BW, kg	----	**0.38**	**0.60**	0.09	-0.02
ADG, kg/d			**0.64**	**-0.64**	-0.02
DMI, kg/d				0.17	**0.65**
F:G ratio					**0.66**
Bunk Visit (BV) traits					
BV frequency, events/d	0.05	0.18	*0.26*	0.05	0.23
BV duration, min/d	-0.01	**0.32**	**0.33**	-0.1	*0.27*
BV eating rate, g/min	0.17	0.01	0.09	0.11	0.03
Meal traits					
Meal criterion, min	0.15	**0.42**	**0.36**	-0.18	0.14
Meal frequency, events/d	*-0.25*	**-0.37**	*-0.27*	-0.27	0.02
Meal duration (MD), min/d	0.07	**0.56**	**0.53**	-0.19	**0.34**
Meal length, min/event	0.18	**0.57**	**0.47**	*-0.24*	0.17
Meal size, kg/event	**0.44**	**0.57**	**0.63**	-0.08	*0.25*
Max non-feeding interval (NFI), min	0.02	-0.01	-0.18	-0.14	-0.27
Intensity traits					
Time to bunk, min	-0.03	*-0.28*	-0.2	0.15	-0.08
Head down (HD) duration, min/d	-0.01	**0.30**	*0.27*	-0.14	0.19
BV per meal, events/meal	0.19	**0.40**	**0.36**	-0.12	0.13
HD per MD ratio	0.08	0.13	0.11	-0.07	0.06
Day-to-day variance traits					
DMI SD[1], kg/d	0.14	-0.15	0.10	**0.31**	0.16
BV frequency SD[1], events/d	-0.12	-0.07	0.08	0.19	*0.23*
BV duration SD[1], min/d	-0.17	0.15	0.09	-0.08	0.14
Max NFI SD[1], min	0.02	-0.01	0.02	0.04	0.03
Meal frequency SD[1], events/d	-0.18	**-0.43**	**-0.34**	0.21	-0.08
Meal duration SD[1], min/d	0.01	**0.29**	0.01	-0.14	0.09
HD duration SD[1], min/d	-0.08	**0.29**	0.23	-0.14	0.19
Time to bunk SD[1], min/d	-0.07	**-0.37**	*-0.28*	0.21	-0.10

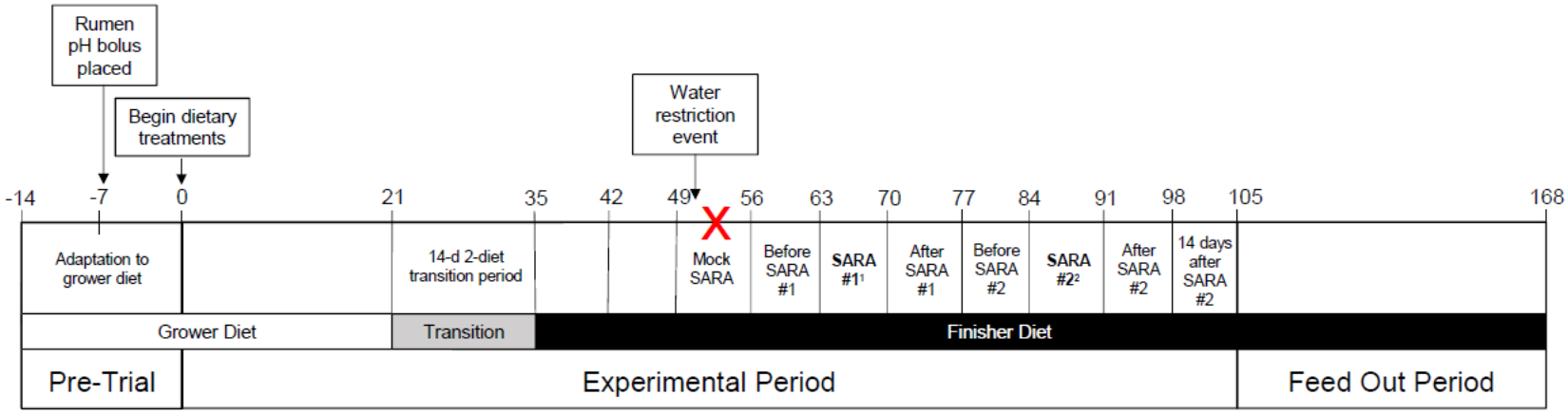
1Correlations in **BOLD** significant at $P < 0.05$; Correlations in *ITALICS* significant at $P < 0.10$

Chapter 3 Figures and Tables

Table 3.8. Ingredient and chemical composition of experimental diet.

Item	Grower	Finisher	Finisher-GW[1]
Ingredient (as-fed basis)			
Steam flaked corn, %	10.0	70.0	50.0
Ground wheat, %	---	---	20.0
Dried distillers' grains, %	8.0	15.0	15.0
Chopped alfalfa, %	50.0	7.0	7.0
Cottonseed hulls, %	22.0	---	---
Molasses, %	5.5	5.5	5.5
Vitamin-mineral premix, %[2]	2.5	2.5	2.5
Dietary treatment premix, %[3]	2.0	2.0	2.0
Chemical composition (dry-matter basis)			
Dry matter, %	85.3	84.1	84.5
CP, %	13.3	11.8	12.7
NDF, %	50.0	17.5	17.1
TDN, %	56.1	78.6	78.9
Starch, %	6.3	52.3	54.6

[1]GW = Ground wheat diet used during SARA challenge #2.
[2]Vitamin-mineral premix contained minimum 15.5% Ca, 2,800 ppm Zn, 1,200 ppm Mn, 12 ppm Se, 14 ppm Co, 30 ppm I, 45.4 KIU/kg Vit-A, 2.3 KIU/kg Vit-D, and 726 IU/kg Vit-E.
[3]Dietary treatment premixes contained limestone, dried distillers' grain (as carrier) and carrier product or LY product.



[1]**SARA 1**: SARA challenge consisting of daily alterations of variable feed delivery from 60 to 140% of baseline intake.
[2]**SARA 2:** SARA challenge consisting of daily alterations of variable feed delivery from 60 to 140% of baseline intake and the inclusion of ground wheat.

Figure 3.3. Experimental timeline of the trial.

Table 3.9. Main-effect means for feed intake, feeding behavior, and rumen bolus parameters of steers during the 14-d adaptation period prior to start of live yeast or SARA challenge treatments.

	Diet		SARA			P-Values		
Item	**Control**	**Live Yeast**	**Non SARA**	**SARA**	**SE**	**Diet**	**SARA**	**Diet x SARA**
DM Intake and feeding behavior:								
DMI, kg/d	7.58	7.21	7.45	7.35	0.20	0.35	0.80	0.85
BV frequency, events/d	78.4	77.2	76.9	78.7	2.4	0.79	0.71	0.17
BV duration, min/d	89.0	95.2	95.8	88.5	3.7	0.40	0.33	0.85
HD duration, min/d	36.2	44.5	42.6	38.1	2.8	0.15	0.44	0.97
Time to bunk, min	23.8	26.7	24.7	25.8	2.8	0.61	0.84	0.16
BV eating rate, g/min	101.5	86.0	90.7	95.8	3.2	**0.03**	0.44	0.89
Feed supply, kg/d	102	88	95	95	----	----	----	----
Mean EBT, min/d	68	76	56	88	----	----	----	----
Rumen bolus measurements:								
Mean pH	6.31	6.36	6.37	6.30	0.10	0.62	0.52	0.46
Maximum pH	6.68	6.72	6.72	6.68	0.09	0.69	0.69	0.27
Minimum pH	5.98	6.06	6.07	5.97	0.10	0.44	0.32	0.58
Variance of pH[1]	0.163	0.155	0.154	0.163	0.010	0.47	0.37	0.18
Duration pH < 5.8,	72.8	16.0	41.8	46.9	22.7	0.23	0.91	0.54
AUC pH < 5.8, pH x	6.76	0.77	3.40	4.13	4.20	0.17	0.86	0.65
Mean temperature, °F	103.5	103.4	103.4	103.5	0.2	0.48	0.81	1.00
Mean activity	6.49	7.57	7.22	6.85	0.57	*0.07*	0.52	0.22

[1]Hour-to-hour variance in pH (SD).

Table 3.10. Subclass means for feed intake, feeding behavior, and rumen bolus parameters of steers during the 14-d adaptation period prior to start of live yeast or SARA challenge treatments.

	Control		Live Yeast			P-Values		
Item	Non SARA	SARA	Non SARA	SARA	SE	Diet	SARA	Diet x SARA
DM Intake and feeding behavior:								
DMI, kg/d	7.67	7.49	7.22	7.20	0.20	0.35	0.80	0.85
BV frequency, events/d	80.9	76.0	73.0	81.4	2.4	0.79	0.71	0.17
BV duration, min/d	93.4	84.7	98.2	92.3	3.7	0.40	0.33	0.85
HD duration, min/d	38.3	34.1	46.9	42.2	2.9	0.15	0.44	0.97
Time to bunk, min	19.3	28.3	30.1	23.3	2.8	0.61	0.84	0.16
BV eating rate, g/min	98.4	103.6	82.9	89.0	3.2	**0.03**	0.44	0.89
Feed supply, kg/d	101	102	89	87	----	----	----	----
Mean EBT, min/d	69	68	42	109	----	----	----	----
Rumen bolus measurements:								
Mean pH	6.38	6.24	6.35	6.36	0.10	0.62	0.52	0.46
Maximum pH	6.75	6.61	6.68	6.75	0.09	0.69	0.69	0.27
Minimum pH	6.06	5.91	6.08	6.04	0.10	0.44	0.32	0.58
Variance of pH[1]	0.165	0.160	0.143	0.167	0.010	0.47	0.37	0.18
Duration pH < 5.8, min/d	56.1	89.4	27.5	4.4	22.7	0.23	0.91	0.54
AUC pH < 5.8, pH x min	5.44	8.08	1.36	0.18	4.20	0.17	0.86	0.65
Mean temperature, °F	103.5	103.5	103.4	103.4	0.2	0.48	0.81	1.00
Mean activity	6.31	6.67	8.13	7.02	0.57	*0.07*	0.52	0.22

[a,b,c]Means within rows with unlike superscripts differ at $P < 0.05$.

[1]Hour-to-hour variance of pH (SD).

Table 3.11. Main-effect means of live yeast and SARA challenge treatments on intake, feeding behavior, and rumen bolus parameters during grower (21 d) and transition (14 d) periods.

	Diet		SARA			P-Values		
Item	**Control**	**Live Yeast**	**Non SARA**	**SARA**	**SE**	**Diet**	**SARA**	**Diet x SARA**
DM Intake and feeding behavior:								
DMI, kg/d	10.52	9.55	10.15	9.93	0.22	**0.03**	0.62	0.71
BV frequency, events/d	76.5	80.1	81.8	74.8	1.7	0.30	**0.05**	**<0.01**
BV duration, min/d	96.8	101.7	87.6	109.9	4.0	0.63	**0.01**	**<0.01**
HD duration, min/d	41.3	47.4	38.3	50.4	2.8	0.28	**0.04**	**0.01**
Time to bunk, min	31.2	23.6	21.8	33.1	2.3	*0.10*	**0.02**	0.14
BV eating rate, g/min	151	127	156	122	5	**0.03**	**<0.01**	**<0.01**
Feed supply, kg/d	120	110	116	114	----	----	----	----
Mean EBT, min/d	286	133	294	125	----	----	----	----
Rumen bolus parameters:								
Mean pH	6.31	6.36	6.38	6.29	0.05	0.64	0.41	0.55
Maximum pH	6.66	6.68	6.74	6.60	0.05	0.89	0.20	0.35
Minimum pH	5.94	6.07	6.02	5.99	0.05	0.28	0.75	0.91
Variance of pH[1]	0.173	0.151	0.174	0.151	0.005	*0.06*	**0.04**	**0.03**
Duration pH < 5.8, min/d	109.1	37.7	75.6	71.2	26.6	0.19	0.93	0.53
AUC pH < 5.8, pH x min	17.22	4.80	12.00	10.02	3.92	0.13	0.82	0.73
Mean temperature, °F	103.7	103.7	103.7	103.7	0.1	0.90	0.81	0.23
Mean activity	6.24	7.00	6.65	6.59	0.31	0.23	0.93	0.35

[1]Hour-to-hour variance of pH (SD)

Table 3.12. Subclass means of live yeast and SARA challenge treatments on intake, feeding behavior, and rumen bolus parameters during grower (21 d) and transition (14 d) periods.

	Control		Live Yeast			P-Values		
Item	Non SARA	SARA	Non SARA	SARA	SE	Diet	SARA	Diet x SARA
DM Intake and feeding behavior:								
DMI, kg/d	10.55	10.49	9.74	9.36	0.22	**0.03**	0.62	0.71
BV frequency, events/d	91.0[a]	62.1[c]	72.6[b]	87.6[a]	1.7	0.30	**0.05**	**<0.01**
BV duration, min/d	71.1[c]	122.0[a]	104[ab]	97.3[b]	4.0	0.63	**0.01**	**<0.01**
HD duration, min/d	28.0[b]	54.6[a]	48.6[a]	46.2[a]	2.8	0.28	**0.04**	**0.01**
Time to bunk, min	22.2	40.2	21.4	25.9	2.3	*0.10*	**0.02**	0.14
BV eating rate, g/min	191[a]	111[b]	120[b]	133[b]	5	**0.03**	**<0.01**	**<0.01**
Feed supply, kg/d	119	121	112	107	----	----	----	----
Mean EBT, min/d	427	145	161	105	----	----	----	----
Rumen bolus parameters:								
Mean pH	6.38	6.23	6.37	6.35	0.05	0.64	0.41	0.55
Maximum pH	6.78	6.55	6.69	6.66	0.05	0.89	0.20	0.35
Minimum pH	5.97	5.92	6.08	6.06	0.05	0.28	0.75	0.91
Variance of pH[1]	0.198[a]	0.148[b]	0.150[b]	0.152[b]	0.005	*0.06*	**0.04**	**0.03**
Duration pH < 5.8, min/d	94.2	124.0	57.0	18.3	26.6	0.19	0.93	0.53
AUC pH < 5.8, pH x min	16.83	17,60	7.16	2.44	3.92	0.13	0.82	0.73
Mean temperature, °F	103.6	103.8	103.8	103.6	0.07	0.90	0.81	0.23
Mean activity	5.97	6.50	7.33	6.68	0.31	0.23	0.93	0.35

[a-,b,]Means within rows with unlike superscripts differ at $P < 0.05$.

[1]Hour-to-hour variance of pH (SD).

Table 3.13. Main-effect means of live yeast and SARA challenge treatments on intake, feeding behavior and rumen bolus parameters during 70-d period (d 35 to 105) following the transition period.

	Diet		SARA			P-Values		
Item	**Control**	**Live Yeast**	**Non SARA**	**SARA**	**SE**	**Diet**	**SARA**	**Diet x SARA**
DM Intake and feeding behavior:								
DMI, kg/d	10.42	9.12	9.93	9.60	0.19	**<0.01**	0.39	0.43
BV frequency, events/d	39.3	45.5	42.2	42.6	4.19	**0.01**	0.85	**0.01**
BV duration, min/d	66.0	57.5	60.4	63.0	9.81	*0.09*	0.60	0.71
HD duration, min/d	30.9	25.6	26.4	30.1	7.20	0.15	0.31	0.91
Time to bunk, min	40.3	38.2	38.8	39.7	3.01	0.74	0.89	**0.04**
BV eating rate, g/min	196	195	199	192	8.14	0.95	0.64	0.45
Feed supply, kg/d	124	107	117	114	----	----	----	----
Mean EBT, min/d	243	241	257	227	----	----	----	----
Rumen bolus parameters:								
Mean pH	6.30	6.34	6.36	6.28	0.05	0.75	0.42	0.36
Maximum pH	6.88	6.83	6.93	6.79	0.05	0.63	0.20	*0.08*
Minimum pH	5.78	5.89	5.87	5.80	0.05	0.32	0.55	0.89
Variance of pH[1]	0.289	0.258	0.274	0.273	0.011	0.17	0.93	0.14
Duration pH < 5.8, min/d	83.4	26.4	54.0	55.8	17.6	0.12	0.96	0.47
AUC pH < 5.8, pH x min	41.37	14.99	27.71	28.65	8.26	0.13	0.96	0.45
Mean temperature, °F	103.7	103.6	103.8	103.6	0.05	0.19	*0.09*	0.17
Mean activity	4.49	4.72	4.47	4.74	0.26	0.66	0.61	0.62

[1]Hour-to-hour variance of ruminal pH (SD)

Table 3.14. Subclass means of live yeast and SARA challenge treatments on intake, feeding behavior and rumen bolus parameters during 70-d period (d 35 to 105) following the transition period.

	Control		Live Yeast			P-Values		
Item	Non SARA	SARA	Non SARA	SARA	SE	Diet	SARA	Diet x SARA
DM Intake and feeding behavior:								
DMI, kg/d	10.73	10.10	9.13	9.11	0.19	**<0.01**	0.39	0.43
BV frequency, events/d	42.2^{b}	36.5^{b}	42.2^{b}	48.7^{a}	4.19	**0.01**	0.85	**0.01**
BV duration, min/d	63.7	68.2	57.1	57.9	9.81	*0.09*	0.60	0.71
HD duration, min/d	28.9	33.0	24.0	27.3	7.20	0.15	0.31	0.91
Time to bunk, min	33.4^{xy}	47.2^{x}	44.3^{xy}	32.2^{y}	3.01	0.74	0.89	**0.04**
BV eating rate, g/min	206	186	193	197	8.14	0.95	0.64	0.45
Feed supply, kg/d	126	121	108	106	----	----	----	----
Mean EBT, min/d	290	195	224	258	----	----	----	----
Rumen bolus parameters:								
Mean pH	6.39	6.22	6.33	6.34	0.05	0.75	0.42	0.36
Maximum pH	7.05	6.72	6.81	6.86	0.05	0.63	0.20	*0.08*
Minimum pH	5.82	5.74	5.92	5.87	0.05	0.32	0.55	0.89
Variance of pH[1]	0.307	0.271	0.242	0.274	0.011	0.17	0.93	0.14
Duration pH < 5.8, min/d	69.6	97.1	38.3	14.4	17.6	0.12	0.96	0.47
AUC pH < 5.8, pH x min	34.48	48.25	20.94	9.04	8.26	0.13	0.96	0.45
Mean temperature, °F	103.8	103.7	103.8	103.4	0.05	0.19	*0.09*	0.17
Mean activity	4.49	4.50	4.46	4.99	0.26	0.66	0.61	0.62

[a,b,c]Means within rows with unlike superscripts differ at $P < 0.05$.

[x,y]Means within rows with unlike superscripts differ at $P < 0.10$.

[1]Hour-to-hour variance of ruminal pH (SD).

Table 3.15. Main-effect means of live yeast, SARA treatments, and day on intake, feeding behavior during the 2 SARA challenge periods (days 56 to 97).

	Diet		SARA			P-Values					
Item	**Control**	**Live Yeast**	**Non SARA**	**SARA**	**SE**	**Diet**	**SARA**	**Diet x SARA**	**Day**	**Diet x Day**	**SARA x Day**
SARA challenge #1 (days 56-76)											
DMI, kg/d	11.34	9.87	10.64	10.57	0.15	**<0.01**	0.66	**<0.01**	**<0.01**	0.58	**<0.01**
BV frequency, events/d	43.8	49.7	46.1	47.4	0.8	**<0.01**	0.12	**<0.01**	**<0.01**	0.65	**<0.01**
BV duration, min/d	72.2	64.1	67.0	69.2	1.6	**<0.01**	0.18	0.72	**<0.01**	0.69	**0.04**
HD duration, min/d	33.0	27.6	28.3	32.3	1.1	**<0.01**	**<0.01**	0.60	**<0.01**	0.82	0.60
Time to bunk, min	30.1	25.9	25.1	30.9	2.5	0.25	0.12	0.12	**<0.01**	*0.10*	**<0.01**
BV eating rate, g/min	177	181	179	180	4.2	0.42	0.80	0.13	**<0.01**	0.38	**<0.01**
HD per BV duration, min	0.441	0.422	0.415	0.449	0.009	**0.05**	**<0.01**	0.25	0.80	0.83	0.99
SARA challenge #2 (days 77-97)											
DMI, kg/d	11.35	9.88	10.76	10.47	0.15	**<0.01**	*0.06*	**<0.01**	**<0.01**	0.56	**<0.01**
BV frequency, events/d	31.7	39.2	36.1	34.8	0.8	**<0.01**	0.11	**<0.01**	**<0.01**	0.45	0.25
BV duration, min/d	70.0	60.1	65.2	64.9	1.6	**<0.01**	0.85	*0.09*	**<0.01**	**<0.01**	0.25
HD duration, min/d	35.8	28.2	31.3	32.7	1.1	**<0.01**	0.23	0.80	**0.01**	0.17	0.77
Time to bunk, min	72.2	57.4	61.8	67.8	4.7	**0.04**	0.38	**<0.01**	**<0.01**	0.37	0.34
BV eating rate, g/min	178	185	178	185	4.4	**0.10**	0.16	**<0.01**	**<0.01**	**0.02**	0.11
HD per BV duration, min	0.506	0.468	0.473	0.501	0.011	**<0.01**	**0.02**	0.22	**<0.01**	0.99	0.26

[1]Feeding behavior traits during SARA challenge #1 and #2 are based on 20/21 d and 16/21 d (including 3/7 d during challenge week), respectively, due to GrowSafe system failure and technical issues.

Table 3.16. Subclass means of live yeast, SARA treatments, and day on intake, feeding behavior during the 2 SARA challenge periods (days 56 to 97).

	Control		Live Yeast			P-Values					
Item	**Non SARA**	**SARA**	**Non SARA**	**SARA**	**SE**	**Diet**	**SARA**	**Diet x SARA**	**Day**	**Diet x Day**	**SARA x Day**
SARA challenge #1 (days 56-76)											
DMI, kg/d	11.63[a]	11.05[b]	9.64[d]	10.09[c]	0.15	**<0.01**	0.66	**<0.01**	**<0.01**	0.58	**<0.01**
BV frequency, events/d	45.0[b]	42.6[c]	47.2[b]	52.2[a]	0.8	**<0.01**	0.12	**<0.01**	**<0.01**	0.65	**<0.01**
BV duration, min/d	71.4	73.0	62.7	65.4	1.6	**<0.01**	0.18	0.72	**<0.01**	0.69	**0.04**
HD duration, min/d	30.7	35.2	25.9	29.3	1.1	**<0.01**	**<0.01**	0.60	**<0.01**	0.82	0.60
Time to bunk, min	24.3	35.9	25.9	25.9	2.5	0.25	0.12	0.12	**<0.01**	*0.10*	**<0.01**
BV eating rate, g/min	180.2	174.7	177.1	184.7	4.2	0.35	0.78	0.12	**<0.01**	0.38	**<0.01**
HD per BV duration	0.419	0.464	0.410	0.433	0.009	**0.05**	**<0.01**	0.25	0.82	0.83	0.99
SARA challenge #2 (days 77-97)											
DMI, kg/d	11.86[a]	10.85[b]	9.67[d]	10.10[c]	0.15	**<0.01**	*0.06*	**<0.01**	**<0.01**	0.56	**<0.01**
BV frequency, events/d	35.9b	27.6c	36.3b	42.0a	0.8	**<0.01**	0.11	**<0.01**	**<0.01**	0.45	0.25
BV duration, min/d	68.8	71.3	61.7	58.6	1.6	**<0.01**	0.85	*0.09*	**<0.01**	**<0.01**	0.25
HD duration, min/d	35.0	36.7	27.6	28.7	1.1	**<0.01**	0.22	0.80	**0.01**	0.17	0.77
Time to bunk, min	56.7[b]	87.7[a]	66.9[b]	47.9[b]	4.7	**0.04**	0.38	**<0.01**	**<0.01**	0.37	0.34
BV eating rate, g/min	186.5[b]	169.1[c]	170.3[c]	200.2[a]	4.4	*0.10*	0.16	**<0.01**	**<0.01**	**0.02**	0.11
HD per BV duration	0.499	0.513	0.447	0.489	0.011	**<0.01**	**0.02**	0.22	**<0.01**	0.99	0.26

Table 3.17. Main-effect means of live yeast, SARA treatments, and day on rumen bolus parameters during the 2 SARA challenge periods (days 56 to 97).

	Diet		SARA			P-Values					
Item	**Control**	**Live Yeast**	**Non SARA**	**SARA**	**SE**	**Diet**	**SARA**	**Diet x SARA**	**Day**	**Diet x Day**	**SARA x Day**
SARA challenge #1 (days 56-76)											
Mean pH	6.33	6.33	6.40	6.27	0.02	0.92	**<0.01**	**<0.01**	**<0.01**	0.99	**0.04**
Maximum pH	6.95	6.86	6.97	6.84	0.02	**0.01**	**<0.01**	**<0.01**	**<0.01**	0.92	**<0.01**
Minimum pH	5.81	5.88	5.91	5.78	0.02	**0.01**	**<0.01**	0.41	**<0.01**	0.99	*0.06*
Variance of pH[2]	0.302	0.274	0.275	0.302	0.006	**0.01**	**0.01**	**<0.01**	**<0.01**	0.78	**<0.01**
Duration pH < 5.8, min/d	201.7	89.4	102.3	188.8	14.8	**<0.01**	**<0.01**	**0.01**	**<0.01**	0.80	0.21
AUC pH < 5.8, pH x min	36.51	10.51	16.10	30.92	3.20	**<0.01**	**<0.01**	**0.04**	*0.07*	0.41	0.38
Mean temperature, °F	104.1	103.8	104.1	103.7	0.03	**<0.01**	**<0.01**	*0.07*	**<0.01**	0.92	**0.02**
Mean activity	4.66	5.11	4.86	4.92	0.16	**0.01**	0.71	0.95	**0.01**	0.93	0.99
SARA challenge #2 (days 77-97)											
Mean pH	6.30	6.36	6.36	6.30	0.02	**0.01**	**0.01**	**<0.01**	0.74	0.99	0.75
Maximum pH	6.83	6.84	6.89	6.79	0.02	0.72	**<0.01**	**<0.01**	*0.08*	0.99	*0.06*
Minimum pH	5.76	5.92	5.88	5.80	0.02	**<0.01**	**0.01**	0.64	0.96	0.98	0.73
Variance of pH[2]	0.280	0.257	0.261	0.275	0.006	**0.01**	*0.10*	**<0.01**	**<0.01**	0.62	**<0.01**
Duration pH < 5.8, min/d	195.7	68.7	122.1	142.3	13.9	**<0.01**	0.31	**0.03**	0.75	0.98	0.35
AUC pH < 5.8, pH x min	41.73	8.72	23.91	26.55	3.57	**<0.01**	0.60	0.20	0.76	0.96	0.35
Mean temperature, °F	103.5	103.6	103.6	103.5	0.03	0.26	**<0.01**	**<0.01**	**<0.01**	0.95	0.98
Mean activity	3.86	3.73	3.60	3.99	0.08	0.25	**<0.01**	**<0.01**	*0.06*	0.99	0.99

[1]Hour-to-hour variance of ruminal pH (SD).

Table 3.18 Subclass means of live yeast, SARA treatments, and day on rumen bolus parameters during the 2 SARA challenge periods (days 56 to 97).

	Control		Live Yeast			P-Values					
Item	Non SARA	SARA	Non SARA	SARA	SE	Diet	SARA	Diet x SARA	Day	Diet x Day	SARA x Day
SARA challenge #1 (days 56-76)											
Mean pH	6.44[a]	6.22[c]	6.35[b]	6.31[b]	0.02	0.92	**<0.01**	**<0.01**	**<0.01**	0.99	**0.04**
Maximum pH	7.14[a]	6.77[c]	6.81[c]	6.91[b]	0.02	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.92	**<0.01**
Minimum pH	5.86	5.75	5.96	5.81	0.02	**<0.01**	**<0.01**	0.41	**<0.01**	0.99	*0.06*
Variance of pH[2]	0.315[a]	0.289[b]	0.233[c]	0.314[a]	0.006	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.78	**<0.01**
Duration pH < 5.8, min/d	131.2[b]	272.3[a]	73.4[b]	105.3[b]	14.8	**<0.01**	**<0.01**	**0.01**	**<0.01**	0.80	0.21
AUC pH < 5.8, pH x min	24.27[b]	48.75[a]	7.93[c]	13.08[bc]	3.20	**<0.01**	**<0.01**	**0.04**	*0.07*	0.41	0.38
Mean temperature, °F	104.2	103.9	104.0	103.6	0.03	**<0.01**	**<0.01**	*0.07*	**<0.01**	0.92	**0.02**
Mean activity	4.64	4.69	5.08	5.15	0.16	**<0.01**	0.71	0.96	**0.01**	0.93	0.99
SARA challenge #2 (days 77-97)											
Mean pH	6.38[a]	6.22[b]	6.35[a]	6.38[a]	0.02	**0.01**	**0.01**	**<0.01**	0.76	0.99	0.75
Maximum pH	6.98[a]	6.69[d]	6.80[c]	6.89[b]	0.02	0.73	**<0.01**	**<0.01**	*0.10*	0.99	*0.06*
Minimum pH	5.81	5.71	5.96	5.89	0.02	**<0.01**	**<0.01**	0.64	0.97	0.98	0.73
Variance of pH[2]	0.290[a]	0.269[a]	0.232[b]	0.281[a]	0.006	**<0.01**	*0.10*	**<0.01**	**<0.01**	0.62	**<0.01**
Duration pH < 5.8, min/d	162.1[b]	229.3[a]	82.1[c]	55.2[c]	13.9	**<0.01**	0.31	**0.03**	0.75	0.98	0.35
AUC pH < 5.8, pH x min	37.04	46.43	10.77	6.67	3.57	**<0.01**	0.60	0.20	0.76	0.96	0.35
Mean temperature, °F	103.5[b]	103.5[bc]	103.7[a]	103.4[b]	0.03	0.27	**<0.01**	**<0.01**	**<0.01**	0.95	0.98
Mean activity	3.93[ab]	3.80[b]	3.28[c]	4.18[a]	0.08	0.26	**<0.01**	**<0.01**	*0.08*	0.99	0.99

[1]Hour-to-hour variance of ruminal pH (SD).

Table 3.19. Summary of DMI and feeding behavior traits during the SARA challenge periods.

			LY[1] vs. control		P-Values		
Item	Non SARA	SARA	Non SARA	SARA	Diet	SARA	Diet x SARA†
SARA challenge #1 (days 56-76)							
DMI, kg/d			↓17%	↓9%	*	NS	*
BV frequency, events/d			**↑5%NS**	↑23%	*	0.12	*
BV duration, min/d	NS#		↓11%+		*	NS	NS
HD duration, min/d	↑14%#		↓16%+		*	*	NS
BV eating rate, g/min	NS#		NS+		NS	NS	0.12
HD:BV duration	↑8%#		↓4%+		*	*	NS
SARA challenge #2 (days 77-97)							
DMI, kg/d			↓18%	↓7%	*	0.06	*
BV frequency, events/d			**↑1.1%NS**	↑52%	*	0.11	*
BV duration, min/d	NS#		↓14%+		*	NS	0.09
HD duration, min/d	NS#		↓21%+		*	NS	NS
BV eating rate, g/min			↓9%	↑18%	0.10	NS	*
HD:BV duration	↑6%#		↓8%+		*	*	NS

[1]LY = Live yeast

†For response variables with significant interactions, LY vs. control diet subclass means compared within SARA treatments; NS = Difference between subclass means not significant at P > 0.05.

*Significant difference at *P*-value < 0.05.

#Main-effect difference between SARA treatments (P < 0.05).

+Main-effect difference between LY treatments (P < 0.05).

Table 3.20. Summary of rumen bolus parameters during the SARA challenge periods.

			LY vs. control		P-Values		
Item	Non SARA	SARA	Non SARA	SARA	Diet	SARA	Diet x SARA†
SARA challenge #1 (days 56-76)							
Mean pH			↓1.4%	↑1.4%	NS	*	*
Maximum pH			↓4.6%	↑2.1%	*	*	*
Minimum pH	↓2.2%#		↑1.2%+		*	*	NS
Variance of pH (SD)			↓26%	↑9%	*	*	*
Duration pH < 5.8, min/d			**↓44%NS**	↓61%	*	*	*
AUC pH < 5.8, pH x min			↓67%	↓73%	*	*	*
Rumen temperature, °F	↓0.38%#		↓0.29%+		*	*	0.07
SARA challenge #2 (days 77-97)							
Mean pH			**↓0.47%NS**	↑2.6%	*	*	*
Maximum pH			↓2.6%	↑3.0%	NS	*	*
Minimum pH	↓1.4%#		↑2.8%+		*	*	NS
Variance of pH (SD)			↓20%	**↑4.5%NS**	*	0.10	*
Duration pH < 5.8, min/d			↓49%	↓76%	*	NS	*
AUC pH < 5.8, pH x min	NS#		↓79%+		*	NS	NS
Rumen temperature, °F	↑0.19%		**↑0.10%ns**		NS	*	*

[1]LY = Live yeast

†For response variables with significant interactions, LY vs. control diet subclass means compared within SARA treatments; NS = Difference between subclass means not significant at P > 0.05.

*Significant difference at *P*-value < 0.05

#Main-effect difference between SARA treatments (P < 0.05).

+Main-effect difference between LY treatments (P < 0.05).

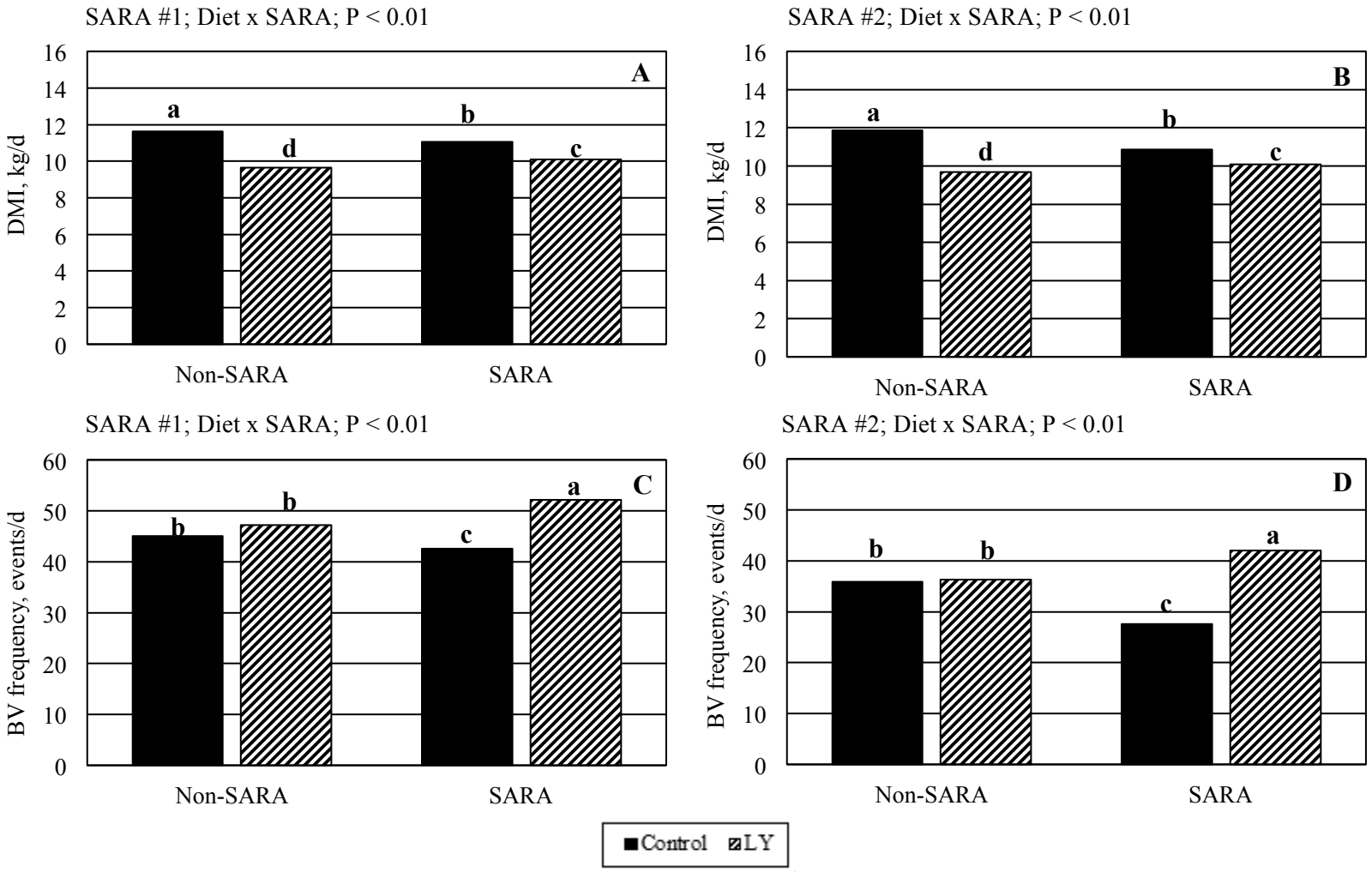


Figure 3.4. Effects of diet and SARA on DMI and BV frequency during SARA challenge #1 (A and C, respectively) and #2 (B and D, respectively).
[a,b,c]Means differ at P < 0.05.

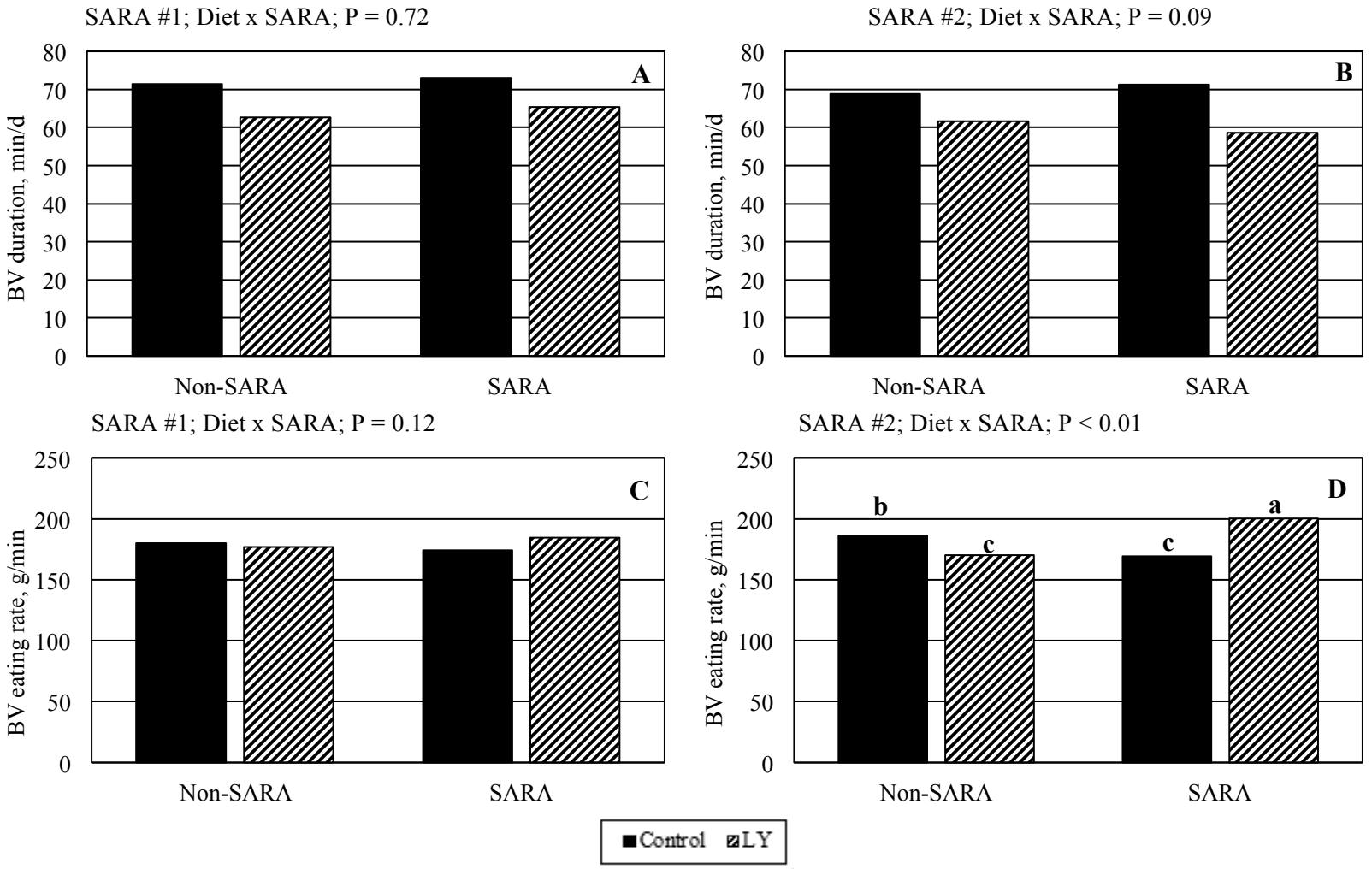


Figure 3.5. Effects of diet and SARA treatment on BV duration and BV eating rate during SARA challenge #1 (A and C, respectively) and #2 (B and D, respectively).
[a,b,c]Means differ at $P < 0.05$.

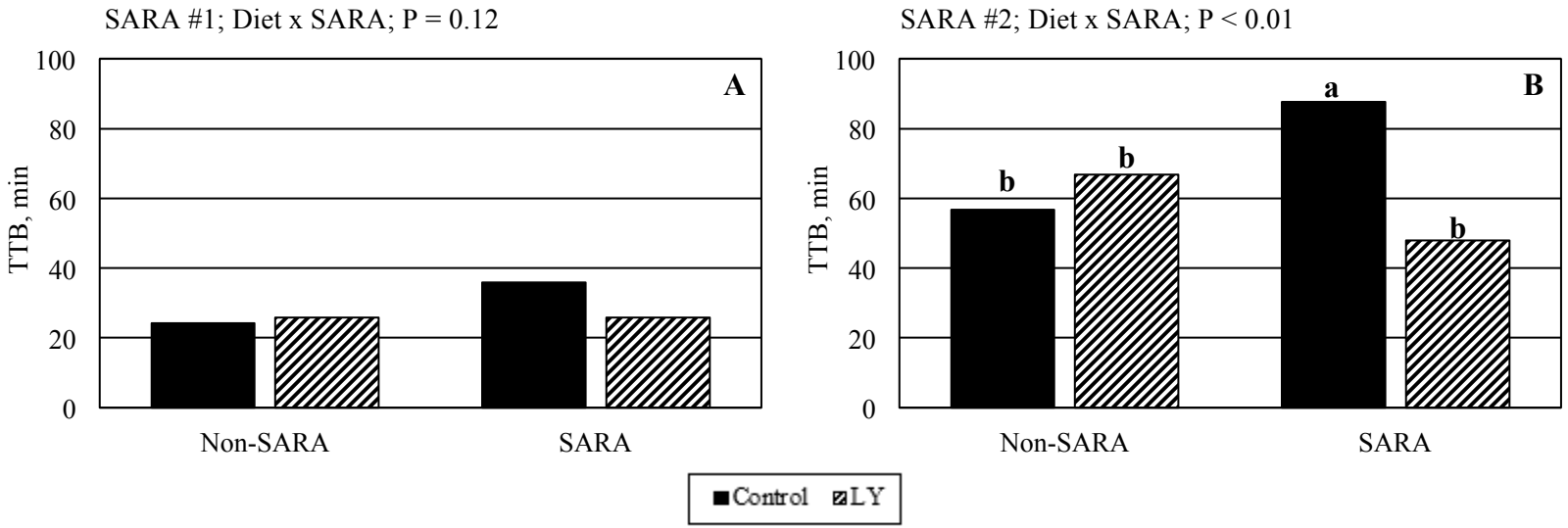


Figure 3.6. Effects of diet and SARA treatment on time to bunk during SARA challenge #1 (A) and #2 (B).
[a,b,c]Means differ at P < 0.05.

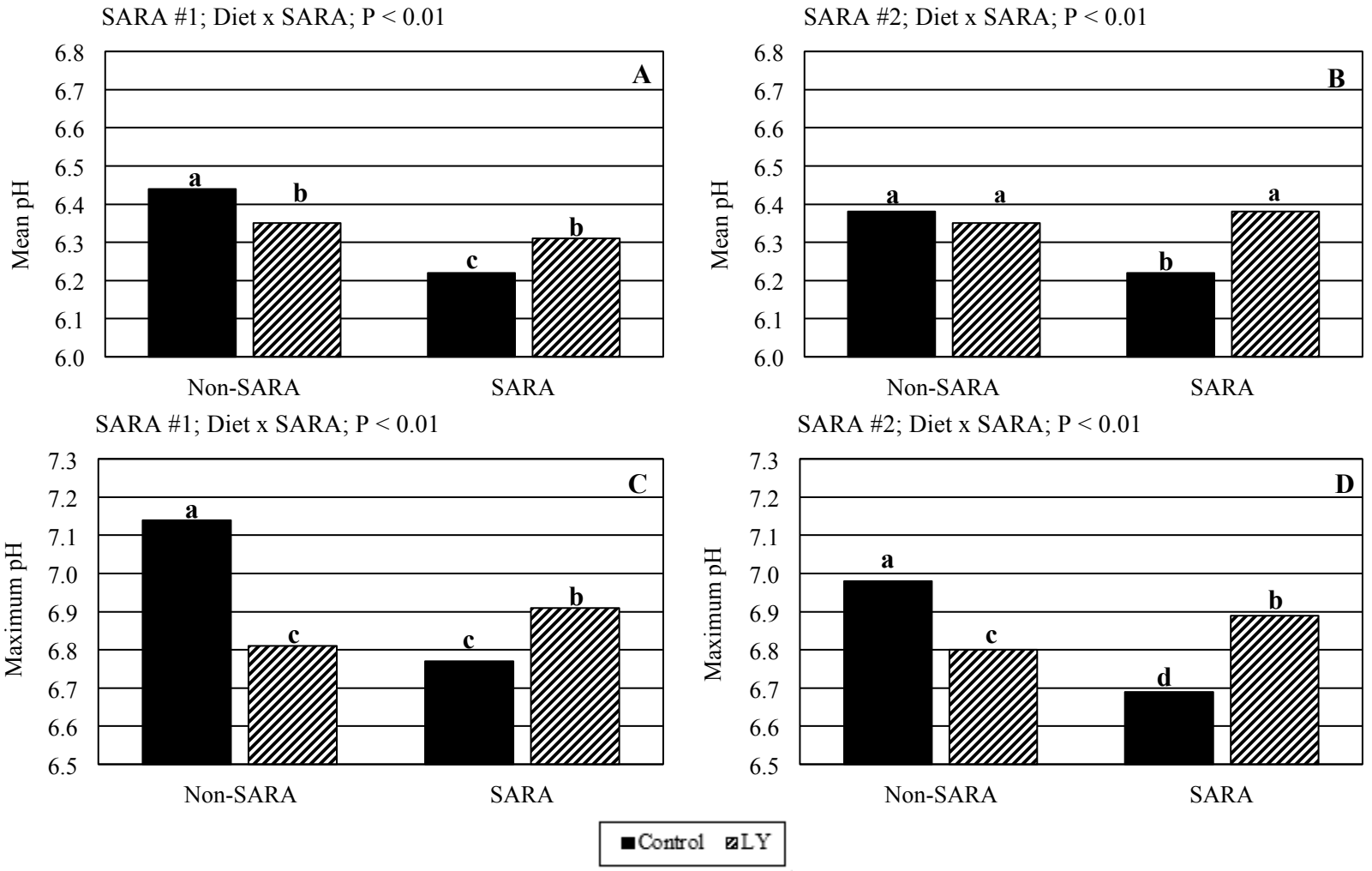


Figure 3.7. Effects of diet and SARA treatments on mean and maximum pH during SARA challenge #1 (A and C, respectively) and #2 (B and D, respectively).
[a,b,c]Means differ at $P < 0.05$.

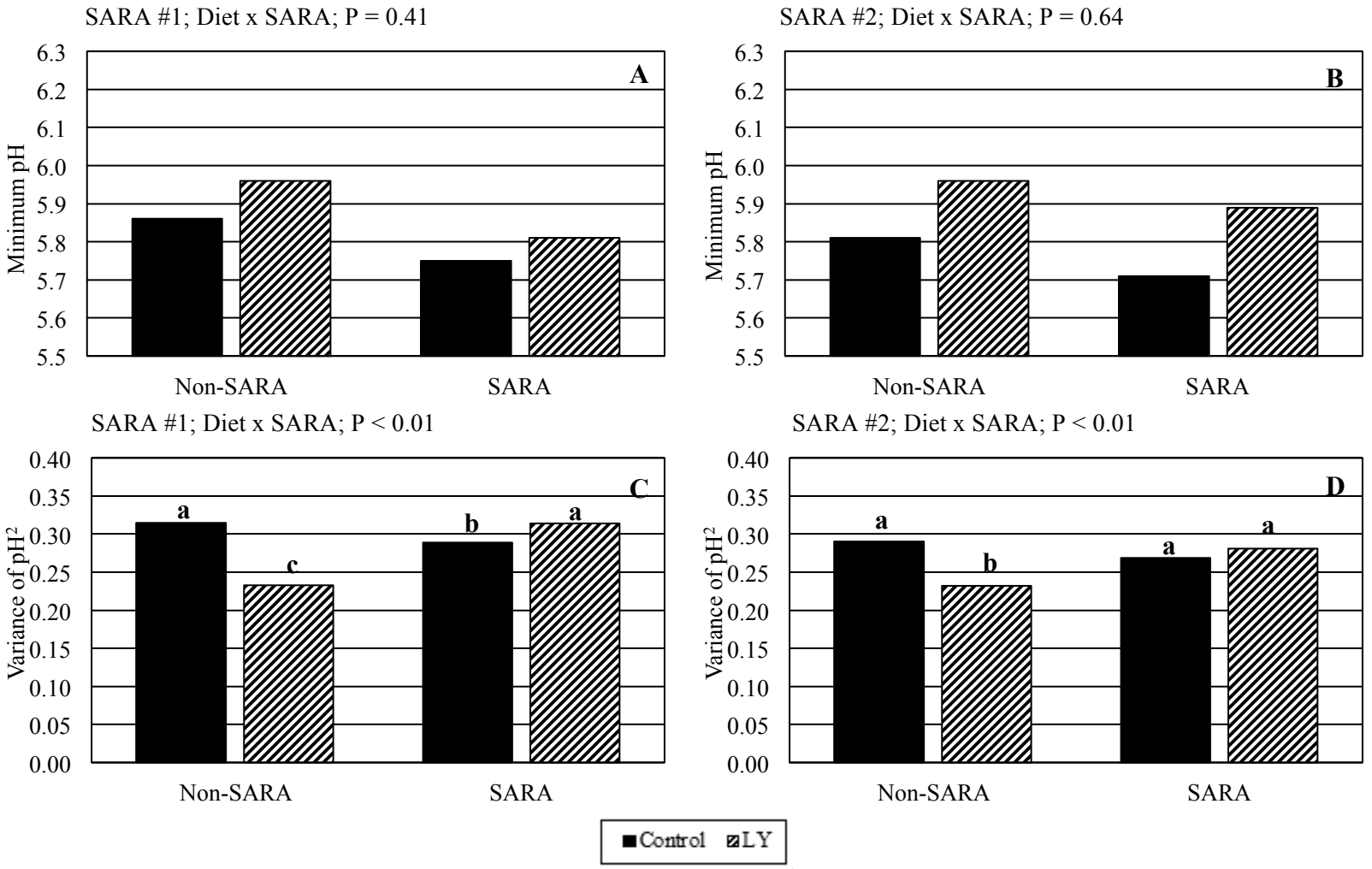


Figure 3.8. Effects of diet and SARA treatments on minimum pH and variance of pH during SARA challenge #1 (A and C, respectively) and #2 (B and D, respectively).
[a,b,c]Means differ at $P < 0.05$; [2]Hour-to-hour variance of ruminal pH (SD)

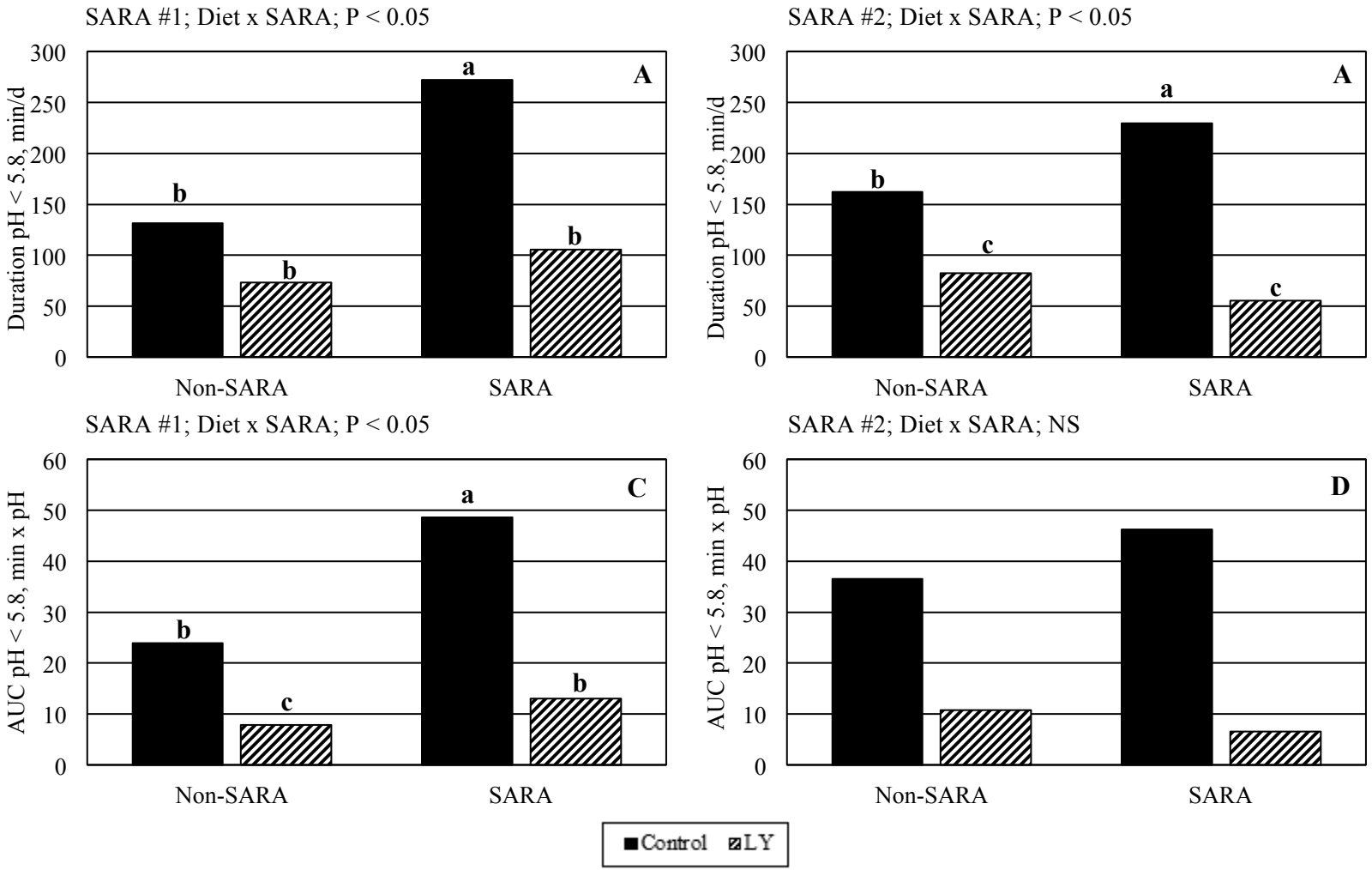


Figure 3.9. Effects of diet and SARA treatments on duration (min/d) and area under the curve (AUC; pH x min) for rumen pH < 5.8 during SARA challenge #1 (A and C, respectively) and #2 (B and D, respectively).
[a,b,c]Means differ at $P < 0.05$.

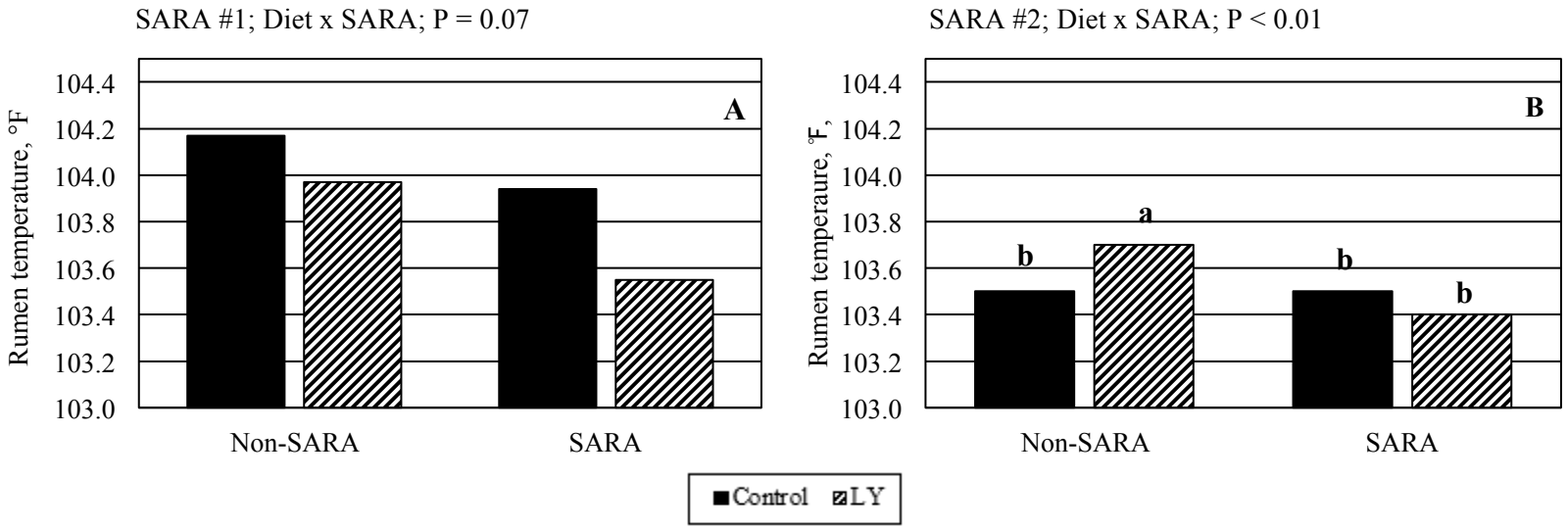


Figure 3.10. Effects of diet and SARA treatments on rumen temperature SARA challenge #1 (A) and #2 (B).
[a,b,c]Means differ at $P < 0.05$.

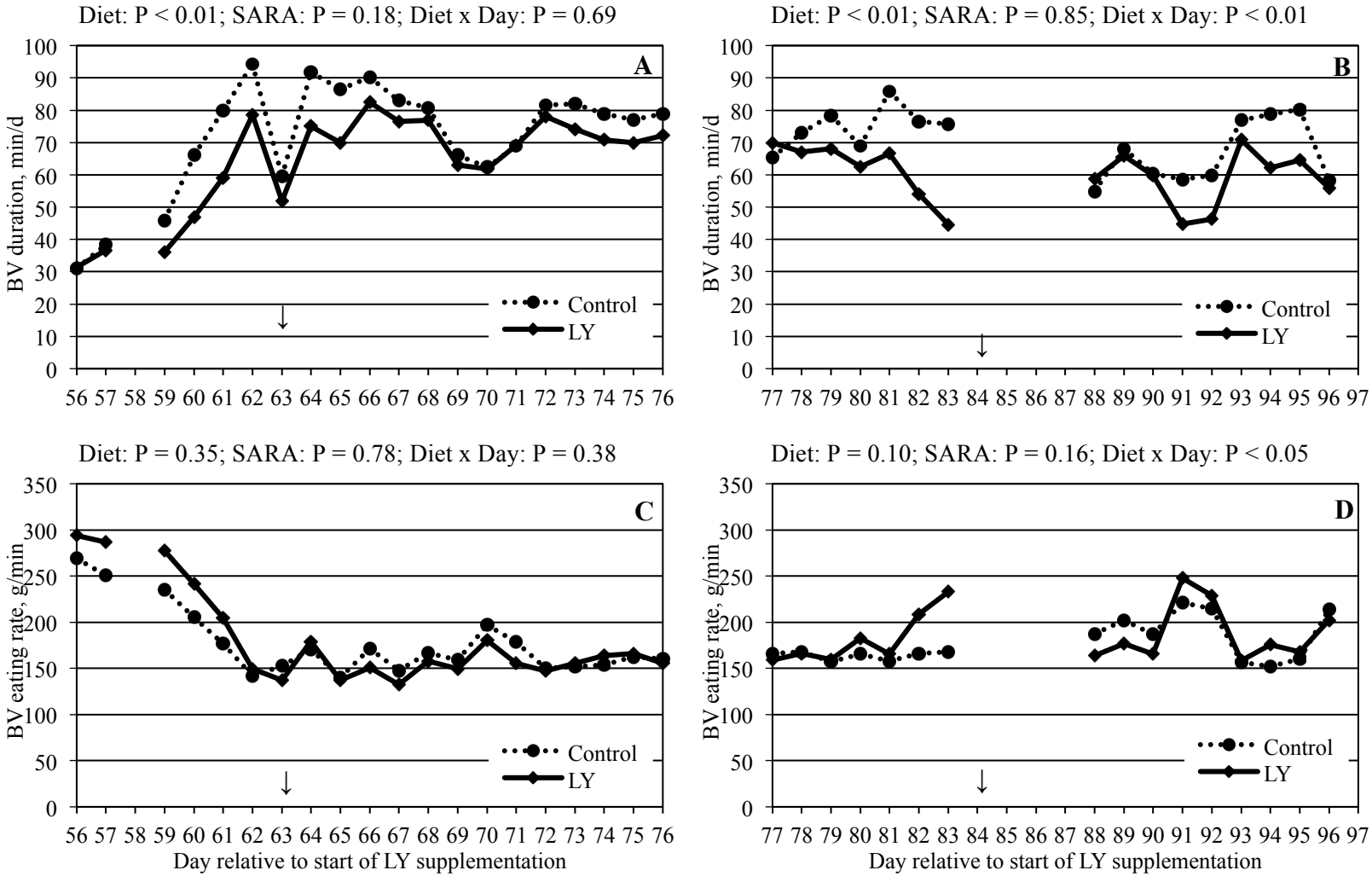


Figure 3.11. Effects of **dietary** treatment on bunk visit (BV) duration and BV eating rate during SARA challenge #1 (A and C, respectively) and #2 (B and D, respectively).
*Means differ at $P < 0.05$; †Means differ at $P < 0.10$; ↓ indicates start of feed intake disruption.

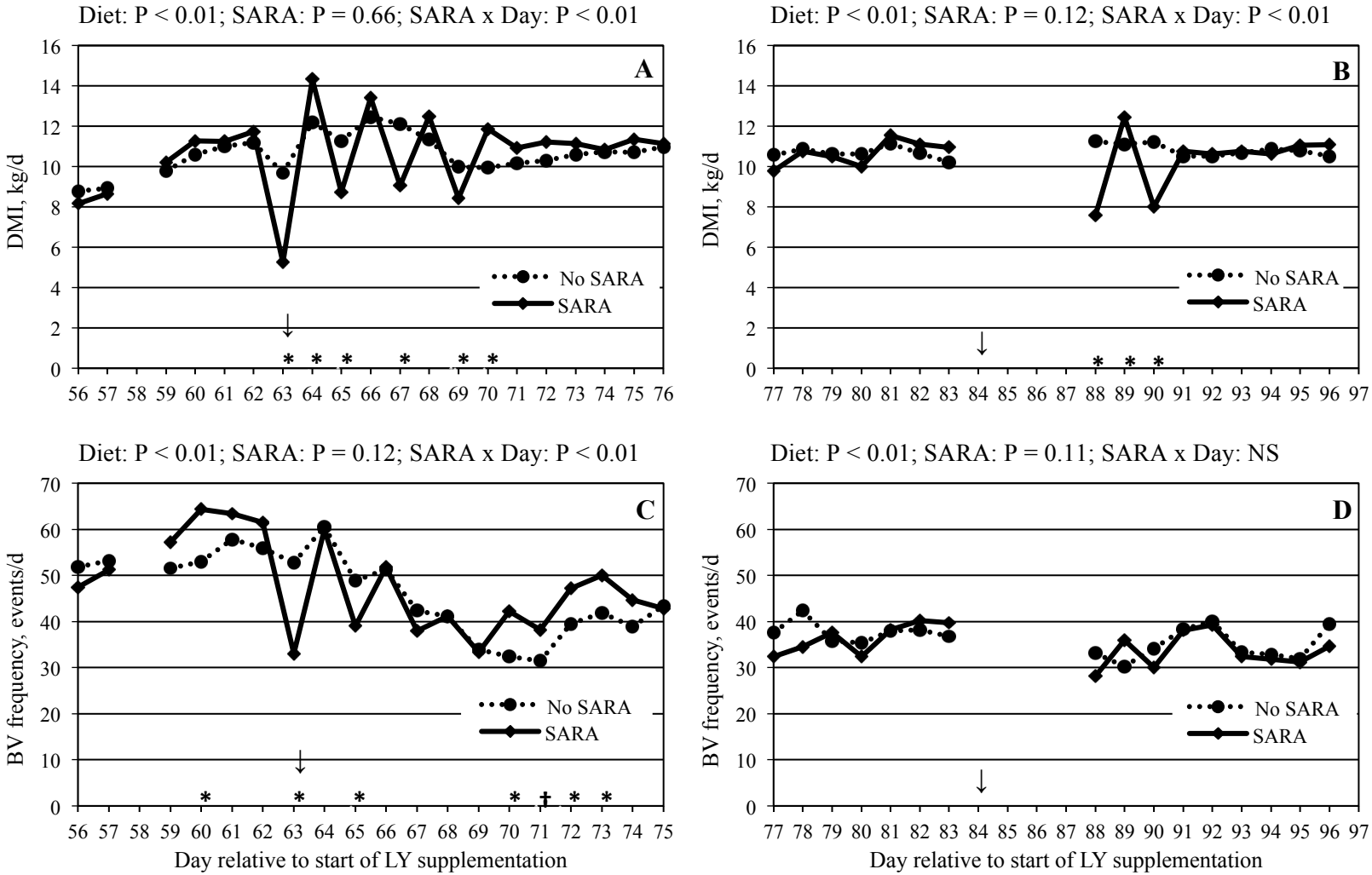


Figure 3.12. Effects of **challenge** treatment on DM intake and bunk visit (BV) frequency during SARA challenge #1 (A and C, respectively) and #2 (B and D, respectively).
*Means differ at $P < 0.05$; †Means differ at $P < 0.10$; ↓ indicates start of feed intake disruption.

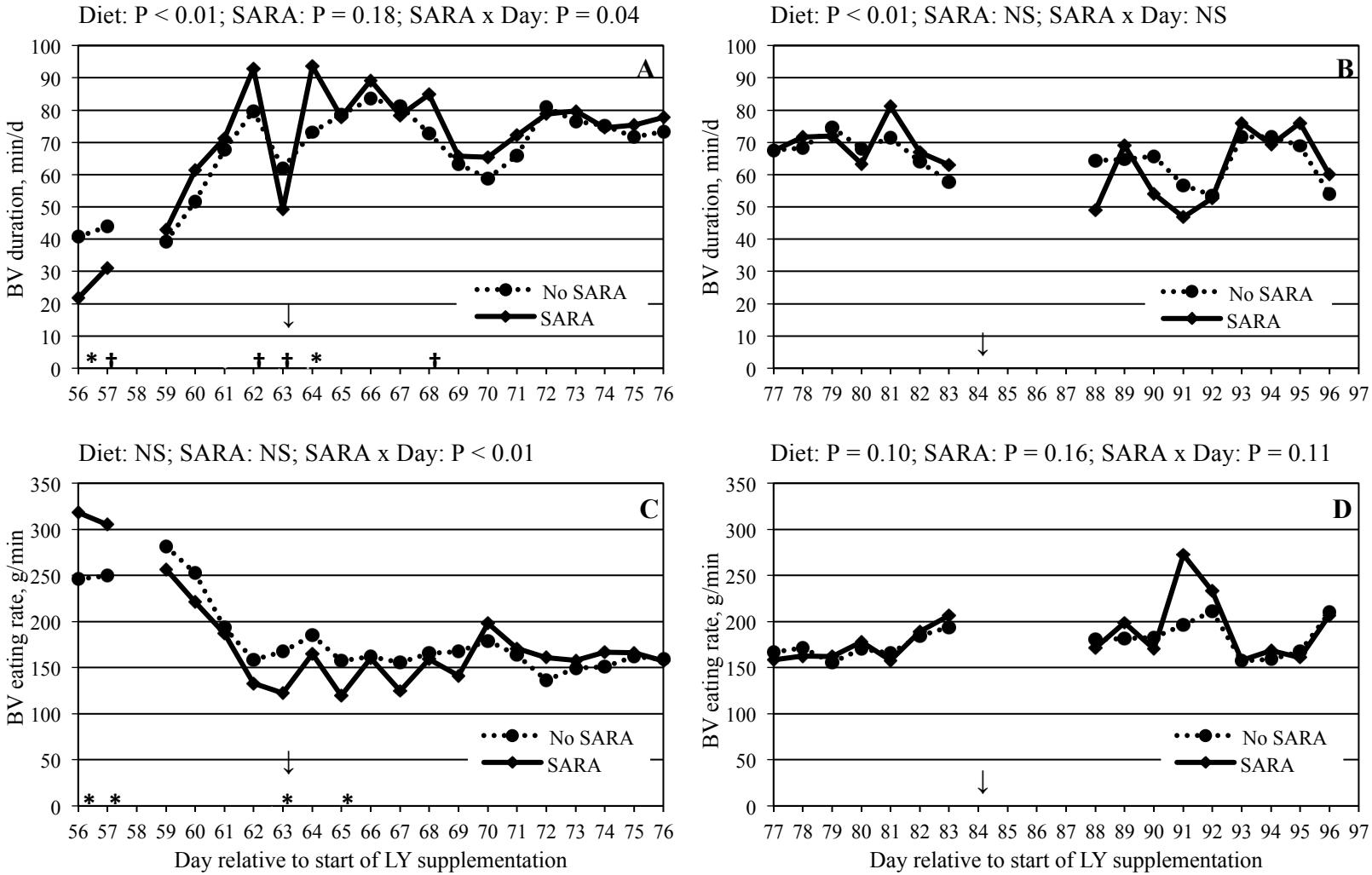


Figure 3.13. Effects of **<u>challenge</u>** treatment on bunk visit (BV) duration and eating rate during SARA challenge #1 (A and C, respectively) and #2 (B and D, respectively).
*Means differ at $P < 0.05$; †Means differ at $P < 0.10$; ↓ indicates start of feed intake disruption

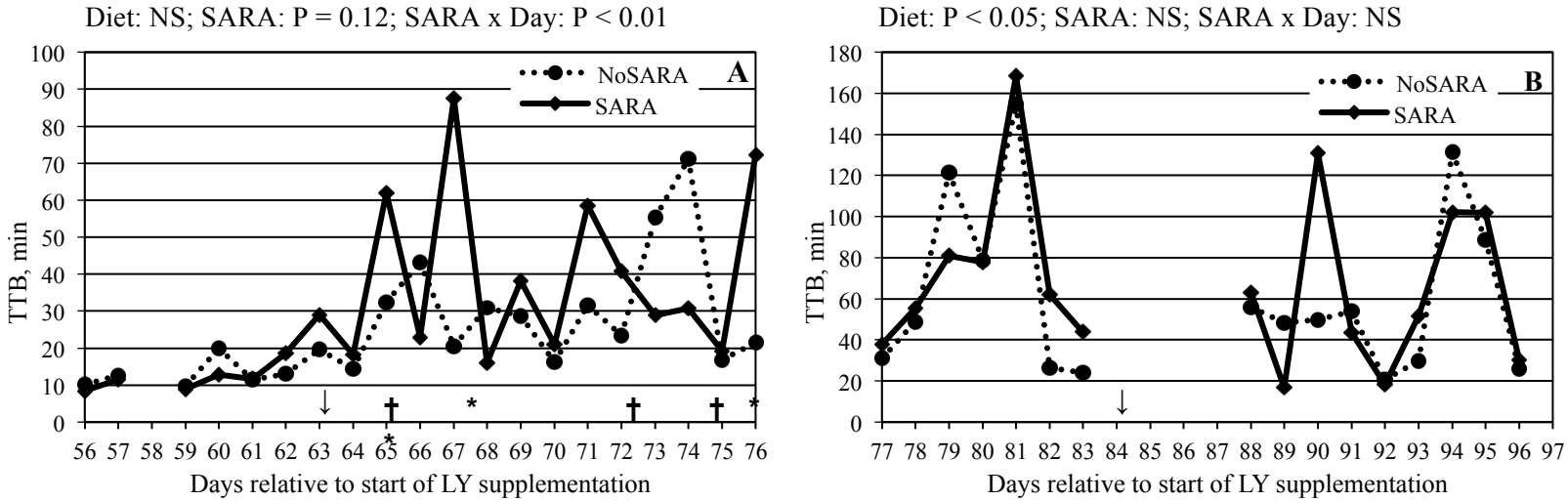


Figure 3.14. Effects of **challenge** treatment on time to bunk during SARA challenge #1 (A) and #2 (B).
*Means differ at $P < 0.05$; †Means differ at $P < 0.10$; ↓ Indicates start of feed intake disruption.

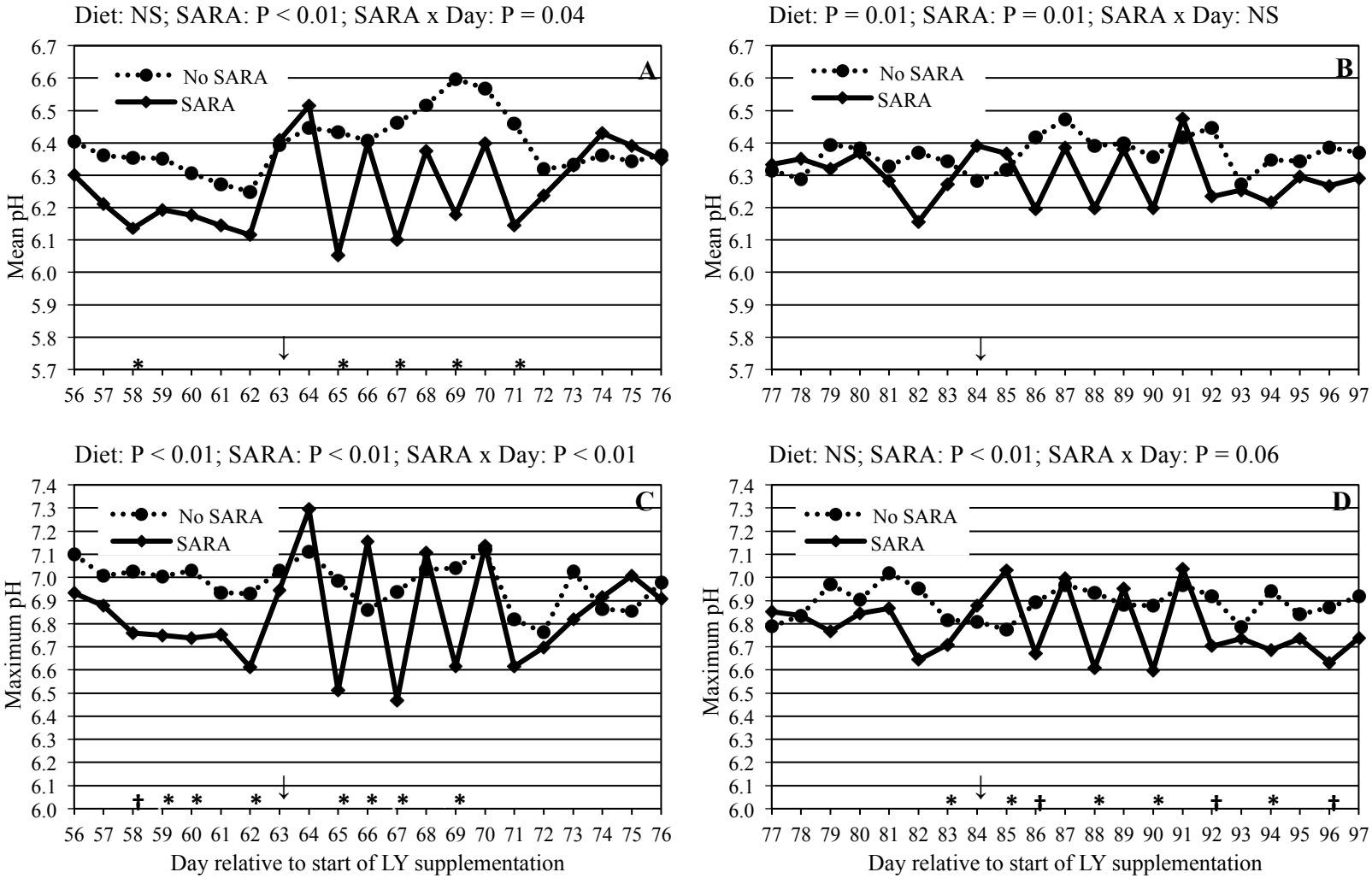


Figure 3.15. Effects of **challenge** treatment on mean and maximum pH during SARA challenge #1 (A and C, respectively) and #2 (B and D, respectively).

*Means differ at $P < 0.05$; †Means differ at $P < 0.10$; ↓ indicates start of feed intake disruption.

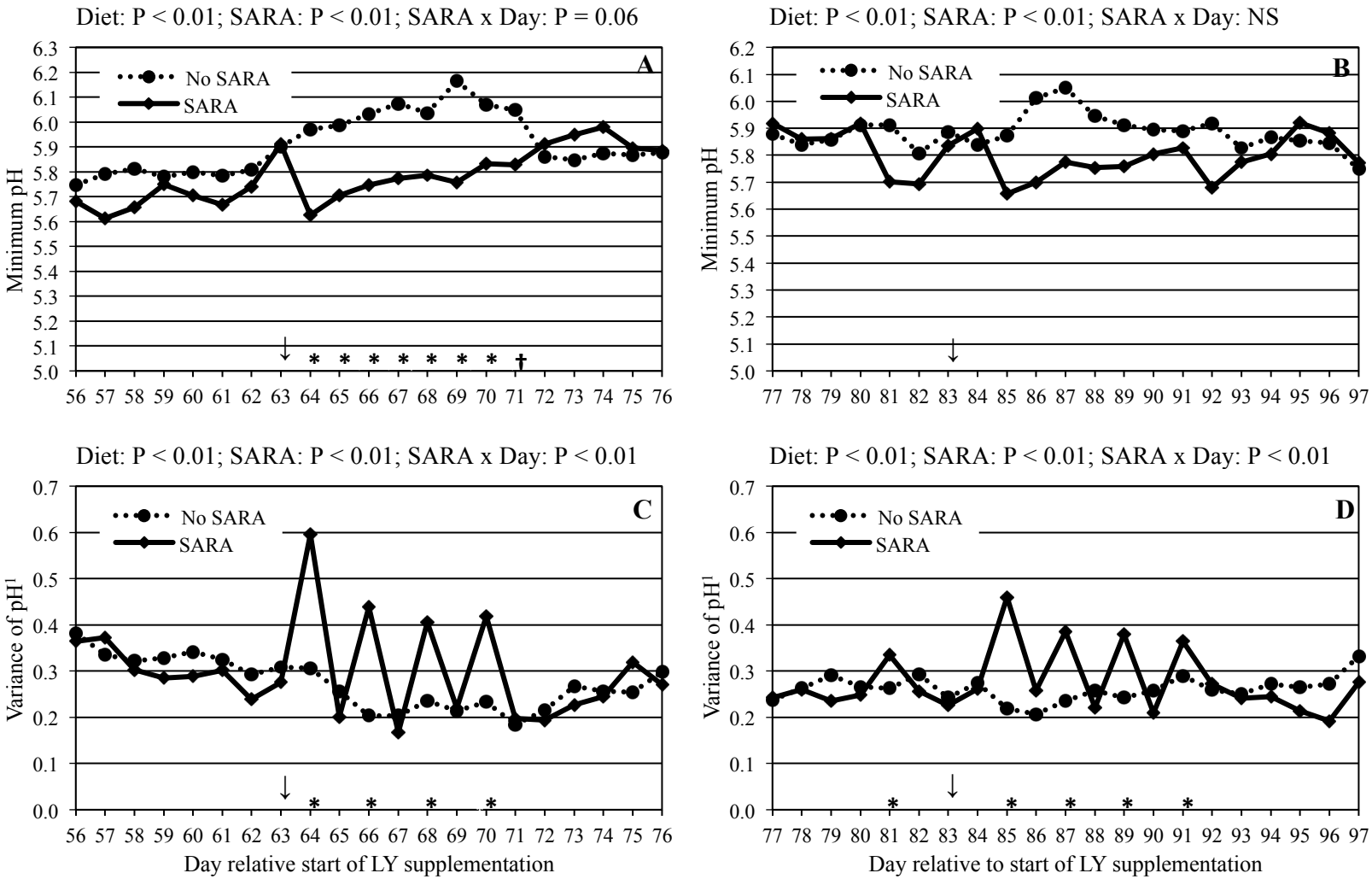


Figure 3.16. Effects of **challenge** treatment on minimum pH and variance of pH during SARA challenge #1 (A and C, respectively) and #2 (B and D, respectively).

*Means differ at $P < 0.05$; †Means differ at $P < 0.10$; ↓ indicates start of feed intake disruption; [1]Hour-to-hour variance of pH (SD).

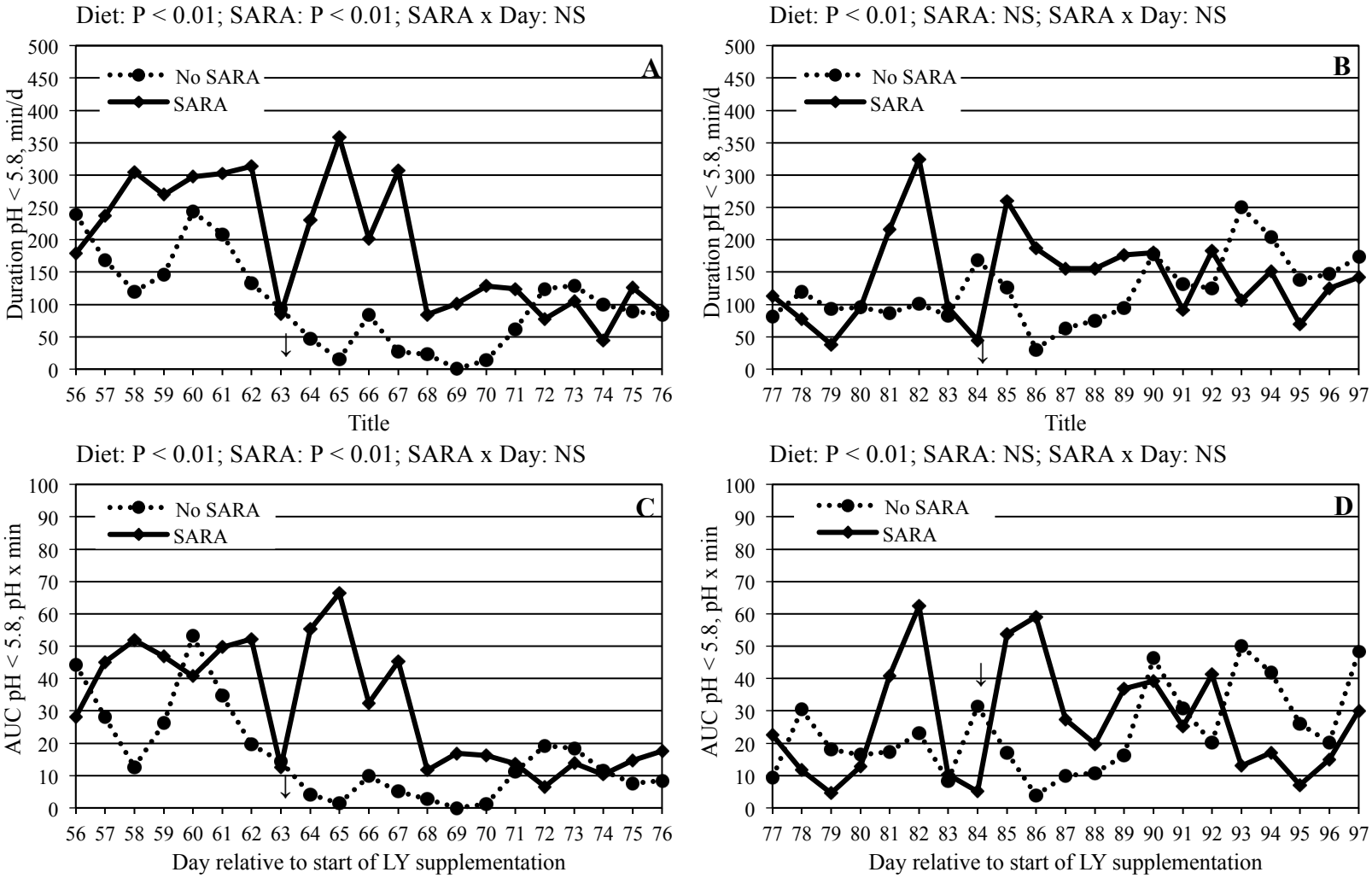


Figure 3.17. Effects of **challenge** treatment on duration (min/d) and area under the curve (AUC; pH x min) for rumen pH < 5.8 during SARA challenge #1 (A and C, respectively) and #2 (B and D, respectively).
*Means differ at $P < 0.05$; †Means differ at $P < 0.10$; ↓ indicates start of feed intake disruption.

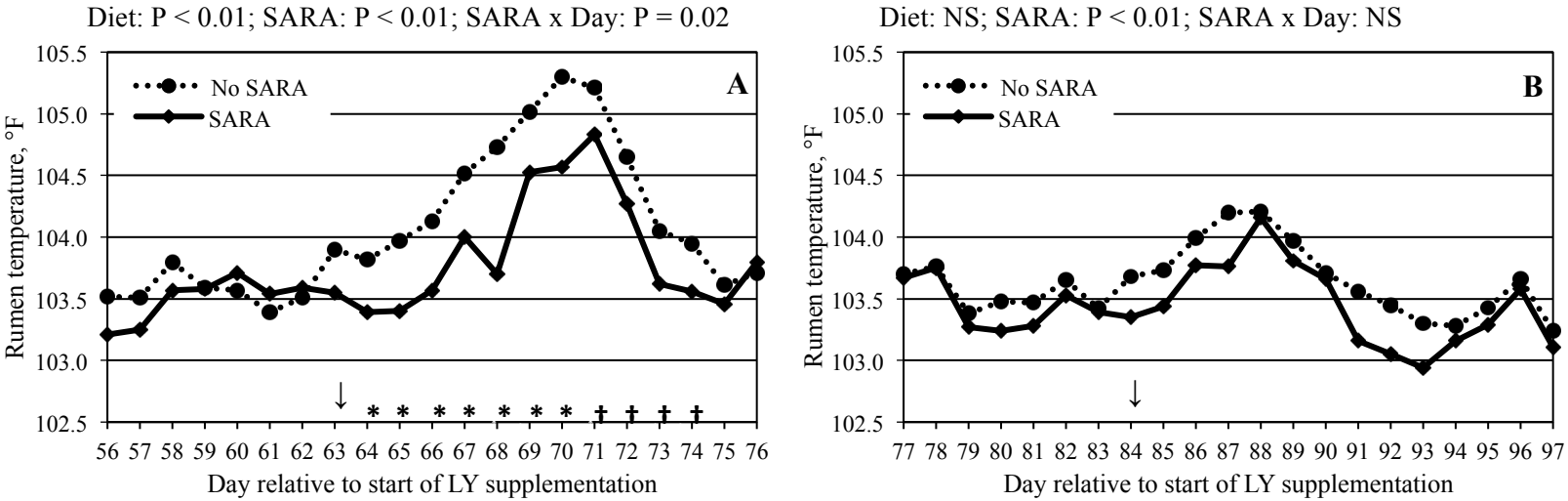


Figure 3.18. Effects of **challenge** treatment on rumen temperature during SARA challenge #1 (A) and #2 (B).
*Means differ at $P < 0.05$; †Means differ at $P < 0.10$; ↓ Indicates start of feed intake disruption.

Table 3.21. Main-effect means of live yeast and SARA treatments on arterial blood measurements.

	Diet		SARA			P-Values		
Item	**Control**	**Live Yeast**	**Non SARA**	**SARA**	**SE**	**Diet**	**SARA**	**Diet x SARA**
Before SARA challenge #1 (day 63)								
No. of steers	8	12	10	10	---	---	---	---
pH	7.46	7.47	7.46	7.47	0.02	0.59	0.70	0.11
HCO3-	24.5	24.8	24.0	25.3	1.0	0.91	0.53	0.35
Base excess	4.00	1.17	3.54	1.63	1.10	0.22	0.40	0.79
Lactate	5.51	4.31	6.27	3.55	0.94	0.53	0.17	0.13
saO2, %	96.4	97.0	96.8	96.6	0.3	0.31	0.84	0.16
After SARA challenge #1 (day 70)								
No. of steers	10	11	9	12	---	---	---	---
pH	7.52	7.50	7.52	7.50	0.01	0.34	0.46	0.31
HCO3-	25.0	23.2	24.4	23.8	0.6	0.12	0.61	0.80
Base excess	2.17	-0.07	1.35	0.75	0.69	0.12	0.67	0.96
Lactate	4.30	4.24	4.50	4.04	0.57	0.95	0.69	0.36
saO2, %	97.0	97.4	96.5	97.8	0.4	0.60	*0.08*	0.16
Before SARA challenge #2 (day 84)								
No. of steers	12	12	12	12	---	---	---	---
pH	7.51	7.50	7.51	7.50	0.01	0.66	0.79	0.34
HCO3-	26.9	26.2	26.7	26.4	0.6	0.61	0.83	0.45
Base excess	3.67	3.08	3.67	3.08	0.73	0.70	0.70	0.40
Lactate	3.50	4.09	4.04	3.54	0.59	0.62	0.67	0.17
saO2, %	97.6	97.1	97.5	97.2	0.3	0.39	0.57	0.16
After SARA challenge #2 (day 91)								
No. of steers	12	11	11	12	---	---	---	---
pH	7.51	7.51	7.49	7.53	0.01	0.87	*0.08*	0.68
HCO3-	26.0	26.2	25.3	26.8	0.5	0.86	0.17	0.48
Base excess	2.92	3.03	1.87	4.08	0.62	0.93	*0.09*	0.45
Lactate	2.89	3.48	4.12	2.24	0.42	0.49	**0.04**	0.33
saO2, %	97.2	97.2	96.5	97.9	0.5	0.96	0.13	0.82

[1]Arterial samples with saO2 levels < 90 were excluded from analysis.

Table 3.22. Main-effect means of live yeast and SARA challenge treatments on performance during grower (21 d) and transition (14 d) periods.

	Diet		SARA			P-Values		
Item	Control	Live Yeast	Non SARA	SARA	SE	Diet	SARA	Diet x SARA
Performance and feed efficiency:								
Initial BW (day 0), kg	343	340	340	344	5	0.44	0.34	0.82
Final BW (day 35), kg	373	375	376	373	3	0.71	0.61	0.90
ADG, kg/d[1]	0.969	1.014	1.059	0.924	0.046	0.62	0.15	0.37
DMI, kg/d	10.52	9.55	10.15	9.93	0.22	**0.03**	0.62	0.71
F:G ratio	11.69	10.48	10.38	11.79	0.64	0.35	0.27	0.42
G:F ratio	0.096	0.110	0.109	0.098	0.005	0.18	0.31	0.66

[1]ADG was computed as the slope of regressed individual BW for the given period.

Table 3.23. Main-effect means of live yeast and SARA challenge treatments on performance during 70-d period (days 35 to 105) following the transition period.

	Diet		SARA			P-Values		
Item	Control	Live Yeast	Non SARA	SARA	SE	Diet	SARA	Diet x SARA
Performance and feed efficiency:								
Initial BW (day 35), kg	373	375	376	373	3	0.71	0.61	0.90
Final BW (day 105), kg	431	431	434	429	9	0.95	0.50	0.84
ADG, kg/d[1]	0.917	0.963	0.967	0.913	0.045	0.61	0.56	0.67
DMI, kg/d	10.42	9.12	9.93	9.60	0.19	**<0.01**	0.39	0.43
F:G ratio	12.44	9.86	11.35	10.95	0.59	**0.03**	0.74	0.92
G:F ratio	0.087	0.107	0.098	0.096	0.005	**0.04**	0.90	0.74

[1]ADG was computed as the slope of regressed individual BW for the given period.

Table 3.24. Main-effect means of live yeast and SARA treatments on carcass traits[1] in finishing.

	Diet		SARA			P-Values		
Item	**Control**	**Live Yeast**	**Non SARA**	**SARA**	**SE**	**Diet**	**SARA**	**Diet x SARA**
No. of steers	24	24	24	24	---	---	---	---
HCW, kg	320	329	327	322	7	0.15	0.41	0.43
Backfat depth, cm	0.513	0.475	0.503	0.485	0.018	0.29	0.61	0.46
LMA, cm^2	12.3	13.0	12. 8	12.5	0.2	*0.08*	0.48	0.19
Yield grade	3.06	2.84	2.95	2.95	0.08	0.16	0.98	0.27
Marbling score	531	512	545	498	18	0.47	*0.08*	*0.06*
QG distribution, %[2]								
Prime	8.3	0.0	8.3	0.0	---	0.99	0.99	0.99
Premium CH	45.8	54.2	50.0	50.0	---	0.57	1.00	0.57
Choice	33.3	41.7	41.7	33.3	---	0.57	0.57	0.57
Select	12.5	4.2	0.0	16.7	---	0.99	0.98	0.99
No. of steers[3]	21	18	18	21	---	---	---	---
Liver score distribution, %[2]								
Edible	0.0	7.1	7.1	0.0	---	0.99	0.99	0.99
A-	85.0	64.9	78.6	71.4	---	0.97	0.98	0.97
A	0.0	16.2	7.1	9.1	---	0.97	0.99	0.99
A+	15.0	11.7	7.1	19.5	---	0.98	0.98	0.97

[1]Carcass traits were determined using instrument grading system; KPH fat % data not presented.
[2]Distribution values were calculated for ordinal data to provide reference for χ^2 test.
[3]Livers that were classified as contaminated or had flukes were excluded from analysis of liver scores