EFFICACY OF FEED-GRADE ANTIBIOTICS, ANTIBIOTIC ALTERNATIVES AND THE IMPACT OF THERMAL STRESS ON THE RUMINAL MICROBIOME, RUMINAL FERMENTATION, FEEDLOT PERFORMANCE, FEEDING BEHAVIOR, AND CARCASS CHARACTERISTICS OF BEEF CATTLE

A Dissertation

by

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

DOCTOR OF PHILOSOPHY

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May 2018

Major Subject: Animal Science

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ABSTRACT

Beef cattle production in the United States is faced with restricted access to previously used feed-grade antibiotics, deemed medically important, under the 2017 Veterinary Feed Directive. Suitable alternatives should be characterized and verified across different feeding scenarios to maintain production efficiency and implementation strategies should be investigated to ensure the best utilization of current technology.

In the first study, continuous versus rotational feeding of two products, monensin and bambermycin, were investigated to determine their effects on volatile fatty acids, potential activity of methane production, and rumen microbial populations of steers. We found no evidence to suggest that rotational feeding schemes were more effective than the continuous feeding of monensin and bambermycin or that rotational regimens delay microbial adaptation.

In the second study, the effects of supplemental yeast (**ADY**) in the diets of finishing steers were investigated under different environmental temperatures (**TN** = 18 ± 0.55°C or **HS** = 35 ± 0.55°C). We concluded that supplementing ADY in the diets of finishing steers improved digestibility, digestible energy, metabolizable energy, and mean ruminal pH under TN conditions, but not under extreme HS conditions, due to depressed intake and energetic demand.

In the third study, we observed the effects of supplementing ADY to feedlot steers (n = 120) fed for 164 days. Final treatment assignments were designated on d 70, followed by a 14 d dietary transition to a finishing diet, resulting in four treatment groups (Balaam's design: two parallel groups and two cross-over groups). There is evidence to suggest that the

addition of ADY in the diets of beef cattle during the dietary transition may aid in ruminal stabilization without affecting growth performance or carcass traits.

A fourth study was initiated to isolate and characterize bacteriophage that target hyper ammonia producing bacteria (**HAB**) in the rumen. HAB had poor bacterial lawn growth to detect phage plaques. Sequential transfer methods of Félix d'Hérelle are recommended to identify phage presence on these obligately anaerobic bacteria. Naturally occurring antimicrobial substances in environmental samples may have interfering effects. Future phage work should look to high impact bacteria that cause easily measurable losses to truly understand efficacy.

DEDICATION

This dissertation is dedicated to those who helped me become the scientist I am today. By now I have figured out that I still have quite a lot to learn. And I can't wait to spend the rest of my career doing that very thing.

To my advisor: Dr. Tedeschi, there aren't enough words to tell you how much I appreciate you for giving me another opportunity to work with you in your research program (and for footing the bill). You have a lot to read here so I'll keep it short. I know the last 4 years have not been easy. Thank you for the letters of recommendation for the many scholarships and applications. You have invested a great deal of time and money into cultivating me as a young scientist and I only hope I can promote that same passion in my own research program. I am proud to call you mentor.

To my committee: Dr. Gill, Dr. Wickersham, Dr. Herring, and Dr. Nagaraja, thank you for allowing me to randomly show up and ask you questions and advice. Your contributions and perspective have given me a well-balanced perception of the intricate ways nutrition is tied to all aspects of beef production and how I should pursue grants in the future.

To my husband, thank you for sticking by me through this venture. Without you, I would have crashed and burned in year 2. It was hard and there are some scars but I think we can both agree we are better people now than we were when we started. And now, through faith and perseverance (or hard-headedness?) we have achieved our goals of returning to the panhandle where we can raise our family in the cattle business. We are so very blessed.

ACKNOWLEDGEMENTS

Thank you to the Department of Animal Science for providing me the opportunity to be the instructor of record for Animal Science 320 and for covering my tuition during those years. Thank you also to the College of Agriculture and Life Science for selecting me to receive and Excellence Fellowship and the Willie Mae Harris Teaching Fellowship. Thank you to the business coordination staff Robbie, Linda, Jennifer, Kori and Sarah for your help with the details that goes with raising a graduate student. Thank you to Kerry Dean, Kendrick LeBlanc and Kenton Krueger for all their help with feed services and facilities.

Thank you to my committee, Dr. Tedeschi, Dr. Gill, Dr. Wickersham, Dr. Herring and Dr. Nagaraja for their guidance and support throughout the course of this research.

Thanks also go to my friends and colleagues and the department faculty and staff for making my time at Texas A&M University a great experience. A huge amount of gratitude to my colleague Aaron Norris for his skill and ability in helping me complete my research projects and for all the laughs we shared doing “Re-re-search”.

Finally, thanks to my encouraging family for their support and patience through my late-blooming desire for a career in academia.

CONTRIBUTORS AND FUNDING SOURCES

This work was supervised by the dissertation committee chair, Dr. Luis O. Tedeschi of the Texas A&M University Department of Animal Science, and committee members: Dr. Jason J. Gill, Dr. Tryon A. Wickersham, Dr. Andy D. Herring of the Texs A&M University Department of Animal Science, and Dr. T. G. Nagaraja of Kansas State University, adjunct member to the Texas A&M Univesity Department Animal Science.

Graduate study was partially supported by the Excellence Fellowship and Willie Mae Harris Fellowship from the Texas A&M University College of Agriculture and Natural Resources. Graduate studies were also partially supported by the Department of Animal Science for teaching and research responsibilities. Graduate studies were also partially supported by the committee chair for research responsibilities.

Research was made possible in part by the Texas A&M AgriLife Beef Initiative (02-513406-20000) and also in part by ABVista LLC (06-407988-91230).

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Beef producers in the United States are a relatively small community (< 0.5% of Americans > 18 years old) according to the 2012 USDA census. Challenges facing this community include, but are not limited to: federal regulations dictating policy in animal production systems, impending climate change and the agricultural contribution to greenhouse gasses, the ever-scrutinizing public perception of animal husbandry, increasing emergence of multiple drug-resistant bacteria and a rising global demand for protein coupled with the largest threat to food and water security. Meanwhile business must remain economically sustainable to deliver a source of safe, high-quality beef protein to consumers. Regarding food security, a large amount of research dollars have been devoted to using the best technologies and practices available to promote sustainability from both an economic and environmental standpoint.

In the beef cattle industry, the many segments and length of the production chain coupled with environmental challenges require the most efficient use of natural resources to be economically and environmentally sustainable. Considerable effort has been made by researchers to establish nutrient requirements of beef cattle (National Academies of Sciences, Engineering, and Medicine, **NASEM**, 2016) at all phases of production and predict their performance using dynamic models for decision-making software (Tedeschi and Fox, 2018). Forecasting tools, will be an important necessity to endure the upcoming challenges

facing the cattle feeding industry: 1) increasing awareness and incidence of antibiotic-resistant bacteria, and 2) producing beef in a warming climate.

Predicting energy requirements and feed efficiency

Energy requirements of beef cattle. Calorimetry has been used to determine animal energy requirements as well as the energy provided by typical feed stuffs. Calorimetry is the measurement of heat and therefore may be used to determine the energetic costs of animal maintenance, growth, and performance, or the energy contained in a diet. The maintenance energy required by animals of a given weight, gender and frame is based on the fasting heat production measured by indirect calorimetry. Comparative slaughter studies, however, have resulted in the equation [1.1] we use to estimate the net energy for maintenance (**NEm**) requirements for beef cattle,

$$NEm = 0.077\times(SBW)^{0.75} \quad [1.1]$$

where SBW= Shrunk body weight in kg, and NEm= Mcal/d (Lofgreen and Garrett,1968; Garrett,1980).

Energy terms and concepts are represented in Figure 1.1 adapted from NASEM (2016). The net energy system has been established for large ruminants and represents the flow of energy losses and energy retained. Gross energy (**GE**) is the energy of the diet derived from the heat of combustion. Energy lost in feces (**FE**) may be deducted from GE resulting in the apparently digestible energy (**DE**). The next sources of energy loss are urinary energy (**UE**) and gaseous energy (**GASE**), primarily CH_4. Deduction of UE and GASE from DE result in the metabolizable energy (**ME**). Heat production or heat energy (**HE**) may be measured via indirect calorimetry. Typically in calorimetry only O_2 and CO_2 are measured, but modifications have been made due to the large quantities of CH_4 produced

by ruminants. The volume of O_2, CO_2, and CH_4 and the urinary nitrogen excretion (g/d) may be used according to the Brouwer (1965) equation [1.2]:

$$HE\ (kcal) = (3.866 \times VO_2) + (1.2 \times VCO_2) - (0.518 \times VCH_4) - (1.431 \times urinary\ N) \quad [1.2]$$

Subtracting the HE from ME will result in retained energy (**RE**) for productive functions such as growth, pregnancy, or lactation. While serial slaughter may be the recommended method for determining RE, for some classes of animals slaughter may not be ideal (i.e., pregnant and lactating cattle) and can be expensive when considering sample size and labor.

There is some variation in energy requirements for maintenance which differ by body weight (BW), breed, sex, age, environment, physiological status and previous nutritional status (NASEM, 2016). In prediction tools like the Cornell Net Carbohydrate and Protein System (**CNCPS**), Large Ruminant Nutrition System (**LRNS**) and the Beef Cattle Nutrient Requirements Model (**BCNRM**, 2016) accounting for variation is key in optimizing prediction accuracy of diets for different classes of cattle experiencing different environments (Tedeschi and Fox, 2018).

One of the top bullets in Chapter 21 *Research Needs* of NASEM (2016), under Maintenance, Growth and Reproduction and Stress is additional research to refine the energetic adjustments associated with changes in environmental temperature. In light of the threat of a warming climate, the effect of increased heat stress in beef cattle is a major concern and is already a relevant topic considering its major drain on animal production in the hot season. Animals adapt to heat stress by decreasing DMI and lowering their metabolic rate (Fox et al., 1988). To maintain core body temperature cattle will invoke measures of evaporative heat loss of sweating and panting. However, when the thermal gradient between core body temperature and environmental temperature decrease, more work (energy) is

required to dissipate heat and prevent heat loading. The only heat stress adjustments made in the current beef cattle models are based on work in *Bos indicus* cattle which correspond to a panting score (Fox et al., 1992). *Bos indicus* cattle exhibiting rapid, shallow panting require approximately a 7 % increase in maintenance energy and an 18 % increase when exhibiting open mouth panting. Tedeschi and Fox (2018) discuss the work that has been done to better predict the energy requirements of heat-stressed *Bos taurus* cattle (technically dairy cattle). Fox and Tylutki (1998) developed current effective temperature index (**CETI**) from published data and models derived from the work in *Bos indicus* cattle. CETI is based on the ambient temperature, hours of exposed sunlight, relative humidity and rate of wind speed to develop a heat stress adjustment factor (HSF) with a coefficient of determination of 0.99. The HSF is a value greater than one that can be multiplied by the basal NEm to predict the energy required for heat dissipation. It has been established that the different genotypes of *Bos indicus*, *Bos taurus* beef and *Bos taurus* dairy cattle have different maintenance requirements (NASEM, 2016). There are no models for HSF adjustments in beef cattle, and current software adjustments use either the CETI for dairy cattle or the panting index for *Bos indicus* cattle. The CETI model has been validated in dairy cattle with great precision and could likely be adapted easily for beef cattle. Research using more extreme temperature and humidity may also be necessary to ensure models are within the scope of realistic environmental factors.

In addition to predicting energy requirements of heat-stressed beef cattle, simultaneous objectives should be investigated regarding rumen digestibility of feeds. It has been suggested that the decreases in DMI observed in cattle under heat stress are offset to some degree by the greater ruminal digestibility due to the increased retention time (Beede

and Collier, 1986). However, this likely varies depending on the total digestible nutrients (**TDN**) of the diet, intake level, temperature of the rumen and liquid dilution effects. To my knowledge, there have not been any beef cattle studies investigating this interaction. Additionally, extreme environmental temperature interactions with dietary ingredients may alter the kinetics of rumen fermentation which is outside the scope of our current models. Theoretically, finishing cattle with slower passage rates may experience greater accumulation of acids in the rumen, resulting in lower pH and impose a greater risk of ruminal dysfunction. Current models include estimations for mean ruminal pH on the basis of physically effective NDF inclusion of the diet. However, mean pH does not elucidate the lower critical pH thresholds associated with the onset of ruminal acidosis. Establishing the effect size of environmental and dietary risk factors related to ruminal acidosis may increase precision diet formulation and offer greater flexibility for different situations. Future research should address rumen kinetics of different diets at gradients of temperatures outside the thermoneutral zone of beef cattle.

Efficient diets & growth-promoting feed additives

In terminal cattle, feedlot diets are nutrient dense, rich in processed grain and readily fermentable. Highly fermentable diets promote greater digestibility and rate of feed conversion over forage-based diets. Finishing cattle typically spend 100 – 180 days on a high grain diet to reach desirable end goals (weight or carcass merit) based on the current market situations. However, there is an inherent risk of feeding highly fermentable diets which can cause ruminal disturbances. Rapid fermentation may result in acid accumulation in the rumen which exceeds the animal ability to properly buffer, resulting in ruminal acidosis. The severity of acidosis (acute or sub-acute) is probably animal-dependent, but in the literature,

critical pH thresholds have been established that cause systemic changes in the rumen microflora (Nagaraja and Titgemeyer, 2007, Nagaraja and Lechtenberg, 2007). Ruminal acidosis results in time-off feed and cattle who experience a bout of acidosis are often subject to repeated occurrences (Dohme et al., 2008). Chronic acidosis may lead to rumenitis, parakeratosis of the rumen wall, laminitis and in extreme cases death from metabolic acidosis (Nocek, 1997; Owens et al., 1998, Nagaraja and Titgemeyer, 2007). Acidosis may also be linked with liver abscesses and which consequently negatively impact animal growth productivity. Paced adaptation to high grain diets is critical for transitioning cattle and ruminal health (Fernando et al., 2010). However, bouts of acidosis may also occur in previously adapted cattle when cattle suddenly consume a larger than normal meal. Environmental conditions may also play a role in ruminal acidosis as temperature may affect feeding behavior and ruminal passage rate which both affect the rate and extent of nutrient fermentation. Much research has been conducted to identify and reduce the incidence of acidosis in feedlot cattle. New technology has been pursued of indwelling rumen monitoring systems that may help to better characterize rumen dysfunction and sub-clinical stress indicators (AlZahal et al., 2008).

Feed additives, specifically antibiotics and ionophores, are rumen modifying technologies currently used to alter fermentation end products to meet the efficiency goals. Manipulation of ruminal fermentation is an attempt to optimize feeding and growth performance and reduce the risks associated with aggressive feeding regimens. Feeding antibiotics and ionophores have been shown to decrease CH_4 and ammonia (NH_3) output and increase rumen efficiency of feed ingredients, reduction of the acetate: propionate ratio, reduce ruminal disorders, improving nutrient utilization, decreasing time on feed (Brown et

al., 1975; Raun et al., 1976, Poos et al., 1979; Fuller and Johnson, 1981, Goodrich et al., 1984; Smith et al., 1989; Morris et al., 1990; Casey et al., 1994; Rogers et al., 1995; de Paula Lana, 1997; Coe et al., 1999; Nagaraja and Chengappa, 1998; Salles et al., 2008; Nickell and White, 2010) and thus optimizing the use of natural resources. The effects of ionophores and antibiotics are caused by altering the populations and productivity of the rumen microbiota. Feed grade antibiotics are intended to target the cell wall or protein synthesis (Madigan et al., 1997) of bacteria (largely Gram-positive) which include the primary offenders of low ruminal pH, *Streptococcus bovis* and *Lactobacillus spp*., which have been observed to have a doubling time of 24 minutes (Russell and Tsuneo, 1985). Antibiotics currently used in the diets of beef cattle include, but are not limited to Bacitracin, Bambermycin, Chlortetracycline, Laidlomycin, Lasalocid, Neomycin (Oxytetracycline), Monensin, Sulfamethazine, and Virginiamycin, all of which are labeled for the *rate of gain* and *feed efficiency* (U.S. FDA, 2017). In some cases, feeding these products result in more favorable carcass traits which benefiting both the live and terminal side of the production chain (Brown et al., 1975; Nagaraja and Chengappa, 1998; Nickell and White, 2010). However, concerns over the increasing emergence of antibiotic-resistant bacteria in human health have caused researchers to seek alternatives (Tedeschi et al., 2011). Greater regulation has already been demonstrated by the implementation of the veterinarian feed directive (**VFD**), and in other countries, these technologies have already been banned. Additionally, there has been evidence of microbial adaptation to daily supplementation of growth-promoting feed additives over time (Guan et al., 2006) and the rumen has been implicated as a reservoir for resistance genes (Auffret et al., 2017; Hitch et al., 2018). This evidence has made the cattle

feeding industry hyper-aware that change is on the horizon and economically viable alternative technologies must be established quickly.

Antibiotic alternatives

There are two primary objectives of using feed-grade antibiotics in the diets of beef cattle fed high energy diets:

1) Alter ruminal fermentation pathways to improve feed conversion,

2) Reduce the risk of disease or feeding disorders

Ideally, products that increase the extent of ruminal (and total tract) digestibility at a rate that does not exceed the buffering capacity of the rumen are preferred. There are several rumen modifiers that may alter rumen fermentation that are naturally occurring in plants and lower organisms.

Plant compounds. There are some secondary plant compounds that have been investigated in the diets of cattle for their antimicrobial properties; tannins, saponins, terpenes, and essential oils. Due to their anti-nutritional factors in the plants, there has been some degree of interest in their ability to alter ruminal fermentation and more specifically their antimicrobial effects.

Tannins are the second largest polyphenolic compound behind lignin. Broadly, the hydroxyl groups of tannins bind proteins and metal ions both in feed particles and the surface receptors of bacteria. Patra and Saxena (2010) offer a comprehensive review on the effects of tannins in the diets of ruminants. Broadly, there is evidence that they may shift the metabolism of N from the rumen to the small intestine, slow the rate of fermentation and reduce overall CH_4 production. Tannins may also exert a health benefit and overall performance increase in small ruminants where an anthelminthic effect can be seen when

grazing tannin-containing foliage (Athanasiadou et al., 2001; Nguyen et al., 2005; Hoste et al., 2006; Iqbal et al., 2007). However, the slower rate of fermentation and decreased methane production is attributed to decreased microbial activity directly impacting overall carbohydrate digestibility in the rumen and therefore may result in decreased VFA production. Yet because of their negative effect on microbial activity, there has been evidence tannins may reduce the incidence and severity of bloat in cattle grazing wheat pasture or legume forages without affected performance (McMahon et la., 2000; Min et al., 2006). Much of the tannin literature has been done in small ruminants and grazing cattle. There have been few studies using tannins in feedlot scenarios that have found any beneficial response of growth performance. Beauchemin et al. (2007) fed 1 and 2 % of the dietary DM of in a 70 % forage diet to growing heifers and found no difference in performance CH_4 emissions and a linear decrease in the apparent digestibility of crude protein, suggesting condensed tannins indeed have a protein binding effect. Mezzomo et al. (2011) fed a low dose of tannins (0.4% DM intake) in a high-grain diet and found that crude protein utilization was improved by increasing the metabolizable protein flux to the duodenum. In 96 head of finishing steers Rivera-Méndez et al. (2017), found that low dose tannin inclusion (0.2, 0.4, and 0.6 % of DM intake) significantly increased ADG and G:F vs. controls by 6.5 and 5.5 %, respectively and saw a tendency for greater final BW. It should be noted that an ionophore was used in the basal diet and therefore may have been a beneficial additive effect combined with tannin inclusion (Rivera-Méndez et al., 2017).

Saponins are steroidal or triterpenoid glycosides and have significant interactions with cellular membranes (Makkar et al., 2007). Similar to tannins, saponins may enhance N retention (Makkar et al., 1999) and exert a bactericidal effect but more specifically affect

Gram-positive bacteria, similar to ionophores (Wang et al., 2000). Others have reported protozoacidal effects (Newbold et al., 1997). However, unlike tannins which are not digestible, some report that both saliva and some rumen bacteria may degrade saponins (Wang et al., 1997; Odenyo et al., 1997). The role of saponins in the diets of beef cattle is probably less promising than tannins, but their abundance in nature makes them worthy of greater research.

A wide variety of research exists for terpenes and essential oils, mostly in vitro. Their modes also revolve around reducing microbial activity, especially for zoonotic pathogens (Vikram et al., 2010; Pittman et al., 2011; Pendleton et al., 2012) and protein sparing (Castillejos et al., 2006). Anthelmintic effects have also been reported against cattle ticks (Ribeiro et al., 2008; Martinez-Velazquez et al., 2011). One study comparing essential oil (thymol, eugenol, vanillin, and limonene) with monensin and control fed cattle receiving a silage-based diet showed that at low doses (2 g/d) essential oil treatment resulted in similar feed efficiency as monensin (Benchaar et al., 2006). However, large-scale studies examining these plant extracts for growth performance are limited, but their use in many cattle feeding scenarios for several purposes seem promising.

Enzymes. Many fiber digesting enzymes have been promoted to enhance digestibility including cellulases, hemicellulases, xylanases, and β-glucanases. Many of these are derived from yeast (*Saccharomyces spp.*), fungal (*Aspergillus spp.*) and bacterial donors. A review by Beauchemin et al. (2003) has indicated that feeding exogenous fibrolytic enzymes has been very successful in improving fiber degradation in the diets of beef cattle for both forage-based and high-grain diet types. However, considerations should be made for each feeding scenario as there is evidence of a grain type by enzyme interaction

specifically between barley and corn pre-treated with cellulase or xylanase, where pre-treated barley increased feed efficiency, but pre-treated corn did not affect animal performance (Beauchemin et al., 1997). McAllister et al. (1999) showed that steers backgrounded with an enzyme-treated silage had significantly improved feed efficiency over control fed steers and when transitioned to a pre-treated finishing diet had greater ADG and tended to have better G:F. The authors also reported greater proportions of cellulolytic bacteria in rumen fluid and no differences in ruminal pH. Although the evidence for improved feed conversion and greater feed use is apparent in the literature, current pricing and availability may not be economically viable.

Direct-fed microbials. The U. S. Food and Drug Administration defines direct-fed microbials (**DFM**) as products that are purported to contain live (viable) microorganisms, typically bacteria and/or yeasts. The most widely used DFM in the cattle feeding industry is yeast products and is hence discussed in detail as it pertains to the following chapters.

Live yeast products, specifically *Saccharomyces cerevisiae*, have been fed in the industry to promote feed digestibility and to help stabilize ruminal pH, primarily in the dairy industry. *S. cerevisiae* is a single-celled eukaryote, can grow in both aerobic and anaerobic environments and has an attractive aroma, making it an easy additive to incorporate in animal diets. Although *Saccharomyces cerevisiae* feed additives have been widely used in the industry for many years and its entire genome has been sequenced, its mode of action in the bovine rumen has yet to be fully elucidated. The mode of action of yeast has been theorized based on both in vitro work and the biological responses of cattle. In vitro comparisons of different forage:concentrate ratios (30:70, 50:50, 70:30) with added yeast showed that there was an additive effect of energy density and yeast for digestibility, VFA production, CH_4

production, increased protozoal population and decreased NH_3 production (Carro et al., 1992). Wallace et al. (1994) found enhanced fiber-degradation of in vitro fermentations with added wild-type yeasts versus some non-respiratory mutants and hypothesized wild-yeast might scavenge oxygen in the rumen and enhance the viability of the existing consortium, improving digestibility. Later work (Newbold et al., 1998) conducted in continuous in vitro fermenters for 21 days revealed when live yeast was supplied at 2.5 % of the diet there was a 25% increase of bacterial mass. A grazing study using ruminally and duodenally cannulated steers reported greater bacterial N flow to the duodenum for yeast fed steers versus control when consuming actively growing summer forage (Olson et al., 1994). However, a more recent study resulted in less bacterial N flow to the duodenum when steers were consuming a 60:40 forage to concentrate TMR and yeast, although digestibility of the diet was still significantly improved over the controls (Lehloenya et al., 2008).

Another potential mode of action is that yeast enhances lactate utilization by *Selenomonas ruminantium* and *Megasphaera elsdenii* (Nisbet and Martin, 1990, 1991, 1993; Rossi et al., 1995). It is not known if live yeasts play a role in this stimulation or if they serve as nutrients themselves. It has been suggested that yeast may be a source of malate (Nisbet and Martin, 1990, 1991) which is a rate-limiting nutrient for *M. elsdenii* (Russell, 2002). Regardless, stimulation of lactate utilizers may increase the conversion activity of lactate to propionate which would be a desirable effect regarding gluconeogenesis by the animal host. This effect may also prevent ruminal pH from dropping into ranges where populations of fiber-loving bacteria may systemically die or become dormant. It is known that pH is positively correlated with fiber degradability (Mould et al., 1983). Therefore, by keeping the fiber-loving bacteria alive and stimulating lactate utilizers, there is positive

effect on ruminal digestibility and a reduced risk of ruminal acidosis (Newbold et al., 1996). Williams et al. (1991) found that feeding yeast at 7.5 g/d (5 x 10^9 CFU/g) in a 50:50 forage to concentrate diet to steers prevented ruminal pH from dropping below 6.0 at peak fermentation (vs. 5.6 for control fed) and also reduced the acetate:propionate ratio to 2.8:1 vs. control fed steers who had 3.3:1. This is indeed indicative of a possible stimulatory effect of yeast for the greater conversion of lactate to propionate. Vyas et al. (2014) addressed the viability (live vs. killed) of yeast in the diets of beef heifers and found that regardless of viability, digestibility was improved, and pH was stabilized more than control fed heifers. Vyas et al. (2014) also investigated differences the treatments invoked on the population of cellulolytic and lactate-utilizing bacteria and found no difference, although population does not equate to activity. Their results may be somewhat supportive of the proposed idea that stimulating effect of yeast on lactate utilizers is malate driven. Another study in steers fed various levels of forage (40, 60 and 80 %) supplemented with 10 g of yeast revealed a linear increase of NDF digestibility and also a linear reduction in the acetate:propionate ratio (Plata et al., 1994), suggesting stimulatory effect of bacterial population and favorable end-product shift.

In the beef cattle literature, dosing of yeast, diet, and animal class have been variable leading to inconsistent outcomes. However, a meta-analysis on the effects of yeast in the diets of dairy cattle has revealed statistically significant effects on digestibility, intake, ruminal pH and milk fat production (Desnoyers et al., 2009). Desnoyers et al. (2009) reported that yeast supplementation increased DMI by 1.1 %, improved milk yield and milk fat content by 2.5 and 1.2 %, respectively, and showed that ruminal pH and VFA concentration increased linearly with yeast dose. However, the authors admit there is large

variation in the data between experiments, and therefore the subtle differences in dairy cow performance parameters may also be difficult to detect in the analogous production parameters in beef cattle. An example of this problem was observed by Johnson and Rops (2000) who found no significant effect on feeding performance when supplementing yeast to receiving steers for 35 days consuming either a corn-based or soybean hull-based diet, although the numerical trends favored diets with yeast. The study by Vyas et al. (2014) also failed to detect any significant effect of feed performance, but the live yeast supplemented heifers were numerically favorable over the control and killed yeast treatments. A feed through slaughter experiment where steers were fed barley and potato processing residue diet and supplemented with yeast (85 g/d for 28 d and 28 g/d for 85 d) improved ADG by (0.10 kg/d) and without affected DMI, which resulted in greater 4.6 % higher G:F but the amount of yeast fed is not realistic for industry (Hinman et al., 1998). Yet the overall consensus among research conducted on beef cattle consuming high-grain diets is that yeast provides no improvement or may negatively impact feed and growth performance compared to control fed cattle (El Hassan et al., 1996; Mutsvangwa et al., 2010; Rodrigues et al., 2013) or even ionophore fed cattle (Mir and Mir, 1994). Mir and Mir (1994) compared the effects of yeast, lasalocid and their interaction on three diet types and found no detectable differences although numerically they performed better than controls. El Hassan et al. (1996) found no significant difference in growth performance of slaughter bulls between control and yeast fed subjects. Rodrigues et al. (2013) reported that yeast fed cattle performed lower than control cattle (ADG, G:F, HCW). Monnerat et al. (2013) found no benefit of feed efficiency or ruminal parameters when feeding yeast to steers consuming high-grain diets. Beauchemin et al. (2003) found no difference in digestibility or significant effect on acidosis

risk of steers fed a high grain diet supplemented with yeast and *Enterococcus faecium*, a known lactate producer. A later study investigating the blood chemistry and immune response of steers consuming an acidosis provocative diet, supplemented yeast and *E. faecium* resulted in a significant increase of acute phage proteins, likely due to the release of endotoxins by the lysis of Gram-negative bacteria at low pH (Emmanuel et al., 2007). Additionally, more research indicates that different strains of S. cerevisiae may have different effects on ruminal fermentation (Newbold et al., 1995). In a study in non-lactating beef cows, two strains of *S. cerevisiae* were compared for their effects on rumen pH and CH_4 production, researchers found that one strain selected for its 'enhanced fiber-degradation', decreased CH_4 production but significantly decreased rumen pH even below control fed cattle (Chung et al., 2011). Although it has been shown that on lower risk diets yeast aides to stabilize ruminal pH (primarily dairy cattle diets), the research indicates that as the energy density of the diet increases there must be some limiting threshold of which can't be circumvented by the effect of yeast.

As Mir and Mir (1994) summarized, yeast supplementation in the diets of feedlot cattle caused a 'consistent but nonsignificant improvement in growth parameters'. Throughout the literature, diet varies greatly, and not many have reported the concentration or strain of yeast used, possibly due to proprietary reasons by the manufacturing company. It may be that the effect size yeast elicits on growth performance is subtle and therefore lost in the animal to animal variation, signifying under-replication of experiments. On the contrary, there may be some diet by strain interactions that we have not yet identified or characterized. Nevertheless, the early work that proved yeast could improve digestibility and

stabilize ruminal pH indicates that larger trials are worth pursuing to identify its best use for growth promotion in the cattle feeding industry.

Bacteriophage Technology

Bacteriophage may be old technology but could be new age feed additive. Bacteriophages (phages) are viruses that infect bacteria. Phage are thought to be the most abundant life form on Earth and are highly pervasive predators of bacteria (Srinivasiah et al., 2008). Phage have been isolated from the ocean and freshwater sources, soil, and the GIT of mammals as part of their normal consortium and have been found to be as numerous as 10^{11} per mL of rumen fluid in the fed state (Klieve & Swain, 1993; Klieve et al., 1996). The history of bacteriophage therapy is well summarized by Atterbury (2009). In animal agriculture, phage have been increasingly looked to as a viable treatment of infectious or zoonotic bacteria in light of greater antibiotic resistance among bacteria.

Phage infection. Phage infection can be either lytic or lysogenic. Steps to successful phage infection are summarized in Hyman and Abedon (2010) and are as follows. The first two steps are the same for all phage types; 1) viral adsorption to host by recognizing host receptors, 2) phage genome ejection into the host cytoplasm. For obligately lytic phage, host take-over begins with the synthesis of viral proteins and DNA necessary for new virions by host ribosomes. Once the viral particles are assembled into complete daughter virions, virus-specific proteins and enzymes work in synchrony to lyse the bacterial host releasing the new virions into the environment to infect new hosts. The number of virions released or *burst size* of a single phage infection varies by phage and may range from as few as 40 in the marine Cyanophage S-PM2 (Brown et al., 2006) to as many as 10,000 in the bacteriophage MS2 of *E. coli* (Grosjean and Fiers, 1982). However, releasing very large numbers of virions

into the environment does not automatically result in a more effective phage. If the phage:bacteria ratio (multiplicity of infection; MOI) is too high, phage may lyse bacteria prematurely in what is known as “lysis from without” (Delbrück, 1940). Still, for the bacteria, the result is the same.

For temperate (lysogenic) phage the viral DNA integrates into the host chromosome, or exists as an unintegrated episomal element, replicating along with the bacterium during cellular division. The infected bacteria is now a lysogen, and the phage is now a prophage providing immunity to obligately lytic phage infections. Under normal bacterial growth conditions, the prophage often provides some fitness advantage to its host. However, lysogenic phage still pose a potential threat to the host bacterium. In some cases, if the bacteria is threatened by the prospect of DNA damage, an induction event could cause the virus to abort the safety of its host, as this threat would also compromise the viral DNA. Exposure to UV light, extreme heat, or Mitomycin C are known to cause the induction of a lysogenic phage to lyse its host. Some lysogenic phage has no known induction signal and seem to spontaneously induce lysis. This makes a lysogenic phage a high-risk, high-reward parasite of bacteria.

Callaway et al. (2008) and Gill and Hyman (2010) agree that temperate phages should be avoided for use in phage therapy. Gill and Hyman (2010) explain their reasoning to be: 1) the fitness advantage provided by the genes of the temperate phage may make the bacterial host a more effective disease-causing agent, 2) the lysogens may be immune to superinfection of the same or related phage, and lastly, 3) lysogenic phage can mediate the movement of bacterial DNA from one bacterium to another *generalized transduction*, contributing the horizontal gene transfer. Research in livestock species proves the latter

theory is merited (Colomer-Lluch et al., 2011). Allen et al. (2011) used a metagenomic approach that suggests that feeding antibiotics to livestock may cause the induction of existing prophage which may aid in conferring horizontal gene transfer of antibacterial resistance factors between bacteria. Another study revealed that phages isolated from antibiotic-treated mice conferred increased drug resistance to their bacterial community (Modi et al., 2013). These authors have suggested that the natural presence of lysogenic phage in animal microbiome may be partially responsible for the decreased efficiency of antibiotics over time.

Bacterial protective measures. Due to the co-evolution of bacteria and phage, it is no surprise that bacteria have evolved mechanisms for phage resistance. These mechanisms have been extensively studied in *Lactococcus* bacteria and are well summarized in a review by Garvey et al., (1995). The most effective mechanisms by which bacteria may resist infection are: 1) Adsorption or uptake blocks, 2) prevention of host take-over by restriction enzymes & Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) immune response, and lastly 3) Abortive infection (Seed, 2015).

Bacteria may employ the production of physical barriers, changes the cell-surface to disguise receptors or even the downregulation or deletion of those surface receptors. The first line of defense is to block phage adsorption and uptake through mediated changes in the cell surface. Bacteria have many surface molecules that play integrally in their survival. However vital they are to the bacterium they may also serve as surface receptors to predatory phage. Bacteria circumvent phage infection by producing an exopolysaccharide capsule and/or be protected in a biofilm, both of which serve as a physical barrier to reduce encounters with phage. Some of the cell-surface fitness changes may be mediated by

plasmids or a prophage (Garvey et al., 1995). A report by Scholl et al., (2005) revealed that the capsule produced form *E. coli* K1 was sufficient to block phage T7 infection, but the artificial degradation of the capsule resulted in successful T7 infection. More work by Scholl and Merril (2005) revealed that phage K1F, likely evolved from a common ancestor with phage T7, was able to recognize and degrade capsule due to a replacement of the C-terminal portion of the tail fiber with an endosialidase domain. Therefore, although the production of capsule may slow the rate of infection of one phage, it may inadvertently become more recognizable by another (Weinbauer, 2004).

The loss of bacterial surface receptors another method to shield itself from adsorption and uptake. Surface molecules serve as essential roles to the survival of the bacterium, and their loss or down-regulation may seriously compromise its persistence in that environment, which still agrees with our mission to reduce their impact. Despite their significant contribution to cell survival, mutations and modifications are likely common. Selective pressure by a predatory phage may result in the succession of mutant strains displaying discreet modification in the structure of receptors, known as phage-resistant mutants. A prime example of this is reported German and Misra (2001) where a mutation of the TolC surface receptor protein of *E. coli* resulted in immunity from a phage of which the parent strain was previously susceptible.

If phage are successful in injecting their DNA into the host cytoplasm the second line of defense is the restriction-modification system (RMS) and CRISPR protein. The RMS system is found in all prokaryotes and broadly acts on non-methylated foreign DNA recognizing and cutting target sequences. However, there is significant evidence that phage have their own adaptions to evade detection (Hoskisson and Smith, 2007) and some

researchers encourage the genetic engineering of phage for this purpose (Skiena, 2001). The CRISPR is an enzyme which recognizes sequences of invading phage DNA that the bacteria has already encountered. Sorek et al. (2008) reported that the CRISPR system has been identified in approximately 40% of all sequenced bacterial genomes. Similarly to RMS evasion, there is genomic evidence that phage may escape CRISPR recognition by rapid recombination of sequence motifs (Andersson and Banfield, 2008).

The last line of defense against phage is an abortive infection. The programmed cell death has been described as *bacterial altruism*, in that, although the bacterium dies as a result, ultimately it protects its community from a similar fate (Chopin et al., 2005). Nevertheless, occasionally intact virions may escape, but they are fewer and have less vigor.

Phage therapy in Livestock. Most phage therapy trials conducted in livestock have been pursuant of known zoonotic pathogens, *Campylobacter spp.*, *E. coli*, *Listeria spp*., *Salmonella spp*., and *Staphylococcus spp.,* especially in regions where there has been increased regulation of feed-grade antibiotics (Atterbury, 2009). Huff et al., (2002) reported a two-phage aerosol was highly successful in reducing mortality (up to 50 %) of broiler chicks experimentally infected with an avian-pathogenic strain of *E. coli*. Follow up studies reported that intramuscular injection of the same phage were also successful in reducing mortality from 48 % to 7 % when higher titers were used which was not different from the mortality of non-challenged, negative controls (Huff et al., 2006). Other research has shown that the oral application of *Salmonella* phage cocktails may persist in the GI tract of broiler chickens, providing long-term control of the foodborne pathogen (Fiorentin et al., 2005). In larger livestock, research has indicated that successful phage therapy is highly dependent on phage application route, titer, and site of colonization by the target bacteria (Gill et al., 2006;

Raya et al., 2006; Sheng et al., 2006; Callaway et al., 2008). Gill et al. (2006) found that, in lactating dairy cows, naturally occurring mastitis caused by *Staphylococcus aureus* was not significantly cured or reduced by phage infusion through the affected gland, and that titers of phage recovery were significantly lower than the titer of the inoculum, suggesting interfering host immunity, or potentially inactivation by milk proteins, to the site of application. Sheng et al. (2006) experimentally infected mice, sheep and cattle with *E. coli* O157:H7 and found that oral administration of phage was sufficient to eliminate infection from the mice, however, oral administration and direct application of phage to the site of colonization in sheep and steers was not sufficient to eliminate bacteria, although it significantly reduced the population. Raya et al. (2006) and Callaway et al. (2008) also found that orally administered phage cocktails significantly reduced but did not eliminate *E. coli* O157:H7 of experimentally infected sheep and steers and deduced the ideal PFU to CFU ratios based on the GIT volume of the experimental subjects. In all three of the previous studies, the lack of complete elimination has been suggested to be the result of phage-resistant mutants. Phage-resistant mutants reiterate the suggestion by Callaway et al. (2008) that the use of phage therapy is not a magic bullet but can be used in a multi-hurdle approach to significantly reduce persistence and population of a target bacterium. In swine, the oral administration of phage prevented the colonization of *Salmonella* Typhimurium, as well as other serovars, of naïve pigs co-mingled with infected pigs compared with mock-treated naïve pigs, although this study was relatively short (Wall et al., 2010). However, this provides an incentive for the use of phage therapy as a preventative measure rather than a cure. There are many situations in the livestock production, especially in the highly fractured

beef production chain, to which preventative measures could be more advantageous than treatment.

Phage as feed additives. Although there is ample evidence that phage therapy reduces morbidity and mortality of livestock, research into their use as growth-promoting feed additives has not been well studied. Behind bolstering the immune system, the secondary benefit of feed grade antibiotics is their alteration of the gut microbiome that promotes efficient feed conversion. There have been few studies comparing the effectiveness of antibiotics versus phage therapy. A study that compared the effectiveness of feed antibiotics or orally administered phage on experimentally infected Roman chicks with enteropathogenic *E. coli* reported that the phage treatment reduced morbidity more than antibiotics (26 vs. 36 % affected, respectively) as well as mortality (1.2 vs. 6%, death loss) (Xie et al., 2005). They also reported that length of morbidity for phage treated chicks was significantly reduced within a week compared to antibiotic-treated chicks (0 vs. 12.4 % affected by week 2). Logically, healthier chicks would result in greater feed conversion rates (Huff et al., 2002) and smaller faster-growing livestock disease reduction has the greatest effect of production efficiency and is the reasoning for all the bacterial challenge studies. However, in beef cattle, the major benefit of feeding antibiotics is the alteration of rumen consortium by broadly affecting many Gram-positive bacteria, decreased intake, and the major shift in volatile fatty acid profile.

The diversity of the rumen microbiota may pose challenges for the use of phage therapy as antibiotic alternatives. Although there have been several isolated from the bovine rumen (Klieve et al., 1996), there are some limiting factors for their use associated with the rumen environment. As mentioned previously, the rumen is a highly competitive

environment and bacterial populations flux throughout the day upon feeding and fasting. McAllister and Newbold (2008) expressed concerns that because phage must come into contact with their host to cause infection, their effectiveness could oscillate due to the dynamics of a reinforcing loop of a predator-prey model. This reiterates the need for the establishment of the mean and variation of target bacterial populations in the bovine rumen to determine that critical threshold for consistent infection and control. Another concern is that because phage are typically very strain specific, even in populations with relative genetic homogeneity, that the elimination of a few strains may not cause a significant impact on ruminal fermentation. Ruminal acidosis is a major problem of feedlot cattle which may decrease feed efficiency and is currently regulated with feeding antibiotics which act broadly against Gram-positive bacteria. Klieve et al. (1999) investigated the genomic diversity of the 37 strains of *Streptococcus bovis,* the primary instigator of lactic acidosis in ruminants, and the host range of some *S. bovis* phage finding that although *S. bovis* strains were closely related even to other *Streptococcus* species, phage were only effective on the strains they were grown on. Klieve et al. (1999) also isolated *S. bovis* phage during this experiment which turned out to be temperate suggesting that wild *S. bovis* likely carry prophage. It is not likely that the removal of one or two strains of bacteria from the bovine rumen will have a very large impact on fermentation. Therefore, suitable candidates should be identified which may have the greatest impact on feed efficiency, those which may impact other organs related to the efficiency of metabolism, or those that are just inherently wasteful to the bovine host. Despite the challenges, Klieve et al. (1999) faced in their pursuit of lytic phage for *S. bovis* and other lactic acid producing bacteria remains a valiant endeavor as non-clinical sub-acute ruminal acidosis plagues the cattle feeding industry causing an unknown amount of

unrealized revenue. *Fusobacterium necrophorum*, along with a few others, found in the rumen but which also colonize the liver creating abscesses may also be pursued. There is growing evidence that liver abscesses negatively affect feedlot performance in beef cattle (McKeith et al., 2012; Rezac et al., 2014; Reinhardt and Hubbert, 2015) and affect both the live and terminal side of beef production. An example of wasteful rumen bacteria are the hyper-ammonia-producing bacteria (**HAB**) of the rumen. The most well studied are *Clostridium aminophilum*, *Clostridium sticklandii* and *Peptostreptococcus anaerobius*, and Russell (2002) indicated that these bacteria deaminate valuable amino-acids and peptides resulting in an α-ketoacid, NH_3 and free H_2 in the rumen. While these bacteria and their related cohorts are not a highly prevalent population in the rumen, the rate at which they deaminate amino acids makes them quantitatively important (Russell, 2002). Consequently, they are wasteful to the host and negatively influence the environment through the increased excretion of NH_3 and, by contributing to the pool of available H_2, potential influence on CH_4 production.

Another approach using phage as a means of bacterial control may be the use of their lytic enzymes. Although it is not recommended to use temperate phage as a means of therapy, in cases like the persistent *Clostridium* species where lytic phage are difficult to find, the lysis enzymes produced by the phage may still be harvested and applied directly to bacteria rather than the phage itself (Zimmer et al., 2002; Mayer et al., 2008; Seal, 2013). Similar to antibiotics, phage lytic enzymes work by disrupting cell membrane and is known as *endolysin therapy*. There is concern that this could produce endolysin resistant bacteria, however, in vitro models have found no such threat (Briers et al., 2014; Herpers, 2015). It could be that phage may offer us a simple more direct tool that acts slightly more broadly on

our target Gram-positive bacteria. This is very promising if it can be harnessed for future use as a feed additive.

Implications for the future

The pursuit of viable antibiotic alternatives is a relatively urgent matter with the threat of greater regulation on the horizon and will be a massive undertaking by researchers and industry leaders. Plant secondary compounds, enzymes, direct-fed microbials and phage therapy all hold promise as growth-promoting feed additives which alter rumen fermentation pathways. There is no one *cure-all* that can span the entirety of the cattle feeding industry to improve health and performance. For our industry to be sustainable without antibiotic feed additives, we will need to develop highly integrated systems that address the most pressing needs for each sector of the production chain. Additionally, these technologies will have to withstand the changes that may occur under a warming climate.

LITERATURE CITED

AlZahal, O., E. Kebreab, J. France, M. Froetschel, and B. McBride. 2008. Ruminal temperature may aid in the detection of subacute ruminal acidosis. Journal of Dairy Science 91: 202-207.

Amachawadi, R. G., and T. G. Nagaraja. 2016. Liver abscesses in cattle: A review of incidence in Holsteins and of bacteriology and vaccine approaches to control in feedlot cattle12. Journal of Animal Science 94: 1620-1632. doi: 10.2527/jas.2015-0261.

Andersson, A. F., and J. F. Banfield. 2008. Virus population dynamics and acquired virus resistance in natural microbial communities. Science 320: 1047-1050.

Athanasiadou, S., I. Kyriazakis, F. Jackson, and R. Coop. 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: in vitro and in vivo studies. Veterinary Parasitology 99: 205-219.

Auffret, M. D. et al. 2017. The rumen microbiome as a reservoir of antimicrobial resistance and pathogenicity genes is directly affected by diet in beef cattle. Microbiome 5: 159.

Beauchemin, K., S. Jones, L. Rode, and V. Sewalt. 1997. Effects of fibrolytic enzymes in corn or barley diets on performance and carcass characteristics of feedlot cattle. Canadian Journal of Animal Science 77: 645-653.

Beauchemin, K., S. McGinn, T. Martinez, and T. McAllister. 2007. Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle1. Journal of Animal Science 85: 1990-1996.

Beauchemin, K. et al. 2003. Effects of bacterial direct-fed microbials and yeast on site and extent of digestion, blood chemistry, and subclinical ruminal acidosis in feedlot cattle. Journal of Animal Science 81: 1628-1640.

Beede, D., and R. Collier. 1986. Potential nutritional strategies for intensively managed cattle during thermal stress. Journal of Animal Science 62: 543-554.

Benchaar, C., J. Duynisveld, and E. Charmley. 2006. Effects of monensin and increasing dose levels of a mixture of essential oil compounds on intake, digestion and growth performance of beef cattle. Canadian Journal of Animal Science 86: 91-96.

Briers, Y. et al. 2014. Art-175 is a highly efficient antibacterial against multidrug-resistant strains and persisters of *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy 58: 3774-3784.

Brouwer, E. 1965. Report of sub-committee on constant factors. In energy metabolism: Proceedings of 3rd Symposium held at Troon, Scotland, May 1964, 441-443. KL Blaxter, ed. EAAP Publ. No. 11. London, UK: Academic Press.

Brown, C. M., J. E. Lawrence, and D. A. Campbell. 2006. Are phytoplankton population density maxima predictable through analysis of host and viral genomic DNA content? Journal of the Marine Biological Association of the United Kingdom 86: 491-498.

Carro, M. D., P. Lebzien, and K. Rohr. 1992. Influence of yeast culture on the in vitro fermentation (Rusitec) of diets containing variable portions of concentrates. Animal Feed Science and Technology 37: 209-220. doi: https://doi.org/10.1016/0377-8401(92)90005-Q.

Castillejos, L., S. Calsamiglia, and A. Ferret. 2006. Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow in in vitro systems. Journal of Dairy Science 89: 2649-2658.

Chopin, M.-C., A. Chopin, and E. Bidnenko. 2005. Phage abortive infection in lactococci: variations on a theme. Current opinion in microbiology 8: 473-479.

Chung, Y.-H., N. Walker, S. McGinn, and K. Beauchemin. 2011. Differing effects of 2 active dried yeast (*Saccharomyces cerevisiae*) strains on ruminal acidosis and methane production in nonlactating dairy cows. Journal of Dairy Science 94: 2431-2439.

Coe, M. et al. 1999. Effect of virginiamycin on ruminal fermentation in cattle during adaptation to a high concentrate diet and during an induced acidosis. Journal of Animal Science 77: 2259-2268.

Colomer-Lluch, M., L. Imamovic, J. Jofre, and M. Muniesa. 2011. Bacteriophages carrying antibiotic resistance genes in fecal waste from cattle, pigs, and poultry. Antimicrobial agents and chemotherapy 55: 4908-4911.

de Paula Lana, R. 1997. Effects of monensin on ruminal bacteria, ruminal fermentation and feedlot performance. Cornell University, August.

Delbrück, M. 1940. The growth of bacteriophage and lysis of the host. The journal of general physiology 23: 643-660.

Desnoyers, M., S. Giger-Reverdin, G. Bertin, C. Duvaux-Ponter, and D. Sauvant. 2009. Meta-analysis of the influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminants. Journal of Dairy Science 92: 1620-1632.

Dohme, F., T. J. DeVries, and K. A. Beauchemin. 2008. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: ruminal pH. Journal of Dairy Science 91: 3554-3567. doi: https://doi.org/10.3168/jds.2008-1264.

El Hassan, S., C. Newbold, I. Edwards, J. Topps, and R. Wallace. 1996. Effect of yeast culture on rumen fermentation, microbial protein flow from the rumen and live-weight gain in bulls given high cereal diets. Journal of Animal Science 62: 43-48.

Emmanuel, D., A. Jafari, K. Beauchemin, J. Leedle, and B. Ametaj. 2007. Feeding live cultures of *Enterococcus faecium* and *Saccharomyces cerevisiae* induces an inflammatory response in feedlot steers. Journal of Animal Science 85: 233-239.

Fernando, S. C. et al. 2010. Rumen microbial population dynamics during adaptation to a high-grain diet. Applied Environmental Microbiology 76: 7482-7490. doi: 10.1128/AEM.00388-10.

Fiorentin, L., N. D. Vieira, and W. Barioni Jr. 2005. Oral treatment with bacteriophages reduces the concentration of Salmonella Enteritidis PT4 in caecal contents of broilers. Avian Pathology 34: 258-263.

Fox, D., and T. Tylutki. 1998. Accounting for the effects of environment on the nutrient requirements of dairy cattle. Journal of Dairy Science 81: 3085-3095.

Fuller, J., and D. Johnson. 1981. Monensin and Lasalocid Effects on Fermentation. Journal of Animal Science 53: 1574-1580.

Garrett, W. 1980. Factors influencing energetic efficiency of beef production. Journal of Animal Science 51: 1434-1440.

Garvey, P., D. Van Sinderen, D. Twomey, C. Hill, and G. Fitzgerald. 1995. Molecular genetics of bacteriophage and natural phage defence systems in the genus Lactococcus. International Dairy Journal 5: 905-947.

German, G. J., and R. Misra. 2001. The TolC protein of *Escherichia coli* serves as a cell-surface receptor for the newly characterized TLS bacteriophage1. Journal of Molecular Biology 308: 579-585.

Gill, J. et al. 2006. Efficacy and pharmacokinetics of bacteriophage therapy in treatment of subclinical *Staphylococcus aureus* mastitis in lactating dairy cattle. Antimicrobial agents and chemotherapy 50: 2912-2918.

Gill, J. J., and P. Hyman. 2010. Phage choice, isolation, and preparation for phage therapy. Current pharmaceutical biotechnology 11: 2-14.

Goodrich, R. et al. 1984. Influence of monensin on the performance of cattle. Journal of Animal Science 58: 1484-1498.

Grosjean, H., and W. Fiers. 1982. Preferential codon usage in prokaryotic genes: the optimal codon-anticodon interaction energy and the selective codon usage in efficiently expressed genes. Gene 18: 199-209.

Guan, H., K. Wittenberg, K. Ominski, and D. Krause. 2006. Efficacy of ionophores in cattle diets for mitigation of enteric methane. Journal of Animal Science 84: 1896-1906. doi: 10.2527/jas.2005-652.

Herpers, B. 2015. Endolysins: redefining antibacterial therapy. Future microbiology 10: 309-311.

Hinman, D. D., S. Sorensen, P. Momont, R. Albin, and N. Cole. 1998. Effect of Yeast Culture on Steer Performance, Apparent Diet Digestibility, and Carcass

Measurements When Used in a Barley and Potato Finishing Diet1. The Professional Animal Scientist 14: 173-177.

Hitch, T. C., B. J. Thomas, J. C. Friedersdorff, H. Ougham, and C. J. Creevey. 2018. Deep sequence analysis reveals the ovine rumen as a reservoir of antibiotic resistance genes. Environmental Pollution 235: 571-575.

Hoskisson, P. A., and M. C. Smith. 2007. Hypervariation and phase variation in the bacteriophage 'resistome'. Current opinion in microbiology 10: 396-400.

Hoste, H., F. Jackson, S. Athanasiadou, S. M. Thamsborg, and S. O. Hoskin. 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. Trends in Parasitology 22: 253-261.

Huff, W., G. Huff, N. Rath, J. Balog, and A. Donoghue. 2002. Prevention of *Escherichia coli* infection in broiler chickens with a bacteriophage aerosol spray. Poultry Science 81: 1486-1491.

Huff, W., G. Huff, N. Rath, and A. Donoghue. 2006. Evaluation of the influence of bacteriophage titer on the treatment of colibacillosis in broiler chickens. Poultry Science 85: 1373-1377.

Hyman, P., and S. T. Abedon. 2010. Bacteriophage host range and bacterial resistance Advances in Applied Microbiology No. 70. p 217-248. Elsevier.

Iqbal, Z. et al. 2007. Direct and indirect anthelmintic effects of condensed tannins in sheep. Veterinary Parasitology 144: 125-131.

Johnson, B., and B. Rops. 2000. The effects of energy source and yeast (Biosaf Sc47) on feedlot performance during the receiving period.

Klieve, A. V., and R. A. Swain. 1993. Estimation of ruminal bacteriophage numbers by pulsed-field gel electrophoresis and laser densitometry. Applied Environmental Microbiology 59: 2299-2303.

Klieve, A., R. Swain, and J. Nolan. 1996. Bacteriophages in the rumen: types present, population size and implications for the efficiency of feed utilisation. In: Proceedings-Australian Society of Animal Production. p 92-94.

Klieve, A., G. Heck, M. Prance, and Q. Shu. 1999. Genetic homogeneity and phage susceptibility of ruminal strains of *Streptococcus bovis* isolated in Australia. Letters in applied microbiology 29: 108-112.

Lehloenya, K., C. Krehbiel, K. Mertz, T. Rehberger, and L. Spicer. 2008. Effects of *propionibacteria* and yeast culture fed to steers on nutrient intake and site and extent of digestion. Journal of Dairy Science 91: 653-662.

Lofgreen, G., and W. Garrett. 1968. A system for expressing net energy requirements and feed values for growing and finishing beef cattle. Journal of Animal Science 27: 793-806.

Madigan, M. T., J. M. Martinko, and J. Parker. 1997. Brock Biology of Microorganisms. Prentice Hall, Upper Saddle River, NJ.

Makkar, H. P., P. Siddhuraju, and K. Becker. 2007. Saponins Plant Secondary Metabolites. p 93-100. Springer.

Martinez-Velazquez, M. et al. 2011. Acaricidal effect and chemical composition of essential oils extracted from *Cuminum cyminum*, *Pimenta dioica* and *Ocimum basilicum* against the cattle tick *Rhipicephalus (Boophilus) microplus (Acari: Ixodidae)*. Parasitology research 108: 481-487.

Mayer, M. J., A. Narbad, and M. J. Gasson. 2008. Molecular characterization of a *Clostridium difficile* bacteriophage and its cloned biologically active endolysin. Journal of Bacteriology 190: 6734-6740.

McAllister, T. et al. 1999. Effect of exogenous enzymes on digestibility of barley silage and growth performance of feedlot cattle. Canadian Journal of Animal Science 79: 353-360.

McGinn, S., K. Beauchemin, T. Coates, and D. Colombatto. 2004. Methane emissions from beef cattle: effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. Journal of Animal Science 82: 3346-3356. doi: 10.2527/2004.82113346x.

McMahon, L. et al. 2000. A review of the effects of forage condensed tannins on ruminal fermentation and bloat in grazing cattle. Canadian Journal of Plant Science 80: 469-485.

Mezzomo, R. et al. 2011. Influence of condensed tannin on intake, digestibility, and efficiency of protein utilization in beef steers fed high concentrate diet. Livestock Science 141: 1-11. doi: https://doi.org/10.1016/j.livsci.2011.04.004.

Min, B., W. Pinchak, R. Anderson, J. Fulford, and R. Puchala. 2006. Effects of condensed tannins supplementation level on weight gain and in vitro and in vivo bloat precursors in steers grazing winter wheat. Journal of Animal Science 84: 2546-2554.

Mir, P., and Z. Mir. 1994. Effect of live-yeast culture and lasalocid supplementation on performance of growing-finishing steers fed alfalfa-silage, corn-silage and high-grain diets sequentially. Canadian Journal of Animal Science 74: 563-566.

Modi, S. R., H. H. Lee, C. S. Spina, and J. J. Collins. 2013. Antibiotic treatment expands the resistance reservoir and ecological network of the phage metagenome. Nature 499: 219.

Monnerat, J. P. I. d. S. et al. 2013. Effects of *Saccharomyces cerevisiae* and monensin on digestion, ruminal parameters, and balance of nitrogenous compounds of beef cattle fed diets with different starch concentrations. Tropical Animal Health and Production 45: 1251-1257. doi: 10.1007/s11250-013-0356-9.

Morris, F. et al. 1990. Effect of rotating monensin plus tylosin and lasalocid on performance, ruminal fermentation, and site and extent of digestion in feedlot cattle. Journal of Animal Science 68: 3069. doi: 10.2527/1990.68103069x.

Mould, F., E. Ørskov, and S. Mann. 1983. Associative effects of mixed feeds. I. Effects of type and level of supplementation and the influence of the rumen fluid pH on cellulolysis in vivo and dry matter digestion of various roughages. Animal Feed Science and Technology 10: 15-30.

Mutsvangwa, T., I. E. Edwards, J. H. Topps, and G. F. M. Paterson. 2010. The effect of dietary inclusion of yeast culture (Yea-Sacc) on patterns of rumen fermentation, food intake and growth of intensively fed bulls. Animal Science 55: 35-40. doi: 10.1017/S0003356100037247.

Nagaraja, T., and M. Chengappa. 1998. Liver abscesses in feedlot cattle: a review. Journal of Animal Science 76: 287-298.

Nagaraja, T., and K. F. Lechtenberg. 2007. Acidosis in feedlot cattle. Veterinary Clinics: Food Animal Practice 23: 333-350.

Nagaraja, T., and E. Titgemeyer. 2007. Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook 1, 2. Journal of Dairy Science 90: E17-E38.

National Academy of Sciences, Engineering, and Medicine. 2016. Nutrient Requirements of Beef Cattle. (8th ed.). Nutrient requirements of domestic animals. National Academy Press, Washington, DC.

Newbold, C., S. El Hassan, J. Wang, M. Ortega, and R. Wallace. 1997. Influence of foliage from African multipurpose trees on activity of rumen protozoa and bacteria. British Journal of Nutrition 78: 237-249.

Newbold, C., F. McIntosh, and R. Wallace. 1998. Changes in the microbial population of a rumen-simulating fermenter in response to yeast culture. Canadian Journal of Animal Science 78: 241-244.

Newbold, C., R. Wallace, X. Chen, and F. McIntosh. 1995. Different strains of *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers in vitro and in sheep. Journal of Animal Science 73: 1811-1818.

Newbold, C. J., R. Wallace, and F. McIntosh. 1996. Mode of action of the yeast *Saccharomyces cerevisiae* as a feed additive for ruminants. British Journal of Nutrition 76: 249-261.

Nguyen, T. M., D. Van Binh, and E. Ørskov. 2005. Effect of foliages containing condensed tannins and on gastrointestinal parasites. Animal Feed Science and Technology 121: 77-87.

Nickell, J. S., and B. J. White. 2010. Metaphylactic antimicrobial therapy for bovine respiratory disease in stocker and feedlot cattle. Veterinary Clinics of North America: Food Animal Practice 26: 285-301.

Nisbet, D., and S. Martin. 1991. Effect of a *Saccharomyces cerevisiae* culture on lactate utilization by the ruminal bacterium *Selenomonas ruminantium*. Journal of Animal Science 69: 4628-4633.

Nisbet, D. J., and S. A. Martin. 1990. Effect of dicarboxylic acids and Aspergillus oryzae fermentation extract on lactate uptake by the ruminal bacterium *Selenomonas ruminantium*. Applied and Environmental Microbiology 56: 3515-3518.

Nisbet, D. J., and S. A. Martin. 1993. Effects of fumarate, l-malate, and an Aspergillus oryzae fermentation extract on d-lactate Utilization by the ruminal bacterium *Selenomonas ruminantium*. Current Microbiology 26: 133-136.

Nocek, J. E. 1997. Bovine acidosis: Implications on laminitis. Journal of Dairy Science 80: 1005-1028.

Olson, K. C., J. S. Caton, D. R. Kirby, and P. L. Norton. 1994. Influence of yeast culture supplementation and advancing season on steers grazing mixed-grass prairie in the northern Great Plains: II. Ruminal fermentation, site of digestion, and microbial efficiency. Journal of Animal Science 72: 2158-2170. doi: 10.2527/1994.7282158x.

Owens, F., D. Secrist, W. Hill, and D. Gill. 1998. Acidosis in cattle: a review. Journal of Animal Science 76: 275-286.

Patra, A. K., and J. Saxena. 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. Journal of the Science of Food and Agriculture 91: 24-37.

Pendleton, S. J., P. G. Crandall, S. C. Ricke, L. Goodridge, and C. A. O'Bryan. 2012. Inhibition of beef isolates of *E. coli* O157:H7 by orange oil at various temperatures. Journal of Food Science 77: 308-311.

Pittman, C. I. et al. 2011. Activity of citrus essential oils against *Escherichia coli* O157:H7 and Salmonella spp. and effects on beef subprimal cuts under refrigeration. Journal of Food Science 76: 433-438.

Plata, F. P., G. D. M. M, J. R. Bárcena-Gama, and S. G. M. 1994. Effect of a yeast culture (*Saccharomyces cerevisiae*) on neutral detergent fiber digestion in steers fed oat straw based diets. Animal Feed Science and Technology 49: 203-210. doi: https://doi.org/10.1016/0377-8401(94)90046-9.

Poos, M., T. Hanson, and T. Klopfenstein. 1979. Monensin effects on diet digestibility, ruminal protein bypass and microbial protein synthesis. Journal of Animal Science 48: 1516-1524.

Raun, A., C. O. Cooley, E. Potter, R. Rathmacher, and L. Richardson. 1976. Effect of monensin on feed efficiency of feedlot cattle. Journal of Animal Science 43: 670-677.

Raya, R. R., P. Varey, R. A Oot, M. R. Dyen, T. R. Callaway, T.S. Edrington, E. M. Kutter, A. D. Brabban. 2006. Isolation and characterization of a new T-even bacteriophage, CEV1, and determination of its potential to reduce *Escherichia coli* O157: H7 levels in sheep. Applied and Environmental Microbiology 72: 6405-6410.

Ribeiro, V. L., V. Rolim, S. Bordiognon, A. T. Henriques, G. G. Dorneles, R. P. Limberger, G. Von Poser. 2008. Chemical composition and larvicidal properties of the essential oils from *Drimys brasiliensis* Miers (Winteraceae) on the cattle tick *Rhipicephalus*

(*Boophilus*) *microplus* and the brown dog tick *Rhipicephalus sanguineus*. Parasitology Research 102: 531-535.

Rivera-Méndez, C., A. Plascencia, N. Torrentera, and R. Zinn. 2017. Effect of level and source of supplemental tannin on growth performance of steers during the late finishing phase. Journal of Applied Animal Research 45: 199-203.

Rodrigues, É. et al. 2013. Performance, carcass characteristics and gain cost of feedlot cattle fed a high level of concentrate and different feed additives. Revista Brasileira de Zootecnia 42: 61-69.

Rogers, J. et al. 1995. Effects of dietary virginiamycin on performance and liver abscess incidence in feedlot cattle. Journal of Animal Science 73: 9-20.

Rossi, F., P. Cocconcelli, and F. Masoero. 1995. Effect of a *Saccharomyces cerevisiae* culture on growth and lactate utilization by the ruminal bacterium *Megasphaera elsdenii*. In: Annales de Zootechnie. p 403-409.

Russell, J. B. 2002. Rumen microbiology and its role in ruminant nutrition. Cornell University.

Russell, J. B., and T. Hino. 1985. Regulation of lactate production in *Streptococcus bovis*: a spiraling effect that contributes to rumen acidosis. Journal of Dairy Science 68: 1712-1721.

Salles, M. S. V., M. A. Zanetti, E. A. L. Titto, and R. M. C. Conti. 2008. Effect of monensin on performance in growing ruminants reared under different environmental temperatures. Animal Feed Science and Technology 147: 279-291.

Scholl, D., S. Adhya, and C. Merril. 2005. *Escherichia coli* K1's capsule is a barrier to bacteriophage T7. Applied and Environmental Microbiology 71: 4872-4874.

Seal, B. S. 2013. Characterization of bacteriophages virulent for *Clostridium perfringens* and identification of phage lytic enzymes as alternatives to antibiotics for potential control of the bacterium. Poultry Science 92: 526-533.

Seed, K. D. 2015. Battling phages: How bacteria defend against viral attack. PLoS Pathogens 11: e1004847.

Sheng, H., H. J. Knecht, I. T. Kudva, and C. J. Hovde. 2006. Application of bacteriophages to control intestinal *Escherichia coli* O157: H7 levels in ruminants. Applied and Environmental Microbiology 72: 5359-5366.

Skiena, S. S. 2001. Designing better phages. Bioinformatics 17: S253-S261.

Smith, M. et al. 1989. The effect of virginiamycin on performance of feedlot cattle. Animal Science Research Report—Agricultural Experiment Station, Oklahoma State University No. MP-127: 137-141.

Sniffen, C. J., J. O'Connor, P. Van Soest, D. Fox, and J. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. Journal of Animal Science 70: 3562-3577.

Sorek, R., V. Kunin, and P. Hugenholtz. 2008. CRISPR—a widespread system that provides acquired resistance against phages in bacteria and archaea. Nature Reviews Microbiology 6: 181.

Srinivasiah, S., J. Bhavsar, K. Thapar, M. Liles, T. Schoenfeld and E. K. Wommack. 2008. Phages across the biosphere: contrasts of viruses in soil and aquatic environments. Research in Microbiology 159: 349-357.

Tedeschi, L. O., and D. G. Fox. 2018. The Ruminant nutrition system: an applied model for predicting nutrient requirements and feed utilization in ruminants, 2nd edition, XanEdu, Acton, MA.

Vikram, A., P. R. Jesudhasan, G. K. Jayaprakasha, B. S. Pillai, and B. S. Patil. 2010. Grapefruit bioactive limonoids modulate *E. coli* O157:H7 TTSS and biofilm. International Journal of Food Microbiology 140: 109-116.

Vyas, D. et al. 2014. The effects of active dried and killed dried yeast on subacute ruminal acidosis, ruminal fermentation, and nutrient digestibility in beef heifers. Journal of Animal Science 92: 724-732. doi: 10.2527/jas.2013-7072.

Wall, S. K., J. Zhang, M. H. Rostagno, and P. D. Ebner. 2010. Phage therapy to reduce preprocessing Salmonella infections in market-weight swine. Applied and Environmental Microbiology 76: 48-53.

Wallace, R. 1994. Ruminal microbiology, biotechnology, and ruminant nutrition: progress and problems. Journal of Animal Science 72: 2992-3003.

Wang, Y., T. McAllister, C. Newbold, P. Cheeke, and K.-J. Cheng. 1997. Effects of Yucca extract on fermentation and degradation of saponins in the Rusitec. In: Proceedings-American Society of Animal Science Western Section. p 149-152.

Wang, Y., T. McAllister, L. Yanke, and P. Cheeke. 2000. Effect of steroidal saponin from *Yucca schidigera* extract on ruminal microbes. Journal of Applied Microbiology 88: 887-896.

Weinbauer, M. G. 2004. Ecology of prokaryotic viruses. FEMS Microbiology Reviews 28: 127-181.

Williams, P., C. Tait, G. Innes, and C. Newbold. 1991. Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. Journal of Animal Science 69: 3016-3026.

Xie, H., X. Zhuang, J. Kong, G. Ma, and H. Zhang. 2005. Bacteriophage Esc-A is an efficient therapy for *Escherichia coli* 3-1 caused diarrhea in chickens. The Journal of General and Applied Microbiology 51: 159-163.

Zimmer, M., S. Scherer, and M. J. Loessner. 2002. Genomic analysis of *Clostridium perfringens* bacteriophage φ3626, which integrates into guaA and possibly affects sporulation. Journal of Bacteriology 184: 4359-4368.

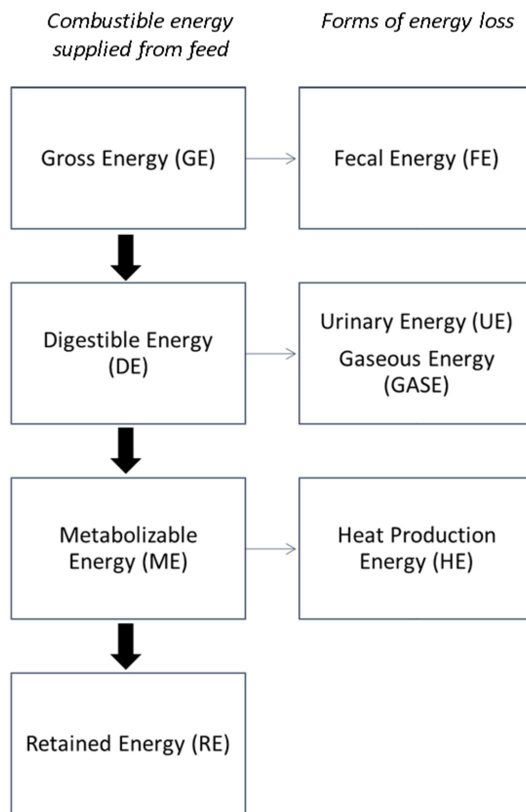

Figure 1.1. Schematic of the flow of energy in the net energy system of beef cattle. Adapted from National Academies of Sciences, Engineering, and Medicine (2016).

CHAPTER II

EFFECTS OF ROTATING ANTIBIOTIC AND IONOPHORE FEED ADDITIVES ON VOLATILE FATTY ACID PRODUCTION, POTENTIAL FOR METHANE PRODUCTION, AND MICROBIAL POPULATIONS OF STEERS CONSUMING A MODERATE-FORAGE DIET*

SYNOPSIS

Ionophores and antibiotics have been shown to decrease ruminal methanogenesis both in vitro and in vivo but have shown little evidence toward a sustainable means of mitigation. Feed additive rotation was proposed and investigated for methane, VFA, and microbial population response. In the present study, cannulated steers (n = 12) were fed a moderate-forage basal diet in a Calan gate facility for 13 wk. In addition to the basal diet, steers were randomly assigned to 1 of 6 treatments: 1) control, no additive; 2) bambermycin, 20 mg bambermycin/d; 3) monensin, 200 mg monensin/d; 4) the basal diet + weekly rotation of bambermycin and monensin treatments (B7M); 5) the basal diet + rotation of bambermycin and monensin treatments every 14 d (B14M); and 6) the basal diet + rotation of bambermycin and monensin treatments every 21 d (B21M). Steers were blocked by weight in a randomized complete block design where the week was the repeated measure.

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Rumen fluid was collected weekly for analysis (n = 13), and results were normalized according to individual OM intake (OMI; kg/d). Potential activity of methane production was not significantly different among treatments ($P > 0.05$). However, treatment tended to affect the CH_4–to-propionate ratio ($P = 0.0565$), which was highest in the control and lowest in the monensin, B21M, and B14M treatments (0.42 vs. 0.36, 0.36, and 0.33, respectively). The CH_4:propionate ratio was lowest in wk 2 and 3 ($P < 0.05$) but the ratio in wk 4 to 12 was not different from the ratio in wk 0. Week also affected total VFA, with total VFA peaking at wk 3 and plummeting at wk 4 (4.02 vs. 2.86 mM/kg OMI; $P < 0.05$). A significant treatment × week interaction was observed for the acetate-to-propionate (A:P) ratio, where bambermycin- and rotationally fed steers did not have a reduced A:P ratio compared with monensin-fed steers throughout the feeding period ($P < 0.0001$). Microbial analysis revealed significant shifts, but several predominant classes showed adaptation between 4 and 6 wk after additive initiation. There was no significant evidence to suggest that rotations of monensin and bambermycin provided additional benefits to steers consuming a moderate-forage diet at the microbial/animal and environmental level versus those continuously fed.

INTRODUCTION

Methane (CH_4) is a natural gaseous end product of anaerobic microbial fermentation of ruminants. The production of enteric CH_4 represents an energetic loss to the animal at the microbe level, directly contributes to greenhouse gas production within the agriculture sector, and has been determined to be alterable with diet management (Pitesky et al., 2009; Place and Mitloehner, 2014; Caro et al., 2016; Tedeschi and Fox., 2016). Several feed additives have been shown to alter the ruminal microbiome of beef cattle, improve feed

efficiency, and decrease enteric methane production (Tedeschi et al., 2003; McGinn et al., 2004; Guan et al., 2006; Van Nevel and Demeyer, 2008; Patra, 2012). Many of these feed additives are commercially available and already widely continuously fed throughout many phases of beef production systems.

Although ionophore and antibiotic feed additives may shift end products toward propionigenesis and initially reduce CH_4, over time, a diminishing response may be seen due to rumen microbial adaptation (Rumpler et al., 1986; Callaway et al., 2003; Guan et al., 2006). To maximize potential effectiveness, some researchers have investigated the usefulness of rotating feed additives on a daily, weekly, or every two weeks basis as a potential management strategy (Hicks et al., 1988; Morris et al., 1990; Duff et al., 1995; Guan et al., 2006). However, most research in this area has focused on effects of rotating different ionophore compounds on cattle fed high-concentrate diets. The long-term effectiveness of many feed additives on VFA and CH_4 production is not well defined in moderate-forage basal diet scenarios, and there have been no studies comparing the long-term rotational effects of monensin and bambermycin.

Therefore, the objective of this trial was to determine the short- and long-term effects of feeding bambermycin and monensin, continuously or rotationally, to steers consuming a moderate-forage diet on fermentation end products, the potential for methane production, and dynamics of rumen microbial populations.

MATERIALS AND METHODS

Animals and Feeding

Twelve ruminally cannulated Angus-cross long yearling steers (approximately 18 mo of age) weighing 344 ± 22 kg were cared for in accordance with approval by the Institutional Animal Care and Use Committee of Texas A&M University (number 2014-0242). Steers were fed in confinement in the Calan gate (American Calan Inc., Northwood, NH) facility at the Animal Science Teaching and Research Extension Center in College Station, TX, for 101 d. Steers were blocked by weight into 2 pens with 6 bunks per pen, and each treatment group was represented once per pen. Steers were bunk trained and adapted to the moderate-forage basal diet (Table 2.1) for 14 d prior to the start of the 13-wk feeding trial. Dry matter intake was restricted to 2% of individual BW, with a target ADG of 0.45 kg/d, and adjusted each week according to increased BW. Steers were randomly assigned to 1 of 6 dietary treatments: 1) control, containing the basal diet and no additive; 2) bambermycin, the basal diet + 20 mg bambermycin/d (Huvepharma, Inc., Peachtree City, GA); 3) monensin, the basal diet + 200 mg monensin/d (Elanco Animal Health, Greenfield, IN); 4) the basal diet + weekly rotation of bambermycin and monensin treatments (B7M); 5) the basal diet + rotation of bambermycin and monensin treatments every 14 d (B14M); and 6) the basal diet + rotation of bambermycin and monensin treatments every 21 d (B21M). After the initial 14-d adaptation period, steers were fed the basal diet without an additive for 1 wk (Week 0) prior to the start of feed additive treatments (wk 1–12). To ensure appropriate animal dosing, feed additives were separately weighed out in the laboratory and top-dressed in the morning feeding event. Basal diet feed samples were taken each week for 13 wk,

homogenized for a representative sample, and analyzed for nutrient content by Cumberland Valley Analytical Services (Hagerstown, MD; Table 2.1).

Animal Sampling and In Vitro Analyses

Rumen fluid collections were taken at the end of each week for 13 wk at 3 h following the morning feeding. Approximately 1 L of rumen fluid was taken from 3 places in the rumen, strained through 8 layers of cheesecloth, and preserved in individual thermoses for each steer. Animal weights were taken prior to the morning feeding to monitor and make feed intake adjustment.

Immediately following collection, rumen fluid was transported to the laboratory and prepared for pH, CH_4, and VFA analyses. The pH of each rumen fluid sample was recorded using a VWR sympHony benchtop meter (VWR International, Radnor, PA). Potential activity of methane production (**PAMP**; Anderson et al., 2006) was determined by in vitro incubation of 5 mL of each steer's respective rumen fluid in 5 mL of an anaerobic dilution media (Bryant and Burkey, 1953). The anaerobic media also contained 60 m*M* sodium formate as a methanogenic substrate and 0.2 g of ground (2 mm) alfalfa hay as the microbial substrate. Tubes were flushed with CO_2 prior to adding rumen fluid from treated steers, sealed, and incubated for 3 h at 39°C. Headspace samples were taken by airtight syringe and methane concentration was determined by gas chromatography (Allison et al., 1992). Next, approximately 8 mL of rumen fluid was transferred to 4 new 2-mL microtubes, which were spun at a centrifugal force of 11,337 × *g* for 3 min at 18° to obtain cell-free supernatant, which was frozen at −20°C. Supernatant VFA concentration was measured by gas chromatography (Hinton et al., 1990).

In Vitro Gas Production Technique

The in vitro gas production technique was used to determine the digestibility and fermentation characteristics of bermudagrass hay using the ionophore- and antibiotic-treated rumen fluid. A medium-quality bermudagrass hay, the energy value of which was similar to that of the basal diet, was selected and fermented for 48 h at intermittent time points throughout the feeding period. In vitro fermentations took place in an incubation chamber equipped with multiplate stirrers at 39°C for 48 h in replicate (Tedeschi et al., 2008; Williams et al., 2010). The in vitro gas production technique has been previously described, but briefly, approximately 200 mg of bermudagrass hay was weighed into 160-mL Wheaton bottles containing equal-sized stir bars. Samples were wetted with 2 mL of deionized water to reduce particle scattering during CO_2addition to maintain an oxygen-reduced atmosphere. Goering and Van Soest's (1970) in vitro buffering media (14 mL) was added to each bottle under constant CO_2 flushing, and the bottles were sealed with a butyl rubber stopper and crimp sealed. Bottles were promptly placed in the incubation chamber to begin heating to rumen temperature. Rumen fluid inoculum was obtained from the treated steers and was filtered through 1 layer of cheesecloth and then, subsequently, filtered through glass wool under a CO_2 atmosphere. Adapted rumen fluid inoculum (4 mL) was injected into predetermined bottles to represent each treatment group. Internal pressure was equilibrated across all bottles after inoculation by inserting needles into the stoppers for approximately 5 s. After 48 h, bottles were set in an ice bath to stop fermentation. Head space samples were removed and analyzed for methane concentration using the same gas chromatography method as previously mentioned (Allison et al., 1992). Rumen fluid pH was measured, and approximately 40 mL of neutral detergent solution (ANKOM Technology,

Macedon, NY) was added to each bottle of fermented feed residue. Bottles were resealed and set in the autoclave for 15 min at 121°C. Samples were then filtered using Whatman 54 paper (Sigma-Aldrich Corporation, St. Louis, MO) to collect the washed feed residue to calculate DM digestibility (**DMD**).

The kinetic analysis of the 48-h fermentation of bermudagrass hay was evaluated using nonlinear functions, and that with the lowest sum of square errors was selected (Schofield et al., 1994). The nonlinear fitting was performed using Gasfit (http://www.nutritionmodels.com/gasfit.html; accessed 20 Feb. 2017), which executes specific R (R Development Core Team, 2014) scripts to perform convergence of gas production data using the *nls* function (Chambers and Bates, 1992) and the *port* algorithm (Fox et al., 1978; Gay, 1990). Preliminary results indicated the exponential with discrete lag nonlinear function had the lowest sum of squared errors and best fitted the fermentation profiles (Williams et al., 2010).

Rumen Microbial DNA Extraction and Sequencing

The remaining rumen fluid sample (approximately 50 mL of fluid) was frozen at −20°C for further microbial DNA analysis. Rumen fluid samples were shipped on dry ice for bacterial diversity analysis (MR DNA [Molecular Research LP], Shallowater, TX). Genomic DNA was extracted using a QIAamp DNA Mini Kit (Qaigen; Hilden, Germany), and concentrations were equalized and prepared for amplification. The 16S rRNA gene (V3–V4 variable region) was analyzed (PCR primers 341F and 805R with barcode on the forward primer) in a 30-cycle PCR (5 cycles used on PCR products) using a HotStarTaq Plus Master Mix Kit (Qaigen; Hilden, Germany) under the following conditions: 94°C for 3 min followed by 28 cycles of 94°C for 30 s, 53°C for 40 s, and 72°C for 1 min, after which a final

elongation step at 72°C for 5 min was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated AMPure XP beads (Beckman Coulter, Inc., Pasadena, CA). Then, the pooled and purified PCR product was used to prepare DNA library by following Illumina TruSeq DNA library preparation protocol (Illumina, Inc. San Diego, CA). Sequencing was performed at MR DNA (http://www.mrdnalab.com [accessed 15 March 2017]; Shallowater, TX) on a MiSeq Illumina System, Illumina, Inc.) following the manufacturer's guidelines. Sequence data were processed using the MR DNA analysis pipeline, a customized pipeline of Qiime (MR DNA [Molecular Research LP]). In summary, sequences were joined and depleted of barcodes. Then, sequences < 150 bp and those with ambiguous base calls were removed. Sequences were then denoised, operational taxonomic units (**OTU**) were generated, and chimeras were removed. Operational taxonomic units were defined by clustering at 3% divergence (97% similarity). Final OTU were taxonomically classified using the nucleotide basic local alignment search tool (BLASTN; https://blast.ncbi.nlm.nih.gov [accessed 4 June 2015) against a curated database derived from Greengenes (DeSantis et al., 2006), RDP release 11 (http://rdp.cme.msu.edu; accessed 4 June 2015), and the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov [accessed 4 June 2017]). The microbial consortium is quantifiably reported as percent relative abundance (% relative abundance). Overall, 5,769,938 cleaned sequences were generated with a read length range of 384 to 446 bp and an average length of 439 bp. No ambiguous bases were detected. The average quality score [Q] score was 39, and 99.31% of sequences had a Q score ≥ 30.

Statistical Analysis

Due to differences in DMI among the steers, raw data of VFA and CH_4measurements were normalized, and data herein is represented as concentration per unit of OM intake (**OMI**; response variable value divided by respective kg of feed OMI). Data were analyzed using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC) as a randomized complete block design and week as a repeated measure. The following model was used:

$$Y_{ijkl} = \mu + T_i + W_j + B_k(T_i) + T_iW_j + e_{ijkl}$$

in which *yijkl* is the response variables (pH, total VFA, acetate, propionate, butyrate, acetate-to-propionate [**A:P**] ratio, PAMP, CH_4:propionate ratio, and microbial guilds), μ is the overall mean, T*i* is the fixed effect of treatment (*i* = control, monensin, bambermycin, B7M, B14M, and B21M), W*j* is the fixed effect of week (*j* = wk 0, 1, …, 12), B*k*(T*i*) is the random effect of treatment within a block (*k* = pen 1 or pen 2 for the *i*th treatment), T*i*W*j* is the interaction of treatment and week, and *eijkl* is the random error associated with the measurement at week *j* on the *l*th subject assigned to treatment *i* within the *k*th block.

An autoregressive covariance structure was chosen when analyzing the rumen fluid measurements (pH, total VFA, acetate, propionate, butyrate, A:P ratio, PAMP, and CH_4:propionate ratio) to convey that weekly variance in repeated measurements taken closer together may be more correlated than those taken farther apart for wk 0 to 12. A spatial powers covariance structure was used for microbial analysis because time points were unequally spaced (0, 3, 4, 5, 6, and 12 weeks).

The in vitro fermentation of bermudagrass hay was analyzed using PROC MIXED of SAS as a 4 × 6 factorial arrangement. The following model was used:

$$Y_{ij} = \mu + T_i + W_j + T_iW_j + e_{ij}$$

in which y_{ij} is the response variables (total gas production, fractional degradation rate, lag time, DMD, CH_4, CH_4:DMD ratio, and pH), μ is the overall mean, T_i is the fixed effect of treatment (i = control, monensin, bambermycin, B7M, B14M, and B21M rumen fluid inoculum), W_j is the fixed effect of week (j = wk 1, 3, 6, or 12), T_iW_j is the interaction of treatment and week, and e_{ij} is the random error associated with the measurement at week j on the hay sample assigned treatment i. Least squares means and SE were reported, and a 95% confidence level was used to determine significant differences among least squares mean multiple comparisons.

RESULTS AND DISCUSSION

Rumen Fluid Analysis

***Rumen Fluid Measurements*.** The pH of the rumen fluid was not different across treatments (Table 2.2) but changed over time, as week effect was significant (P = 0.0221; Table 2.3). During wk 3, pH was lowest (6.23) and was different from that in wk 0, 4, and 5 (6.45, 6.42, and 6.47, respectively). This is likely a result of the differences in VFA production from week to week. Volatile fatty acids and PAMP are expressed as the millimole concentration and millimoles per kilogram OMI to normalize across different intakes. Total VFA (m*M* of acetate + propionate + butyrate and isobutyrate) were not affected by treatment but were affected by week (P < 0.0001). Total VFA production (expressed as both m*M* and m*M*/kg OMI) was greatest during wk 3 and was different from all other weeks. When expressed as millimoles, VFA for wk 0 was not different from that of wk 6 and 12 but, when normalized for intake wk 0, was not different from that of wk 6 but was greater than that of wk 12. It is interesting to note the increase of total VFA toward wk 3 followed by the sharp

drop in wk 4. There was a significant interaction of the A:P ratio (P = 0.0007; Figure 2.1). From wk 0 to 3, the A:P ratio increased for the control, bambermycin, and B21M treatments whereas it decreased for the monensin, B7M, and B14M treatments. This is likely the effect of the duration of monensin being fed in the continuous and more frequently rotated treatments. After wk 3 control, the bambermycin and monensin treatments showed an increase of the A:P ratio toward wk 6 whereas the B7M, B14M, and B21M treatments tended to fluctuate between the values of the continuous treatments. Continuous treatments did decrease the A:P ratio more than the control treatment, but rotational treatments (B7M, B14M, and B21M) did not reduce the A:P ratio more than the continuous feeding of monensin.

The PAMP ranged from 2.68 to 10.24 mM (0.0742 to 0.274 mM/kg OMI) throughout the trial, similar to the range of other reports (Hu et al., 2005). There was no effect of treatment on PAMP, likely due to the methodology for quantification. For the purposes of this study, we were interested in the maximum potential for methane production based on methanogen population rather than dietary substrate availability to methanogens at a given point in time. There was an effect of the week on PAMP (P < 0.0001). Normalized for intake, PAMP was greatest during wk 0, as expected. Least squares means of PAMP were not different for wk 3, 4, 5, or 6 and were significantly lower (23 to 32%) for wk 0. This reduction in PAMP is similar to other results of feeding antibiotics and ionophores (Guan et al., 2006; Van Nevel and Demeyer, 2008). However, PAMP in wk 0 was not different from that in wk 12 (0.234 vs. 0.224 mM CH_4/kg OMI), indicating that the reduction in PAMP was not sustained long term. This result is the same when PAMP is expressed as millimoles. This finding is consistent with previous research where methane production of cattle consuming

high-forage diets showed an adaptive response (approximately 6 wk) to feed additives (Johnson et al., 1997; Guan et al., 2006). It is known that propionate formation competes with the CH_4 formation (Russell and Strobel, 1989), and therefore, we examined the ratio of these measurements. The potential activity of the CH_4:propionate ratio was not affected by treatment but was affected by week ($P < 0.0001$). The CH_4:propionate ratio significantly decreased from wk 0 to wk 3 but, although lower still, was no longer significantly different by wk 4. The CH_4:propionate ratio during wk 4 through 6 was 15 to 18% lower than during wk 0, but the CH_4:propionate ratio during wk 12 was about 8% greater than during wk 0. Other researchers have reported the lowest A:P ratio and greatest CH_4suppression to occur between 2 and 6 wk when cattle were treated with ionophores before returning to near-baseline levels (Rumpler et al., 1986; DelCurto et al., 1998; Guan et al., 2006). Our findings suggest a reduction in methane production in the short term but possibly due to a reduction in overall VFA production.

In Vitro Gas Production Technique. When bermudagrass hay was fermented with the adapted rumen fluid inoculum, a treatment × week interaction was revealed for DMD, CH_4, and the CH_4:DMD ratio ($P = 0.0014$, $P = 0.0138$, and $P = 0.0078$, respectively; Figures 2.2a, 2.22b, and 2.2c). Only data from wk 1, 3, and 6 are presented. Week 12 collection was eliminated from the analysis as the inoculum was compromised during extreme weather events and, therefore, deemed to be unreliable. Dry matter digestibility of bermudagrass hay varied little when using the control inoculum (52.74, 55.27, and 57.92% for wk 1, 3, and 6, respectively). Bambermycin-adapted inoculum decreased DMD from wk 1 to 3 and significantly increased DMD at wk 6 (69.3, 38.25, and 72.92%, respectively). The DMD for monensin-adapted inoculum did not differ between wk 1 and 3 but also significantly

increased during 6 (44.67, 46.80, and 90.87%, respectively). Rotationally adapted inoculum decreased DMD from wk 1 to 3 but the DMD remained below that of the control through wk 6 (69.4 vs. 43.66% pooled average for wk 1 and 6, respectively). Interestingly, bermudagrass hay fermented with the control inoculum produced much less CH_4 during wk 3 than other treatments but was not different during wk 1 and 6 (Figure 2.3). When expressed as a CH_4:DMD ratio, the interaction is more clear, where bermudagrass hay fermented with bambermycin- and rotationally treated inoculum resulted in greater CH_4 per unit of DM digested versus the control and monensin treatments when decreased over time, albeit at different rates (Figure 2.3). These findings may suggest a short-term inefficiency in the fermentation kinetics of the bambermycin and monensin treatments as the microbial consortium are adjusting. This outcome also confirms the previously mentioned thought that reduced methane production might be a result of lower digestibility and thus lower VFA production as observed in the rumen fluid analysis.

Microbial Analysis

Archaea. The 16s RNA analysis returned 6 genera of archaea identified in the rumen fluid samples, which accounted for 1.35% of the total bacterial and archaeal OTU classified: *Methanobacterium spp*.; *Methanobrevibacter spp*.; *Methanomicrobium spp*.; *Methanosphaera spp*.; *Nitrosoarchaeum limnia*, an ammonia oxidizer (Mosier et al., 2012); and *Thermoplasma spp*., a genus specialized in respiration of sulfur (Hedderich et al., 1998; Carberry et al., 2014). The predominant archaeal genus (<90% relative abundance) was *Methanobrevibacter*, which is consistent with several reports (Tokura et al., 1999; Janssen and Kirs, 2008; Wright et al., 2008; Zhou and Hernandez-Sanabria, 2009; Tan et al., 2011).

There were no treatment effects on archaeal genera (Table 2.6). Week affected the population of *Methanobacterium* and *Methanobrevibacter* as well as *Methanosphaera*, *Nitrosoarchaeum limnia*, and *Thermoplasma* (Table 2.7). Although it is unlikely that the treatment directly affected the archaea, it is clear that there were significant shifts in population throughout the feeding period, even among the control-fed steers, indicating that duration of treatment (week) may have indirectly affected archaea. Relative abundance of *Methanobacterium* was greatest during wk 0 and, although numerically lower, this relative abundance was not different from that during wk 3 and 6 (1.881, 1.070, and 1.351%, respectively) but was significantly different from that during wk 4, 5, and 12 (0.510, 0.536, and 0.528%, respectively). This is likely due to gaps in the cross-feeding efficiency between archaea and the shifting bacterial consortium. *Methanobacterium spp*. have been shown to feed solely on H_2 and CO_2 and formate (Paynter and Hungate, 1968). Many of the predominant rumen bacteria produce formate as well as contribute to the pool of available H_2 and CO_2, and therefore, bacterial population changes or changes in their activity may have reduced the substrate available to this archaeon (Russell, 2002). This is further supported by the correlation of *Methanobacterium* with total VFA production and acetate production ($R^2 = 0.25$, $P = 0.0313$ and $R^2 = 0.30$, $P = 0.0109$, respectively). Populations of *Methanobrevibacter* were significantly higher during wk 4 than during wk 3, 5, and 6 (96.82 vs. 90.69, 90.55, and 90.52%, respectively) but were not different from those during wk 0 or 12. Populations of *Methanosphaera spp*. were greatest during wk 0 and were significantly reduced (>50%) during all other weeks (1.32 vs. 0.493, 0.427, 0.025, 0.470, and 0.536%, respectively). It is not surprising to see this low prevalence, as *Methanosphaera* has a metabolism restricted to H_2 and methanol and is typically not as prevalent in higher-forage

diets as compared with concentrate diets (Carberry et al., 2014). Additionally, it is likely that treatments affected bacteria capable of fermenting pectin and therefore reduced methanol availability to this genus, likely accounting for the difference here between wk 0 and other weeks (McFeeters et al., 2001). Populations of *Nitrosoarchaeum limnia* significantly increased between wk 4 and 5 (0.042 vs. 0.930%, respectively) but were not different from those during other weeks. Because *Nitrosoarchaeum limnia* predominately oxidizes ammonia into nitrite, it may be that there was a population shift of proteolytic bacteria occurring between wk 4 and 5, thereby providing more substrate to the archaea. Populations of *Thermoplasma spp*. significantly shifted among wk 0, 3, 4, and 5 (2.943, 6.297, 2.202, and 6.929%, respectively), but those during wk 0, 4, and 12 were not different. Little is known about the role *Thermoplasma* plays in the rumen, but its presence has been detected by several researchers (Irbis and Ushida, 2004; Wright et al., 2006; Tan et al., 2011; Carberry et al., 2014) and it has been described as a scavenger of the decomposing elements of other microorganisms and has the ability to tolerate extreme acidity (Ruepp et al., 2000) and temperature (Reysenbach and Brileya, 2014) as well as respire sulfur (Carberry et al., 2014). Due to its relative abundance and its unknown role in the rumen, more research should be conducted on this genus of archaea.

Bacteria. Gram-positive bacteria have been shown to be more susceptible to monensin and bambermycin than Gram-negative bacteria (Chen and Russell, 1989; Russell and Strobel, 1989; Butaye et al., 2003; Pfaller, 2006). Therefore, it was expected that treatment would negatively affect the relative abundance of those bacteria that have an exposed cell wall and create a competitive advantage for those bacteria with an outer cell membrane. Unexpectedly, there was a considerable variation on percent relative abundance

of bacteria among individuals steers during wk 0 (Figure 2.3), even after being adapted to the diet for 14 d, whereas the average percent relative abundance of Gram-positive bacteria in the rumen of all steers was 51.07% during wk 0 and populations ranged between 18.5 and 87% for individuals. This finding is echoed in other reports where the animal-to-animal variation may be just as great as or greater than the dietary treatment variation (Li et al., 2009; Jami et al., 2014).

Overall, 27 phyla were identified in the samples; however, a combined 23 phyla accounted for less than 3.3% of those detected. The 2 dominate phyla identified were *Firmicutes* (42.6%) and *Bacteroidetes* (49.3%), as expected based on previous research reports, followed by *Spirochaetes* (2.93%) and the candidate phylum TM7 *Candidatus Saccharibacteria* (1.88%). At the phylum level, there were no significant interactions of treatment × week on the total Gram-positive bacteria population, Gram-negative bacteria population, or *Firmicutes*-to-*Bacteroidetes* (**F:B**) ratio. There were, however, significant treatment and time effects on these variables, as shown in Table 2.8 and Table 2.9. The B21M treatment resulted in the greatest F:B ratio, differing from all other treatments. Control-treated rumen fluid had the lowest F:B ratio and was not different from monensin or bambermycin treatments but was significantly lower than rotationally treated rumen fluid. In general, the percent relative abundance of Gram-positive bacteria was less than the Gram-negative bacteria across all treatments, contrary to other findings for high-forage diets (Fernando et al., 2010). Treatment effects revealed that the control and bambermycin treatments had the lowest percent relative abundance of Gram-positive bacteria (36.28 and 36.55%, respectively); the monensin, B7M, and B21M treatments were intermediate (44.12, 45.50, and 47.78%, respectively); and the B14M treatment had the greatest (53.07%) but

was not different from the B21M treatment. The percent relative abundance of Gram-negative bacteria shows the counter mean separation and is shown in Table 2.8. Week effects indicate that the F:B ratio was highest during wk 0 before treatments were applied and lowest during wk 5 and 6 (1.762 vs. 0.742 and 0.654, respectively; Table 2.9). The F:B ratio was not statistically different during wk 0, 4, and 12. Gram-positive bacteria were affected by week, being significantly lower during wk 3, 5, and 6 but not different among wk 0, 4, and 12. Gram-negative bacteria were, therefore, the counteractivity of the Gram-positive bacterial populations and were also affected by week.

In total, 41 classes of bacteria were shared across all samples, 11 of which accounted for 97.35% of bacteria detected: *Actinobacteria*, *Bacilli*, *Candidatus Saccharibacteria* (TM7), *Clostridia*, *Erysipelotrichia*, *Bacteroidia*, *Cytophagia*, *Flavobacteriia*, *Negativicutes*, *Sphingobacteriia*, and *Spirochaetia*. For the purposes of our objectives, only those classes are reported, because their prevalence was greater than 1% relative abundance or, as in the case of *Actinobacteria*, proved to be moderately correlated with other measurements; this is discussed later. At the class level, there was a significant treatment × time interaction for the Gram-positive bacterial class *Erysipelotrichia* ($P < 0.0001$; Fig. 4). During wk 0, there was major diversity in the population of this class, ranging from 2.64 to 24.30% relative abundance among the treatment groups. However, by wk 3, the population significantly decreased, ranging from 0.320 to 2.601%, and continued to stay below 2.5% for all treatments for the remainder of the trial. The interaction occurred between wk 3 and 4, where *Erysipelotrichia* decreased in B7M-treated rumen fluid when all other treatments increased. Another interaction occurred between wk 5 and 6, where the *Erysipelotrichia* population declined in the B7M and B21M treatments when other treatments were showing

increased populations. And finally, there was an interaction between wk 6 and 12, where populations in the B7M treatments did not change, the B21M treatments decreased, and all other treatments increased. Although the interaction is intriguing, the bigger picture is that steers that had a greater initial population of *Erysipelotrichia* showed a dramatic response to treatment and showed no signs of adaptation to treatment. It could be that *Erysipelotrichia* was highly affected by treatments due to several members of its class having displayed a unique loss of spore-forming genes and was, therefore, unable to cope with the environment (Davis et al., 2013; Galperin, 2013; Tang et al., 2016). This hypothesis also may explain the significant effects of week observed for the other classes of Gram-positive bacteria. The relative abundance of *Actinobacteria*, *Bacilli*, and *Clostridia* at wk 5, 6, and 12 were not significantly different than their initial population at wk 0 (Table 2.9). Each of these classes of bacteria has spore-forming capabilities and, in the case of *Actinobacteria*, has different levels of dormancy and activities based on stress stimulus (especially among Streptomyces spp.; Robinson and Batt, 2014). It may be that any favorable response of VFA production traits occurs between wk 1 and 3 and that a subsequent shift in population between wk 4 and 6 is the reemergence of previously prominent bacterial communities, minus those that lack genes to combat molecules that permeate the cell wall. This thought may also be supported by the significant shift in total VFA and the A:P ratio from wk 3 to 4, as previously mentioned.

One class of Gram-positive bacteria was affected by treatment, where percent relative abundance of *Clostridia* was lowest in the control and bambermycin-treated rumen fluid and was greatest for the B14M-treated rumen fluid (30.76, 31.10, and 45.54, respectively). Monensin-, B7M-, and B21M-treated rumen fluid had a similar percent of

Clostridia and were intermediate compared with other treatments. The *Clostridia* class made up the largest portion of the detected Gram-positive group and similarly follows the treatment effects on Gram-positive bacteria relative abundance. Gram-negative classes *Cytophagia* and *Flavobacteriia* were affected by treatment. The percent of *Cytophagia* was greatest for the B21M treatment and lowest for the B14M treatment (1.638 vs. 0.982, respectively), whereas other treatments were intermediate and not significantly different. In general, treatments that had greater percent of *Clostridia* had lower *Flavobacteriia* and vice versa. The class *Flavobacteriia* was most prevalent in the control and bambermycin treatments and less evident in the monensin and B14M treatments, with the B7M and B21M treatments being intermediate. Although the true relationship between these 2 classes is unclear at this time, it would appear that *Clostridia* and *Flavobacteriia* may share a common niche based on their antagonistic behavior observed here. There was an effect of week on Gram-negative classes *Bacteroidia*, *Cytophagia*, *Negativicutes*, and *Spirochaetia* (Table 2.9). As was observed in the Gram-positive classes, although there were significant differences among weeks, there was no difference among wk 0, 4, and 12 for *Bacteroidia*, *Cytophagia*, and *Negativicutes*. Week 0 differed from wk 4, 5, and 12, however, for *Spirochaetia* (3.922 vs. 1.976, 1.678, and 1.976%, respectively). At this time, this result is not clear but may be a consequence of greater competitiveness for substrate due to increased needs for bacterial energy to maintain functionality in an assaulting environment.

Archaea and Bacteria Correlations

Methanobrevibacter spp. were correlated with Gram-positive bacteria (R2 = 0.497, $P < 0.0001$), especially the *Actinobacteria* and *Clostridia* classes (R2 = 0.496, $P < 0.0001$ and R2 = 0.381, $P < 0.001$, respectively). Contrarily, *Methanomicrobium* was correlated

with Gram-negative bacteria (R^2= 0.271, $P < 0.05$), especially *Sphingobacteriia* and *Spirochaetia* ($R^2 = 0.472$, $P < 0.0001$ and $R^2 = 0.308$, $P < 0.05$). Interestingly, *Methanosphaera* was correlated with *Erysipelotrichia* and *Candidatus Saccharibacteria* ($R^2 = 0.320$, $P < 0.05$ and R2 = 0.381, $P < 0.001$, respectively). As previously mentioned, pectate lyase genes can be found in the genome assemblies of several subspecies belonging to the class *Erysipelotrichia* (Ogawa et al., 2011; Tang et al., 2016). Therefore, reduction of these bacteria and their end products due to susceptibility to antibiotics and/or ionophores likely restricted the growth of *Methanosphaera spp*., which feeds exclusively on H_2 and methanol (Pol and Demeyer, 1988). *Nitrosoarchaeum limnia* was correlated with the class *Flavobacteriia* ($R^2 = 0.235$, $P < 0.05$). *Thermoplasma spp*. was correlated with Gram-negative bacteria ($R^2 = 0.502$, $P < 0.0001$), primarily *Flavobacteriia* ($R^2 = 0.373$, $P < 0.001$), *Sphingobacteriia* ($R^2 = 0.378$, $P < 0.001$), and *Spirochaetia* ($R^2 = 0.306$, $P < 0.01$).

CONCLUSION

Although ionophores and antibiotics may have different modes of action, there is no significant evidence that rotating bambermycin and monensin would alter the VFA profile or decrease methane potential more than continuous feeding, but rather, it may dilute the efficacy of the ionophore when continuously fed. Neither did we find evidence to suggest that rotating additives delays rumen microbial adaptation better than continuous feeding, which has also been reported in other research (Guan et al., 2006). In general, it appears that bacterial adaptation occurred between 4 and 6 wk after initiation of additives. Therefore, based on our findings, feeding antibiotics or ionophores or rotating the 2 may lose its efficacy in the long term, regarding target microbial populations, when cattle are consuming a

moderate-forage diet. Future research may look to a pulse-feeding approach to feed additives as a means to circumvent microbial adaptation when considering a long-term feeding plan.

LITERATURE CITED

Allison, M. J., W. R. Mayberry, C. S. McSweeney, and D. A. Stahl. 1992. *Synergistes jonesii*, gen. nov., sp. nov.: a rumen bacterium that degrades toxic pyridinediols. Systematic and Applied Microbiology 15: 522-529. doi: 10.1016/S0723-2020(11)80111-6.

Anderson, R. et al. 2006. Effect of oral nitroethane and 2-nitropropanol administration on methane-producing activity and volatile fatty acid production in the ovine rumen. Bioresource Technology 97: 2421-2426. doi: 10.1016/j.biortech.2005.10.013.

Bryant, M., and L. A. Burkey. 1953. Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. Journal of Dairy Science 36: 205-217. doi: 10.3168/jds.S0022-0302(53)91482-9.

Butaye, P., L. A. Devriese, and F. Haesebrouck. 2003. Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on gram-positive bacteria. Clinical microbiology reviews 16: 175-188.

Callaway, T. et al. 2003. Ionophores: their use as ruminant growth promotants and impact on food safety. Current issues in intestinal microbiology 4: 43-51.

Carberry, C. A., S. M. Waters, D. A. Kenny, and C. J. Creevey. 2014. Rumen methanogenic genotypes differ in abundance according to host residual feed intake phenotype and diet type. Applied and Environmental Microbiology 80: 586-594. doi: 10.1128/AEM.03131-13.

Caro, D., E. Kebreab, and F. M. Mitloehner. 2016. Mitigation of enteric methane emissions from global livestock systems through nutrition strategies. Climatic Change 137: 467-480. doi: 10.1007/s10584-016-1686-1.

Chambers, J., and D. Bates. 1992. Nonlinear models. Statistical models in S. Pacific Grove, California: Wadsworth & Brooks/Cole Advanced Books & Software: 432-433.

Chen, G., and J. Russell. 1989. More monensin-sensitive, ammonia-producing bacteria from the rumen. Applied and Environmental Microbiology 55: 1052-1057.

Davis, J. J., F. Xia, R. A. Overbeek, and G. J. Olsen. 2013. Genomes of the class *Erysipelotrichia* clarify the firmicute origin of the class Mollicutes. International journal of systematic and evolutionary microbiology 63: 2727-2741. doi: 10.1099/ijs.0.048983-0.

DelCurto, T., A. Earley, T. May, and W. Nichols. 1998. Comparison of bambermycins, lasalocid, and momensin on the nutritional physiology of beef cattle consuming concentrate and forage base diets. Agricultural Research Center Annual Report 1998: 101.

Duff, G., M. Galyean, and M. Branine. 1995. Effects of adaptation to lasalocid, monensin or a daily rotation of lasalocid and monensin on in vitro fermentation of a 90% concentrate diet. Canadian Journal of Animal Science 75: 129-134. doi: 10.4141/cjas95-017.

Fernando, S. C. et al. 2010. Rumen microbial population dynamics during adaptation to a high-grain diet. Appl Environ Microbiol 76: 7482-7490. doi: 10.1128/AEM.00388-10.

Fox, P., A. Hall, and N. L. Schryer. 1978. The PORT mathematical subroutine library. ACM Transactions on Mathematical Software (TOMS) 4: 104-126.

Galperin, M. Y. 2013. Genome diversity of spore-forming Firmicutes. Microbiology spectrum 1: TBS-0015-2012. doi: 10.1128/microbiolspectrum.TBS-0015-2012.

Gay, D. M. 1990. Usage summary for selected optimization routines. Computing science technical report 153: 1-21.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, prcedures, and some applications). USDA Agr Handb.

Guan, H., K. Wittenberg, K. Ominski, and D. Krause. 2006. Efficacy of ionophores in cattle diets for mitigation of enteric methane. Journal of Animal Science 84: 1896-1906. doi: 10.2527/jas.2005-652.

Hedderich, R. et al. 1998. Anaerobic respiration with elemental sulfur and with disulfides. FEMS microbiology reviews 22: 353-381. doi: 10.1111/j.1574-6976.1998.tb00376.x.

Hicks, R., F. Owens, D. Gill, J. Hartin, and C. Strasia. 1988. Effects of weekly or bi-weekly rotational feeding of lasalocid and monensin on performance of feedlot steers. Okla. Agr. Exp. Sta. Res. Rep. MP-125 171.

Hinton Jr, A. et al. 1990. Biological control of Salmonella typhimurium in young chickens. Avian diseases: 626-633.

Hu, W.-L., J.-X. Liu, J.-A. Ye, Y.-M. Wu, and Y.-Q. Guo. 2005. Effect of tea saponin on rumen fermentation in vitro. Animal Feed Science and Technology 120: 333-339. doi: 10.1016/j.anifeedsci.2005.02.029.

Irbis, C., and K. Ushida. 2004. Detection of methanogens and proteobacteria from a single cell of rumen ciliate protozoa. The Journal of general and applied microbiology 50: 203-212. doi: 10.2323/jgam.50.203.

Jami, E., B. A. White, and I. Mizrahi. 2014. Potential role of the bovine rumen microbiome in modulating milk composition and feed efficiency. PLoS One 9: e85423. doi: 10.1371/journal.pone.0085423.

Janssen, P. H., and M. Kirs. 2008. Structure of the archaeal community of the rumen. Applied and Environmental Microbiology 74: 3619-3625. doi: 10.1128/AEM.02812-07.

Johnson, K., H. Westberg, B. Lamb, and R. Kincaid. 1997. Quantifying methane emissions from ruminant livestock and examination of methane reduction strategies. In: Ruminant Livestock Efficiency Program Conference Proceedings, EPA, USDA

Li, M., G. Penner, E. Hernandez-Sanabria, M. Oba, and L. Guan. 2009. Effects of sampling location and time, and host animal on assessment of bacterial diversity and fermentation parameters in the bovine rumen. Journal of applied microbiology 107: 1924-1934. doi: 10.1111/j.1365-2672.2009.04376.x.

McFeeters, R., L. Hankin, and G. Lacy. 2001. Pectinolytic and pectolytic microorganisms. Compendium of methods for the microbiological examination of foods. 4th ed. Washington, DC: American Public Health Association: 195-199.

McGinn, S., K. Beauchemin, T. Coates, and D. Colombatto. 2004. Methane emissions from beef cattle: effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. Journal of Animal Science 82: 3346-3356. doi: 10.2527/2004.82113346x.

Morris, F. et al. 1990. Effect of rotating monensin plus tylosin and lasalocid on performance, ruminal fermentation, and site and extent of digestion in feedlot cattle. Journal of Animal Science 68: 3069. doi: 10.2527/1990.68103069x.

Mosier, A. C., M. B. Lund, and C. A. Francis. 2012. Ecophysiology of an ammonia-oxidizing archaeon adapted to low-salinity habitats. Microbial ecology 64: 955-963. doi: 10.1007/s00248-012-0075-1.

Ogawa, Y. et al. 2011. The genome of *Erysipelothrix rhusiopathiae*, the causative agent of swine erysipelas, reveals new insights into the evolution of *Firmicutes* and its intracellular adaptations. Journal of bacteriology. doi: 10.1128/JB.01500-10.

Patra, A. K. 2012. Enteric methane mitigation technologies for ruminant livestock: a synthesis of current research and future directions. Environmental monitoring and assessment 184: 1929-1952. doi: 10.1007/s10661-011-2090-y.

Paynter, M., and R. Hungate. 1968. Characterization of *Methanobacterium* mobilis, sp. n., isolated from the bovine rumen. Journal of Bacteriology 95: 1943-1951.

Pfaller, M. A. 2006. Flavophospholipol use in animals: Positive implications for antimicrobial resistance based on its microbiologic properties. Diagnostic Microbiology and Infectious Disease 56: 115-121. doi: https://doi.org/10.1016/j.diagmicrobio.2006.03.014.

Pitesky, M. E., K. R. Stackhouse, and F. M. Mitloehner. 2009. Clearing the Air. Advances in Agronomy 103: 1-40. doi: http://dx.doi.org/10.1016/S0065-2113(09)03001-6.

Place, S. E., and F. M. Mitloehner. 2014. The nexus of environmental quality and livestock welfare. Annual Review of Animal Biosciences 2: 555-569. doi: 10.1146/annurev-animal-022513-114242.

Pol, A., and D. Demeyer. 1988. Fermentation of methanol in the sheep rumen. Applied and Environmental Microbiology 54: 832-834.

Reysenbach, A.-L., and K. Brileya. 2014. The Family Thermoplasmataceae The Prokaryotes. p 385-387. Springer.

Robinson, R. K., and C. A. Batt. 2014. Encyclopedia of food microbiology. Academic press.

Ruepp, A. et al. 2000. The genome sequence of the thermoacidophilic scavenger *Thermoplasma* acidophilum. Nature 407: 508-513. doi: 10.1038/35035069.

Rumpler, W., D. Johnson, and D. Bates. 1986. The effect of high dietary cation concentration on methanogenesis by steers fed diets with and without ionophores. Journal of Animal Science 62: 1737-1741. doi: 10.2527/jas1986.6261737x.

Russell, J. B. 2002. Rumen microbiology and its role in ruminant nutrition. Cornell University.

Russell, J. B., and H. Strobel. 1989. Effect of ionophores on ruminal fermentation. Applied and Environmental Microbiology 55: 1.

Schofield, P., R. Pitt, and A. Pell. 1994. Kinetics of fiber digestion from in vitro gas production. Journal of Animal Science 72: 2980-2991. doi: 10.2527/1994.72112980x.

Tan, H. Y. et al. 2011. Diversity of bovine rumen methanogens in vitro in the presence of condensed tannins, as determined by sequence analysis of 16S rRNA gene library. The Journal of Microbiology 49: 492-498. doi: 10.1007/s12275-011-0319-7.

Tang, H.-b., J. Xie, L. Wang, F. Liu, and J. Wu. 2016. Complete genome sequence of Erysipelothrix rhusiopathiae strain GXBY-1 isolated from acute swine erysipelas outbreaks in south China. Genomics data 8: 70-71. doi: 10.1016/j.gdata.2016.04.006.

Team, R. C. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2013.

Tedeschi, L. O., D. G. Fox, and T. P. Tylutki. 2003. Potential environmental benefits of ionophores in ruminant diets. Journal of environmental quality 32: 1591-1602.

Tedeschi, L., P. Schofield, and A. Pell. 2008. Determining feed quality for ruminants using in vitro gas production technique. Building an anaerobic fermentation chamber. In: The 4th Workshop on Modeling in Ruminant Nutrition. Application of the Gas Production Technique, Juiz de Fora, Brazil. p 16.

Tedeschi, L. O., and D. G. Fox. 2016. The Ruminant Nutrition System: An Applied Model for Predicting Nutrient Requirements and Feed Utilization in Ruminants.

Tokura, M., I. Chagan, K. Ushida, and Y. Kojima. 1999. Phylogenetic study of methanogens associated with rumen ciliates. Current microbiology 39: 123-128. doi: 10.1007/s002849900432.

Van Nevel, C., and D. Demeyer. 2008. Feed additives and other interventions for decreasing methane emissions. Biotechnology in animal feeds and animal feeding: 329.

Williams, W. et al. 2010. Evaluation of in vitro gas production and rumen bacterial populations fermenting corn milling (co) products. Journal of Dairy Science 93: 4735-4743. doi: 10.3168/jds.2009-2920.

Wright, A.-D. G., X. Ma, and N. E. Obispo. 2008. *Methanobrevibacter* phylotypes are the dominant methanogens in sheep from Venezuela. Microbial Ecology 56: 390-394. doi: 0.1007/s00248-007-9351-x.

Wright, A.-D. G., A. F. Toovey, and C. L. Pimm. 2006. Molecular identification of methanogenic archaea from sheep in Queensland, Australia reveal more uncultured novel archaea. Anaerobe 12: 134-139. doi: 10.1016/j.anaerobe.2006.02.002.

Zhou, M., and E. Hernandez-Sanabria. 2009. Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. Applied and Environmental Microbiology 75: 6524-6533. doi: 10.1128/AEM.02815-08.

Table 2.1. Ingredient and chemical composition of basal diet fed to cannulated angus-cross steers and Bermudagrass hay fermented *in vitro*

Item	Basal Diet, control %	Bermudagrass Hay
Corn	29.92	
Soybean meal	3.17	
Cottonseed hulls	28.02	
Bermudagrass hay, chopped	33.15	
Molasses	3.72	
Urea	1.00	
Trace mineral	0.01	
Limestone	0.50	
Salt	0.12	
Vitamin E-20[1]	0.39	
Dry Matter, % of Diet	91.6	93.2
CP, % DM	11.6	10.5
Soluble protein, % CP	38.1	21.3
NDICP, % CP[2]	18.6	45.5
ADICP, % CP[3]	20.6	13.6
NDF, % DM	53.8	75.9
ADF, % DM	39.3	40.1
Lignin, % DM	10.7	5.12
Crude fat, % DM	2.0	2.36
Sugar, % DM	5.9	4.7
Starch, % DM	16.9	2.4
Ash, % DM	4.5	6.82
TDN,%	55.1	55.6
NEm, Mcal/kg	1.15	1.17
NEg, Mcal/kg	0.59	0.59

[1]Chemical analysis of diet evaluated by Cumberland Valley Analytical Services (Hagerstown, MD).
[2]NDICP= neutral detergent insoluble crude protein.
[3]ADICP= acid detergent insoluble crude protein.

Table 2.2. Effect of treatment on rumen fluid pH, VFA profile and potential methane production

Items[1]	Treatment[2]						SEM	P-value
	Control	Monensin	Bambermycin	B7M	B14M	B21M		
pH	6.41	6.25	6.36	6.57	6.36	6.45	0.100	0.4395
Total VFA, mM/kg of OMI	3.23	3.46	3.06	2.87	3.37	3.04	0.242	0.5607
Acetate, mM/kg of OMI	2.35	2.39	2.21	2.06	2.40	2.18	0.163	0.6369
Propionate, mM/kg of OMI	0.486	0.669	0.486	0.482	0.592	0.522	0.061	0.3097
Acetate:Propionate	4.93[a]	3.72[c]	4.69[ab]	4.40[abc]	4.16[bc]	4.26[abc]	0.208	0.0567
PAMP, mM/kg of OMI	0.198	0.220	0.193	0.191	0.186	0.178	0.019	0.7409
CH_4:Propionate	0.424	0.361	0.420	0.416	0.331	0.358	0.052	0.7140
Total VFA, mM	108.2	113.5	109.1	99.02	113.1	100.8	8.25	0.7355
Acetate, mM	78.78	78.27	78.83	71.06	80.68	72.22	5.05	0.6926
Propionate, mM	16.30	21.97	17.29	16.60	19.86	17.29	2.25	0.5003
PAMP, mM	6.629	7.187	6.911	6.604	6.279	5.974	0.470	0.5702

[a-h] LSM within a row without a common superscript differ at $P < 0.05$.

[1] Items are the pH of rumen fluid samples, Total VFA = acetate, propionate, butyrate & isobutyrate concentration of rumen fluid samples, PAMP= maximum potential methane production based on methanogen population in rumen fluid samples. Items expressed as mM concentrations in rumen fluid and also the normalized concentration per kg of organic matter intake (OMI) of the individual steers.

[2] Treatments were rumen fluid inoculum adapted to diets: Control = basal diet with no additive, Monensin = basal diet + monensin 200 mg/hd/day (Elanco Animal Health, Greenfield, IN), Bambermycin = basal diet + bambermycin 20 mg/hd/day (Huvepharma, Inc., Peachtree City, GA); B7M= basal diet + weekly rotation of bambermycin and monensin, B14M = basal diet + rotation of bambermycin and monensin every two weeks, B21M = basal diet + rotation of bambermycin and monensin every three weeks.

Table 2.3. Effect of week on rumen fluid pH, VFA profile and potential activity of methane production

Items[1]	Week[2]						SEM	*P*-value
	0	3	4	5	6	12		
pH	6.45[ab]	6.23[d]	6.42[bc]	6.47[ab]	6.37[bcd]	6.39[bcd]	0.070	0.0221
Total VFA, mM/kg OMI	3.31[cde]	4.01[a]	2.85[f]	2.80[f]	3.03[ef]	2.76[f]	0.155	<0.0001
Acetate, mM/kg OMI	2.36[cd]	2.76[a]	2.05[ef]	2.03[ef]	2.21[de]	1.94[f]	0.106	<0.0001
Propionate, mM/kg OMI	0.584[b]	0.708[a]	0.494[cd]	0.468[d]	0.493[cd]	0.490[cd]	0.038	<0.0001
Acetate:Propionate	4.23[efg]	4.06[gh]	4.26[defg]	4.46[cdef]	4.61[bc]	4.01[gh]	0.134	<0.0001
PAMP, mM/kg of OMI	0.234[a]	0.178[cde]	0.167[de]	0.159[e]	0.170[cde]	0.224[ab]	0.014	<0.0001
CH_4:Propionate	0.425[bcde]	0.270[fg]	0.348[ef]	0.349[e]	0.360[de]	0.462[abc]	0.035	<0.0001
Total VFA, mM	106.5[cde]	131.5[a]	94.73[g]	95.13[fg]	101.5[efg]	100.5[efg]	5.10	<0.0001
Acetate, mM	76.06[def]	90.70[a]	68.10[g]	68.84[fg]	73.69[defg]	70.61[efg]	3.34	<0.0001
Propionate, mM	18.80[bcd]	23.21[a]	16.40[de]	15.89[e]	16.36[de]	17.81[bcde]	1.31	<0.0001
PAMP, mM	7.576[ab]	5.800[cd]	5.466[d]	5.391[d]	5.685[cd]	8.129[a]	0.440	<0.0001

[a-h] LSM within a row without a common superscript differ at $P < 0.05$.
[1] Items are the pH of rumen fluid samples, Total VFA = acetate, propionate, butyrate & isobutyrate concentration of rumen fluid samples, PAMP= maximum potential methane production based on methanogen population in rumen fluid samples. Items expressed as mM concentrations in rumen fluid and also the normalized concentration per kg of organic matter intake (OMI) of the individual steers.
[2] Weeks were week 0 = basal diet and no treatment, week 3 through 12 = basal diet plus treatment for the respective number of weeks.

Table 2.4. Treatment effects of in vitro fermentation characteristics of Bermudagrass hay inoculated with adapted rumen fluid

Item[2]	Treatment[1]						SEM	*P*-value
	Control	Bambermycin	Monensin	B7M	B14M	B21M		
Total gas, mL	21.29	19.16	21.45	16.41	18.35	21.37	1.411	0.1121
Fermentation rate, h^{-1}	5.85	6.00	5.23	7.17	5.33	4.87	0.764	0.3961
Lag time, h	1.89[b]	3.48[a]	1.78[b]	4.71[a]	4.46[a]	3.39[a]	0.528	0.0026
DMD, %	55.31	60.15	60.78	52.78	49.33	52.93	3.503	0.3158
CH_4, mM	16.27[b]	19.96[a]	20.49[a]	20.43[a]	20.15[a]	19.11[a]	0.811	0.0010
CH_4:DMD	0.3009[b]	0.3689[a]	0.4088[a]	0.3800[a]	0.4162[a]	0.3743[a]	0.019	0.0083
pH	6.58	6.43	6.43	6.48	6.45	6.45	0.049	0.8925

[a-b] LSM within a row without a common superscript differ at $P < 0.05$.
[1]Treatments were rumen fluid inoculum adapted to diets: Control = basal diet with no additive, Monensin = basal diet + monensin 200 mg/hd/day (Elanco Animal Health, Greenfield, IN), Bambermycin = basal diet + bambermycin 20 mg/hd/day (Huvepharma, Inc., Peachtree City, GA); B7M= basal diet + weekly rotation of bambermycin and monensin, B14M = basal diet + rotation of bambermycin and monensin every two weeks, B21M = basal diet + rotation of bambermycin and monensin every three weeks.
[2] Items are the fermentation characteristics of Bermudagrass hay using the exponential with discrete lag nonlinear function, DMD=Dry matter digestibility.

Table 2.5. Week effects of in vitro fermentation characteristics of Bermudagrass hay inoculated with adapted rumen fluid

Item[1]	Week			SEM	P-value
	1	3	6		
Total gas, mL	23.75[a]	18.18[b]	17.08[b]	1.219	0.0083
Fermentation rate, h^{-1}	4.78	6.44	6.00	0.649	0.2309
Lag time, h	2.49[b]	2.83[b]	4.53[a]	0.418	0.0023
DMD, %	62.44[a]	44.42[a]	58.78[b]	2.175	< 0.0001
CH_4, mM	27.71[a]	20.86[b]	9.63[c]	0.750	< 0.0001
CH_4:DMD	0.4585[a]	0.4837[a]	0.1824[b]	0.017	< 0.0001
pH	6.49	6.42	6.47	0.051	0.2602

[a-b] LSM within a row without a common superscript differ at $P < 0.05$.

[1]Items are the fermentation characteristics of Bermudagrass hay using the exponential with discrete lag nonlinear function, DMD=Dry matter digestibility, Methane concentration, Methane concentration to DMD ratio and final pH.

Table 2.6. Effect of treatment on relative abundance of archaeal genera

Items[1]	Treatment[2]						SEM	*P*-value
	Control	Monensin	Bambermycin	B7M	B14M	B21M		
Methanobacterium spp., %	1.099	1.159	0.843	0.615	1.276	0.883	0.45	0.9050
Methanobrevibacter spp., %	91.47	93.11	92.16	93.22	93.80	92.71	1.67	0.9290
Methanomicrobium spp. , %	0.265	1.133	1.103	0.160	0.254	0.421	0.60	0.7392
Methanosphaera spp. , %	0.506	0.680	0.562	0.515	0.415	0.593	0.28	0.9883
Nitrosoarchaeum limnia, %	0.731	0.188	0.137	0.448	0.291	0.270	0.22	0.4528
Thermoplasma spp. , %	5.924	3.726	5.192	5.045	3.969	5.122	1.23	0.8070

[1]Items are the archaeal genera expressed in percent relative abundance.
[2]Treatments: control = basal diet with no additive; monensin = basal diet + monensin 200 mg/d (Elanco Animal Health, Greenfield, IN); bambermycin = basal diet + bambermycin 20 mg/d (Huvepharma, Inc., Peachtree City, GA); B7M = basal diet + weekly rotation of bambermycin and monensin treatments; B14M = basal diet + rotation of bambermycin and monensin treatments every 14 d; B21M = basal diet + rotation of bambermycin and monensin treatments every 21 d.

Table 2.7. Effect of week on relative abundance of archaeal genera

Items[1]	Week						SEM	*P* -value
	0	3	4	5	6	12		
Methanobacterium spp., %	1.881^{a}	1.070^{abc}	0.510^{c}	0.536^{bc}	1.351^{ab}	0.528^{bc}	0.33	0.0287
Methanobrevibacter spp., %	93.71^{ab}	90.69^{b}	96.82^{a}	90.55^{b}	90.52^{b}	94.19^{ab}	1.59	0.0557
Methanomicrobium spp.,%	0.019	1.347	0.000	1.029	0.561	0.380	0.53	0.3165
Methanosphaera spp.,%	1.320^{a}	0.493^{b}	0.427^{b}	0.025^{b}	0.470^{b}	0.536^{b}	0.23	0.0062
Nitrosoarchaeum limnia,%	0.127^{b}	0.104^{b}	0.042^{b}	0.929^{a}	0.627^{ab}	0.235^{b}	0.21	0.0290
Thermoplasma spp.,%	2.943^{c}	6.297^{ab}	2.202^{c}	6.929^{a}	6.477^{a}	4.130^{abc}	1.21	0.0340

[a-c] LSM within a row without a common superscript differ at $P < 0.05$.
[1] Items are the archaeal genera expressed in % relative abundance of total archaea.

Table 2.8. Effect of treatment on the relative abundance of predominant ruminal bacteria

Items[1]	Treatment[2]						SEM	*P*-value
	Control	Monensin	Bambermycin	B7M	B14M	B21M		
Firmicutes/Bacteroidetes ratio	0.635^{d}	1.047^{bcd}	0.664^{cd}	1.131^{bc}	1.199^{b}	1.845^{a}	0.16	0.0001
Gram Positive Phyla, %	36.28^{c}	44.12^{b}	36.55^{c}	45.50^{b}	53.07^{a}	47.78^{ab}	2.24	<0.0001
Gram Positive Class								
Actinobacteria, %	0.3692	0.5861	0.1787	0.3559	0.4879	0.3397	0.10	0.2432
Bacilli, %	1.752	1.969	1.463	1.801	1.808	2.097	0.23	0.5078
Candidatus sacc., %	2.020	2.922	1.067	0.903	3.558	0.831	1.75	0.8129
Clostridia, %	30.76^{c}	36.43^{b}	31.10^{c}	37.53^{b}	45.54^{a}	39.38^{b}	1.76	<0.0001
Erysipelotrichia, %	1.188^{b}	1.893^{b}	2.544^{b}	4.680^{a}	1.304^{b}	4.770^{a}	0.44	0.0002
Gram Negative Phyla, %	61.72^{a}	52.90^{b}	61.14^{a}	51.62^{b}	43.92^{c}	49.45^{bc}	2.32	<0.0001
Gram Negative Class								
Bacteroidia, %	32.59	29.32	33.16	30.34	27.68	30.44	2.20	0.5528
Cytophagia, %	1.085^{bc}	1.315^{abc}	1.102^{bc}	1.432^{ab}	0.928^{c}	1.638^{a}	0.15	0.0332
Flavobacteriia, %	11.21^{ab}	4.169^{c}	11.53^{a}	6.330^{bc}	4.878^{c}	6.603^{abc}	1.45	0.0427
Negativicutes, %	0.790	1.411	1.084	1.399	1.137	1.718	0.20	0.1362
Sphingobacteriia, %	12.61	12.98	11.81	8.606	7.625	6.275	2.78	0.4603
Spirochaetia, %	3.440	3.708	2.460	3.507	1.664	2.783	0.62	0.2221

[a-c] LSM within a row without a common superscript differ at $P < 0.05$.

[1] Items are the phylum ratio of Firmicutes to Bacteroidetes and predominant (> 1 % relative abundance) taxonomic classes of bacteria in % relative abundance organized by typical gram stain results. Actinobacteria was included due to its moderately positive correlation to predominant Archaea.

[2] Treatments were Control = basal diet with no additive, Monensin = basal diet + monensin 200 mg/hd/day (Elanco Animal Health, Greenfield, IN), Bambermycin = basal diet + bambermycin 20 mg/hd/day (Huvepharma, Inc., Peachtree City, GA); B7M= basal diet + weekly rotation of bambermycin and monensin, B14M = basal diet + rotation of bambermycin and monensin every two weeks, B21M = basal diet + rotation of bambermycin and monensin every three weeks.

Table 2.9. Effect of week on the relative abundance of predominating bacterial classes

Items[1]	Week						SEM	*P*-value
	0	3	4	5	6	12		
Firmicutes/Bacteroidetes ratio	1.762^{a}	0.821^{bc}	1.597^{ab}	0.742^{c}	0.654^{c}	0.945^{ab}	0.21	0.0031
Gram Positive Phyla, %	51.07^{ab}	39.39^{c}	56.29^{a}	37.51^{c}	35.06^{c}	43.96^{bc}	3.14	<0.0001
Gram Positive Class								
Actinobacteria, %	0.458^{ab}	0.213^{c}	0.629^{a}	0.340^{bc}	0.355^{bc}	0.319^{bc}	0.07	0.0045
Bacilli, %	1.738^{b}	1.584^{b}	2.477^{a}	1.551^{b}	1.519^{b}	2.019^{ab}	0.21	0.0077
Candidatus sacc. , %	4.387	1.859	2.114	0.962	0.636	1.341	1.11	0.1861
Clostridia, %	33.24^{bc}	34.31^{bc}	49.32^{a}	33.55^{bc}	31.43^{c}	38.87^{b}	2.46	<0.0001
Erysipelotrichia, %	10.94^{a}	1.245^{b}	1.203^{b}	0.914^{b}	0.972^{b}	1.094^{b}	0.67	<0.0001
Gram Negative Phyla, %	46.93^{bc}	57.80^{a}	40.96^{c}	59.41^{a}	61.58^{a}	54.08^{ab}	3.18	0.0002
Gram Negative Class								
Bacteroidia, %	23.72^{c}	31.40^{ab}	26.00^{bc}	34.13^{ab}	38.02^{a}	30.25^{bc}	2.43	0.0063
Cytophagia, %	1.012^{b}	1.587^{a}	0.928^{b}	1.603^{a}	1.265^{ab}	1.103^{b}	0.16	0.0345
Flavobacteriia, %	5.500	7.728	6.702	10.70	7.718	6.369	1.34	0.1428
Negativicutes, %	0.337^{c}	1.361^{b}	0.590^{c}	2.321^{a}	2.316^{a}	0.612^{c}	0.19	<0.0001
Sphingobacteriia, %	12.43	10.53	4.759	8.967	9.434	13.76	2.77	0.3528
Spirochaetia, %	3.922^{ab}	5.183^{a}	1.976^{c}	1.678^{c}	2.821^{bc}	1.976^{c}	0.66	0.0119

[a-c] LSM within a row without a common superscript differ at $P < 0.05$.

[1] Items are the phylum ratio of Firmicutes to Bacteroidetes and predominant (> 1 % relative abundance) taxonomic classes of bacteria in % relative abundance organized by typical gram stain results. Actinobacteria was included due to its moderately positive correlation to predominant Archaea.

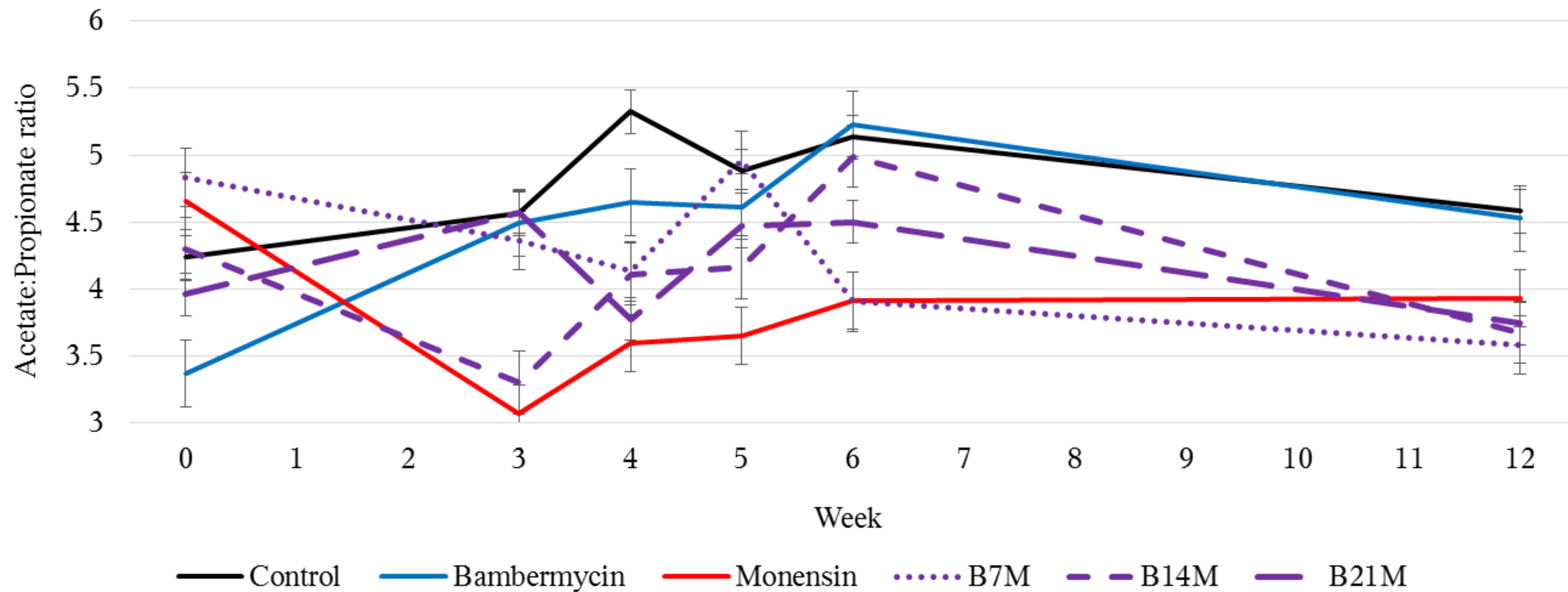

Figure 2.1 Interaction of treatment and week on the acetate-to-propionate ratio in the rumen fluid of steers. Values are least squares means ± SEM. Treatments: control = basal diet with no additive; monensin = basal diet + monensin 200 mg/d (Elanco Animal Health, Greenfield, IN); bambermycin = basal diet + bambermycin 20 mg/d (Huvepharma, Inc., Peachtree City, GA); B7M = basal diet + weekly rotation of bambermycin and monensin treatments; B14M = basal diet + rotation of bambermycin and monensin treatments every 14 d; B21M = basal diet + rotation of bambermycin and monensin treatments every 21 d.

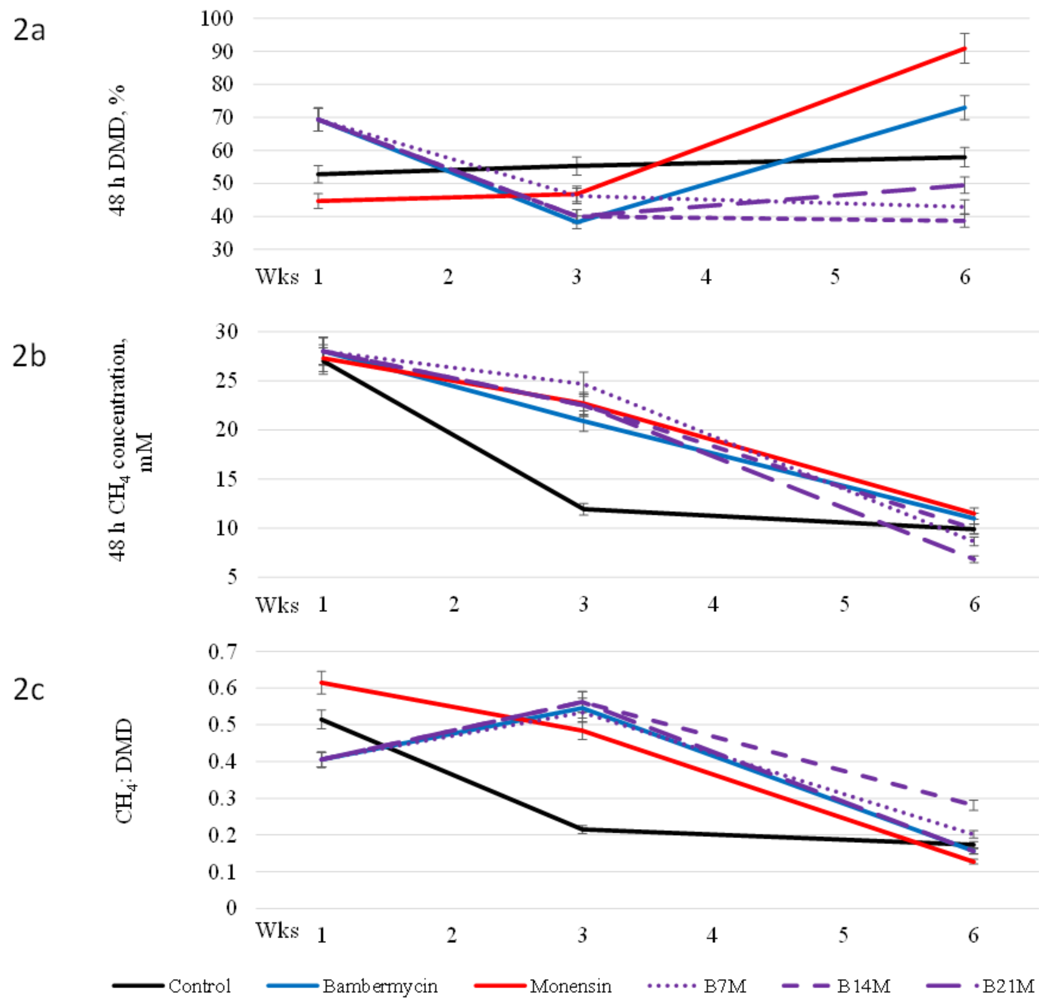

Figure 2.2. Interaction of treatment and week on DM digestibility (DMD; %) (a), methane concentration (m*M*) (b), and the methane-to–DM digestibility ratio (c) of bermudagrass hay fermented using the in vitro gas production technique and adapted rumen fluid. Values are least squares means ± SEM. Treatments were rumen fluid inoculum adapted to diets: control = basal diet with no additive; monensin = basal diet + monensin 200 mg/d (Elanco Animal Health, Greenfield, IN); bambermycin = basal diet + bambermycin 20 mg/d (Huvepharma, Inc., Peachtree City, GA); B7M = basal diet + weekly rotation of bambermycin and monensin treatments; B14M = basal diet + rotation of bambermycin and monensin treatments every 14 d; B21M = basal diet + rotation of bambermycin and monensin treatments every 21 d.

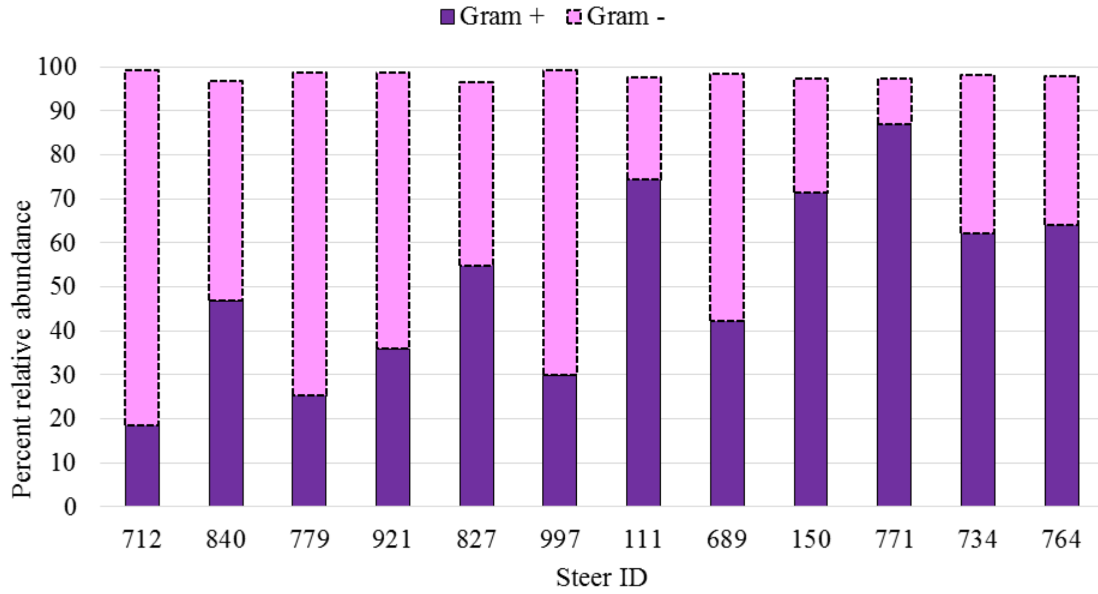

Figure 2.3. Relative abundance of Gram positive and Gram negative bacteria during week 0 collection 3 h after the morning feeding. Steers were adapted to a basal diet with no treatment for 14 d prior to the collection, but an animal-to-animal variation of the bacterial population is apparent.

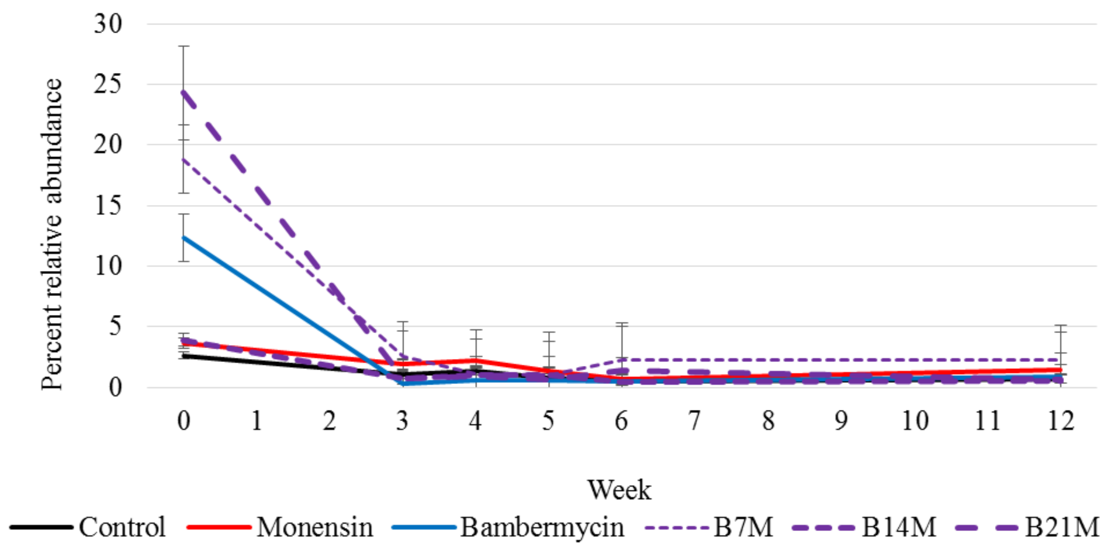

Figure 2.4. Interaction of treatment and week on the relative abundance of the Gram positive class *Erysipelotrichia* in the rumen fluid of steers. Values are LSM ± SEM. Treatments were Control = basal diet with no additive, Monensin = basal diet + monensin 200 mg/hd/day (Elanco Animal Health, Greenfield, IN), Bambermycin = basal diet + bambermycin 20 mg/hd/day (Huvepharma, Inc., Peachtree City, GA), B7M= basal diet + weekly rotation of bambermycin and monensin, B14M = basal diet + rotation of bambermycin and monensin every two weeks, B21M = basal diet + rotation of bambermycin and monensin every three weeks.

CHAPTER III

EFFECTS OF ACTIVE DRY YEAST ON RUMINAL PH CHARACTERISTICS AND ENERGY PARTITIONING OF FINISHING STEERS UNDER THERMONEUTRAL OR HEAT-STRESSED ENVIRONMENT

SYNOPSIS

The objective of this trial was to determine the effects of supplementing active dried yeast (**ADY**) in the diets of finishing steers on energy and nitrogen metabolism and ruminal pH characteristics under thermoneutral (**TN**) or heat-stressed (**HS**) conditions. Eight British-cross steers received one of two TRT (either a control finishing diet (**CON**) or supplemented with 3 g/d (6×10^{10} CFU/d) of ADY) under one of two temperatures (**TEMP**: TN = 18 ± 0.55°C and 20 ± 1.2% RH or HS = 35 ± 0.55°C and 42 ± 6.1% RH). Steers were orally administered an indwelling rumen pH and temperature recording bolus. Steers were adapted for 12 d before data collections. Data collection occurred for 48 consecutive hours inside two calorimetry chambers, and daily means were computed. Data were analyzed as in a 4 × 8 Latin rectangle design with fixed effects of TRT and TEMP, and random effects of steer and period. There were no TRT by TEMP interactions for metabolism or calorimetric measurements. In vivo DM digestibility (DMD) was greater for ADY- than CON-fed steers (77.1 vs. 75.3%, respectively; $P = 0.0311$). Nitrogen retention was not different between CON- and ADY-fed steers (42.1 vs. 47%, respectively; $P = 0.30$) but tended to be different between steers under TN vs. HS conditions (47.8 vs. 41.3%, respectively; $P = 0.18$). Energy

partitioning showed DE and ME (Mcal/kg) were greater for ADY- than CON-fed steers ($P = 0.0097$ and 0.0377, respectively) and retained energy (**RE**) tended to be greater for ADY- vs. CON-fed steers (1.56 vs. 1.43 Mcal/kg, respectively; $P = 0.11$). Steers under HS had reduced DMI, and as expected, greater DMD than TN steers (77.1 vs. 75.3 %, respectively; $P = 0.0316$) and greater CH_4 per unit of DM (12.9 vs. 9.8 L/kg, respectively; $P < 0.05$). Although DE was greater for HS than TN (3.16 vs. 3.06 Mcal/kg, respectively; $P = 0.0123$) heat production (**HE**) was considerably greater for HS than TN (9.96 vs 8.46 Mcal/d, respectively; $P = 0.0013$), resulting in a less RE (1.39 vs. 1.63 Mcal/kg; $P = 0.0043$). There was a tendency for an interaction for mean ruminal pH ($P = 0.12$) in which pH of ADY-fed steers was greater than CON-fed steers under TN conditions (5.81 vs. 5.57, respectively), but not under HS conditions (5.37 vs. 5.41, respectively). Duration and area under the curve for pH > 5.6 had similar tendencies in which under TN conditions the DUR and AUC pH >5.6 of ADY-fed steers was greater than CON-fed steers ($P = 0.07$ and $P = 0.09$, respectively), but under HS conditions there was no difference between ADY and CON. We conclude that supplementing ADY in the diets of finishing steers improved DMD, DE, ME and RE, and mean ruminal pH under TN conditions, but not in extreme HS conditions likely because of their reduced DMI and greater HE requirements.

INTRODUCTION

The effects of including an active dry yeast (**ADY**; i.e., *Saccharomyces cerevisiae*) in the diets of dairy cattle have been extensively studied (Desnoyers et al., 2009), but its effects on beef cattle under feedlot conditions are not well defined. Improvements in DM

digestibility (**DMD**), stabilization of ruminal pH, and feed efficiency are translational variables of interest in finishing cattle supplemented with ADY. The role of ADY in ruminant diets has been suggested to alter fermentative pathways from lactate to propionate by stimulating populations of lactate-utilizing and cellulolytic bacteria populations thereby decreasing the risk of low ruminal pH and increasing ruminal digestibility (Chaucheyras et al., 1996; Lila et al., 2004).

Some research in dairy cattle has even shown positive impacts when supplementing ADY during hotter months when productive functions tend to decline (Moallem et al., 2009; Salvati et al., 2015). Heat stress is a substantial drain on feed energy in beef cattle and may increase maintenance requirements by up to 18 % (National Academies of Sciences, Engineering, and Medicine, **NASEM**, 2016). As thermal heat index increases, animal energy expenditure increases in an effort to maintain core body temperature within physiological limits. Additionally, heat stress may affect feeding behavior, DMI, cause ruminal disturbances that may lead to acute ruminal acidosis (Collier et al., 2006). During extreme weather in the United States and more commonly in sub-tropical latitudes, heat load and stress may have significant adverse impacts on beef production, reducing economic sustainability, and potentially prejudicing animal welfare.

Therefore, the objective of this study evaluated the effects of supplemental ADY in the diets of finishing steers on the metabolism of energy and nitrogen using indirect calorimetry, and ruminal pH characteristics under thermoneutral (**TN**) or heat-stressed (**HS**) conditions within respiration chambers.

MATERIALS AND METHODS

Animals, Feeding Regimens, and Dietary Treatments

Eight British crossbred steers (389 ± 30 kg) were cared for according to the approved animal use protocol (IACUC: 2016-0267) and housed individually in metabolism stalls in a climate controlled barn (18 ± 0.55°C; 35 ± 6 % RH). The Large Ruminant Nutrition System (**LRNS**; http://www.nutritionmodels.com/lrns.html; Accessed on January 21, 2018; Tedeschi and Fox, 2018) was used to formulate the high-concentrate control diet (**CON**) using the following ingredients: cracked corn, dried distiller's grain, a medium chopped alfalfa hay, and mineral supplement, as detailed in Table 3.1. Steers were stepped up over a period of 21 d to the CON diet and further adapted for another 14 d prior to the trial. To compensate for expected drops in the voluntary intake due to extreme environmental temperature and ensure proper dosage and full consumption of feed treatment steers were limit-fed (1.5% of shrunk BW, daily DMI) at 0700 and 1700 h each day, but with unrestricted access to water. An active dry yeast (**ADY**) supplement (VistaCell; ABVista, Marlborough, UK) was top dressed and thoroughly hand mixed (1.5 g) at each feeding to assigned treated steers within each period to allow a total of 3 g/d (6×10^{10} CFU/d). Viability was ensured before and after the trial for quality control purposes.

Experimental Design, Equipment, and Data Collection

A 4×8 Latin rectangle design was used to determine the effects of two feed treatments (CON and ADY) and two environmental temperatures (**TN** vs. **HS**) so that within a period each interaction of feed treatment and the temperature was replicated by two steers. Because we used two respiration chambers side-by-side, only two steers could be collected at one

time; steers were paired together to begin the trial and each period in a stepwise progression being 2 d apart between each pair. Steers were adapted to their feed treatment for 12 d before any measurements. Within each period, on d 13 for a pair of steers, a shrunk BW (**SBW**) was taken before the morning feeding. Each steer was placed in a single stall open-circuit respiration calorimetry chamber system using a mass flow system (Flowkit model FK-500; Sable System Int., Henderson, NV) for a 48-h data collection period. Chambers were designated as either TN (18 ± 0.55°C; 20 ± 1.2 % RH) or HS (35 ± 0.55°C; 42 ± 6.1 % RH). Ambient air (baseline) and air from each chamber were sampled by a multiplexer (Respirometry Multiplexer V 2.0, Sable System Int., Henderson, NV) rotating every 2 min and measured O_2, CO_2 and CH_4 (FC-1B O2 analyzer, CA-2A CO2 analyzer and MA-10 Methane analyzer; Sable System Int., Henderson, NV). The SBW, dietary energy density, and the known dimensions of the calorimetry chambers were used to calculate the proper bank time and flow rate needed before data recording. The assumed gas concentrations of baseline ambient air (O_2 =20.95 %, CO_2=0.04% and CH_4 =0.00 %) were used to calibrate O_2, CO_2 and CH_4 analyzers using known gasses, SPAN, and nitrogen, before each steer entry for data collection. The measured gas was scrubbed of water vapor using fresh drierite desiccant (Hammond Drierite Co LTD, Xenia, OH) for each 48 h collection and the rate of O_2, CO_2 and CH_4 production (VO_2, VCO_2, and VCH_4) were determined (L/min) (Lighton, 2008). Prior to each period an oxygen recovery trial was performed using the gravimetric nitrogen injection technique (Cooper et al., 1991), where expected (20.95 % × volume of N) and observed VO_2 uptake were verified with recovery being no less than 100 % ($O_2 < 0.001$ L/min).

Each chamber was preloaded with 4 rations of designated feed TRT, equipped with a line voltage thermostat (Ranco Enterprises, Inc., Model# ETC-111000-000), dehumidifier (Hisense USA, Model# DH-70K1SDLE), water meter (Neptune Technology Group, Inc., Model# T10-DR-075-G-F), digital HOBO temperature and humidity data loggers (Onset Computer Corporation, Model# UX100-003), and security cameras (FLIR Lorex Inc., Model# LBV1511W) for monitoring animal activity within the chambers. Additionally, each chamber was equipped with a metabolism stand to allow for the collection of total urine and fecal output. After 48 h in the chambers, steers were restrained in a squeeze chute to collect approximately 1 L of rumen fluid via esophageal tubing. Animals were allowed to rest for approximately 1 h in an open pen before returning to the stalls in the climate controlled barn to begin d 1 of their next experimental period with a different diet.

Water, Feed, Fecal, and Urine Analyses

Water intake was manually recorded from the analog water meter. During each period, batch feed samples were taken and homogenized into representative samples (n = 4) to determine the chemical analysis of DM, NDF, ADF, lignin, CP, soluble protein, NDIN, ADIN, starch, sugars, and minerals at Cumberland Valley Analytical Services (Hagerstown, MD). Residual orts during a data collection days were weighed, and a sub-sample was cataloged to be analyzed for DM, GE, and N to be used for calorimetry adjustments.

Total fecal output for the 48-h data collection period was weighed, homogenized, subsampled, dried, and analyzed for DM, GE, and N. Total urine collection was achieved using a large transmission funnel and, to eliminate fecal contamination, two non-splatter filters fitted beneath the metabolism stand and over the catch tub. The catch tub was linked

to an external holding tub and vacuum pump system that remained primed to eliminate gaseous escape from the chamber. To prevent volatilization of N, 600 mL of 3 molar HCl was added to each catch tub at the beginning of each recorded steer entry. Urine was vacuumed as necessary into the external holding tub from the catch tub. Total urine was weighed and homogenized for subsampling at the end of each 48 h data collection. Urine samples were analyzed for GE and N.

All GE analyses were conducted by a bomb calorimeter (Parr adiabatic calorimeter; Parr Instruments Co., Moline, IL) and Total N analysis was performed by Servi-Tech Laboratories (Amarillo, TX) using the Dumas combustion method.

Rumen Boluses

Rumen pH and temperature were recorded using a wireless, indwelling rumen bolus which communicated via radio-transmission to a base station inside the barn (smaXtec animal care, GMbH, Graz, Austria). Because manufacturer guaranteed lifespan was limited to 50 d, boluses were activated, calibrated and inserted into the steers serially one week prior to their first chamber collection days within the first period. Boluses were inserted orally using the manufacturer provided balling gun. Continuous recordings of the reticulo-ruminal pH and temperature were averaged and plotted for every 10 min for the duration of the trial automatically transmitting data to the base station radio system which stored data in the cloud for real-time monitoring. Data was serially downloaded to reflect the relative information for a given steer's stay in the calorimetry chamber. Rumen variables were chosen to reflect the time (**DUR**=duration, h/d or %/d) and magnitude (area under the curve; **AUC**) below pH thresholds of biological importance. Rumen pH variable thresholds of 5.0

and 5.6 were assigned based on work by Nagaraja et al., (2007) where ruminal pH below 5.0 was considered to be acutely acidotic and between 5.0 and 5.6 were considered to be sub-acutely acidotic. Rumen pH variables were therefore calculated as mean pH, DUR above pH 5.6 and the area above the curve (**AAC**), DUR of SARA (pH 5.0-5.6) (h/d and %/d), AUC of SARA, DUR of acute ruminal acidosis (**ARA**; pH < 5.0, h/d and %/d) and AUC of ARA. Rumen temperature variables were calculated to detect changes in the normal rumen temperature above the typical heat of fermentation as a threshold of 40°C, resulting in the variables mean rumen temperature, DUR above 40°C (h/d and %/d), and AAC of 40°C. The AUC and AAC variables were determined with a script that used the definite integral approach and the *rootSolve* and *Spline* functions in the CRAN package of the R (R Core Team, 2017) where y-base thresholds of pH 5.0 and 5.6 were established for rumen pH data and an y-base threshold of 40°C was used for rumen temperature calculations.

Energy Partitioning & Nitrogen Balance

Gross energy intake (**GEI;** Mcal/d) was determined by multiplying the GE of the representative diet by the kilograms of feed offered (Mcal × kg/d) minus the energy contained in the residual orts. Fecal energy (**FE**; Mcal/d) was calculated by multiplying the energy density of the feces by daily fecal output. Urinary energy (**UE**; Mcal/d) and urinary nitrogen (**UN;** g/d) were calculated by multiplying the energy (Mcal/kg) and nitrogen density (% N) of the urine by the daily urinary output (kg/d), respectively. Gaseous energy (**GASE**) was determined by multiplying the L/d of CH_4 produced in the chamber by the density of CH_4 (0.6556 g/L at 25 °C) and its energy density (13.3 Mcal/g) to yield GASE in Mcal/d. Heat production energy (**HE**) was calculated as: HE (Mcal/d) = $(3.866 \times VO_2) + (1.2 \times$

VCO_2) – (0.518 × VCH_4) – (1.431× UN) (Brouwer, 1965). Final values of energy partitioning were calculated as: DE = GEI – FE; MEI = DE – (UE +GASE); and retained energy (**RE**) assumed to be RE=MEI – HE, where ME intake (MEI) was calculated as the observed dietary ME content (Mcal/kg) multiplied by the DMI (kg/d) of diet. The net energy for maintenance (**NEm**, Mcal/kg) of the diet was calculated as $NEm = 1.37 \times ME - 0.138 \times ME^2 + 0.0105 \times ME^3 - 1.12$ and the partial efficiency of the use of ME for maintenance (***km***) = NEm/ME. The net energy for gain (**NEg**, Mcal/kg) of the diet was calculated as $NEg = 1.42 \times ME - 0.174 \times ME^2 + 0.0122 \times ME^3 - 1.65$ and the partial efficiency of the use of ME for gain (***kg***) = NEg/ME. Shrunk weight gain was calculated according to Tedeschi and Fox (2018) where $SWG = 13.91 \times RE^{0.9116} \times SBW^{-0.6837}$. Nitrogen intake was determined by multiplying the total N (%) of the representative diet by the kg of feed offered (kg/d), less the total N (kg) contained in the residual orts. Fecal N was calculated as the % N of the feces (DM) by the daily fecal excretion (kg/d, DM). Dietary N retained was calculated by subtracting fecal and urinary N from N intake.

In Vitro Fermentation

As steers were removed from the calorimetry chambers, rumen fluid inoculum was obtained via esophageal tubing. Rumen fluid samples were collected in a 1 L thermos and filtered through 8 layers of cheese-cloth to remove any feed particles. The in vitro gas production (**IVGP**) technique has been described previously in detail (Tedeschi et al., 2009), but briefly, approximately 200 mg of the CON diet (ground to 2 mm) was weighed into 160 mL Wheaton bottles, containing equal sized magnetic stir bars. Samples were wetted with 2 mL of deionized water to reduce particle scattering during CO_2 flushing to maintain an

oxygen reduced atmosphere. Goering and Van Soest's (1970) in vitro buffering media (14 mL) was added to each bottle under constant CO_2 flushing, sealed with a butyl rubber stopper and crimp sealed. Bottles were promptly placed in the incubation chamber to begin heating to rumen temperature (39°C). Rumen inoculum from the treated steers was filtered through glass wool under a CO_2 atmosphere. The treatment & temperature adapted rumen fluid inoculum (4 mL) was injected into the Wheaton bottles which contained either a blank, alfalfa standard or the representative CON diet in triplicate, respectively. Internal pressure was equilibrated across all bottles after inoculation by inserting needles into the stoppers for approximately 5 s, and pressure sensors were inserted. The pressure was recorded at 5 min intervals for 48 h plotting the fermentation profile over time for each sample. After 48 h, bottles were set in an ice bath to cease fermentation. Head space samples (1 mL) were removed and analyzed for methane concentration using the gas chromatography method (Allison et al., 1992). Final rumen fluid pH was measured, and approximately 40 mL of neutral detergent solution (ANKOM Technology, Macedon, NY) was added to each bottle of fermented feed residue. Bottles were resealed and set in the autoclave for 15 minutes at 121°C. Samples were then filtered using Whatman 54 paper to collect the washed feed residue to calculate dry matter digestibility.

The kinetic analysis of the 48 h fermentation of the CON diet using treated rumen fluid was evaluated using nonlinear functions, and that with the lowest sum of square errors was selected (Schofield et al., 1994). The nonlinear fitting was performed using Gasfit (http://www.nutritionmodels.com/gasfit.html; Accessed on Jan 21, 2018), which executes specific R scripts to perform convergence of gas production data using the *nls* function

(Chambers and Bates, 1992) and the *port* algorithm (Fox et al., 1978; Gay, 1990). Preliminary results indicated the exponential with discrete lag nonlinear function had the lowest SSE and best fit of the fermentation profiles (Williams et al., 2010). The Gasfit provides the total gas production (mL), the fermentation rate (h^{-1}), and the lag time (h).

Data analyzed from the IVGP technique included: Total gas production (mL), the rate of fermentation (h^{-1}), and lag time (h), apparent TDN and ME assuming passage rate of 2, 4, 6 and 8 %/h. Apparent TDN was computed using the fractional degradation rate of NDF obtained from the IVGP technique with the most likely fractional passage rate, using Eq. [3.1] to [3.6] (Tedeschi and Fox, 2018).

$$aTDN = 0.98 \times (100 - NDF - CP - EE - Ash) + dCP + dEE + dNDF - 7 \quad [3.1]$$

$$aCP = CP \times \left(1 - 0.004 \times \left(100 \times \frac{ADIP}{CP}\right)\right) \quad [3.2]$$

$$aEE = 2.25 \times (EE - 1) \quad [3.3]$$

$$aNDF = NDF \times \left(\frac{kd}{kd+kp} + IDNDF - IDNDF \times \frac{kd}{kd+kp}\right) \quad [3.4]$$

$$DE = aTDN \times 4.409 \quad [3.5]$$

$$ME = \frac{0.82 \times DE}{DMI} \quad [3.6]$$

where *ADIP* is acid-detergent insoluble (crude) protein, %DM; *aTDN* is apparent total digestible nutrients, % DM; *CP* is crude protein, %DM; *EE* is ether extract, %DM; *IDNDF* is intestinal digestibility of NDF (assumed to be 20 % (Sniffen et al., 1992)); *kd* is fractional degradation rate of NDF, %/h; *kp* is fractional passage rate of NDF, %/h, and *NDF* is neutral

detergent fiber, %DM; *DE* is digestible energy, Mcal/d; *ME* is Metabolizable energy, Mcal/kg.

Statistical Analysis

Data collected during the chamber stay (indirect calorimetry variables, rumen bolus variables, feed and water intake, and urine and fecal output variables) were analyzed according to the Latin rectangle design using the PROC Mixed of SAS (SAS Inst. Inc., Cary, NC) using the following model:

$$Y_{ijkl} = \mu + steer_i + period_j + TRT_k + TEMP_l + TRT_k TEMP_l + e_{ijkl}$$

Where Y_{ijkl} = response variables (Heat Production, Respiratory Quotient, Methane production, GE efficiency, mean rumen temperature and pH, AUC/AAC and time spent above 5.6, subacute, acute and above 40°C, feed intake, water intake, fecal energy output & urinary energy and nitrogen output), μ= overall mean, $steer_i$= random effect of the steer within a column (i = 1, 2, … 8 steers), $period_j$= random effect of the period within a row (j =1, 2, 3, 4), TRT_k= the fixed effect of feed treatment (k = CON or ADY), $TEMP_l$= the fixed effect of temperature (l= TN or HS), $TRT_k \times TEMP_l$= interaction of treatment and temperature, and e_{ijkl} = random error associated with the measurement of the *i*th steer within period *j* assigned to treatment *k* and temperature *l*.

Data collected for the IVGP technique was analyzed as a CRD. Inoculum was taken only once from each of the 8 steers at given periods so that each treatment and temperature combination was represented twice. The following model was used in the PROC Mixed of SAS (SAS Inst. Inc., Cary, NC):

$$Y_{ij} = \mu + TRT_i + TEMP_j + TRT_i \times TEMP_j + e_{ij}$$

Where Y_{ij} = response variables (DMD, total gas production, fractional degradation rate, lag time, TDN, ME, CH_4, inoculum pH and final pH), μ= overall mean, TRT_i = fixed effect of treatment (i = CON or ADY rumen fluid inoculum), $TEMP_j$= fixed effect of temperature (j = TN or HS), $TRT_i \times TEMP_j$= interaction of treatment and temperature, and e_{ij} = random error associated with the measurement of the feed sample assigned to temperature *j* and treatment *i*.

RESULTS AND DISCUSSION

Digestion and Metabolism Analyses

Table 3.2 shows the effects of TRT and TEMP on water consumption, metabolism of the diet, dietary N, and methane output. There were no significant TRT by TEMP interactions for these variables.

Treatment effects. There were no significant TRT effects on daily water intake although the ADY supplemented steers tended to consume more than CON-fed steers (15 vs. 12.1 kg/d, respectively; $P = 0.25$; Table 3.2). Dietary TRT did not significantly affect DMI ($P = 0.97$). This result is consistent with other reports in beef cattle fed ADY supplements (McGinn et al., 2004; Zerby et al., 2011). There was a tendency for TRT to affect fecal DM excretion when expressed either as kg/d or as % of SBW basis ($P = 0.07$). In general, ADY steers tended to excrete less fecal DM than CON steers (0.321 vs. 0.348% of SBW), indicating greater apparent DMD when receiving ADY. In fact, DMD was

significantly affected by TRT (*P* =0.0311) in which DMD was greater for ADY-supplemented steers than for CON-fed steers (77.1 vs. 75.3%, respectively). This finding is supported by a meta-analysis of research conducted in dairy cattle (Desnoyers et al., 2009). This result suggested that ADY may increase available DE to steers receiving high-concentrate diets (i.e., finishing diets). There was no effect of TRT on urine excretion (*P* =0.8387). There was considerable variance in methane production (L/d) but no significant effects of TRT (% of DMI) (*P* = 0.5519). There was no significant effect of TRT on N intake, fecal N excretion, or urinary N excretion and consequently, no effect on % N retained was observed (*P* = 0.3032).

Temperature effects. There were no significant TEMP effects on daily water intake (Table 3.2). It was expected that TEMP would increase water consumption in an effort to regulate body temperature. However, the temperature of the available water was similar to the temperature of the chamber and may have only provided minimal relief. Additionally, there has been evidence that ambient temperatures >30 °C have a low negative correlation with water intake (Rouda et al., 1994).There was a significant effect of TEMP on DMI (kg/d and % of SBW; *P*< 0.0005). Though steers were restricted to consuming only 1.5% of their SBW as DM, HS steers still had reduced DMI versus TN steers (7.10 vs. 6.43 kg/d; *P* < 0.0005). This 9.4% reduction in DMI is slightly less than that predicted by the LRNS model (12.5%) for DMI adjustment due to current effective temperature index (**CETI**) of 35.3°C with no night time cooling (Fox et al., 2004; Tedeschi and Fox, 2018) but this is likely because animals where not consuming at their voluntary intake level.

There was also a significant effect of TEMP on fecal DM excretion and DMD (P < 0.005 and < 0.05, respectively). It was expected that since steers experiencing HS had lower DMI, they would excrete significantly less DM than TN steers. Indeed HS steers excreted significantly less fecal DM than TN steers, resulting in a 15% difference (0.306 vs. 0.363% SBW, respectively). The net effect of decreased DMI and fecal DM excretion still resulted in significantly greater DMD for HS steers than TN steers (77.1 vs. 75.3%, respectively). High ambient temperature is known to decrease ruminal passage rate, potentially increasing the diet digestibility (Miaron and Christopherson, 1992; Bernabucci et al., 1999).

There was a significant effect of TEMP on urine excretion (P < 0.05) where steers under HS produced more total urine than TN steers (14.8 vs. 10.7 kg/d, respectively) at a greater percentage of their SBW (3.07 vs. 2.23% SBW, respectively). Interestingly, the urinary output of HS steers exceeded their water consumption (3.07 vs. 2.84% of SBW) indicating negative water balance.

Due to a decreased DMI, there was a significant effect of TEMP on N intake where HS steers consumed less N than TN steers (0.122 vs. 0.134 kg/d, respectively; P < 0.0005). This manifested into significantly less total N excreted in the feces for HS vs. TN steers (0.0036 vs. 0.0039 kg/d) with a tendency to be less concentrated (2.91 vs. 3.01% N, respectively; P = 0.12). There was no significant TEMP effect of urinary N excretion, although as expected, there was a tendency for TN steers to produce urine with a greater concentration of N than HS steers (0.731 vs. 0.581 %, respectively; P = 0.08), likely due the overall difference in N intake. Although there are obvious differences in % N retained, there was no significant effect of TEMP (P = 0.1833).

There was considerable variance in methane production (L/d), and no significant effects of TRT and TEMP were detected. However, when CH_4 was expressed per kg of DMI, a significant effect of TEMP was observed. Steers that experienced HS produced significantly more CH_4 than TN steers (12.9 vs. 9.8, L/kg DMI, respectively; $P < 0.05$). The greater CH_4 output per kg of intake is very likely due to the slower ruminal passage rate previously suggested to contribute to the greater DMD of HS steers. By this logic, it is interesting to note that no observed TRT difference observed for CH_4 production (L/kg of DMI) considering the TRT effect on DMD, suggesting that a post-rumen digestibility rather than ruminal digestibility might have occurred for ADY-fed steers.

Indirect Calorimetry

Table 3.3 shows the results of the indirect calorimetry analysis. There were no significant TRT by TEMP interactions. The respiratory quotient (**RQ**) of CO_2/O_2 was above 1.0 for all steers indicative of possible lipogenesis (Ferrannini, 1988) regardless of TRT or TEMP. This is also validated by the SWG and the *kg* (mean= 0.79, based on regression), indicative that the composition of gain from RE was likely due to accumulation of much more fat than protein (Tedeschi et al., 2004; Chizzotti et al., 2008; Tedeschi et al., 2010; Marcondes et al., 2013), likely influenced by the maturity of the steers in this trial. Tedeschi and Fox (2018) summarize these studies to reveal that the efficiency of use of ME for growth is inversely related to the percentage of RE in the form of protein, and at zero protein deposition, *kg* is between approximately 0.6 and 0.8, depending on the ME concentration of the diet. There was no significant effect of TRT or TEMP on RQ, although there was a tendency for an effect of TEMP where TN steers had greater RQ than HS (1.11 vs. 1.09,

respectively; $P = 0.07$), which may indicate that TN steers were more efficient at depositing energy as fat than HS steers. The indirect calorimetry technique consistently yields greater RE values than the comparative slaughter technique when animals are fed at production levels (Larson and Johnson, 1997). Likewise, comparative slaughter techniques may inflate the HE of cattle in production settings. Due to the inability to effectively separate energy used to support maintenance from that used to support growth requirements at different levels of energy intake, either technique may result in errors between predicted and observed *kg*, which is also affected by dietary energy concentrations and the composition of the gain. Tedeschi and Fox (2018) discussed the needs for the next generation of growth models highlighting a more integrated system to better predict NEm requirements and *kg* at different levels of MEI. This is highlighted in Figure 3.1 where the HE and RE of steers (kcal/kg MBW) are plotted with a line indicating the threshold for NEm with adjustments for no physical activity (NEm = 70 kcal/kg MBW) according to NASEM (2016). Using the regression of RE on MEI and the partial efficiency of use of ME for maintenance (***km***) and ME of the diet, the predicted NEm is lower for both TN and HS compared with expected values (38.9 and 46.4 vs. 70 kcal/kg$^{0.75}$ of SBW; NASEM, 2016). However, when regressing log HE on MEI, the antilog of the intercept resulted in values closer, but still lower, to this threshold where NEm for TN steers was lower than HS steers (55.0 vs. 62.2 kcal/kg$^{0.75}$ of SBW) indicating the nonlinear regression of HE on MEI may be more precise at predicting NEm than regressions from RE (Chizzotti et al., 2008). Deviations in the observed performance from predicted dietary NEm and NEg contents may also be due to the inherited errors when using the assumed DE to ME ratio of 0.82 rather than being directly measured (Galyean et al., 2016). The impact of inconsistent use of DE-to-ME and ME-to-NE

efficiency calculations was illustrated by Tedeschi and Fox (2018) where they compare the relationships of ME-to-DE ratio and NEm-to-ME ratio versus DE intake using empirical equations recommended by NRC (1996, 2000), NRC (2001), Galyean, et al., 2016 and NASEM (2016). In summary, the steers in this study exhibited NEm values that were lower than the NASEM (2016) recommended maintenance energy requirement likely due to major differences in the *km* and *kg* of steers fed at production levels versus fasted or maintenance levels, calorimetry technique, physical activity, and dietary factors.

Treatment effects. Since there was no difference in DMI between TRT, the GEI was also not different (P = 0.97). However, there was a significant effect of TRT on FE in which ADY steers excreted roughly 10% less fecal energy than CON steers (6.77 vs. 7.44 Mcal/d, respectively; P = 0.0320). There was no effect of TRT on DE (Mcal/d; P = 0.19) however, the conversion of GE to DE was significantly greater for ADY- vs. CON- fed steers (76 vs. 73.6% of GE). There was no significant effect of TRT on UE (P = 0.83) or GASE (P = 0.52) in Mcal/d. GASE losses as a % of GE were not significantly different (P = 0.55). Therefore, there was no significant effect of TRT on Mcal/d of ME (P = 0.21) and the conversion of ME to DE was similar for CON and ADY (92.6 and 92.8 %, respectively; P = 0.73). As anticipated, these values are much higher than the used ME:DE conversion of 0.82 (NASEM, 2016). Studies conducted by Hales et al., (2012 and 2013) feeding Jersey steers dry-rolled or steam-flaked corn and different levels of wet distiller's grain plus solubles have also observed greater ME:DE ratios (91.9% to 96 %) than that predicted by the NASEM (2016) equations. Tedeschi and Fox (2018) provide a very good discussion of this matter and suggest that because the current models predicting TDN and DE are not discounted for

diet type or level of intake, true ME may be underestimated, especially for high concentrate diets. In fact, when we computed the NEm of the diet based on observed ME from steers we calculated a 7.4% greater NEm than what was predicted by the feed analysis; 1.88 Mcal/kg for TN steers vs. the predicted 1.75 Mcal/kg. Heat production was not affected by TRT (P = 0.92). Overall, there was a tendency for RE to be greater for ADY- than CON-fed steers (1.56 vs. 1.43 Mcal/kg, respectively; P = 0.11) but RE:ME was not significantly different (53.2 vs. 50.9 %, respectively; P = 0.27).

Temperature effects. Differences in DMI resulted in differences of GEI in which there was a significant reduction GEI of HS steers vs. TN steers (29.5 vs. 26.7 Mcal/d, respectively; P = 0.0004). Temperature also had a significant effect on FE excretion where HS steers excreted roughly 17 % less FE than TN steers (6.45 vs. 7.75 Mcal/d, respectively; P = 0.0003). This resulted in a significant effect of TEMP on DE availability where TN retained more energy than HS (21.8 vs. 20.3 Mcal/d; P = 0.0096), but interestingly the conversion of GE to DE was greater for HS than for TN (75.9 vs. 73.7 % of GE, respectively; P = 0.0123).

Although the HS steers produced more urine, there was no significant effect of TEMP on urinary energy (P = 0.49). There was a tendency for HS steers to produce more GASE than steers in TN conditions (0.719 vs. 0.606 Mcal/d, respectively; P = 0.13) and convert significantly more GE to GASE (2.70 vs. 2.07 % of GE, respectively; P = 0.0176). Regardless of the dietary restriction imposed the conversion of GE to CH_4 for steers under TN conditions was on the lower end of the 2006 Intergovernmental Panel on Climate Change

inventory report (3.0 ± 1.0 % of GE for feedlot cattle consuming a 90% concentrate diet) whereas those under HS were much closer to this value.

As expected, the remaining ME was greater for steers in TN conditions than HS conditions (20.3 vs. 18.8 Mcal/d, respectively; $P = 0.0191$), however there was a tendency for ME to be greater for steers under HS than for those in TN conditions when expressed as Mcal/kg of DM (2.93 vs. 2.85 Mcal/kg DM; $P = 0.10$) due to the lack of significant differences in UE and GASE losses. The conversion of DE to ME was similar for steers under TN and HS conditions indicating that effects of TEMP on UE and GASE losses are minor when steers are consuming a restricted amount of finishing diet.

As expected, HE was significantly affected by TEMP ($P = 0.0013$). Steers experiencing HS lost significantly more HE than TN steers due to increased respiration in attempt to maintain core body temperature (9.96 vs. 8.46 Mcal/d, respectively). This equates to roughly a 17.7% increase in maintenance energy for heat dissipation and is consistent with the heat stress adjustment factor (**HSF**) of 1.18 for open-mouth panting (NASEM, 2016) although panting was not analyzed. Using the CETI of the environment within the HS chamber (35 ± 0.55 °C and 42 ± 6.1 RH %), assuming a wind speed and sunlight exposure of 0, respectively, HSF would range from 1.15 to 1.24 with a mean of 1.19 (Tedeschi and Fox, 2018). Using the CETI to account for NEm requirements under HS conditions slightly over-predicted individual requirements, but offers greater control of differing environmental variables than the panting index which only designates two adjustments, 1.07 or 1.18 times NEm. Overall, steers under TN conditions retained 3.3 Mcal/d more than steers experiencing HS ($P = 0.0002$), as well as per kg of DM (1.63 vs. 1.39 Mcal/kg DM, respectively; $P =$

0.0043) and a greater conversion of ME to RE (57.5 vs. 46.6%, respectively; $P < 0.0001$). Using equations reported by Tedeschi and Fox (2018), the calculated SWG was roughly 21.7% less for steers under HS conditions vs. TN conditions (1.48 vs.1.89 kg/d, respectively; $P = 0.0003$).

Rumen Parameters

Feed treatment and environmental temperature effects on rumen parameters are shown in Table 3.4. There was a tendency for an interaction for mean ruminal pH ($P = 0.13$; Figure 3.1a). Under TN conditions ADY steers had significantly greater mean pH than CON steers (5.81 vs. 5.57, respectively), but under HS conditions, ruminal pH was much lower for CON and ADY and not different (5.42 vs. 5.37, respectively). Slower ruminal passage rate due to TEMP likely resulted in decreased acid clearance leading to overall lower mean pH in the rumen. The results of mean pH in a TN environment are clear that supplementing ADY promotes higher mean pH, notably above the SARA threshold. Evaluations made on the effects of feeding ADY and its role in modulating ruminal pH suggest shifting fermentation pathways from lactate to propionate (Desnoyers et al., 2009; Humer et al., 2017), however, it would seem that under extreme HS conditions this effect may be lost. There was no significant effect of TRT or TEMP on ruminal pH variation.

There was a tendency for an interaction of TRT and TEMP on DUR > pH 5.6 ($P =$ 0.07; Figure 3.3a). Steers supplemented with ADY spent greater DUR > pH 5.6 than CON-fed steers under TN conditions (863 vs. 619 min/d, respectively), but under HS conditions this was reversed (509 vs. 698 min/d, respectively). The DUR of time spent in SARA range was not significantly affected by TRT (450 vs. 376 min/d for CON and ADY respectively)

or TEMP (432 vs. 394 min/d for TN and HS, respectively). However, there was a tendency for a significant interaction of TRT and TEMP on DUR of time spent in ARA range (*P* = 0.14; Figure 3.3b) in which steers experiencing HS and supplemented ADY spent more time in ARA range than CON (485 vs. 307 min/d, respectively). For TN conditions and CON diet, however, steers spent more time in ARA range than ADY-supplemented steers (246 vs. 143 min/d, respectively). The main effect of TEMP on DUR of ARA was quite significant, (*P* = 0.0079), steers experiencing HS spent twice as much time in ARA than steers in TN conditions (396 vs. 195 min/d, respectively).

There was a tendency for interaction of TRT by TEMP for AUC > pH 5.6 (*P* = 0.10). Similar to the DUR > pH 5.6, the AUC > pH 5.6 indicated that not only did steers supplemented with ADY under TN conditions spend more time above pH 5.6 but they also reached higher pH during the day compared to CON under TN conditions (17.9 vs. 8.39 h/d, respectively). There was no difference between TRT under HS conditions (8.31 vs. 8.34 h/d, respectively). The main effects also revealed tendencies for TRT and TEMP effects in which ADY-fed steers had greater AUC > pH 5.6 than CON fed steers (13.1 vs. 8.36 h/d, respectively; *P* = 0.10) and steers in TN conditions were greater than when experiencing HS (13.2 vs. 8.32 h/d, respectively; *P* = 0.09). There was a significant effect of TEMP on the AUC in the SARA range in which TN steers had greater AUC than HS steers (20.8 vs. 15.2 h/d, respectively; *P* < 0.005). However, for AUC in ARA range, there was a significant effect of TEMP where steers in HS were nearly seven times greater than TN steers (4.67 vs. 0.70 h/d, respectively; *P* < 0.05).

There were no significant effects of TRT on mean ruminal temperature, temperature variation, DUR > 40°C or AUC > 40°C, however, as expected TEMP significantly affected these variables (Figure 3.2b). Mean ruminal temperature, and temperature variation was much greater for steers in HS than in TN conditions (40.73 ± 0.638 vs. 39.33 ± 0.241°C, respectively; $P < 0.0001$). The DUR and AUC > 40°C was also much greater for steers in HS than in TN conditions (1,124 vs. 91 min/d and 41.5 vs. 0.6 °C/h, respectively; $P < 0.0001$). Time spent above 40°C for the TN observations was likely due to the heat of fermentation while the steers undergoing HS were simply unable to properly dissipate heat resulting in severe heat loading.

The effect of rumen temperature greatly affects ruminal pH and the duration and magnitude of subclinical and acute ruminal acidosis. We believe it is primarily due to the reduced passage rate and increased ruminal digestibility, this risk of acidosis could be compounded by sudden shifts in the rumen microbiome. There has been evidence of microbial population shifts under heat stressed conditions in dairy heifers, notably the genus *Streptococcus* (Uyeno et al., 2010), which is accepted as the major culprit of the onset of ruminal lactic acidosis. Research on the growth and metabolism of *S. bovis* isolated from the rumen has indicated that peak growth occurs when media is maintained at a pH of 5.0 – 6.2 and at a temperature of 39°C (Russell and Hino, 1985; Yuwono and Kokugan, 2008). However, peak enzyme production seems to occur between 40 and 44°C (Bailey, 1959). Therefore, it could be that the interaction of decreasing pH and increasing temperature of the rumen creates exceptionally favorable conditions for *S. bovis* and other lactic acid producers to proliferate and out compete pH sensitive bacteria.

In Vitro Gas Production Technique

The IVGP technique provided valuable information into the fermentative capacity of the adapted rumen fluid inoculant (Table 3.5 and Figure 3.4).

Treatment effects. Inoculum taken from CON and ADY treated steers had similar pH, likely due to being taken in the fasted state (6.23 vs 6.28, respectively; P = 0.70). However, after 48 h of in vitro fermentation, there was a tendency for those feeds inoculated with ADY adapted rumen fluid to have lower final pH than CON adapted rumen fluid (6.12 vs. 6.23, respectively; P = 0.10). There were no significant differences observed for total gas production, the rate of fermentation or lag time between TRT (P = 0.44, 0.23, and 0.98, respectively). Numerically, ADY inoculum resulted in greater NDFd over CON, but due to variation between fermentation batches this was not significant (46.4 vs. 36.4%, respectively; P = 0.54), still, the numerical means agree with the DMD observed in the steer's metabolism where ADY resulted in greater digestion than CON feed TRT. Similar to the TRT means of CH_4 obtained from the calorimetry chambers differences in CH_4 (g/L) tended to be lower for ADY than CON (0.068 vs. 0.143 g/L, respectively; P = 0.21) and when expressed per kg of DM followed a similar trend (8.7 vs. 18.8 g/kg DM, respectively; P = 0.27), although the IVGP technique resulted in larger values than in vivo due to the complete fermentation of the diet. While the numerical differences are suggestive, the variation between fermentations was larger than expected. The IVGP technique is known to be subject to variation especially when different rumen fluid donors are utilized, but this may be overcome with greater replication (Tedeschi and Fox, 2018). Replication in this

study, however, was limited due to timing and logistics of obtaining rumen fluid from non-cannulated animals.

Temperature effects. Similar to the mean pH of the bolus data, the inoculum was also significantly affected by TEMP where TN steers had much higher pH than HS steers (6.70 vs. 6.16, respectively; P = 0.0339). The final pH after 48 h fermentation was not affected by TEMP (P = 0.39). There was no effect TEMP on the fermentation pattern of the in vitro feed samples; Total gas production, the rate of fermentation, and lag time of fermentation were similar ($P > 0.20$). There were no significant effects of TEMP on NDFd (P = 0.62) although the means were greater for TN than ADY (45.5 vs. 37.3 %, respectively).

Interactions. There was a tendency for a TRT by TEMP interaction regarding the aTDN and ME estimations at a passage rate of 4 and 6 %/h (Table 3.5) where inoculum taken from steers adapted to the CON diet tended to result in greater in vitro aTDN and ME under HS than TN but inoculum from ADY adapted steers was greater under TN than HS. This trend was in contrast to the in vivo derived values for ME and worthy of further investigation. Interestingly this interaction was not observed when using passage rate of 2 %/h. When we compared the observed ME of the steers with that predicted from the IVGP technique using 2, 4, 6, and 8 %/h fractional passage rates, the IVGP predicted ME at 2 and 4%/h was closer to the observed mean values for the HS animals (2.93 Mcal/kg) whereas the predicted ME at 6%/h was closer to TN animals (2.85 Mcal/kg), confirming our hypothesis that ruminal escape may have been significantly reduced with increased heat load (Figure 3.4). Previous research using a similar HS environment reported ruminal passage rate to be closer to 4 ± 0.02%/h in dairy cattle as well (Bernabucci et al., 1999). Moreover,

although it was not an objective of the research we may conclude that if the expected ruminal passage rate is carefully considered, the IVGP technique may be able to accurately estimate the biological value of a diet for animals under HS, even when using rumen inoculum from non-HS steers.

CONCLUSION

Under TN conditions supplemental ADY in the diets of finishing steers under feedlot conditions may significantly improve DM digestibility, DE, ME and possibly RE without affecting DMI. Additionally, supplemental ADY may significantly increase mean ruminal pH, above the SARA threshold under thermoneutral conditions. However, heat stress remains an environmental risk to finishing cattle by significantly affecting feed energy efficiency, methane production and acute ruminal acidosis of which we observed to be significantly different from TN conditions. There have been reports that live yeast supplemented to dairy cows during the hot season improved DMI, ruminal pH and productivity (Moallem et al., 2009; Salvati et al., 2015). However, the dietary characteristics of lactation diets are much different from typical finishing diets of beef cattle regarding energy density and minimum effective fiber. Under HS conditions we could not detect a significant benefit of ADY for feed efficiency or ruminal pH, likely due to the antagonism between slower ruminal passage rate and increased digestibility. It may be that supplemental ADY optimizes fermentation characteristics within a certain scope of energy density, effective fiber, and ruminal temperature. Future work should focus on the long-term feeding

of ADY at different energy densities and combine rumen parameters with performance traits of beef cattle.

LITERATURE CITED

Bailey, R. 1959. Transglucosidase activity of rumen strains of *Streptococcus bovis*. 2. Isolation and properties of dextransucrase. Biochemical Journal 72: 42.

Bernabucci, U., P. Bani, B. Ronchi, N. Lacetera, and A. Nardone. 1999. Influence of short- and long-term exposure to a hot environment on rumen passage rate and diet digestibility by Friesian heifers. Journal of Dairy Science 82: 967-973.

Brouwer, E. 1965. Report of sub committee on constant factors. In energy metabolism: Proceedings of 3rd Symposium held at Troon, Scotland, May 1964, 441-443. KL Blaxter, ed. EAAP Publ. No. 11. London, UK: Academic Press.

Chambers, J., and D. Bates. 1992. Nonlinear models. Statistical models in S. Pacific Grove, California: Wadsworth & Brooks/Cole Advanced Books & Software: 432-433.

Chaucheyras, F., G. Fonty, P. Gouet, G. Bertin, and J.-M. Salmon. 1996. Effects of a strain of *Saccharomyces cerevisiae* (Levucell® SC), a microbial additive for ruminants, on lactate metabolism in vitro. Canadian Journal of Microbiology 42: 927-933.

Chizzotti, M. L., L. O. Tedeschi, and S. C. Valadares Filho. 2008. A meta-analysis of energy and protein requirements for maintenance and growth of Nellore cattle. Journal of Animal Science 86(7):1588-1597.

Collier, R. J., G. E. Dahl, and M. J. VanBaale. 2006. Major Advances Associated with Environmental Effects on Dairy Cattle. Journal of Dairy Science 89: 1244-1253. Doi: https://doi.org/10.3168/jds.S0022-0302(06)72193-2.

Desnoyers, M., S. Giger-Reverdin, G. Bertin, C. Duvaux-Ponter, and D. Sauvant. 2009. Meta-analysis of the influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminants. Journal of Dairy Science 92: 1620-1632.

Ferrannini, E. 1988. The theoretical bases of indirect calorimetry: a review. Metabolism 37: 287-301.

Fox, D.G., L. O. Tedeschi, T. P. Tylutki, J. B. Russell, M. E. Van Amnburgh, L. E. Chase, A. N. Pell, T. R. Overton. 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Animal Feed Science and Technology 112: 29-78.

Fox, P., A. Hall, and N. L. Schryer. 1978. The PORT mathematical subroutine library. ACM Transactions on Mathematical Software (TOMS) 4: 104-126.

Gay, D. M. 1990. Usage summary for selected optimization routines. Computing science technical report 153: 1-21.

Galyean, M. L., N. A. Cole, L. O. Tedeschi, and M. E. Branine. 2016. BOARD-INVITED REVIEW: Efficiency of converting digestible energy to metabolizable energy and reevaluation of the California Net Energy System maintenance requirements and

equations for predicting dietary net energy values for beef cattle. Journal of Animal Science 94(4):1329-1341.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). USDA Agr Handb.

Hales, K. E., N. A. Cole, and J. C. MacDonald. 2012. Effects of corn processing method and dietary inclusion of wet distillers grains with solubles on energy metabolism, carbon-nitrogen balance and methane emissions of cattle. Journal of Animal Science 90 (9):3174-3185.

Hales, K., N. Cole, and J. MacDonald. 2013. Effects of increasing concentrations of wet distillers grains with solubles in steam-flaked, corn-based diets on energy metabolism, carbon-nitrogen balance, and methane emissions of cattle. Journal of Animal Science 91: 819-828.

Humer, E. R. M. Petri, J. R. Aschenbach, B.J. Bradford, G. B. Penner, M. Tafaj, K-H Südekum, Q. Zebeli. 2017. Invited review: Practical feeding management recommendations to mitigate the risk of subacute ruminal acidosis in dairy cattle. Journal of Dairy Science.

Intergovernmental Panel of Climate Change. 2006. IPCC Guidelines for national greenhouse gas inventories. Vol. 4: Agriculture, forestry and other land use. Pp 10.1-10.87.

Lighton, J. R. 2008. Measuring Metabolic Rates: A Manual for Scientists. Oxford University Press.

Lila, Z. A., N. Mohammed, T. Yasui, Y. Kurokawa, S. Kanda, H. Itabashi. 2004. Effects of a twin strain of *Saccharomyces cerevisiae* live cells on mixed ruminal microorganism fermentation in vitro1. Journal of Animal Science 82: 1847-1854. Doi: 10.2527/2004.8261847x.

McGinn, S., K. Beauchemin, T. Coates, and D. Colombatto. 2004. Methane emissions from beef cattle: Effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. Journal of Animal Science 82: 3346-3356.

Miaron, J. O., and R. Christopherson. 1992. Effect of prolonged thermal exposure on heat production, reticular motility, rumen-fluid and-particulate passage-rate constants, and apparent digestibility in steers. Canadian Journal of Animal Science 72: 809-819.

Moallem, U., H. Lehrer, L. Livshitz, M. Zachut, and S. Yakoby. 2009. The effects of live yeast supplementation to dairy cows during the hot season on production, feed efficiency, and digestibility. Journal of Dairy Science 92: 343-351.

National Academy of Sciences, Engineering, and Medicine. 2016. Nutrient Requirements of Beef Cattle. (8th ed.). Nutrient requirements of domestic animals. National Academy Press, Washington, DC.

R Core Team. 2017. R A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org. Accessed on: January 27, 2018.

Rouda, R., D. Anderson, J. Wallace, and L. Murray. 1994. Free-ranging cattle water consumption in southcentral New Mexico. Applied Animal Behaviour Science 39: 29-38.

Russell, J. B., and T. Hino. 1985. Regulation of lactate production in *Streptococcus bovis*: a spiraling effect that contributes to rumen acidosis. Journal of Dairy Science 68: 1712-1721.

Salvati, G.S., N. N. Junior, A. C. S. Melo, R. R. Vilela, F. F. Cardoso, M. Aronovich, R.A.N. Pereira, M. N. Pereira. 2015. Response of lactating cows to live yeast supplementation during summer. Journal of Dairy Science 98: 4062-4073.

Schofield, P., R. Pitt, and A. Pell. 1994. Kinetics of fiber digestion from in vitro gas production. Journal of Animal Science 72: 2980-2991. Doi: 10.2527/1994.72112980x.

Sniffen, C. J., J. O'Connor, P. Van Soest, D. Fox, and J. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. V70: 3562-3577.

Tedeschi, L., P. J. Kononoff, K. Karges, and M. Gibson. 2009. Effects of chemical composition variation on the dynamics of ruminal fermentation and biological value of corn milling (co) products. Journal of Dairy Science 92: 401-413.

Tedeschi, L. O., and D. G. Fox. 2018. The Ruminant nutrition system: an applied model for predicting nutrient requirements and feed utilization in ruminants. 2nd edition, XanEdu, Acton, MA.

Uyeno, Y., Y. Sekiguchi, K. Tajima, A. Takenaka, M. Kurihara, Y. Kamagata. 2010. An rRNA-based analysis for evaluating the effect of heat stress on the rumen microbial composition of Holstein heifers. Anaerobe 16: 27-33. Doi: 10.1016/j.anaerobe.2009.04.006.

Williams, W. L., L. O. Tedeschi, P. J. Kononoff, T.R. Callaway, S. E. Dowd, K. Karges, M. L. Gibson. 2010. Evaluation of in vitro gas production and rumen bacterial populations fermenting corn milling (co) products. Journal of Dairy Science 93: 4735-4743. Doi: 10.3168/jds.2009-2920.

Yuwono, S. D., and T. Kokugan. 2008. Study of the effects of temperature and pH on lactic acid production from fresh cassava roots in tofu liquid waste by *Streptococcus bovis*. Biochemical Engineering Journal 40: 175-183. Doi: https://doi.org/10.1016/j.bej.2007.12.004.

Zerby, H.N., J. L. Bard, S. C. Loerch, P. S. Kuber, A. E. Radunz, F.L. Fluharty. 2011. Effects of diet and Aspergillus oryzae extract or *Saccharomyces cerevisiae* on growth and carcass characteristics of lambs and steers fed to meet requirements of natural markets1, 2. Journal of Animal Science 89: 2257.

Table 3.1. Ingredient and chemical analysis of the basal finishing diet fed to beef steers

Items[1]	Finishing Diet %
Ingredients	
Alfalfa hay, medium chop	15
Cracked corn	70
Dried Distiller's Grain	5.5
Molasses	6.8
Mineral Supplement	1
Limestone	1
Urea	0.7
Chemical Analysis	
DM, % of diet	82.2
CP, % DM	11.8
Soluble protein, % CP	19.1
NDF, % DM	21.7
ADF, % DM	12.4
Lignin, % DM	2.7
Crude fat, % DM	3.5
Sugar, % DM	4.6
Starch, % DM	49.2
Ash, % DM	4.9
Calcium	0.7
Phosphorus	0.4
TDN, %	82
NEm, Mcal/kg	1.74
NEg, Mcal/kg	1.14
peNDF, %[2]	10
GE, Mcal/kg[3]	4.16

[1]Items are feed ingredients and chemical composition of diets evaluated by Cumberland Valley Analytical Services (Hagerstown, MD).
[2]peNDF is physical effective fiber; method by Penn State Particle Size separator 4 mm sieve.
[3]GE measured by bomb calorimetry.

Table 3.2. Effect of yeast treatment and environmental temperature on the water consumption and metabolism of finishing steers

Items[1]	TRT[2]		TEMP[3]			*P*-value		
	CON	ADY	TN	HS	SEM	TRT	TEMP	TRT x TEMP
Water intake, kg/d	12.1	15.0	13.4	13.8	2.40	0.2514	0.8692	0.6165
Water intake, % SBW	2.51	3.09	2.76	2.84	0.444	0.2719	0.8809	0.6479
Water intake:DMI	1.78	2.25	1.89	2.13	0.37	0.2062	0.5149	0.5698
DMI, kg/d	6.77	6.77	7.10[a]	6.43[b]	0.197	0.9786	0.0004	0.6615
DMI, % SBW	1.41	1.40	1.47[a]	1.34[b]	0.0445	0.8568	0.0016	0.8740
Fecal DM, kg/ d	1.67	1.55	1.75[a]	1.48[b]	0.120	0.0766	0.0005	0.7721
Fecal DM, % SBW	0.348	0.321	0.363[a]	0.306[b]	0.0246	0.0730	0.0007	0.8793
DMD, %	75.3[b]	77.1[a]	75.3[b]	77.1[a]	1.90	0.0311	0.0305	0.9296
Urine, kg/d	12.5	13.0	10.7[b]	14.8[a]	2.12	0.8252	0.0495	0.6350
Urine, % SBW	2.61	2.69	2.23[b]	3.07[a]	0.448	0.8387	0.0438	0.7259
Feed N, kg/d	0.128	0.128	0.134[a]	0.122[b]	0.0037	0.9786	0.0004	0.6615
Fecal N, kg/d	0.0037	0.0038	0.0039[a]	0.0036[b]	0.0001	0.4578	0.0172	0.1510
Fecal N, %	2.93	2.99	2.91	3.01	0.072	0.3456	0.1238	0.1788
Urinary N, kg/d	0.0694	0.0630	0.0656	0.0668	0.004	0.2539	0.8356	0.6555
Urinary N, %	0.700	0.613	0.731	0.581	0.117	0.2887	0.0772	0.7584
N retained, %	42.1	47.0	47.8	41.3	3.65	0.3032	0.1833	0.5119
CH_4, L/d	78.7	73.3	69.5	82.5	8.98	0.5175	0.1280	0.3500
CH4, g/kg DM	7.68	7.22	6.46[b]	8.44[a]	0.935	0.5519	0.0174	0.3604

[a-b]Least squares means of the main effects in a row with different superscripts differ at $P < 0.05$

[1]Items are metabolism variables; SBW=shrunk body weight; DMI=Dry matter intake; N=Nitrogen; DMD=Dry matter digestibility.

[2]Treatment is control fed (**CON**) or control + 3 g/d of an active dried yeast (**ADY**).

[3]Temperature is thermoneutral (**TN;** 18 ± 0.55°C; 20% RH) or heat stressed (**HS;** 35 ± 0.55°C; 42% RH).

Table 3.3 Effect of treatment and temperature on energy metabolism of finishing steers using indirect calorimetry

Items[1]	TRT[2]		TEMP[3]		SEM	*P*-value		
	CON	ADY	TN	HS		TRT	TEMP	TRT x TEMP
RQ	1.11	1.10	1.11	1.09	0.009	0.6295	0.0734	0.4324
GEI, Mcal/d	28.1	28.1	29.5[a]	26.7[b]	0.819	0.9797	0.0004	0.6604
FE, Mcal/d	7.44[a]	6.77[b]	7.75[a]	6.45[b]	0.513	0.0320	0.0003	0.5638
DE, Mcal/d	20.7	21.4	21.8[a]	20.3[b]	0.946	0.1886	0.0096	0.8239
DE, Mcal/kg DM	3.06[b]	3.16[a]	3.06[b]	3.16[a]	0.082	0.0097	0.0123	0.6485
DE, % GE	73.6[b]	76.0[a]	73.7[b]	75.9[a]	1.96	0.0094	0.0136	0.6543
UE, Mcal/d	0.882	0.896	0.912	0.866	0.061	0.8279	0.4941	0.8978
GASE, Mcal/d	0.686	0.639	0.606	0.719	0.078	0.5175	0.1280	0.3500
GASE, % GE	2.46	2.31	2.07[b]	2.70[a]	0.299	0.5522	0.0176	0.3598
ME, Mcal/d	19.2	19.9	20.3[a]	18.8[b]	0.929	0.2055	0.0191	0.9181
ME, Mcal/kg DM	2.84[b]	2.94[a]	2.85	2.93	0.099	0.0377	0.0976	0.5740
ME, % DE	92.6	92.8	92.9	92.5	0.888	0.7294	0.5886	0.5889
HE, Mcal/d	9.23	9.19	8.46[b]	9.96[a]	0.293	0.9180	0.0013	0.4880
RE, Mcal/d[4]	9.73	10.7	11.5[a]	8.82[b]	0.683	0.1339	0.0002	0.4629
RE, Mcal/kg DM	1.43	1.56	1.63[a]	1.39[b]	0.081	0.1131	0.0043	0.3290
RE, % ME	50.9	53.2	57.5[a]	46.6[b]	1.49	0.2748	<0.0001	0.5185
SWG, kg/d	1.62	1.75	1.89[a]	1.48[b]	0.179	0.1793	0.0003	0.4281

[a-b]Least squares means within a row with different superscripts differ at $P < 0.05$

[1]Items are variables representative of the net energy system for beef cattle, indirect calorimetry procedures and combustion analysis. RQ=Respiratory Quotient (CO_2/O_2); GEI=Gross energy intake (total feed energy); DE=Digestible energy (GEI – Fecal energy (**FE**)); ME= Metabolizable energy (DE – Urinary energy (**UE**) and Methane energy (**GASE**)); RE=Retained energy (ME – Heat production energy (**HE**)). Shrunk weigt gain (**SBW**)= $13.91 \times RE^{0.9116} \times SBW^{-0.683}$

[2]Treatment is control fed (**CON**) or control + 3 g/d of an active dried yeast (**ADY**).

[3]Temperature is thermoneutral (**TN;** 18 ± 0.55°C; 20% RH) or heat stressed (**HS;** 35 ± 0.55°C; 42% RH).

Table 3.4. Effects of treatment and temperature on rumen pH characteristics of finishing steers

Items[1]	TRT[2]		TEMP[3]		SEM	*P*-value		
	CON	ADY	TN	HS		TRT	TEMP	TRT×TEMP
Mean pH	5.50	5.59	5.69[a]	5.39[b]	0.16	0.2901	0.0029	0.1279
pH variation	0.11	0.13	0.13	0.11	0.02	0.3328	0.3500	0.5440
DUR pH >5.6, min/d	658	686	741	603	167	0.8093	0.2407	0.0726
DUR of SARA, min/d	450	376	432	394	101	0.5519	0.7604	0.5925
DUR of ARA, min/d	277	314	195[b]	396[a]	141	0.6816	0.0381	0.1353
AUC pH >5.6, h	8.36	13.1	13.2	8.32	3.3	0.0976	0.0923	0.0954
AUC in SARA, h	18.1	17.8	20.8[b]	15.2[a]	3.2	0.8482	0.0028	0.2997
AUC in ARA, h	2.75	2.62	0.70[b]	4.67[a]	1.72	0.9255	0.0079	0.8862
Mean temperature, ˚C	40.04	40.01	39.33[b]	40.73[a]	0.15	0.7616	<0.0001	0.6289
Temperature variation, ˚C	0.463	0.417	0.241[b]	0.638[a]	0.076	0.5831	0.0001	0.9342
DUR > 40 ˚C, min/d	624	591	91[b]	1,124[a]	65	0.5422	<0.0001	0.4778
AUC >40, ˚C/h	21.9	20.2	0.6[b]	41.5[a]	4.9	0.7222	<0.0001	0.7641

[a-b]Least squares means within a row with different superscripts differ at $P < 0.05$

[1]Items are variables derived from indwelling ruminal pH and temperature boluses; DUR=duration, SARA=Sub-acute ruminal acidosis (pH= 5.0 to 5.6); ARA=Acute ruminal acidosis (pH < 5.0); AUC=area under the curve (pH units × time, h).

[2]Treatment is control fed (**CON**) or control + 3 g/d of an active dried yeast (**ADY**).

[3]Temperature is thermoneutral (**TN;** 18 ± 0.55°C; 20% RH) or heat stressed (**HS;** 35 ± 0.55°C; 42% RH).

Table 3.5. In vitro fermentation of a finishing diet using donor rumen fluid inoculum from treatment and temperature adapted steers

Items[1]	TRT[2]		TEMP[3]		SEM	*P*-value		
	CON	ADY	TN	HS		TRT	TEMP	TRT×TEMP
Inoculum pH	6.23	6.28	6.59[a]	5.91[b]	0.087	0.6991	0.0116	0.6473
Final pH	6.23	6.12	6.20	6.15	0.063	0.0986	0.3910	0.6681
Total Gas, mL	25.1	27.1	24.5	27.6	4.57	0.4420	0.2580	0.6946
Fermentation rate, h-1	11.9	16.7	15.8	12.8	2.31	0.2320	0.4189	0.5541
Lag time, h	0.524	0.520	0.586	0.458	0.195	0.9806	0.4607	0.2629
NDFd, %	36.4	46.4	45.5	37.3	12.0	0.5448	0.6165	0.3988
TDN,%								
kp 2 %/h	80.8	80.9	80.4	81.3	0.436	0.7869	0.1743	0.7244
kp 4 %/h	79.2	79.3	79.2	79.3	0.323	0.8726	0.7655	0.1692
kp 6 %/h	78.2	78.2	78.1	78.3	0.466	0.8752	0.8189	0.1651
ME, Mcal/kg								
kp 2 %/h	2.92	2.92	2.91	2.94	0.019	0.7944	0.1861	0.7944
kp 4 %/h	2.86	2.87	2.86	2.87	0.017	0.9093	0.7177	0.1411
kp 6 %/h	2.83	2.83	2.83	2.83	0.017	0.9052	0.9052	0.1474
CH_4, g/L gas	0.143	0.069	0.110	0.102	0.033	0.2106	0.8712	0.8866
CH_4, g/kg DM	18.8	8.70	13.7	13.8	5.28	0.2684	0.9855	0.9445

[a-b]Least squares means within a row with different superscripts differ at $P < 0.05$

[1]Items are variables which represent the rumen fluid inoculum or its fermentation effects of a finishing diet. Inoculum is initial pH from the donor steer. Final pH is after 48 h of fermentation in vitro. Total gas production from fermentation of 0.2 g of the finishing diet. NDFd= NDF digestibility. ME is shown with passage rates (kp) of 2, 4 and 6 %/h.

[2]Treatment is control fed (**CON**) or control + 3 g/d of an active dried yeast (**ADY**).

[3]Temperature is thermoneutral (**TN;** 18 ± 0.55°C; 20% RH) or heat stressed (**HS;** 35 ± 0.55°C; 42% RH).

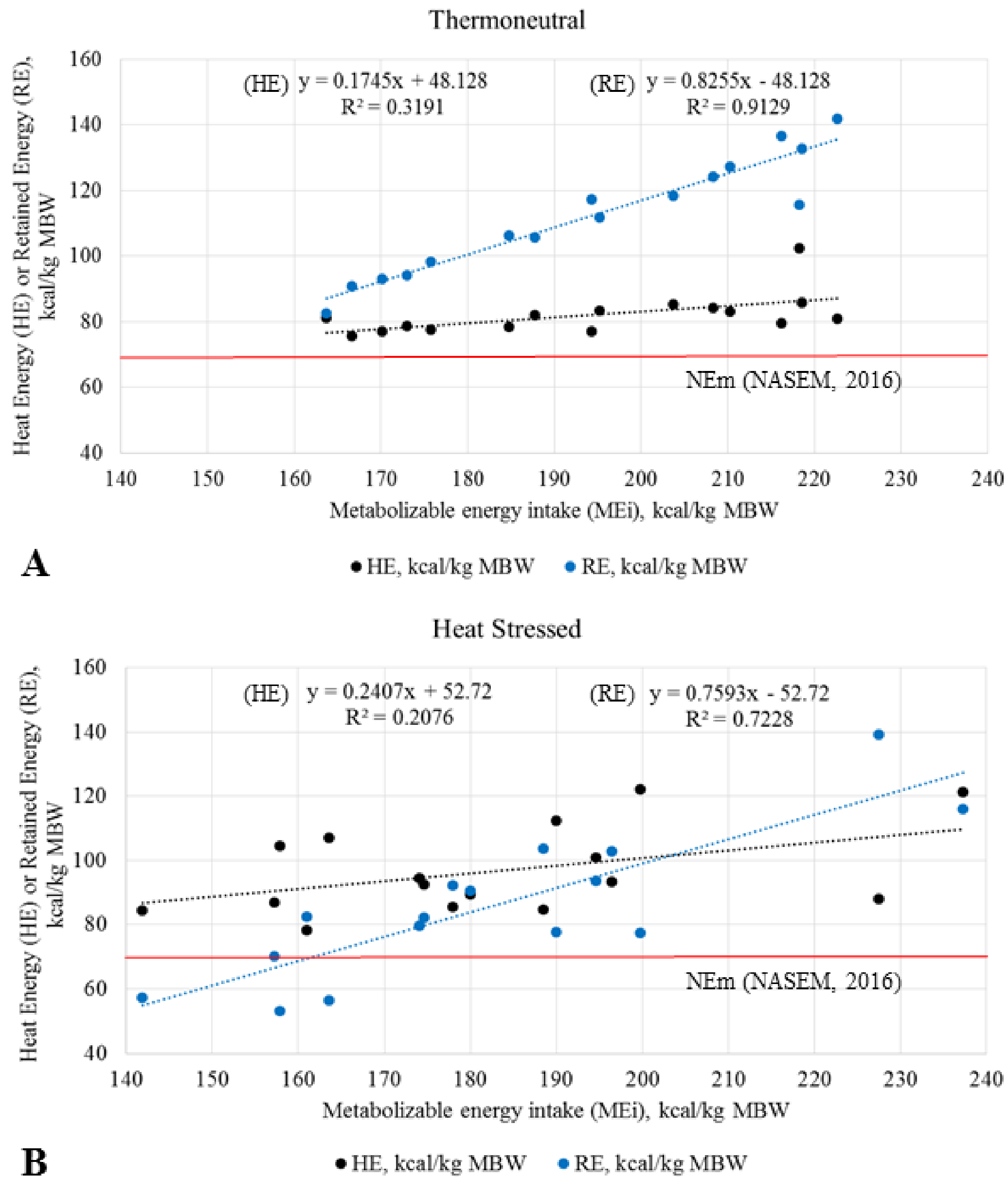

Figure 3.1 Regression of Heat Energy(HE) and Retained Energy (RE) on Metabolizable Energy intake (ME*i*) of steers using indirect calorimetry. Panel A) under thermoneutral (18 ± 0.55°C; 20% RH) or B) heat stressed (35 ± 0.55°C; 42% RH) conditions. Net Energy for maintenance (NEm) of 70 kcal/kg MBW recommended by NASEM (2016) is provided as a reference threshold.

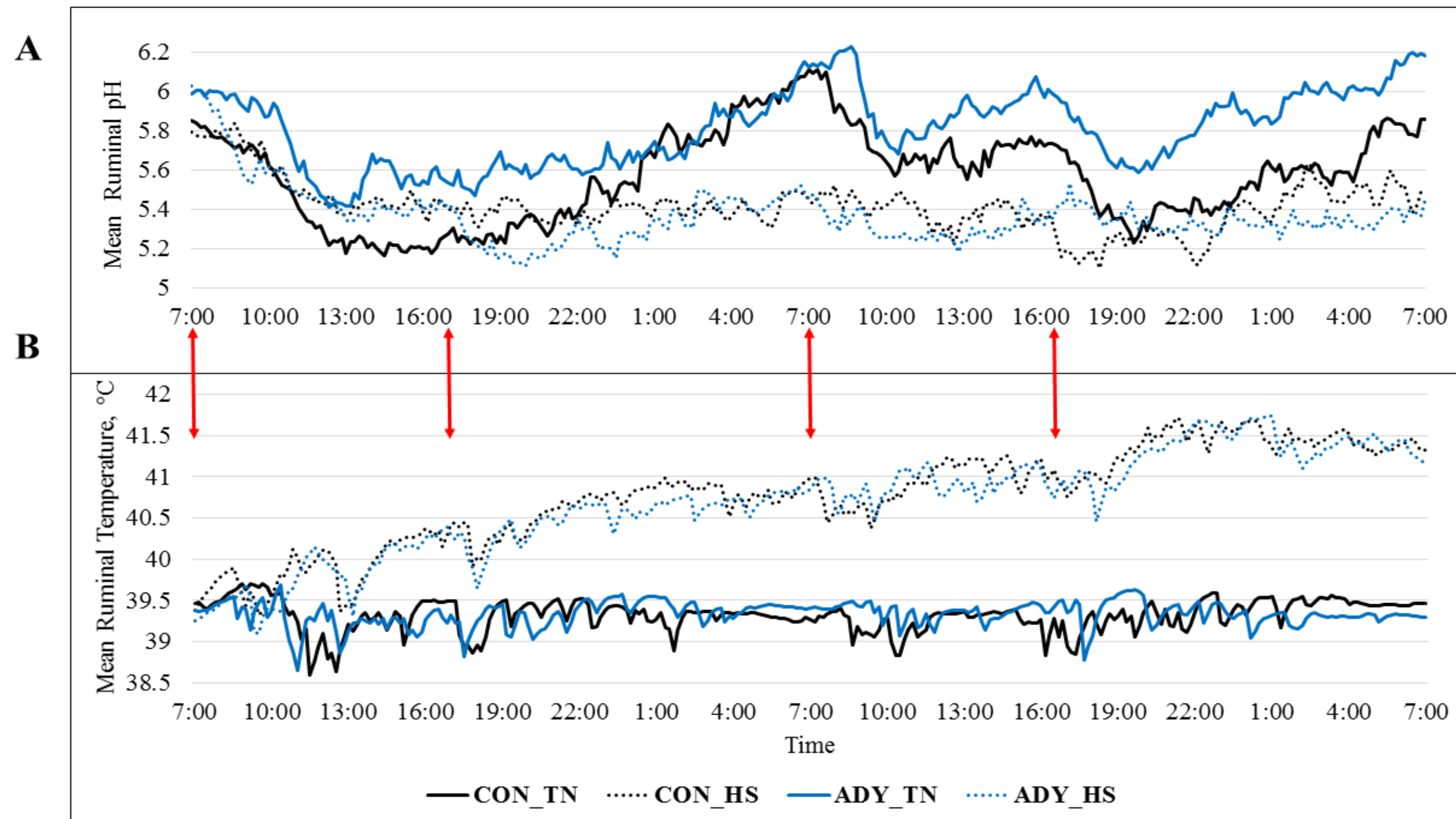

Figure 3.2. Representation of the mean diurnal fluctuation of ruminal pH (a) and ruminal temperature (b) over 48 h consuming a finishing diet. Treatments were control diet (**CON**) or control diet + 3 g/d of an active dried yeast (**ADY**). Temperatures were thermoneutral (**TN**; 18 ± 0.55°C; 20% RH) or heat stressed (**HS**; 35 ± 0.55°C; 42% RH). Interaction of TRT and Red arrows indicate feeding events. The mean pH represents the average of 8 replications per treatment combination, respectively over 48 h at 10 min intervals.

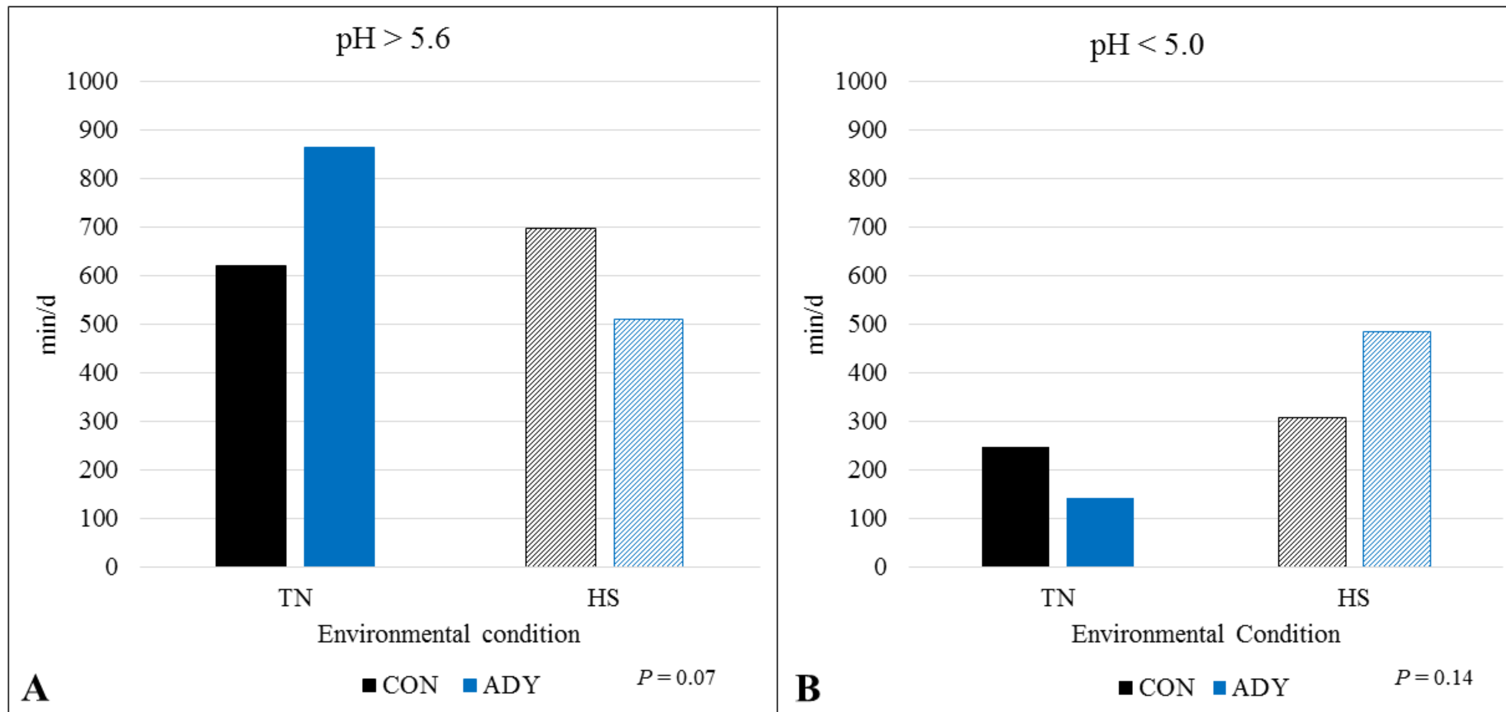

Figure 3.3. Interaction of treatment and temperature on ruminal pH variables; a) duration of time spent above pH 5.6 (min/d), b) duration of time spent in acute ruminal acidosis range, pH < 5.0 (min/d). Treatments were control diet (**CON**) or control diet + 3 g/d of an active dried yeast (**ADY**). Temperatures were thermoneutral (**TN**; 18 ± 0.55°C; 20% RH) or heat stressed (**HS**; 35 ± 0.55°C; 42% RH) indicated by the broken lines. Red arrows indicate feeding events.

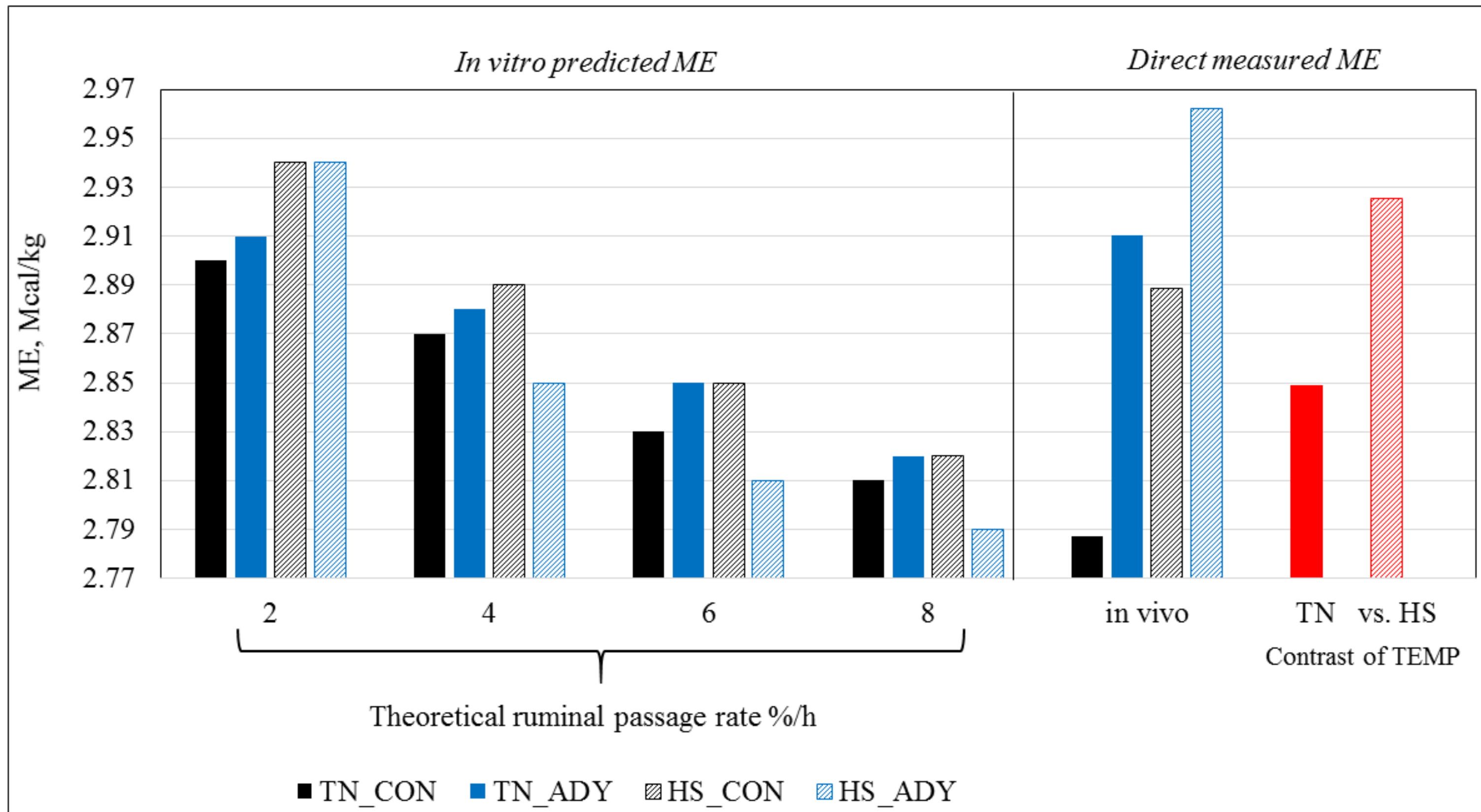

Figure 3.4. Interaction of TRT and TEMP on the predicted ME and comparison of predicted ME by the in vitro gas production technique (**IVGP**) and the direct measure ME of steers consuming a finishing diet. Treatments were control diet (**CON**) or control diet + 3 g/d of an active dried yeast (**ADY**). Temperatures were thermoneutral (**TN**; 18 ± 0.55°C; 20% RH) or heat stressed (**HS**; 35 ± 0.55°C; 42% RH). Contrasts of TN and HS (red) are included to denote possible effect of TEMP on passage rate and accuracy of IVGP predictions.

CHAPTER IV

EFFECTS OF ACTIVE DRIED YEAST ON GROWTH PERFORMANCE, RUMEN PH AND CARCASS CHARACTERISTICS OF GROWING BEEF STEERS

SYNOPSIS

The objective of this trial was to determine the benefits of supplementing active dried yeast (**ADY**) on ruminal pH, growth performance, and carcass characteristics to diets of growing and finishing beef cattle. Growing beef steers (n = 120) were blocked by BW and allocated to one of four pens fitted with an automated intake feeding system. Animals were fed either control (**CON**; no ADY), or ADY supplemented diets. Steers were fed four sequential diets: grower diet (**GROW**) for 70 d, two step up diets (**STEP1** and **STEP2**) for 7 d each, and finishing diet (**FIN**) from d 85 to 164. Indwelling rumen boli were orally administered to monitor rumen pH during d 56 to 106 during the dietary transition. A re-sort occurred on d 70 and resulted in four final TRT assignments: steers fed CON before and after the re-sort (**CC**; n = 30), steers fed CON before and ADY after the re-sort (**CY**; n = 30), steers fed ADY before and CON after the re-sort (**YC**; n = 30), and steers fed ADY (**YY**; n = 30). Dependent variables were analyzed as a Balaam's design, and ruminal parameters were analyzed as a repeated measures design using two approaches: raw preliminary analysis or drift analysis. Growth performance traits (BW, ADG, DMI, G:F, and residual feed intake) were not significantly affected by TRT ($P > 0.05$). Feeding behavior within the transition period revealed that CY steers ate more frequently than CC steers (9.3 vs. 8.5 meals/d, respectively; $P < 0.005$), and YY steers had larger meals than CY and YC

(1.45, 1.31, and 1.34 kg/meal, respectively; $P < 0.005$). Rumen pH analysis indicated that ruminal pH duration (DUR) below 6.0 ($P < 0.05$) and 5.8 ($P < 0.05$) was greater for CY steers than CC steers. Acidosis bout prevalence and DUR was greater for CY than other TRT ($P < 0.05$). The drift analysis indicated the ruminal pH values of CC and YC steers drifted further from basal pH values than CY and YY steers during the dietary transition ($P < 0.05$). Carcass characteristics were not different among TRT ($P > 0.1$). Percentile ranks of steers within performance traits resulted in a significant relationship between ADG and G:F rankings, the number of days steers experienced bouts (**DEB**) ($P < 0.05$), and a tendency for liver abscess severity ($P = 0.079$). Liver abscess severity was not affected by DEB ($P = 0.90$). There is evidence to suggest that the addition of ADY in the diets of beef cattle during the dietary transition may aid in ruminal stabilization without affecting growth performance and carcass traits.

INTRODUCTION

Increased scrutiny over feeding low-dose antimicrobial feed additives for growth promotion in livestock has prompted greater research discovery of antibiotic alternatives. Active dried yeast (**ADY**), specifically *Saccharomyces cerevisiae*, when added to the diets of ruminants seems to moderate ruminal pH, improve digestibility, and increase performance in intensive management systems. Research results of ADY inclusion to dairy cattle diets seem promising (Humer et al., 2018), but its effectiveness in feedlot cattle have not been as well established. A meta-analysis addressing some of these issues indicated that even the direct effects of live *S. cerevisiae* supplementation on OM digestibility, rumen pH, and fermentation end-products are subtle (Desnoyers et al., 2009). Thus, performance traits

measured at the pen level may not be sensitive enough to detect these subtleties, but the use of automated intake systems may help to characterize ADY effects on performance at the animal level.

Practical areas in which ADY supplementation may be useful for diets of growing beef cattle include the high-risk transition from a low to high concentrate diets where cattle may experience bouts of ruminal acidosis. It has been indicated that animals that experience a bout of acidosis may be more susceptible to future challenges (Dohme et al., 2008). Chronically low ruminal pH has been implicated in decreased efficiency of growth performance and linked to liver abscesses (Brink et al., 1990; Owens et al., 1998; Nagaraja and Lechtenberg, 2007). Supplementation of ADY may modulate ruminal pH during dietary transition leading to subtle changes in measurable production traits such as ADG and G:F.

The objective of this study was to determine the benefits of adding ADY to feedlot diets in an automated intake system favorably increased: 1) feedlot performance or feeding behavior traits, 2) stabilization of ruminal pH through the transition period, and 3) carcass characteristics and liver health.

MATERIALS AND METHODS

Animals and Care

All animals were cared for under the Institutional Animal Care and Use Committee of Texas A&M University approved Animal Use Protocol (number 2016-0269). One hundred twenty steers (¾ *Bos taurus*, ¼ *Bos indicus*) born and raised at the McGregor AgriLife Research center, McGregor, TX, were randomly selected after 30 d of weaning, with a weight range of 253 ± 40 kg. Steers were assigned an RFID tag (Allflex USA INC.,

Dallas, TX) and adapted to the GrowSafe feeding system (GrowSafe Systems LTD., Airdrie, Alberta Canada) for 21 d consuming a backgrounding diet before the commencement of the 164-d trial. Facilities included four pens, each equipped with four GrowSafe feed bunks (n = 16 feed bunks). Upon d 0, steers were blocked by BW according to the median BW resulting in 2 pens of light and 2 pens of heavy BW steers (215 ± 20 and 249 ± 16 kg, respectively). Within blocks, steers were randomly assigned to one of two pens.

Diets and Feeding

Steers were fed four diets over the course of the trial (Table 4.1) in 2 phases, growing (d 0 to 70) and finishing (d 71 to 164). Chemical analysis of the diet was performed by Cumberland Valley Analytical Services (Hagerstown, MD) on composite batch samples within diets. The physically effective NDF (peNDF) was determined by Penn State Particle Size separator and multiplying the proportion of particles > 4 mm by the proportion of NDF in the diet. The Large Ruminant Nutrition System (**LRNS**; http://www.nutritionmodels.com/lrns.html; Accessed on January 21, 2018; Tedeschi and Fox, 2018) was used to formulate the diets using the following ingredients: cracked corn, dried distiller's grain, a medium chopped alfalfa hay, molasses, and mineral supplements, as detailed in Table 4.1.

The grower diet (**GROW**; NEg of 1.0 Mcal/kg) was fed for 70 d to yield a mean BW of 365 kg at the time of dietary transition. The first and second transition diets (**STEP1** and **STEP2**, respectively) were fed for 7 d each (d 71 to 85) and the finishing diet (**FIN**; NEg of 1.14 Mcal/kg) was fed from d 86 to 164 to yield a final mean BW of 500 kg. Feed delivery occurred at approximately 0800 and 1700 h and feed calls allowed for 5 % residual orts.

Treatment Assignment

Pens, within a weight block, were assigned to receive either control (**CON**) or diets supplemented daily with approximately 1.5 g/hd (3 x 10^{10} CFU/d) of ADY, *Saccharomyces cerevisiae* (Vistacell, AB Vista, Marlborough, UK). To ensure proper daily dosage of ADY, individual intake was downloaded weekly from the GrowSafe Feed Intake System, and the inter-quartile mean was used to formulate a fresh premix to allocate approximately 1.5 g/d of ADY to be consumed by each animal in the following week. Treatment was the sequence of ADY supplementation. On d 70, 15 steers from each pen were re-sorted within their block to the opposite pen to proceed through the transition with or without ADY. This re-sort yielded four TRT assignments: steers fed CON before and after the re-sort (**CC**), steers fed CON before and ADY after the re-sort (**CY**), steers fed ADY before and CON after the re-sort (**YC**), and steers fed ADY before and after the re-sort (**YY**). The method of animal re-sorting caused equal social order disruption across all TRT groups.

Data Measurements

Steers were weighed on d 0 and every two weeks after that before the morning feeding, except for the final weight in which only 10 d elapsed between weighing events. Data from the GrowSafe Feed Intake System was used to model growth rates by linear regression against the day of trial with an average coefficient of determination of 0.986. The regression coefficients were used to compute initial and final BW, ADG and metabolic BW. As-fed feed intake data was converted to DM based on the chemical analysis of the diet. Residual feed intake (**RFI**) was computed as the difference between actual DMI and the predicted DMI from its regression on metabolic BW and ADG. Gain-to-feed ratio was calculated as ADG divided by DMI. Feeding behavior was modeled using the Meal Criterion

Calculation software (**MCC**) (http://nutritionmodels.tamu.edu/mes.html; accessed on January 12, 2018), which uses the R software (R Core Team, 2017) and the *mixdist* package to determine meals within bunk visits (Bailey et al., 2012).

On d 56, a random sample of steers (n = 66) within each block and TRT were chosen to receive a wireless, indwelling rumen pH, temperature and activity bolus (smaXtec Premium Bolus, smaXtec Animal Care GmbH, Graz, Austria). Bolus ID was assigned to each subject's visual ID tag, calibrated using buffer solution of pH 7 for a minimum of 5 min and inserted orally using the manufacturer provided balling gun. The bolus produced an average pH reading on 10-min intervals, automatically transmitting data when in range of the base station radio system or repeaters. Individual steer data was downloaded from smaXtec messenger for the selected days during the dietary transition. The analysis was limited to 50 d per the manufacturer's guarantee (trial d 56 to 106). Daily summary statistics were computed for each steer: pH minimum, mean, maximum, range, variance, the accrued variance over time (cumulative variance). Bouts of acidosis were considered to be frames of bolus readings displaying 180 consecutive minutes below pH of 5.6. Bouts were summarized by prevalence within a treatment group and the mean DUR of those bouts. The area under the curve (**AUC**) and duration (**DUR**) below pH thresholds were calculated with an R script, which used the rootSolve and Spline functions, of the R software (R Core Team, 2017). All AUC and DUR variables were summarized by diet.

Growth performance and feeding behavior were computed for d 0 to 164 and the transition period d 56 to 106, and included ADG, BW, G:F, meal frequency, meal size and bunk visit DUR.

Statistical Analysis

Three steers in the heavy block (2 from the YY group, 1 of which contained a bolus, and 1 from the YC group) were removed from the analysis due to illness. Therefore, n=64 for the rumen bolus variable analysis and n=117 for growth performance, feeding behavior and carcass characteristics. Due to a sorting error, the CY group contained 1 additional steer intended to be in the CC group. However, this did not exceed the recommended number of head per bunk due to the removal of other animals.

Growth performance, feeding behavior, and carcass characteristics. The feeding trial was designed as a four sequence (CC, CY, YC, YY) Balaam's design with a random variable of arrival weight block (heavy, **HVY,** and light, **LT**; n = 2) using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). It was unknown if the effect of yeast would elicit any carry-over effects. Therefore the Balaam's design was chosen to be the most suitable statistical design as it is strongly balanced so that the treatment difference is not aliased with differential first-order carryover effects (Chow and Liu, 1992). Additionally, the uniformity of equal representation of TRT within the period (growing or finishing phases) of the Balaam's design allows for the removal of the period effect to ascertain the effect of sequence resulting in the final model:

$$Y_{ij} = \mu + TRT_i + b_j + e_{ij},$$

where Y is the variable of interest for Growth performance and feeding behavior (initial BW, mid-test BW, final BW, DMI, ADG, G:F, RFI, meal frequency, mean meal size, bunk visit duration) and carcass characteristics (final shrunk BW, BW shrink percentage, HCW, dressing percentage, 12th rib back fat thickness, longissimus muscle area, calculated YG, and marbling score), i = is the treatment (CC, CY, YC and YY), j = is the weight block (HVY

or LT), μ and TRT_i are fixed parameters such that the mean for the ith treatment is $\mu_i = \mu + TRT_i$, b_j is the random effect associated with the jth block, and e_{ij} is the random error associated with the experimental unit in block j that received treatment i.

Continuous variables of growth performance and feeding behavior were analyzed for the total trial (n = 164) and the days spanning the dietary transition (n = 50 d) corresponding to the guaranteed lifespan of the rumen boli (d 56 – 106).

Ordinal data (USDA Quality Grade, USDA Yield Grade, liver abscess prevalence and severity and lung score) was analyzed using the chi-square test of probability using the FREQ procedure of SAS (SAS Inst. Inc., Cary, NC), observing the Mantel-Haenszel statistic. The distribution of data count is provided for reference and was discussed as significant at $P < 0.05$ and tendencies discussed at $P < 0.10$.

Preliminary rumen pH analysis. The primary drawback of Balaam's design is that it inflates the variance of an estimate of the treatment effect which reduces statistical power for detecting more subtle effects (Mori and Kano, 2015). Rumen pH data between subjects is known to be highly variable and, therefore, an additional analysis was pursued. Because steers were fed different diets and the re-sort occurred during this window of time, a randomized complete block design (**RCBD**) with repeated measures design analysis was deemed appropriate. Rumen bolus data was summarized within each steer by diet. The variables pH variation, AUC, and DUR displayed a Poisson distribution, and the non-parametric procedure (e.g., PROC RANK) was used within diet to produce a Gaussian distribution for parametric analysis (Mitchell et al., 1994; Narinç and Aygün, 2017). Respective means and ranks are reported. Rumen variables were analyzed in the MIXED

procedure of SAS (SAS Inst. Inc., Cary, NC) as an RCBD with diet as a repeated measure within animal subject resulting in the final model:

$$Y_{ijk} = \mu + TRT_i + Diet_j + TRT_i \times Diet_j + b_k + e_{ijkl},$$

where Y is the variable of interest (rumen pH variables: minimum pH, maximum pH, Mean pH, variance, cumulative variance, AUC and DUR < pH 6, 5.8, 5.6 and 5.4 and bout duration), i = is the treatment (CC, CY, YC, and YY), j = is the Diet (GROW, STEP1, STEP2, FIN), k = is the weight block (HVY or LT), μ and TRT_i are fixed parameters such that the mean for the ith treatment is $\mu_i = \mu + T_i$, $Diet_j$ is the fixed effect associated with the jth Diet, b_k is the random effect associated with the kth block, and e_{ijkl} is the random error associated with the experimental unit l, consuming Diet k, in block j that received treatment i.

An autoregressive variance-(co)variance structure was chosen for the repeated measures design to account for the potential of greater correlation between measurements taken closer together than those that were further apart. Acidosis bout prevalence was treated as a categorical variable and analyzed using the chi-square test of probability in PROC FREQ of SAS (SAS Inst. Inc., Cary, NC), observing the likelihood ratio statistic for statistical significance. Acidosis bout duration analysis included only those steers who were experiencing bouts.

Rumen pH variable drift analysis. More sensitive analysis of rumen variables involved a similar repeated measures design in which the days consuming the GROW diet served to establish baseline values for each subject before the re-sort and dietary transition. Deviations from each subject's basal values were then computed for each remaining day. In the drift analysis *days* refers to the days of the guaranteed lifespan of the bolus (d 1 -50)

where days 1 – 14 correspond to the GROW diet, d 15 – 21 correspond to the STEP1 diet, d 22 – 28 correspond to the STEP2 diet, and d 29 – 50 correspond to the FIN diet. Two new variables are introduced here to aid in the characterization of the wave of the diurnal fluctuation of ruminal pH, the upper amplitude (**Up_amp**) and the lower amplitude (**Low_amp**) as amplitude may not be evenly distributed across the daily mean pH. The amplitude variables were calculated by measuring the distance from the upper and lower peak to the mean within each subject per day. Baseline variables were analyzed similarly to the feeding trial analysis in which TRT was fixed and the block was random. The drift data were normally distributed, and the following model was analyzed in the PROC MIXED of SAS (SAS Inst. Inc., Cary, NC) in what is henceforth known as the *drift analysis*:

$$Y_{ijkl} = u + TRT_i + DAY_j + TRT_i \times DAY_j + b_k + e_{ijkl},$$

where Y is the variable of interest (Rumen pH variable change from baseline: Minimum pH (**MinΔ**), Maximum pH (**MaxΔ**), Mean pH (**MeanΔ**), variance (**VarΔ**), range (**RangeΔ**), Up_amp (**Up_ampΔ**), Low_amp (**Low_ampΔ**), i = is the treatment (CC, CY, YC, and YY), j = is the DAY (15, 16…,50), k = is the weight block (HVY or LT), μ and TRT_i are fixed parameters such that the mean for the ith treatment is $\mu_i = \mu + T_i$, $Diet_j$ is the fixed effect associated with the jth Diet, b_k is the random effect associated with the kth block, and e_{ijkl} is the random error associated with the experimental unit l, consuming Diet k, in block j that received treatment i.

Similarly, the first order auto-regressive variance-(co)variance structure was chosen for the time-series analysis. In this analysis, two steers were removed for receiving their boluses late and therefore failed to establish baseline variable (n=63).

RESULTS AND DISCUSSION

Growth Performance and Feeding Behavior

The effects of TRT on the growth performance and feeding behavior for the total feeding period and transition period are shown in Table 4.2. Analysis of the total feeding period revealed the growth performance variables initial BW, midtest BW, final BW, ADG, DMI, G:F, and RFI were similar across TRT groups and there were no significant TRT differences observed for feeding behavior traits. Analysis of the transition period yielded similar results for growth performance variables however there were significant TRT effects on feeding behavior for meal frequency and meal size. Steers in TRT group CY had significantly greater meal frequency than steers in the CC group (9.3 vs. 8.5 meals/d, respectively), while estimates for the YC and YY groups were intermediate and not different (9.0 and 8.8, respectively; $P < 0.005$). Mean meal size was also affected by TRT in which YY steers had the greatest meal size (1.45 g/meal), CY and YC steers had the least (1.31 and 1.34 g/ meal, respectively), and CC steers had intermediate meal size (1.38 g/meal; $P < 0.005$). Bunk visit DUR was not different between TRT groups. Although meal frequency and size was different across TRT groups during the transition period, it was not clear if this behavior was biologically or economically relevant since it did not manifest into any significant differences of growth performance traits.

Rumen pH Characteristics

Preliminary analysis. In the preliminary repeated measures analysis of rumen pH variables, one interaction of TRT and DIET was observed for maximum pH. Figure 4.1 shows the interaction plot over the course of the dietary transition. After steers were re-sorted there was a brief divergence of maximum daily pH appears between the CON (CC and YC)

and ADY-fed (CY and YY) steers during the STEP1 and STEP2 diets before reconvening during the FIN diet. Over the transition, the maximum daily pH achieved by steers not consuming yeast increases incrementally as dietary energy increases versus those consuming yeast do not show any significant change until they have reached the FIN diet. We would expect steers of similar BW to have the similar buffering capacity and the biological significance of a difference in maximum pH is difficult to interpret. This difference could be related to feeding behavior as the CY ate more frequently and the YY group ate the largest meals either of which may reduce the maximum pH achieved in a day. Another reason could be due to differences in digestibility, and VFA concentration in the rumen of ADY fed steers as has been identified in several studies (Desnoyers et al., 2009). We believe that one or more factors may have contributed to this separation of means. There was a tendency for the interaction of TRT and DIET on acidosis bout prevalence ($P = 0.09$). The distribution of bout prevalence is shown in Figure 4.2 and shows that while the prevalence amongst the CC group was steady throughout the transition, prevalence increased for the CY group through the FIN diet, but was greatest during the STEP2 diet for YC and YY groups. The most valuable information to be taken from Figure 4.2 is that steers supplemented ADY when consuming the GROW diet (groups YY and YC) had fewer bouts of acidosis than those who were not supplemented at this time.

The main effects of TRT on ruminal activity are shown in Table 4.3. Daily minimum pH (5.39, 5.25, 5.33, and 5.33 for CC, CY, YC, and YY, respectively; $P = 0.4061$) was not significantly affected by TRT. Average daily mean pH was affected by TRT in which the CC and YC groups had the greatest average pH and were different from the CY group, with the YY group being intermediate and not different (6.26 and 6.23 vs. 6.09 and 6.19,

respectively; P =0.05). Neither average daily range, variance, nor cumulative variance was detected to be significantly different between TRT groups according to their ranks in the population (P = 0.74, 0.95, and 0.67, respectively).

There was a tendency for the AUC < pH 6.0 to be different across TRT being the greatest for the CY group and different from CC, YC, and YY (31.6 vs. 18.1, 19.9, and 19.7, respectively; $P < 0.10$). However, TRT did not significantly affect the AUC < pH 5.8, 5.6, and 5.4. There was a very strong tendency for TRT to affect the DUR < pH 6.0 and 5.8 (P = 0.0528 and 0.0536, respectively). In both cases, steers in the CY group spent significantly more time below the thresholds than those in the CC group while the YC and YY groups were intermediate. Interestingly, DUR < 5.6 was not different between TRT groups ($P > 0.2$) but acidosis bout prevalence was different ($P < 0.05$). It should be noted here that DUR below a threshold in a day does not indicate that the minutes are consecutive but rather the summation of minutes. A greater percentage of steers in the CY group experienced bouts of acidosis each day than CC, YC and YY groups (12.4 vs. 7.9, 8.3, and 8.8 % of subjects/d, respectively). Mean bout duration was different between TRT groups CY and YC (352 vs. 260 min/bout), but CC and YY were intermediate (327 and 346 min/bout, respectively; $P <$ 0.05). The estimates for mean bout duration are larger than DUR < pH 5.6 because bout duration only included those steers experiencing bouts versus DUR < pH 5.6 includes all 65 fit with a bolus including steers which may have never crossed the pH threshold.

Table 4.4 shows the main effects of DIET on rumen pH variables. It was expected that as the percentage of concentrate increased in the diet the minimum daily pH achieved would decrease. While there was a significant effect of DIET on minimum daily pH only STEP1 and FIN were significantly different (5.40 vs. 5.24, respectively) in which GROW

and STEP2 where intermediate and not different from the FIN diet (5.37 and 5.34, respectively; $P < 0.0001$). The daily mean pH was as expected to be greater for GROW than STEP1, STEP2, and FIN (6.22 vs. 6.19, 6.18, and 6.19, respectively; $P < 0.05$). The daily range in pH was greatest on the FIN diet versus the GROW, STEP1, and STEP2 diets (1.91 vs. 1.50, 1.49, and 1.64 pH units, respectively; $P < 0.0001$). Although numerically the daily pH variance and cumulative pH variance increased over the transition they were not statistically different between diets according to their ranks in the population ($P = 0.99$ and 0.62, respectively). There were no significant effects of DIET on AUC or DUR variables likely due to significant between-subject variation. As expected, acidosis bout prevalence increased over the transition where the number of steers experiencing bouts increase by nearly twice from GROW to STEP1 (3.91 to 7.03 %/d) and tripled by STEP2 and FIN (12.31 and 12.24 %/d, respectively; $P < 0.0001$). The bout duration, however, was not statistically different between DIETS ($P = 0.27$).

Although there were significant differences in feeding behavior and some ruminal pH variables between TRT groups during the transition period, these differences did not manifest into any biologically significant feeding performance trait. Yet because we did not detect a difference in growth performance, it is unclear if the observed differences in feeding behavior or ruminal characteristics are necessarily positive or negative outcomes. As mentioned earlier, the between-subject variation in the sample population for rumen pH variables was extremely high and greater than expected but concurs with other research (Dohme et al., 2008). Many trials profiling the effect of feed additives on ruminal pH parameters have done so on quite large (>450 kg) or mature animal subjects which may account for a great deal of variance in a data set (Bach et al., 2007; Chung et al., 2011; Vyas

et al., 2014). However, the summary statistics of rumen variables for young growing beef calves (Table 4.5), on a single diet (GROW) highlight this matter. When considering the CV % between subjects for daily pH variation, AUC and DUR variables acquiring the appropriate sensitivity to detect subtle differences is largely unattainable due to the required number of necessary and the relative expense of the bolus technology. It also reiterates the necessity of using a repeated measures analysis and, regarding data distribution (in our case, Poisson), the need for data transformation for meaningful comparison that does not violate parametric analysis assumptions. To account for non-Gaussian distributed data, some experiments reporting significant differences in AUC have done so by blocking experimental animals based on their pre-trial tendency to spend time below a pH threshold (Chung et al., 2011). Other researchers have used log transformations to enhance distribution normality and decrease variation (Dohme et al., 2008). However, log transformations may not always result in a normal distribution and should not be conducted for variables when observations may contain values of zero or values less than 1 (e.g., Duration below or AUC of given thresholds, pH variation) (Sileshi, 2012; Changyong et al., 2014), and moreover, may not be appropriate for variables already on a log scale, such as pH. More recent research suggests that data derived from continuous monitoring systems should focus on deviations from the expected outcomes based on individual animal patterns rather than pre-defined pH thresholds (Denwood et al., 2018). This concept may eventually be the right approach, but currently, the lifespan of the technology is limited, the analysis is complex and for feed additive studies, may not be economically feasible for the necessary replication requirements. However, the following drift analysis is based on the idea of deviations from

the expected values within a subject over time and offers its unique value to our knowledge of rumen pH characteristics.

***Drift analysis*.** The daily pH fluctuation and corresponding coefficient of determination for the basal period (GROW diet) and each TRT group are shown in Figure 4.3. The drift analysis does not include the AUC or DUR variables due to the extreme CV % of the sample population mentioned previously. In the drift analysis, based on the claims that supplemental ADY may help stabilize ruminal pH over the dietary transition, there were certain expectations for the outcome of TRT groups; 1) that changes from baseline would be lower for YY than for CC and 2) that changes for YC would be greater than CY.

There were no significant TRT by DAY interactions observed in the drift analysis. Table 4.6 and Figures 4 and 5 have the effects of TRT and day on the deviations of rumen variables from their basal values. In Table 4.6, the mean basal values are given as a reference, and the direction of the variable Δ is shown below. There was no significant difference between TRT groups MinΔ ($P = 0.46$) or MeanΔ ($P = 0.27$). The finding for MinΔ is similar to the preliminary data analysis. However, the finding for MeanΔ is in contrast. The difference here is that the preliminary analysis compared group means of the raw pH values, the drift analysis compares how far each subject's mean pH drifted from its baseline established on the GROW diet. Analysis of the baseline mean pH shows that there was a slight tendency ($P = 0.15$) for TRT group CY to be different from CC, YC and YY (6.13 vs. 6.26, 6.25, and 6.23, respectively) and this condition may have been inflated over the transition. However, in the drift analysis, deviations from baseline values offer greater accounting for the between-subject variation and therefore may be more meaningful to isolating the TRT effect, which in this case was different between TRT groups. Similar to

the preliminary analysis there was a significant effect of TRT on MaxΔ where the YC group had the greatest increase from basal values and were different from CY and YY groups, while the CC group was intermediate and not different (0.165 vs. 0.089, 0.087 and 0.120, respectively; $P < 0.01$). This likely contributed to the similar pattern for RangeΔ where again the YC group was significantly greater than CY and YY, and CC was intermediate and not different (0.310 vs. 0.208, 0.194 and 0.226, respectively; $P < 0.01$). This indicates that there was less change in daily fluctuation of CY and YY groups than the YC group and agrees with the presumptive claim of pH stabilization. There was a significant TRT effect on VarΔ where the YC group was significantly greater than CC, CY and YY (0.058 vs. 0.035, 0.038 and 0.039, respectively; $P < 0.01$). This finding is interesting because it may indicate that when ADY is removed from the diet, the variance may increase within a subject whereas the addition of ADY (i.e., CY treatment) may aid in reducing the variance. Since the CC and YY groups were not subjected to the ADY supplement change, we would expect to not see a difference in their variance from their baseline values, but we would expect to potentially see a difference in the cross-over groups. There was a significant TRT effect on the Up_ampΔ where YC was greatest and significantly different from CC and YY but was not different from CY which was intermediate (0.194 vs. 0.137, 0.135 and 0.141 respectively; $P < 0.05$). There was also a significant TRT effect on the Low_ampΔ in which again the YC group drifted further downward from the mean more significantly than CY and YY but was not different from CC which was intermediate (0.116 vs 0.068, 0.059 and 0.089, respectively; $P = 0.005$). This confers with the RangeΔ outcome but gives a better perspective of the influence of direction.

As expected, DAY significantly affected the Δ in all rumen pH variables. Figures 4 and 5 illustrate the general direction of the change over the transition. For each figure, the basal value for each variable is represented by the y-base of 0. Figure 4.4 shows the effect of DAY on the maximum, minimum, variance and range which shows that days that were most affected corresponded to the STEP2 diet through the first week of the FIN diet. Interestingly a second assault during the last 7 d of measurement reveals significant changes for these variables which demonstrate that even after adaptation to a diet external factors may cause rumen disturbances. Figure 4.5 displays drift of the mean ruminal pH and the upper and lower amplitude across the mean. Again the most significant days are during the STEP2 and the first week of the FIN diet with a second assault in the last week of measurements. Over the transition, the drift in low_amp is 0.1 pH unit on average lower than the baseline value while the up_amp drifts nearly twice as far from its baseline causing an overall increase in mean ruminal pH. It is suspected that this change in up_amp is due to an increasing bunk visit interval over time (time between visits; data not shown) and greater time spent ruminating.

In summary, the findings of the drift analysis, specifically the main effects of TRT, offered a different perspective of the effect of supplemental ADY during dietary transition than the preliminary analysis. It also empowered the case for measuring deviations within subject rather than raw or transformed data and holding on to arbitrary pH thresholds for meaning. The drift analysis reveals that the addition of ADY during the dietary transition may help stabilize daily variation of ruminal pH during the transition from a low to high energy diet.

Carcass Characteristics

Carcass characteristics of the finished steers are shown in Table 7. Cattle were pre-shrunk before shipment (18 h without feed, 12 h without water) and this data is included in this section. Final shrunk body weight (**SBW**) was not affected by TRT. Interestingly, the percentage of BW shrink was affected by TRT group in which CC steers had a significantly greater loss than YY steers (3.7 vs. 2.7 %, respectively; $P = 0.05$), and CY and YC were intermediate and not different (3.3 and 3.1 %, respectively). Although there was an effect of TRT on the percentage of BW shrink, this did not manifest into any meaningful differences in carcass characteristics. Still, this finding may be of use to other sectors of the beef production chain, especially where hydration may be a key factor in health status such as receiving cattle (Schaefer et al., 1997).There was no TRT effect ($P > 0.1$) on HCW, dressing percent, 12th rib fat thickness, REA or overall YG. Quality Grade distribution and marbling scores were similar across TRT groups. Liver abscess frequency was 19.6 %, but liver abscess prevalence and abscess severity were not different between TRT groups. There were no TRT differences in the distribution of lung scores.

Relationship of Growth Performance, Acidosis Bouts, and Liver Abscesses

There has been considerable speculation (Owens et al., 1998; Nagaraja and Lechtenberg, 2007) that bouts of ruminal acidosis may result in liver abscesses. It also stands to reason that any liver damage may decrease overall metabolic efficiency and thus hinder feeding performance traits. In a post-hoc Chi-Square analysis, feeding performance traits DMI, ADG or G:F were used to detect any relationships with bouts of acidosis and liver abscess occurrence. Steers were assigned a categorical status label based on their relative position within the population quartiles. Steers ranking in the top 25%, 26 to 50%, 51 to75%

and 75 to 100% of the population were labeled *highest*, *high*, *low*, and *lowest*, respectively, for each feed performance trait. Only steers equipped with rumen boluses (n = 65) were used in assessments which included acidosis bout assessment but their original population rank within a feed performance trait was retained and not scaled to the bolus population. Since acidosis bout occurrence displayed a Poisson distribution labels were assigned for meaningful interpretation rather than the distribution in which steers were grouped by the number of days experiencing bouts (**DEB**) of 0, 1 to 10, 11 to 20 or > 20 d. While this separation resulted in unequal group sizes, it provided practical meaning for observation.

Growth performance and acidosis bouts. Feedlot performance traits and DEB relationships are illustrated in the panels of mosaic plots in Figure 4.6. There was no significant relationship between DMI status and DEB (P = 0.78). However, there was a significant relationship between ADG status and DEB in which those steers with the highest ADG never experienced more than ten days of acidosis during the 50 d measured (P = 0.034). Similarly, there was a significant relationship between G:F status and DEB in which those steers with greatest feed efficiency had fewer DEB than those with the lowest feed efficiency. This finding is consistent with suggestions of repeated bouts of acidosis having negative impacts on performance due to decreased short-chain fatty acid absorption (Schwaiger et al., 2013).

Growth performance and liver abscesses. There was no significant relationship between DMI status and liver abscess prevalence or severity (P = 0.83 and 0.65, respectively; Figure 4.7). There was, however, a tendency for ADG status to be related to abscess severity (P = 0.078). Steers within low and lowest ADG status groups had a greater frequency of A+ livers than steers in the high or highest ADG groups. It could be that the presence and

severity of liver abscesses contributed to the lower performance of steers which is supported by earlier research (Brink et al., 1990). While the relationship of G:F status and liver abscesses were not significant the mosaic plot shows a similar trend in the pattern of occurrence and severity as the ADG status outcome.

Acidosis bouts and liver abscesses. There was no significant relationship between liver abscess prevalence or severity and DEB (P = 0.98 and 0.90, respectively; Figure 4.8). This conflicts with the expected outcome and also general assumptions of chronic bouts predisposing cattle to an increased probability of rumenitis, rumen lesions and consequently liver abscesses (Nagaraja and Chengappa, 1998). Although the post-mortem rumen was not examined for lesions or keratinization, our findings showed that steers which never experienced a bout of acidosis during the transition still had a 10 % abscess occurrence (2 of 19 steers). Even more interesting, four steers with the most extreme number of DEB (21, 28, 39 and 42 d of 50 d respectively) yielded only healthy livers. While we cannot provide bouts experienced for the entire feeding period some have suggested that the transition sets the tone for the remaining days on feed (Dohme et al., 2008). Here we may only provide evidence that there is not necessarily a linear relationship between DEB during the dietary transition and liver abscess prevalence or severity if any at all.

CONCLUSION

Characterizing the effects of ADY on beef production efficiency traits may improve our understanding of the relationship between health and nutrition in the feedlot setting. In the current study, we provided a comprehensive feed-through-slaughter assessment of the effects of feeding supplemental active dried yeast to growing beef steers. Additionally,

valuable insight into population variance and approaches to meaningful data analysis of continuous rumen monitoring systems were analyzed. There is evidence to suggest that at the current dosage (3×10^{10} CFU/d) supplemental ADY *Saccharomyces cerevisiae* may aid in the stabilization of ruminal pH characteristics during the dietary transition. However, beyond the direct action in the rumen, it was unclear if ADY effects were sufficient to improve animal performance. Further research should address the potential for dose-dependent effects (in the context of CFU/d) and impacts on water intake.

LITERATURE CITED

Bach, A., C. Iglesias, and M. Devant. 2007. Daily rumen pH pattern of loose-housed dairy cattle as affected by feeding pattern and live yeast supplementation. Animal Feed Science and Technology 136: 146-153.

Bailey, J. C., L. O. Tedeschi, E. D. M. Mendes, J. E. Sawyer, and G. E. Carstens. 2012. Technical note: Evaluation of bimodal distribution models to determine meal criterion in heifers fed a high-grain diet. Journal of Animal Science 90: 2750-2753. doi: 10.2527/jas.2011-4634.

Brink, D., S. Lowry, R. Stock, and J. Parrott. 1990. Severity of liver abscesses and efficiency of feed utilization of feedlot cattle. Journal of Animal Science 68: 1201-1207.

Changyong, F., W. Hongyue, L. Naiji, C. Tian, L. Ying. 2014. Log-transformation and its implications for data analysis. Shanghai Archives of Psychiatry 26: 105.

Chow, S.-C., and J.-P. Liu. 1992. On assessment of bioequivalence under a higher-order crossover design. Journal of Biopharmaceutical Statistics 2: 239-256.

Chung, Y.-H., N. Walker, S. McGinn, and K. Beauchemin. 2011. Differing effects of 2 active dried yeast (*Saccharomyces cerevisiae*) strains on ruminal acidosis and methane production in nonlactating dairy cows. Journal of Dairy Science 94: 2431-2439.

Denwood, M., J. Kleen, D. Jensen, and N. Jonsson. 2018. Describing temporal variation in reticuloruminal pH using continuous monitoring data. Journal of Dairy Science 101:233-245.

Desnoyers, M., S. Giger-Reverdin, G. Bertin, C. Duvaux-Ponter, and D. Sauvant. 2009. Meta-analysis of the influence of *Saccharomyces cerevisiae* supplementation on ruminal parameters and milk production of ruminants. Journal of Dairy Science 92: 1620-1632.

Dohme, F., T. J. DeVries, and K. A. Beauchemin. 2008. Repeated Ruminal Acidosis Challenges in Lactating Dairy Cows at High and Low Risk for Developing Acidosis: Ruminal pH1. Journal of Dairy Science 91: 3554-3567. doi: https://doi.org/10.3168/jds.2008-1264.

Humer, E, R. M. Petri, J. R. Aschenbach, B. J. Bradford, G. B. Penner, M. Tafaj, K-H. Südekum, Q. Zebeli. 2018. Invited review: Practical feeding management recommendations to mitigate the risk of subacute ruminal acidosis in dairy cattle. Journal of Dairy Science 101: 872-888.

Mitchell, I. d. G., N. Amphlett, and R. Rees. 1994. Parametric analysis of rank transformed data for statistical assessment of genotoxicity data with examples from cultured mammalian cells. Mutagenesis 9: 125-132.

Mori, J., and Y. Kano. 2015. A powerful test for Balaam's design. Pharmaceutical statistics 14: 464-470.

Nagaraja, T., and M. Chengappa. 1998. Liver abscesses in feedlot cattle: a review. Journal of Animal Science 76: 287-298.

Nagaraja, T., and K. F. Lechtenberg. 2007. Liver abscesses in feedlot cattle. Veterinary Clinics of North America: Food Animal Practice 23: 351-369.

Narinç, D., and A. Aygün. 2017. A non-parametric data transformation technique for quantitative genetic analyses: The rank transformation. In: AIP Conference Proceedings. p 020060.

Owens, F., D. Secrist, W. Hill, and D. Gill. 1998. Acidosis in cattle: a review. Journal of Animal Science 76: 275-286.

R Core Team. 2012. R A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org. Accessed on: January 27, 2018.

Schaefer, A. L., S. D. Jones, and R. W. Stanley. 1997. The use of electrolyte solutions for reducing transport stress. Journal of Animal Science 75: 258-265. doi: 10.2527/1997.751258x.

Schwaiger, T., K. Beauchemin, and G. Penner. 2013. Duration of time that beef cattle are fed a high-grain diet affects the recovery from a bout of ruminal acidosis: Short-chain fatty acid and lactate absorption, saliva production, and blood metabolites. Journal of Animal Science 91: 5743-5753.

Sileshi, G. W. 2012. A critique of current trends in the statistical analysis of seed germination and viability data. Seed Science Research 22: 145-159.

Vyas, D., A Uwizeye, R. Mohammed, W. Z. Yang, N. D. Walker, K. A. Beauchemin. 2014. The effects of active dried and killed dried yeast on subacute ruminal acidosis, ruminal fermentation, and nutrient digestibility in beef heifers1. Journal of Animal Science 92: 724-732. doi: 10.2527/jas.2013-7072

Table 4.1. Ingredient and chemical composition of diets fed to growing steers with or without the inclusion of an active dried yeast.

Items[1]	Diets[2]							
	GROW		STEP1		STEP2		FIN	
	CON	ADY	CON	ADY	CON	ADY	CON	ADY
% of diet, DM								
Alfalfa hay, medium chop	30	30	25	25	20	20	15	15
Cracked corn	40	40	50	50	60	60	70	70
Dried distiller's grain	20	17	15.5	12.5	10.5	7.5	5.5	2.5
Molasses	6.8	6.8	6.8	6.8	6.8	6.8	6.8	6.8
Mineral	2.5	2.5	1	1	1	1	1	1
Limestone	0	0	1	1	1	1	1	1
Urea	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Premix[3]		3		3		3		3
DM, % of diet	82.7		82.5		82.3		82.2	
CP	16.3		14.9		13.3		11.8	
Soluble protein, % CP	21.4		20.7		19.9		19.1	
NDF	30.1		27.4		24.5		21.7	
ADF	20.2		17.6		15.0		12.4	
Lignin	4.4		3.8		3.3		2.7	
Crude fat	4.1		3.9		3.7		3.5	
Sugar	4.9		4.8		4.7		4.6	
Starch	29.0		35.8		42.5		49.2	
Ash	7.2		6.1		5.5		4.9	
Calcium	0.7		0.8		0.8		0.7	
Phosphorus	0.6		0.4		0.4		0.4	
peNDF[4]	16		14		12		10	
TDN	76		78		80		82	
NEm, Mcal/kg	1.57		1.63		1.68		1.74	
NEg, Mcal/kg	1.00		1.05		1.10		1.14	

[1]Items are feed ingredients and chemical composition of diets (DM basis) evaluated by Cumberland Valley Analytical Services (Hagerstown, MD).
[2]Diets are GROW = grower (fed d 1 - 70), STEP1 = transition step 1 (fed d 71 - 77), STEP2 = transition step 2 (fed d 78 - 85), FIN = finisher (fed d 86 to 164), CON = no active dried yeast supplement, ADY = control diet + 1.5 g/ d of an active dried yeast (3×10^{10} CFU per day).
[3]Premix was included in 3 % of the diet where DDG was the carrier ingredient. The additive was Vistacell (ABVista, Marlborough, UK) and its amount (g) was based on the average of the inner quartile range of the previous week's DMI to allow for feed delivery containing 1.5 g of Vistacell per subject each day.
[4]peNDF (physically effective NDF = proportion of particles larger than 4 mm×NDF proportion of the diet).

Table 4.2. Effect of feed treatment regimen on feedlot performance and eating behavior of growing steers

Items[1]	Treatments[2]				SEM	*P*-value
	CC	CY	YC	YY		
Transition Period[3]						
n=50 d						
Initial BW, kg	374	366	370	372	36.7	0.7260
Final BW, kg	447	444	445	449	33.3	0.9381
ADG, kg/d	1.34	1.46	1.38	1.44	0.088	0.6000
DMI, kg/d	11.6	11.6	11.4	11.6	0.376	0.8020
G:F	0.115	0.122	0.111	0.127	0.013	0.3690
RFI, kg/d	0.026	0.095	-0.026	-0.063	0.144	0.8660
Meal frequency	8.5 [b]	9.3 [a]	9.0 [ab]	8.8 [ab]	0.197	0.0030
Meal size, kg/meal	1.38 [ab]	1.31 [b]	1.34 [b]	1.45 [a]	0.065	0.0020
Bunk visit DUR, min/d	92.5	94.0	92.7	89.8	2.72	0.3901
Total Feeding Period						
n=164 d						
Initial BW, kg	234	229	232	235	17.5	0.4980
Mid-test BW, kg	429	424	425	432	33.4	0.7011
Final BW, kg	500	492	490	500	30.7	0.5990
ADG, kg/d	1.62	1.60	1.58	1.62	0.083	0.7680
DMI, kg/d	11.2	11.1	11.1	11.2	0.338	0.9210
G:F	0.146	0.144	0.143	0.145	0.004	0.8240
RFI, kg/d	0.026	0.095	-0.026	-0.053	0.141	0.8780
Meal Frequency	9.07	9.25	9.57	9.40	0.350	0.7576
Meal size, kg/meal	1.40	1.35	1.33	1.38	0.055	0.7998
Bunk visit DUR, min/d	100.3	100.4	95.4	91.5	3.23	0.1418

[1]Items are performance variables of growing steers. Mid BW= BW on day 84; F:G= feed-to-gain conversion; RFI = residual feed intake, kg/d; Sum = the summation of daily meals; DUR = duration of feeding time.
[2]Treatements are CC = control, no ADY fed throughout the transition; YY = yeast fed throughout the entire transition; CY = control fed during the grower diet and transitioned onto diets containing yeast; YC = yeast fed during the grower diet and transitioned onto diets without yeast.
[3]Transition period is d 56 to d 106 during dietary transition from grower to finisher diets.

Table 4.3. The effects of feed treatment regimen on rumen characteristics of growing steers during transition from a grower diet to a finishing diet.

Items[1]	Treatments[2]				SEM	*P*-value
	CC	CY	YC	YY		
Ruminal pH						
Minimum	5.39	5.27	5.36	5.34	0.087	0.4465
Maximum	7.00	6.89	7.04	6.96	0.063	0.2045
Mean	6.26 [a]	6.09 [b]	6.23 [a]	6.19 [ab]	0.061	0.0587
Range	1.61	1.63	1.68	1.62	0.063	0.8400
Variance	0.095	0.101	0.105	0.099	0.013	
Rank	31.7	33.6	34.3	32.0	5.10	0.9512
Cumulative variance	1.62	1.75	1.83	1.70	0.251	
Rank	28.8	35.3	33.6	32.8	5.69	0.7152
AUC pH 6.0	18.1	31.6	19.9	19.7	8.14	
Rank	26.8	39.4	32.3	32.2	5.52	0.0965
DUR < 6.0, min/d	316.4	533.8	387.5	367.8	74.6	
Rank	26.5 [b]	40.6 [a]	31.7 [ab]	31.7 [ab]	4.81	0.0528
AUC pH 5.8	8.27	14.4	6.91	8.38	5.69	
Rank	27.8	39.0	32.0	32.1	5.97	0.1449
DUR < 5.8, min/d	158.4	277.2	162.1	166.4	64.2	
Rank	26.9 [b]	40.5 [a]	31.7 [ab]	31.5 [ab]	5.72	0.0536
AUC pH 5.6	3.56	6.25	2.52	3.51	3.18	
Rank	28.7	37.4	32.4	32.5	5.73	0.3295
DUR < 5.6, min/d	75.9	119	63.1	75.2	42.83	
Rank	28.6	38.6	31.9	31.8	5.98	0.2151
AUC pH 5.4	1.43	2.88	0.795	1.31	1.64	
Rank	29.3	35.7	32.1	33.9	4.93	0.5017
DUR < 5.4, min/d	37.9	53.1	22.9	35.6	25.2	
Rank	29.6	36.1	31.7	33.7	5.43	0.5163
Acidosis bouts[3]						
Prevalence, %/d	7.9	12.4	8.3	8.8		0.0056
DUR, min/bout	327 [ab]	352 [a]	260 [b]	346 [ab]	36.1	0.0462

[a-b]Least squares means within a row with different superscripts differ at $P < 0.06$

[1]Variables AUC = area under the curve (dimensionless) and DUR = duration (min/d), under the given pH threshold. Rank = the relative position of a data-point in the given data set. Bout = an instance of a subject having ruminal pH <5.6 for 180 consecutive minutes.

[2]Treatements are CC = control, no ADY fed throughout the transition; YY = yeast fed throughout the entire transition; CY = control fed during the grower diet and transitioned onto diets containing yeast; YC = yeast fed during the grower diet and transitioned onto diets without yeast.

[3]Bout prevalence was analyzed by the χ^2 analysis. Percentage of subjects displaying bouts are reported within TRT.

Table 4.4. The effect of diet on rumen characteristics of growing steers during transition from a grower diet to a finishing diet.

Items[1]	Diets[2]				SEM	*P*-value
	GROW	STEP1	STEP2	FIN		
Ruminal pH						
Minimum	5.37 [ab]	5.40 [a]	5.34 [ab]	5.24 [b]	0.075	< 0.0001
Maximum	6.87 [b]	6.89 [b]	6.98 [ab]	7.14 [a]	0.049	< 0.0001
Mean	6.22 [a]	6.19 [b]	6.18 [b]	6.19 [b]	0.053	0.0293
Range	1.50 [b]	1.49 [b]	1.64 [b]	1.91 [a]	0.044	< 0.0001
Variance	0.058	0.089	0.121	0.120	0.012	
Rank	33.0	33.1	33.1	32.4	4.32	0.9901
Cumulative variance	0.548	1.00	1.66	3.69	0.269	
Rank	33.0	33.0	33.1	31.4	6.68	0.6185
AUC pH 6.0	17.5	12.2	14.7	44.9	6.85	
rank	33.0	32.9	32.9	31.9	4.76	0.9459
DUR < 6.0, min/d	334	389	443	440	58.8	
rank	32.9	32.9	32.9	31.7	3.90	0.9171
AUC pH 5.8	5.38	5.44	7.00	20.14	4.77	
rank	33.0	32.9	32.9	31.9	5.40	0.9601
DUR < 5.8, min/d	120	177	233	234	53.3	
rank	32.9	32.9	32.9	31.8	5.06	0.9407
AUC pH 5.6	2.04	2.57	3.23	7.99	2.67	
rank	33.0	33.0	32.9	31.9	5.12	0.9641
DUR < 5.6, min/d	43	81	105	104	37.1	
rank	33.0	32.9	32.9	31.9	5.35	0.9600
AUC pH 5.4	0.76	1.21	1.50	2.94	1.36	
rank	33.0	32.9	33.0	31.9	4.39	0.9674
DUR < 5.4, min/d	18	40	51	41	21.6	
rank	33.0	33.0	33.0	31.9	4.88	0.9626
Acidosis bouts[3]						
Prevalence, %/d	3.91	7.03	12.3	12.2		< 0.0001
DUR, min/bout	283	329	350	324	36.7	0.2694

[a-b]Least squares means within a row with different superscripts differ at *P < 0.06*

[1]Variables represent rumen characteristics of growing steers over the dietary transition; AUC=Area Under the Curve (unit-less) & DUR= Duration (min/d), under the given pH threshold. Rank = the relative position of a data-point in the given data set. Bout= an instance of a subject having ruminal pH <5.6 for 180 consecutive minutes.

[2]Diets are GROW=Growing diet (30% roughage), STEP1= transition 1 diet (25% roughage), STEP2=transition 2 diet (20% roughage), and FIN= Finisher diet (15% roughage).

[3]Bout Prevalence was analyzed by χ^2 analysis. Percentage of subjects displaying bouts are reported at the diet level (n=65).

Table 4.5. Summary statistics of ruminal pH characteristic variables of growing steers fed a 30 % roughage grower diet.

Items[1]	Summary Statistics					
n=63	Mean		SD	Minimum	Maximum	CV, %[2]
Basal pH values						
Minimum	5.75	±	0.23	5.01	6.13	4.04
Mean	6.21	±	0.18	5.77	6.57	2.91
Maximum	6.64	±	0.15	6.31	7.02	2.23
Range	0.89	±	0.16	0.65	1.47	18.41
Variation	0.06	±	0.03	0.02	0.17	55.17
Cumulative variation	0.56	±	0.30	0.24	1.89	52.93
Up_amp	0.42	±	0.08	0.31	0.71	17.75
Low_amp	-0.47	±	0.10	-0.75	-0.32	21.90
AUC <6.0	17.93	±	26.75	0.04	149.34	149.19
AUC <5.8	5.57	±	10.61	0.00	58.24	190.33
AUC <5.6	2.11	±	5.48	0.00	31.96	259.03
DUR <6.0, min/d	23.67	±	23.41	0.13	93.19	98.88
DUR <5.8, min/d	8.54	±	11.59	0.00	52.73	135.72
DUR <5.6, min/d	3.12	±	6.09	0.00	32.71	195.16

[1]Variables are the population average of ruminal pH values during the 14 d prior to dietary transition while consuming a 30% Grower diet. Cumulative variation = the accrued variation over 14 d, Up_amp= the upper amplitude and maximum distance above the daily mean, Low_amp= the lower amplitude and maximum distance below the daily mean, AUC= area under the curve of the given pH threshold, DUR= the duration of time spent below the given pH threshold in min/d.

Table 4.6. Effects of TRT and DAY on ruminal characteristic variable drift from baseline values

Items[1]	Treatment[2]				SEM	*P*-value		
	CC	CY	YC	YY		TRT	DAY[4]	TRT × Day
Basal min pH	5.80	5.68	5.78	5.76	0.091	0.4112		
MinΔ	-0.107	-0.118	-0.143	-0.107	0.019	0.4604	<0.0001	0.8525
Basal mean, pH	6.26	6.13	6.25	6.23	0.057	0.1573		
MeanΔ	0.017	0.051	0.029	0.047	0.014	0.2756	<0.0001	0.1825
Basal max, pH	6.67	6.58	6.66	6.65	0.040	0.2252		
MaxΔ	0.120^{ab}	0.090^{b}	0.165^{a}	0.088^{b}	0.018	0.0081	<0.0001	0.6256
Basal range, pH	0.86	0.90	0.88	0.90	0.064	0.9234		
RangeΔ	0.227^{ab}	0.208^{b}	0.310^{a}	0.194^{b}	0.029	0.0058	<0.0001	0.9853
Basal variance, pH	0.041	0.047	0.041	0.044	0.008	0.8684		
VarΔ	0.035^{b}	0.038^{b}	0.058^{a}	0.039^{b}	0.005	0.0076	0.0007	0.9524
Basal up_amp, pH	0.409	0.442	0.413	0.423	0.024	0.5780		
Up_ampΔ	0.137^{b}	0.141^{ab}	0.194^{a}	0.135^{b}	0.016	0.0185	<0.0001	0.8507
Basal low_amp, pH[3]	-0.454	-0.457	-0.472	-0.473	0.042	0.9310		
Low_ampΔ	-0.089^{ab}	-0.068^{b}	-0.116^{a}	-0.059^{b}	0.020	0.0053	0.0051	0.8023

[a-b]Least squares means within a row with different superscripts differ at $P < 0.05$.
[1]Variables represent rumen characteristics of growing steers over the dietary transition; Basal values indicate the average of the 14 d period steers received a Grower diet (30 % roughage) and subsequent Δ in pH units from basal values in which the sign indicates the distance and direction of the change.
[2] Treatments are CC=Control, no ADY fed throughout the transition; YY=Yeast fed throughout the entire transition; CY=Control fed during the Grower diet and transitioned onto diets containing Yeast; YC= Yeast fed during the Grower diet and transitioned onto diets without Yeast.
[3] Up_amp=Upper amplitude and greatest average distance above the mean pH, Low_amp= Lower amplitude and greatest average distance below the mean pH.
[4]Main effects of Day are shown in figure format.

Table 4.7. Effects of treatment on carcass characteristics of growing beef steers

Items[1]	Treatment[2]				SEM	*P*-value
	CC	CY	YC	YY		
N=	29	31	29	28		
SBW, kg	498	492	489	499	21.4	0.6119
BW shrink, %	3.7 [a]	3.3 [ab]	3.1 [ab]	2.7 [b]	0.549	0.0525
HCW, kg	313	307	310	313	14.0	0.7025
Dressing %	62.9	62.4	63.4	62.8	0.466	0.5243
12th rib backfat thickness, cm	1.9	1.8	1.6	1.8	0.103	0.2004
Calculated yield grade	3.2	3.1	2.9	3.1	0.138	0.6165
LM area, cm^2	12.0	11.9	11.9	12.1	0.220	0.8677
Quality grade distribution, %						0.995
Prime	0.0	0.0	3.4	3.6		
Choice	62.1	67.7	65.5	71.4		0.8639
Modest/Moderate	24.1	22.6	20.7	14.3		
Small	37.9	45.2	44.8	57.1		
Select	37.9	32.3	31.0	25.0		
Yield grade distribution, %						0.5686
Yield grade 1	6.9	6.5	10.3	7.1		
Yield grade 2	24.1	29.0	41.4	28.6		
Yield grade 3	62.1	54.8	37.9	50.0		
Yield grade 4	6.9	9.7	10.3	14.3		
Abscess prevalence						
Healthy	69.0	87.1	82.8	82.1		0.3515
Abscessed	31.0	12.9	17.2	17.9		0.3431
A	17.2	9.7	6.9	10.7		
A+	13.8	3.2	10.3	7.1		
Lungs score						0.6661
1, %	20.7	38.7	51.7	32.1		
2, %	65.5	54.8	34.5	50.0		
3, %	13.8	6.5	13.8	17.9		

[a-b] Least squared means within a row without a common superscript differ at $P < 0.06$.

[1] Items are carcass characteristics; SBW=shrunk BW.

[2] Treatments are CC = control, no ADY fed throughout the transition; YY = yeast fed throughout the entire transition; CY = control fed during the grower diet and transitioned onto diets containing yeast; YC = yeast fed during the grower diet and transitioned onto diets without yeast.

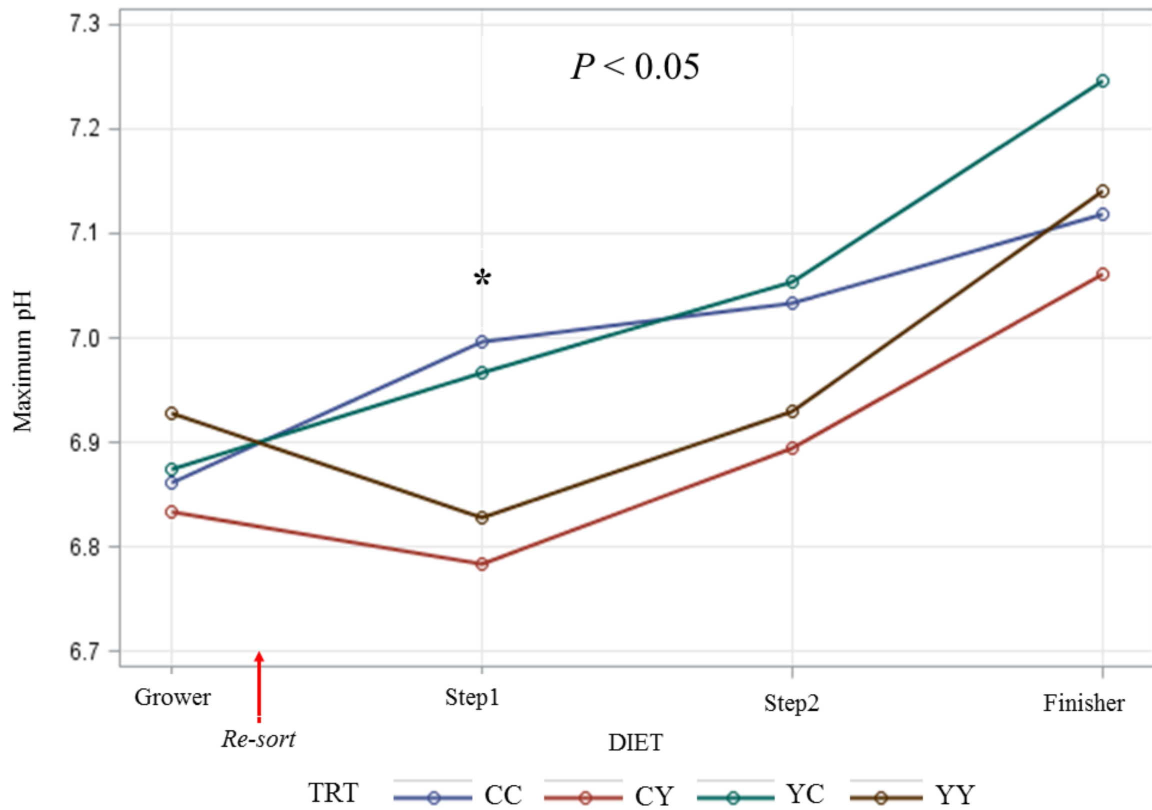

Figure 4.1. Interaction of treatment and diet on the average daily maximum ruminal pH achieved by growing steers. Values are least squares means and (*) indicate estimates that differ at $P < 0.05$. Treatments are CC = control, no ADY fed throughout the transition; YY = yeast fed throughout the entire transition; CY = control fed during the grower diet and transitioned onto diets containing yeast; YC= yeast fed during the grower diet and transitioned onto diets without yeast. Diets are grower = growing diet (30% roughage), STEP1 = transition 1 diet (25% roughage), STEP2 = transition 2 diet (20% roughage), and finisher= finisher diet (15% roughage).

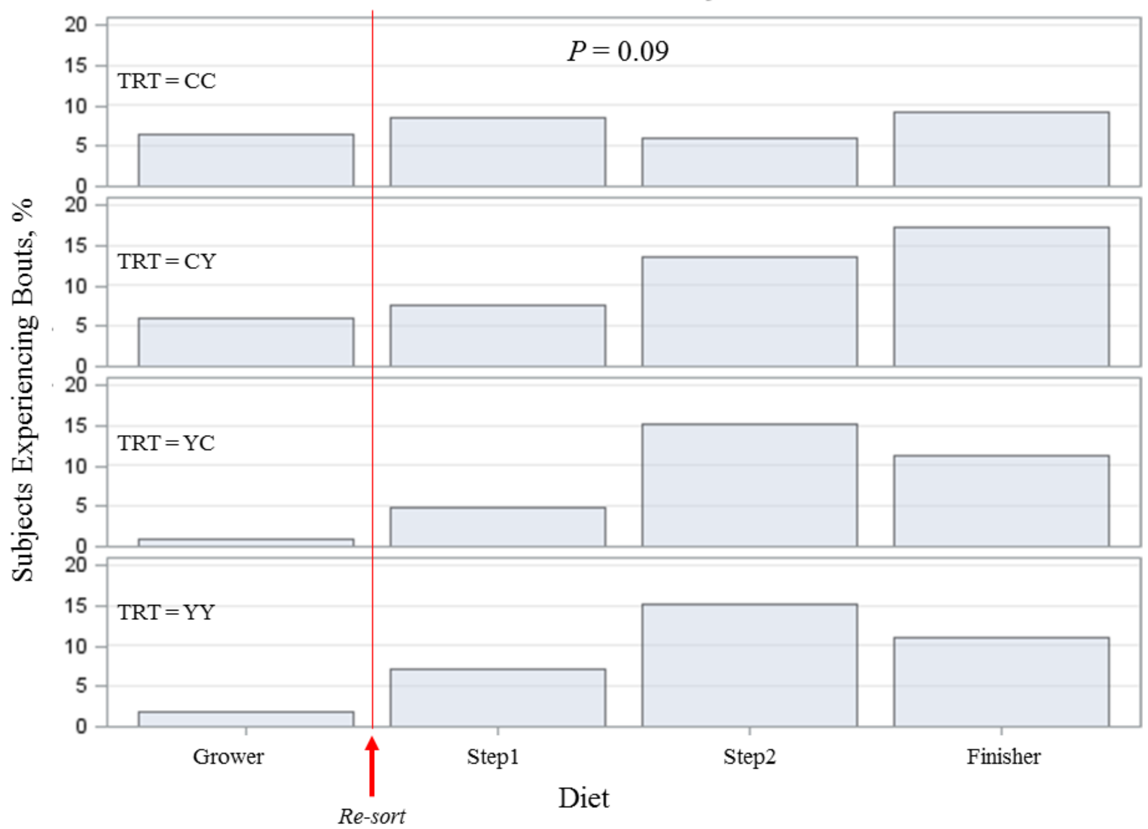

Figure 4.2. Distribution of growing steers experiencing bouts of acidosis during dietary transition with or without an active dried yeast. Treatments are CC = control, no ADY fed throughout the transition; YY = yeast fed throughout the entire transition; CY = control fed during the grower diet and transitioned onto diets containing yeast; YC= yeast fed during the grower diet and transitioned onto diets without yeast.

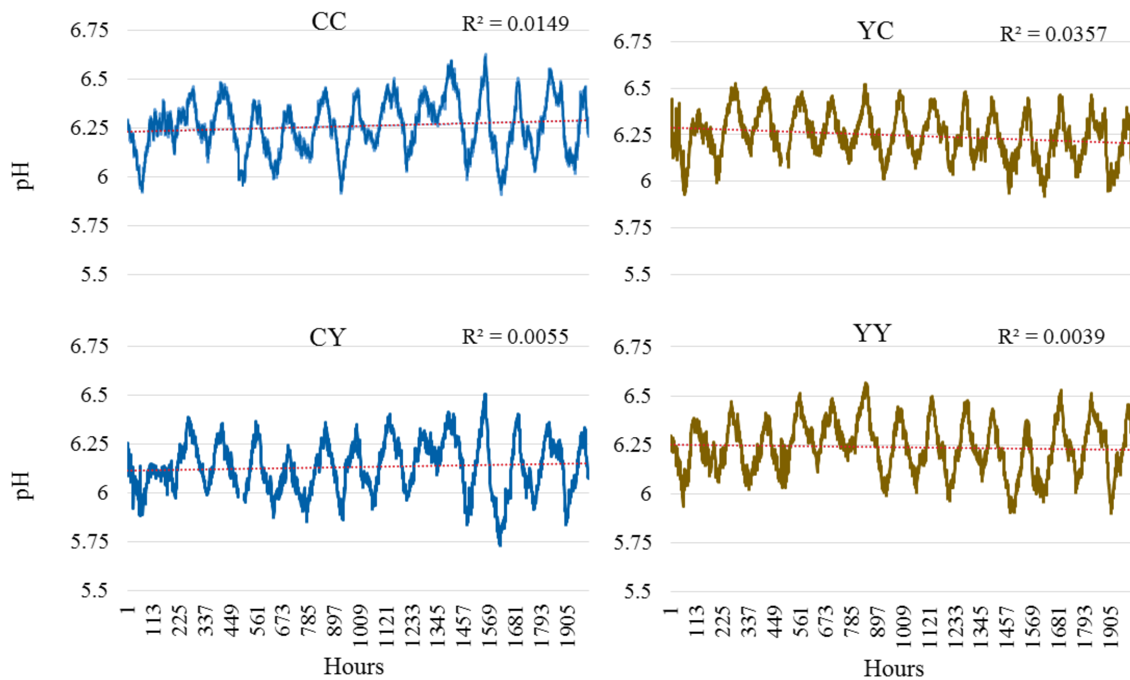

Figure 4.3. Diurnal fluctuations in ruminal pH of steers fed a grower diet for 14 d prior to dietary transition. Treatments are CC = control, no ADY fed throughout the transition; YY = yeast fed throughout the entire transition; CY = control fed during the grower diet and transitioned onto diets containing yeast; YC= yeast fed during the grower diet and transitioned onto diets without yeast. Blue (left panels) and gold (right panels) series represent steers consuming either control or yeast, respectively. Red dashed series indicates the fit line of the unadjusted R-squared estimate.

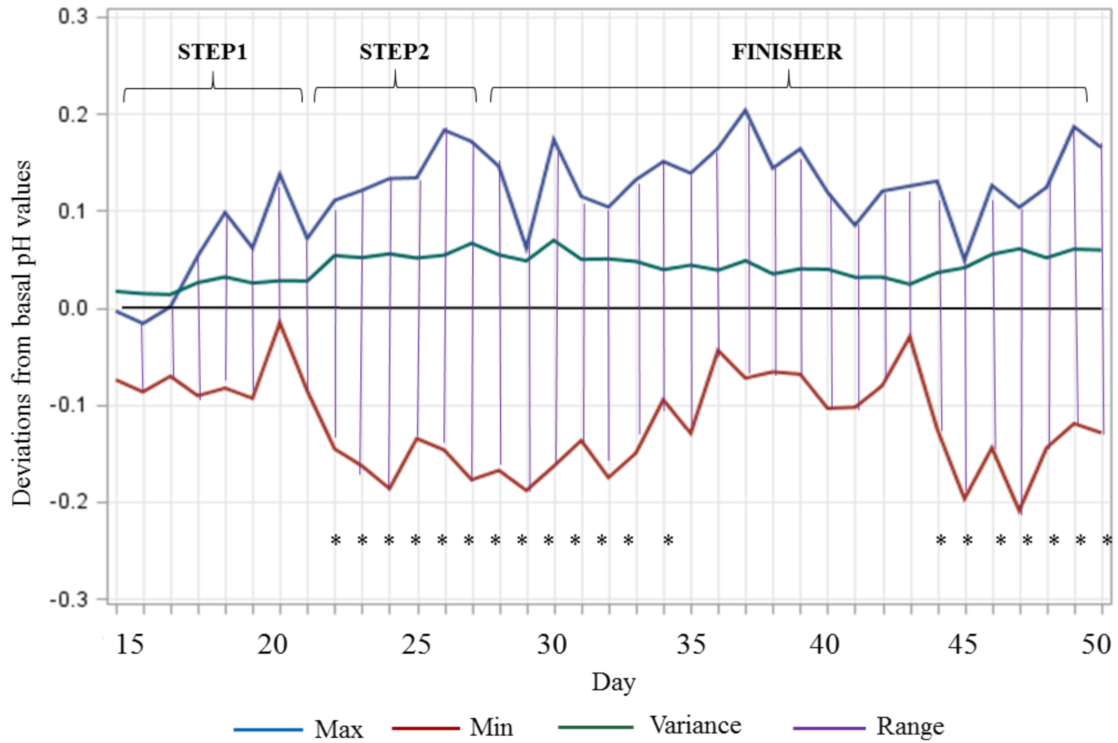

Figure 4.4. Effect of day on the maximum, minimum, variance and range of ruminal pH. Distance from 0 is the distance from the baseline value in pH units. Max = drift of average daily maximum pH form baseline maximum, min = drift of average daily minimum pH form baseline minimum, variance = drift of variance from baseline variance, range = drift distance of the range between maximum and minimum pH from baseline. *Denote days that were different at $P < 0.05$.

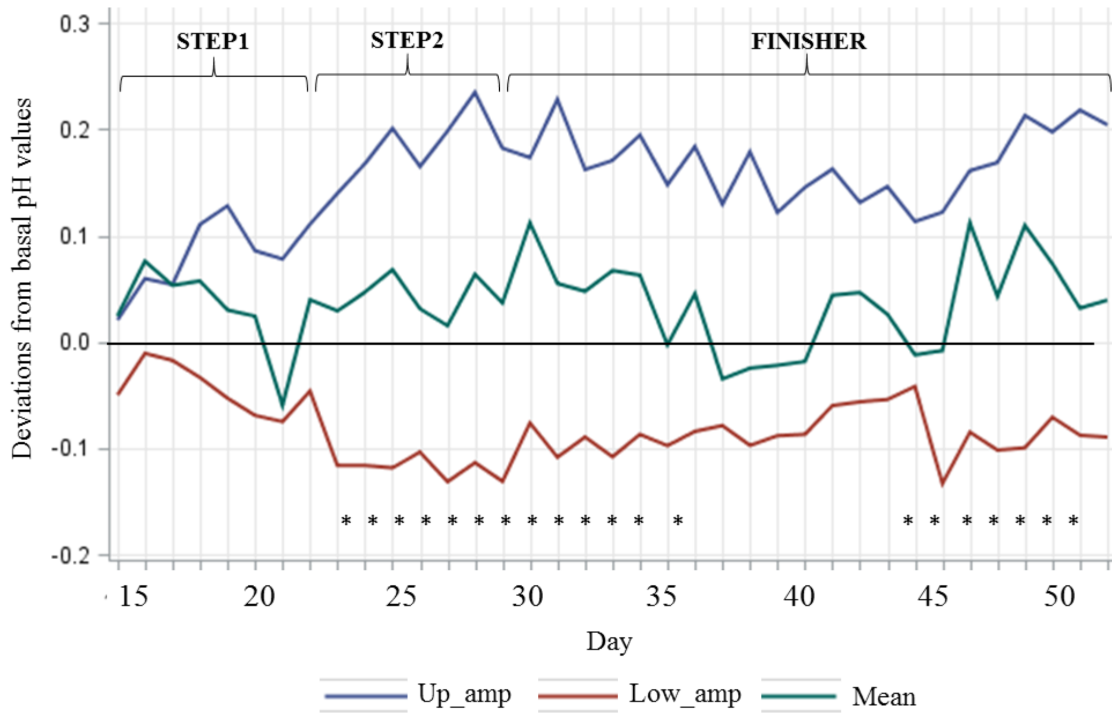

Figure 4.5. Effect of day on the mean, upper and lower amplitude drift from basal values. Distance from 0 is the distance from the baseline value in pH units. Up_amp=upper amplitude distance from the mean, Low_amp= Lower amplitude distance from the mean.

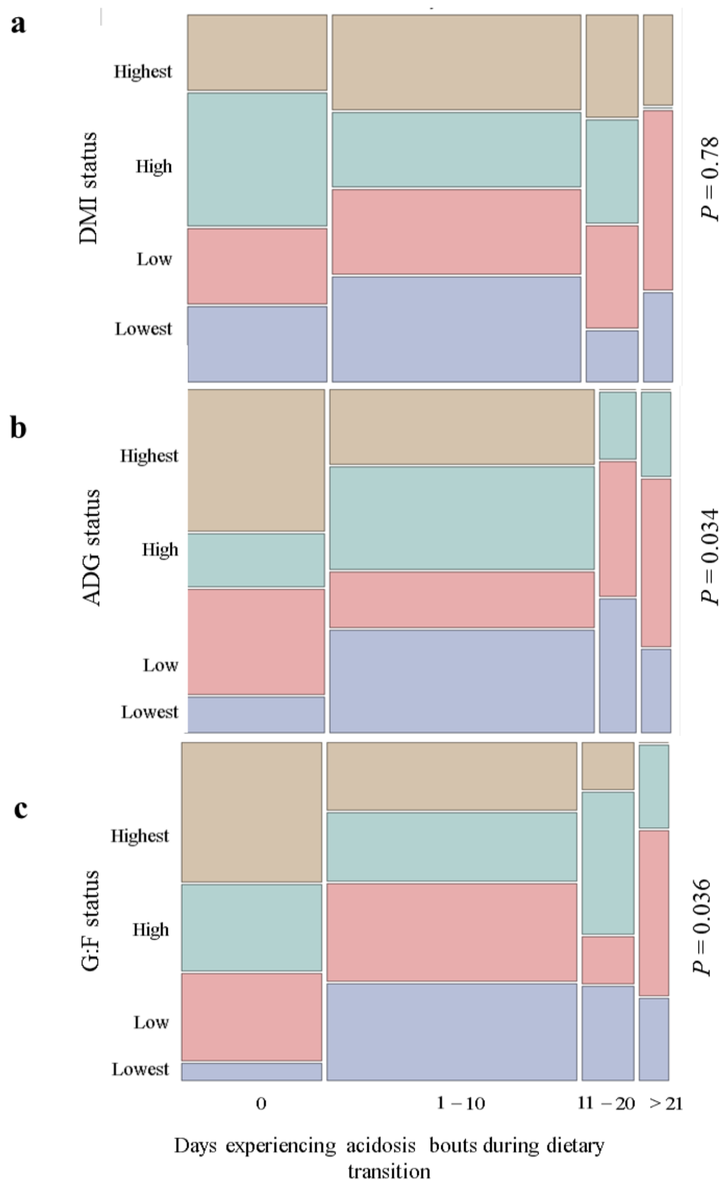

Figure 4.6. Mosaic displays represent the frequency of days experiencing bouts of acidosis and their relationship to feedlot performance traits DMI, ADG and G:F. Cell colors represent the quartile status of subjects within a performance trait; Gold =1-25th percentile, Green = 26-50th percentile, Pink = 51st-75th percentile, Purple = 76th – 100th percentile. Cell width represents the probability of steers within the grouping of days experiencing bouts. Cell height represents the probability of being within the designated population quartile for the respective performance trait within a column.

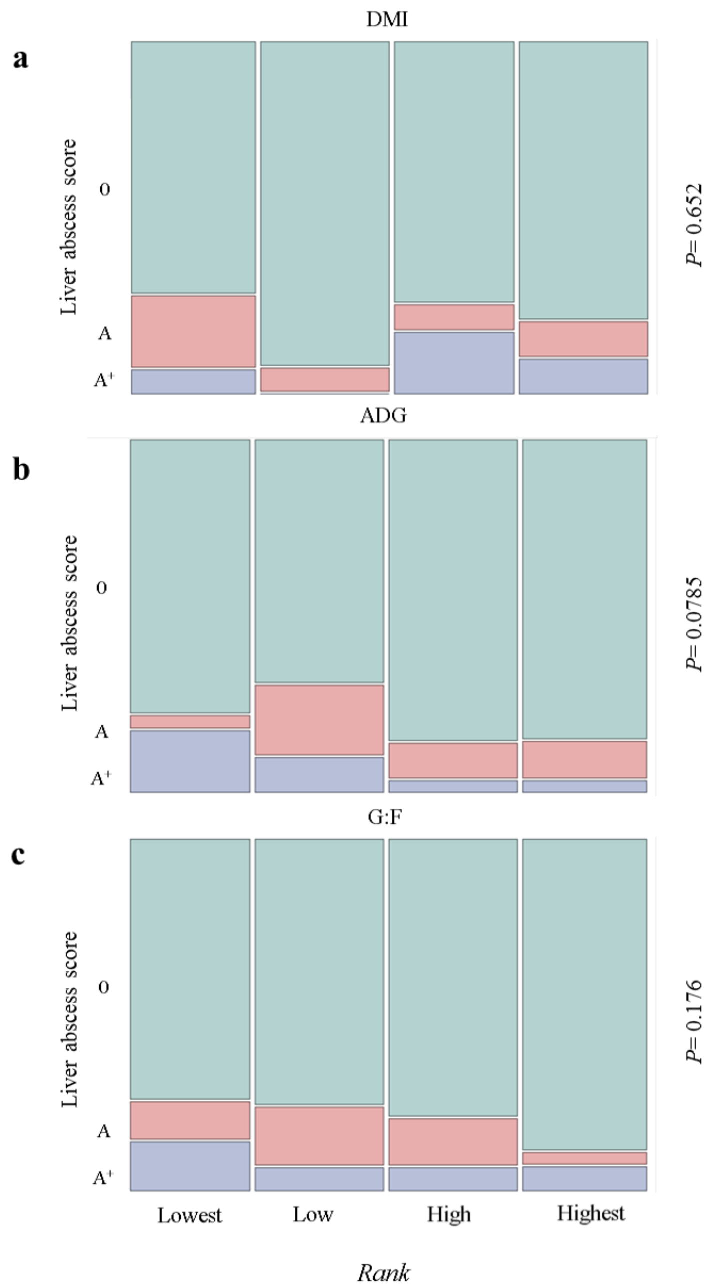

Figure 4.7. Distribution of liver abscess severity by percentile rank within performance traits DMI, ADG, and G:F. Liver abscess scores are categorized on the Elanco scoring system. Colors represent y-axis liver score values of 0 = green, A = red and A+ = blue. Column width and performance trait status are based on n=117 subjects and their rank within performance trait quartiles. Cell height is the probability of a subject portraying respective liver abscess traits within the column.

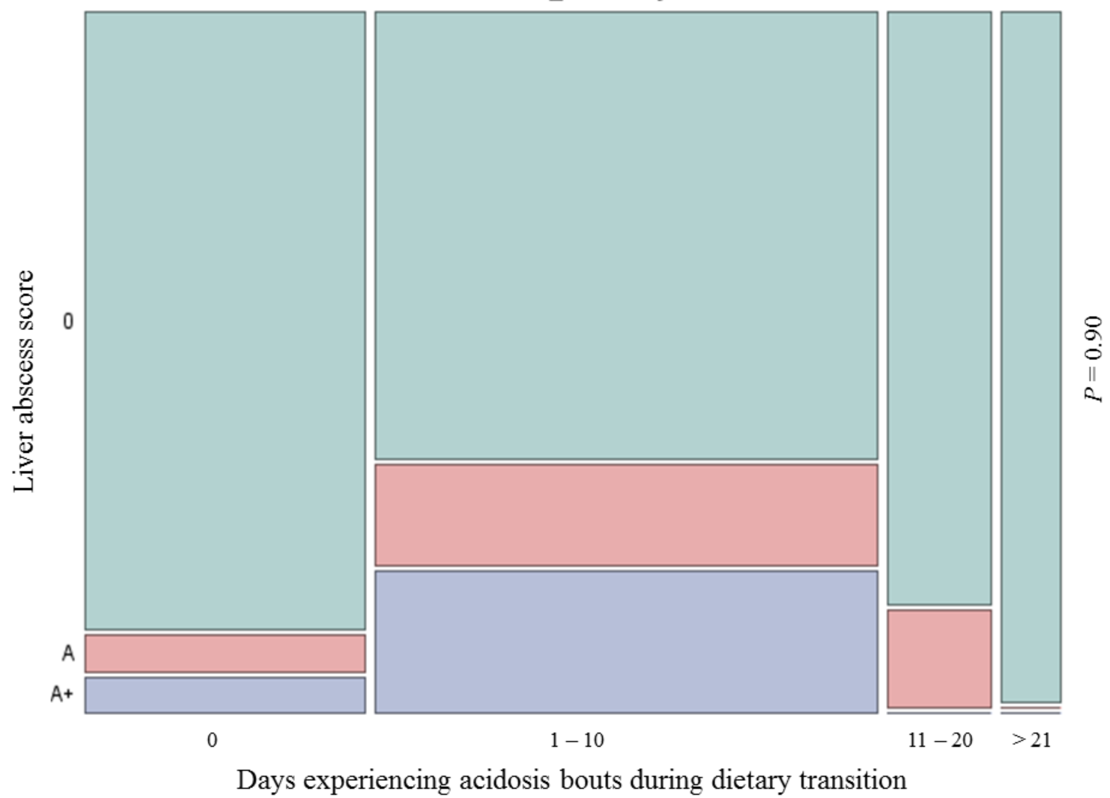

Figure 4.8. Distribution of liver abscess score by days experiencing bouts (**DEB**) of acidosis. Liver abscess scores are categorized on the Elanco scoring system. Colors represent y-axis liver score values of 0 = green, A =red and A+=blue. Column width of DEB are based on n=64 subjects and their associated DEB grouping. Cell height is the probability of a subject portraying respective liver abscess traits within the column.

CHAPTER V

IDENTIFYING BACTERIOPHAGES THAT TARGET HYPER AMMONIA-PRODUCING BACTERIA OF THE BOVINE RUMEN

INTRODUCTION

The ruminal ecosystem contains a microbial consortium that plays a vital role in converting plant matter into usable energy to the bovine host. Some bacteria, however, are known to occupy an ecological niche that may negatively influence the efficiency of using high-quality feed sources and contribute to ammonia (**NH_3**) emissions. Obligate amino acid fermenting bacteria, also known as hyper-ammonia-producing bacteria (**HAB**), may rapidly deaminate essential and growth rate-limiting amino acids, resulting in excessive NH_3 production and reduce N retention of the ruminant. This process is wasteful to the ruminant from a feed protein perspective and may also contribute to the available hydrogen pool in the rumen potentially resulting in higher enteric methane (**CH_4**) production. Antibiotic and ionophore feed additives have shown to be effective against HAB in the short term, but frequent subjectivity to non-selective antimicrobials through the feed has proved to be less effective long-term across many diet types (Guan et al., 2006; Crossland et al., 2017).

In light of greater awareness of widespread bacterial resistance to antibiotics, some researchers have reverted to nature's bacterial predators, the bacteriophages (phage), which are viruses ubiquitous in nature. One attractive component of phage is their narrow host selectivity and prolific self-replication upon each successful bacterial infection allowing for a more targeted approach to be used as a feed additive. Phages have been shown to be

effective at controlling a wide variety of pathogenic bacteria (Atterbury et al., 2007; Johnson et al., 2008; Atterbury, 2009) and have been shown to be a normal part of the rumen microflora (Klieve et al., 1996). Therefore, the objective of this trial was to isolate and characterize bacteriophage that selectively targets HAB. Phage culture could potentially be a viable antibiotic alternative in the diets of livestock that may simultaneously improve beef and milk production efficiency and reduce the environmental contribution of NH_3 and CH_4 from the livestock sector.

MATERIALS AND METHODS

Media and Bacteria

Three known obligate amino acid fermenting bacteria frequently isolated from the bovine rumen were obtained from ATCC: *Clostridium aminophilum*, Paster et al.(ATCC® 49906), *Clostridium sticklandii* Stadtman and McCLUng (ATCC® 49905), and *Peptostreptococcus anaerobius* (ATCC® 27337) (ATCC, Manassas, VA).

Two media types were used based on ATCC and literature recommendations, brain heart infusion (**BHI**) and reinforced clostridial media + casamino acids (**RCM+C**) (Becton Dickson, Sparks, MD). Media was prepared as per label directions, boiled and pre-reduced with L-cysteine. Media (9 mL) was dispensed into 16 mL Belco tubes using a gassing cannula with 100% CO_2 to produce an anaerobic headspace, capped with a sterile butyl rubber stopper, crimp-sealed and steam pressure sterilized at 121°C for 15 min. The broth medium pH for BHI and RCM+C were 6.62 and 6.89, respectively. Nutrient agar (BHI and RCM+C) was prepared as directed and was maintained at 4°C until use.

Bacteria were rehydrated from ampoules following ATCC recommendations for anaerobes. Both BHI and RCM+C were used to hydrate lyophilized bacteria. Rehydrated cultures were then transferred into anaerobic BHI and RCM+C broth. Alcohol swabs and flame from a bunsen burner were used to prevent pure culture contamination. Bacteria were also streaked on nutrient agar plates for purity detection and incubated both aerobically and anaerobically at 37°C. The GasPak EZ Anaerobe Container System (ref: 260001) was used for small batch anaerobic plate incubations (Becton Dickson and Company, Sparks, MD). The purity of bacterial cultures was not clear from plate growth indicating the need for DNA confirmation. Broth cultures were serially transferred to monitor bacterial growth behavior.

Bacterial identification. Once tubes reached an OD of 0.1 broth cultures were plated on Brucella agar (AnaeroGRO, Hardy Diagnostics, Santa Maria, CA) and incubated for 72 h at 39°C in an anaerobic chamber. Each bacteria produced a small entire, glistening, circular, smooth, grey, and raised colonies. A single colony was picked from each bacterial growth plate for DNA extraction to confirm purity and identity. Bacterial DNA extraction was achieved by depositing picked colonies in 20 µL of a lysis buffer (0.25%w/v SDS, 50 mM NaOH) in a 2 mL micro-centrifuge tube. Tubes were set in a heat block at 95°C for 15 minutes and subsequently centrifuged 14000 × g for 5 minutes. One hundred eighty µL of ddH_2O was added to each DNA sample and centrifuged again at 14000 × g for 5 minutes. The supernatant (50 µL) was transferred to a sterile micro-centrifuge tube and stored at -20°C to be used as bacterial DNA template.

Bacterial DNA was prepared for amplification using the following mixture in a 0.2 mL PCR tube: 11.5 µL ultrapure H_2O, 10 µL of Phusion Master Mix (New England Biolabs,), 1.25 µL of forward 8F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R

(5'- GGT TAC CTT GTT ACG ACT T-3') universal 16s rRNA primers respectively, and 1.0 µL of bacterial DNA template. Thermocycler conditions: Denature (98°C for 30 s), Annealing and Elongation (98°C for 10 s, 52°C for 30 s, 72°C for 45 s; repeated 27×), Elongation (72°C for 10 min). Ladder (6 µL; manufacturer) and PCR product (4 µL mixed with 2 µL loading dye) was loaded into the wells of a 1 % agarose gel. Electrophoresis was conducted at 95 V for 1.5 h. The PCR product was sent to Eton BioScience for sequencing. Bacterial identification was confirmed by homology of the forward and reverse primer products with the 16S ribosomal RNA gene of known strains using the NCBI BLASTn suite.

The DNA isolated from *C. aminophilum* was 93% identical to the *Clostridium aminophilum* strain F 16S rRNA complete gene sequence (sequence ID: NR_118651.1) with 96 % query coverage and an e-value of 0. The *C. sticklandii* was 98% identical to *Clostridium sticklandii* strain DSM 519 16S rRNA complete gene sequence (sequence ID: NR_102880.1) with 94 % query coverage and e-value of 0. The *P*. anaerobius strain was shared 98 % identity with the ATCC 27337 strain (NR_118652.1) with 99 % coverage and an e-value of 0.

Bacterial growth. Cultures (100 µL) were transferred to fresh nutrient broth (9ml) using alcohol and flame technique and incubated for 48 h at 39°C. Final pH and redox were measured at after 48 h of incubation. Growth was measured by light absorbance (OD) at 0, 2, 4, 6, 12, 24, 27, 30, and 48 h, and plating 10-fold serial dilutions in PBS. Growth preferences were compared between BHI and RCM+C by maximum OD and CFU/mL on respective nutrient agar.

Environmental Samples and Enrichment Cultures

Six environmental samples were taken from several locations at Texas A&M University in College Station, TX: the Animal Science Teaching, Research and Extension Center (3), the Beef Cattle Research Unit (2), and the Poultry Science Research, Teaching and Extension Center (1). Rumen fluid (**RF**) was collected from two ruminally cannulated British-cross steers (**RF1**) and orally from two non-cannulated British-cross steers (**RF2**) (IACUC # 2016-0267) into two thermoses by source, respectively. Beef cattle and swine lagoon (**BSLG**) sample (1 L) was obtained from ASTREC with permissions from the complex manager. Feedlot pen floor (**FDLT**) samples (approx. 300 g) were taken from pens containing British-cross heifers (n=15) from near the water trough (**FDLT1**) and the wettest area of the pen (**FDLT2**). Poultry cecal (**PC**) fluid (50 mL) was donated from the poultry science department taken from broiler chickens (~15 birds) from a non-treatment group at the time of harvest.

Pond water (**PW**) (500 mL) donated by a local cattle operation from a natural run-off pond frequented by 5 Red Angus yearling bulls. A dairy cattle lagoon (**DLG**) sample (3 L) was donated from a dairy located within 150 miles of College Station, TX. Corn snake (**SNAKE**) feces (~ 60 g) was obtained from a local breeder and the composite of two male and two female specimens.

Environmental samples presented different volumes and viscosity. All samples which were primarily liquid (rumen fluid, beef cattle and swine lagoon, dairy cattle lagoon, and pond water) were individually mixed on a stir plate for approximately 20 min to elute potential phage and a sub-sample volume of 350 mL was saved for filtration. Those samples which were primarily solid or of low sample size were diluted with ddH_2O into a final

volume of 350 mL and individually mixed for 20 min on a stir plate. Samples were then placed in a centrifuge at 8000 × g for 20 minutes to separate particulate from the supernatant. The supernatant was vacuumed overnight through a 0.22 µm pore filter flask (ref: 431097, Corning Filter System, Corning, NY) and a final volume of ~150 mL of filtrate was saved at 4°C.

Enrichment cultures. Broth culture media was concentrated 4 times due to the slow growth nature of the bacteria. For all enrichments, 40 mL of 4× media was dispensed into 150 mL Wheaton screw-cap bottles and sterilized. For each respective bacteria, 100 µL of a fresh overnight culture was added to a Wheaton bottle, and 10 mL of each sterile environmental sample was added under a flame on the benchtop. Enrichment bottles were transferred to the anaerobic chamber and gently swirled un-capped in the anaerobic gas atmosphere (90% N_2, 5% CO_2 and 5% H_2). Caps were loosely screwed and set in the chamber incubator 37°C and checked at 24 and 48 h for culture growth. Regardless of culture clearing, enrichments were centrifuged at 13,000 × g for 5 min and then filtered through a 0.22 µm pore and stored at 4°C as a potential lysate.

Bacterial lawns and lysate spotting. Regular strength BHI and RCM+C agar were used for bacterial lawn development. Plates were pre-dried in a 37°C incubator over-night in the anaerobic chamber. Fresh overnight cultures and the prospective lysate were brought to the flame to reduce contamination of pure cultures. Sterilized micro-centrifuge tubes (2 mL) were labeled, and 900µL of phage buffer (25 mM of 1M Tris-HCl, 100mM NaCl, 8 mM $MgSO_4$, 0.01% gelatin, 47.5% dH_2O) was added for 10-fold dilution of lysate. Molten soft-agar (5 mL) was maintained at 50°C in a heat block and cooled in hand before addition of 100µL of bacterial stock culture. Inoculated soft-agar was briefly vortexed and spread

over designated nutrient agar. Plates, diluted lysate and 1 tube of undiluted lysate and related supplies were transferred to the anaerobic chamber and allowed to dry. Once dried, starting with the most dilute, lysate (10µL) was spotted on the soft agar at 10^{-10}, 10^{-8}, 10^{-6}, 10^{-4}, 10^{-2}, and 10^{1}. Spots were allowed to dry for approximately 1 h then plates were transferred to the chamber incubator for 24 to 48 h.

RESULTS AND DISCUSSION

Bacterial growth. Growth curves of *C. aminophilum*, *C. sticklandii*, and *P. anaerobius* on BHI or RCM+C are presented in Figure 5.1 and based on the most frequently observed growth pattern. Figure 5.1a shows that both *C. aminophilum* and *C. sticklandii* were able to utilize nutrients in BHI broth with doubling times of 9.33 and 3.11 h^{-1}, respectively. However, *C. sticklandii* resulted in greater cell density than *C. aminophilum* (9.2×10^{9} vs. 3.4×10^{10} CFU/mL). Thus BHI broth and agar was a suitable growth medium for *C. aminophilum* and *C. sticklandii*, but *P. anaerobius* showed no apparent growth in 48 h period.

Figure 5.1b showed that *C. aminophilum* grew faster on RCM+C than on BHI (3.08 vs. 9.33 h^{-1}) and indicated exponential growth sometime between 12 and 24 h. This result is similar to that reported in the early cultivation of the bacterium in which it was ultimately named for its preference of free amino acids versus peptides, although it could grow on both (Paster et al., 1993). Similarly, *P. anaerobius* experienced exponential growth between 12 and 24 h after inoculation with a doubling time of 4.74 h^{-1}. Interestingly, *C. sticklandii* showed no apparent growth in RCM+C broth. When determining bacterial density on RCM+C agar, it was noticed that a biofilm, appeared on the agar before any visible colony

growth being greatest for *C. aminophilum*. Bacterial numbers were greater than expected for *C. aminophilum* (TNTC at 10^{-10}) and difficult to count due to the viscous extracellular substance production. Contrarily, *P. anaerobius* bacterial numbers were less than expected (7.6×10^4 CFU/mL). We noted that *P. anaerobius* produced a significant viscous extracellular substance in broth culture (Figure 5.2) and this could potentially interfere with the efficacy of OD measurements causing an over-estimation of bacterial growth. It has also been established that *P. anaerobius* prefers an anaerobic gas mixture similar to that of the anaerobic gas chamber and the 100% CO_2 headspace of the culture tubes may have been a limiting growth factor. However, biofilm formation has been suggested to be in response to several stimuli (Jefferson, 2004). Incubation for 48 h did not yield any growth from *C. sticklandii*. It was determined that either BHI or RCM+C could be used for cultivation of *C. aminophilum*. In contrast, the BHI media may be more appropriate for laboratory work with *C. sticklandii,* and *P. anaerobius* preferred RCM+C. Final pH (Figure 5.3) reveals a marked difference between *C. aminophilum* and *C. sticklandii* vs. *P. anaerobius* which was quite low and maybe the cause for the excessive frothy substance produced in broth culture.

There was high variation in lag time among all three bacteria in broth culture and agar medium. Lag times were observed to be anywhere from 6 h to 48 h in their preferred media, and this made the onset of exponential growth challenging to predict. Age and OD of the sub-sampled stock culture were observed to be the primary determinant of lag time in broth. The spore-forming bacteria varied greatly when stock-cultures were greater than 48 h old. Therefore, growth curves in Figure 5.1 are the results of media inoculated with sub-samples taken from stock cultures less than 24 h old. It can be expected that all three bacteria

will enter exponential growth phase sometime between 12 and 24 h on their respective preferred media.

Heat activation technique. Not being able to predict the onset of early exponential growth makes for difficult experimental planning. Moreover, because diminishing nutrient in a broth culture may stimulate sporulation pathways, frequent culture transfer is necessary. Frequent transfer just to keep cells vegetative is wasteful from laboratory resources perspective. In other spore-forming species where the rate of spore germination is of interest, heat activation is often used to return spores to a vegetative state. Therefore it was proposed that heat-activation of sub-samples taken from cultures > 72 h old may; 1) reduce unnecessary supply use and 2) aide in the prediction of exponential phase growth prediction.

Three different methods in the literature are typically used to induce germination of dormant bacterial spores of Clostridial species; 1) heating at 70°C for 30 min, 2) heating at 75°C for 10 min and 3) heating at 80°C for 10 min. Each of these methods was tested in triplicate in double strength BHI broth, and the results are presented in Figure 5.4. It was observed that heating *C. aminophilum* at either 70°C for 30 min or 75°C for 10 min resulted in equally predictable OD, but 80°C for 10 min may have resulted in cell death. It is clear that by using heat activation, growth will occur reliably between 12 and 24 h. However, this method does not improve the lag time for growth. Interestingly heating *C. sticklandii* at 70°C for 30 min significantly reduced lag time and reached stationary phase at approximately 12 h. This strategy may be recommended for future work with *C. sticklandii*. Boiling at either 75°C or 80°C for 10 min resulted in growth between 12 and 24 h, but interestingly the OD at 24 h was greater for samples boiled at the higher temperature. The results of the heat activation technique indicate that frequent transfer of *C. aminophilum* and *C. sticklandii* is

not necessary for fresh cultures and that older cultures may be successfully used to reduce resource wastage. Additionally, boiling dormant sub-samples of *C. sticklandii* at 70°C for 30 min may reduce lag time enabling more rapid progress in the experimental timeline.

***Preparing bacterial lawns*.** Confluent lawns for *C. aminophilum* and *C. sticklandii* could be achieved in 24 h at 37°C but was dependent on subsamples being taken when broth culture was in an exponential growth phase. All processes could be conducted on the benchtop under a flame and were most successful when the soft agar overlay was allowed to dry in the anaerobic chamber. However, *P. anaerobius* failed to form a confluent lawn and only resulted in spotty colonies even after 72 h in the anaerobic chamber incubator. This could be due to greater O_2 sensitivity of the bacterium versus these Clostridium species. A study using a strain of *P. anaerobius* revealed the strains extreme sensitivity to oxygen (90 % kill rate in 1 h) when growing on the surface of a BHI agar plate or aerated in trypticase soy broth (Carlsson et al., 1977; Frölander and Carlsson, 1977). It is likely that during vortexing the bacterium in the molten soft agar as well as pouring the agar overlay on the benchtop likely resulted in O_2 exposure, resulting in significant cell death. All procedures and future work with *P. anaerobius* should likely be performed inside an anaerobic gas chamber. The laboratory requirements of this strict anaerobe make it a difficult candidate for phage discovery.

Although lawns were achievable for *C. aminophilum* and *C. sticklandii*, they were not always guaranteed. Scattered colonies or no growth on BHI agar were not uncommon resulting in lawn failure, even when the OD of broth culture indicated normal concentration for exponential growth. Confluent lawn formation success rates were 42.8 and 85.7 % on BHI for *C. aminophilum* and *C. sticklandii* (n= 21 and 14 attempts respectively), and no

growth on RCM+C agar. Possible reasons may be temperature sensitivity to soft agar or potential hindrance of the soft agar for bacteria to access growth medium or, as in the case of *P. anaerobius*, the timing of O_2 exposure, causing the initiation of sporulation pathway. The relative difficulty in reliable lawn formation indicated that original techniques by Felix d'Herelle and sequential clearing in broth culture might be more appropriate for these bacteria.

Enrichment of lysates. Results of enrichment cultures are presented in Table 5.1. After 24 h, clearing was observed in the *C. aminophilum* culture inoculated with both RF1, RF2 and DLG samples. However, when enrichments were filter sterilized the resulting potential phage lysate failed to clear subsequent broth cultures. Several samples resulted in the clearing of *C. sticklandii* including RF1 and RF2, PC, DLG, and PW. Again, the resulting lysate was unable to clear subsequent broth cultures, and no plaques were observed on bacterial lawns. Only the PC resulted in the clearing of *P. anaerobius,* but again lysate was not able to clear subsequent cultures.

Reasons for lack of growth of these samples is not definitively known but likely the result of chemical substances in the environmental samples. Presence of antimicrobial chemicals may cause cell lysis. It has been determined that > 99 % of cultivated bacteria produce at least one bacteriocin to inhibit competition, being of the narrow or broad spectrum (Riley and Wertz, 2002). Considering that the taxonomic class of *Clostridia* may occupy more than 10 % of the ruminal consortium of beef cattle (Crossland et al., 2017), it is not surprising that environmental samples taken in the vicinity of ruminants would result in cellular lysis due to the presence of unknown antimicrobial chemicals or compounds. This complicates matters, however, when searching for potential phage from these sources.

Although our pursuit was not successful in identifying any HAB bacteriophage, the valuable groundwork has been established for these bacteria regarding their behavior in the laboratory and the complications with environmental sources.

Implications for Future Works

Other bacteria are known for their adverse effects on feed efficiency of cattle that may be more suitable candidates for phage control. One bacteria which is both predictable and hardy in the laboratory is *Fusobacterium necrophorum* and is also the primary colonizer of ruminant liver abscesses (Nagaraja & Lechtenberg, 2007). Liver abscesses are seen at rates between 13.7 and 32% depending on the class of cattle, are known to be linked with feed efficiency, reductions in carcass weight and quality (McKeith et al., 2012; Rezac et al., 2014) and these rates may be increasing (Reinhardt and Hubbert, 2015). This may be due to decreased efficacy of current antibiotic feed additives currently used in industry. It is unknown how many cattle are affected by liver abscesses because they are asymptomatic and abscesses may heal before slaughter. A characteristic that makes this bacterium attractive for phage work is that it travels through the bloodstream and localizes itself, rather than floating in the vast rumen. The fact that this bacterium affects both the live and terminal side of the production chain indicates its importance in animal health and production efficiency that would likely make a more significant impact than the elimination of HAB.

CONCLUSION

Although *C. aminophilum*, *C. sticklandii* and *P. anaerobius* typically exist at low relative abundance in the rumen, the amount and rate of NH_3 they are capable of producing makes them quantitatively important (Russell et al., 2002). Bacteriophages are an appealing

biological control weapon against HAB offering selectivity without severely affecting the remaining bacterial community. Unfortunately, we do not report the presence of any lytic phage for *C. aminophilum*, *C. sticklandii* or *P. anaerobius* from the environmental samples tested although the presence of antimicrobial substances was apparent. Even among the most well studied Clostridial bacteria, there have been few reports of lytic phage discovery. However, temperate phages are well documented and more recent research has determined that lytic enzymes of temperate phage can be harvested and applied directly as a means of targeted bacterial control (Zimmer et al., 2002; Mayer et al., 2008; Seal, 2013).

It should be addressed that the three bacteria used in this study are not the only HAB in the rumen and others with greater specific activity of NH_3 have been detected (Attwood et al., 1998). Moreover, it follows that in a mixed culture the likely scenario is that the removal of one species simply opens the niche for another to occupy. Phage effectiveness depended on many factors and based on previous work highlighting the prevalence of ruminal bacterial lysogens (Klieve et al., 1996), in a highly competitive environment such as the bovine rumen the odds of successful phage infection do not seem favorable. Although the prospect of a self-replicating 'antibiotic' is enticing, moving forward, it seems that lytic phage enzymes may be a more direct approach in regards to defining suitable options for ruminant feed additives to control HAB, whereas phage stock may be more appropriate for infectious bacteria which may concentrate in an area.

LITERATURE CITED

Attwood, G. T., A. V. Klieve, D. Ouwerkerk, and B. K. Patel. 1998. Ammonia-hyperproducing bacteria from New Zealand ruminants. Applied and Environmental Microbiology 64: 1796-1804.

Carlsson, J., F. Frölander, and G. Sundquist. 1977. Oxygen tolerance of anaerobic bacteria isolated from necrotic dental pulps. Acta Odontologica Scandinavica 35: 139-145.

Crossland, W.L., L. O. Tedeschi, T. R. Callaway, M.D. Miller, W.B. Smith, M. Cravey. 2017. Effects of rotating antibiotic and ionophore feed additives on volatile fatty acid production, potential for methane production, and microbial populations of steers consuming a moderate-forage diet. Journal of Animal Science 95: 4554-4567.

Frölander, F., and J. Carlsson. 1977. Bactericidal effect of anaerobic broth exposed to atmospheric oxygen tested on *Peptostreptococcus anaerobius*. Journal of Clinical Microbiology 6: 117-123.

Guan, H., K. Wittenberg, K. Ominski, and D. Krause. 2006. Efficacy of ionophores in cattle diets for mitigation of enteric methane. Journal of Animal Science 84: 1896-1906. doi: 10.2527/jas.2005-652.

Jefferson, K. K. 2004. What drives bacteria to produce a biofilm? FEMS microbiology letters 236: 163-173.

Klieve, A., R. Swain, and J. Nolan. 1996. Bacteriophages in the rumen: types present, population size and implications for the efficiency of feed utilisation. In: Proceedings-Australian Society of Animal Production. p 92-94.

Mayer, M. J., A. Narbad, and M. J. Gasson. 2008. Molecular characterization of a *Clostridium difficile* bacteriophage and its cloned biologically active endolysin. Journal of Bacteriology 190: 6734-6740.

McKeith, R., G. D. Gray, D. S. Hale, C. R. Kerth, D. B. Griffin, J. W. Savell, C. R. Raines, K. E. Belk, D. R. Woerner, J. D. Tatum. 2012. National Beef Quality Audit-2011: Harvest-floor assessments of targeted characteristics that affect quality and value of cattle, carcasses, and byproducts. Journal of Animal Science 90: 5135-5142.

Nagaraja, T., and K. F. Lechtenberg. 2007. Liver abscesses in feedlot cattle. Veterinary Clinics of North America: Food Animal Practice 23: 351-369.

Paster, B. J., J. B. Russell, C. M. J. Yang, J. M. Chow, C. R. Woese, R. Tanner. 1993. Phylogeny of the Ammonia-Producing Ruminal Bacteria *Peptostreptococcus anaerobius*, *Clostridium sticklandii*, and *Clostridium aminophilum* sp. nov. International Journal of Systematic Bacteriology 43: 107-110.

Reinhardt, C., and M. Hubbert. 2015. Control of liver abscesses in feedlot cattle: A review. The Professional Animal Scientist 31: 101-108.

Rezac, D. J., D. U. Thomas, S. J. Bartle, J. B. Osterstock, F. L. Prouty, C. D. Reinhardt. 2014. Prevalence, severity, and relationships of lung lesions, liver abnormalities, and rumen health scores measured at slaughter in beef cattle. Journal of Animal Science 92: 2595-2602.

Riley, M. A., and J. E. Wertz. 2002. Bacteriocins: Evolution, Ecology, and Application. Annual Review of Microbiology 56: 117-137. doi: 10.1146/annurev.micro.56.012302.161024.

Russell, J. B. 2002. Rumen microbiology and its role in ruminant nutrition. Cornell University.

Seal, B. S. 2013. Characterization of bacteriophages virulent for Clostridium perfringens and identification of phage lytic enzymes as alternatives to antibiotics for potential control of the bacterium. Poultry Science 92: 526-533.

Sonenshein, A. L. 2006. Bacteriophages: How Bacterial Spores Capture and Protect Phage DNA. Current Biology 16: R14-R16. doi: 10.1016/j.cub.2005.12.007.

Zimmer, M., N. Vukov, S. Scherer, and M. J. Loessner. 2002. The murein hydrolase of the bacteriophage φ3626 dual lysis system is active against all *tested Clostridium perfringens* strains. Applied and Environmental Microbiology 68: 5311-5317.

Table 5.1. Results of environmental sample screening for lysate enrichment of three hyper-ammonia-producing bacteria of the bovine rumen.

Items[1]	Bacterium[2]		
	C. aminophilum	*C. sticklandii*	*P. anaerobius*
Ruminal fluid #1	×	×	normal
Ruminal fluid #2	×	×	normal
Poultry Cecal Fluid	normal	×	×
Beef cattle & Swine lagoon	normal	normal	normal
Dairy cattle lagoon	×	×	normal
Pond water	normal	×	normal
Feedlot pen floor, #1	normal	normal	normal
Feedlot pen floor, #2	normal	normal	normal
Corn snake fecal matter	normal	normal	normal

[1]Environmental samples were obtained with permission of authorized personnel or by animal use protocol approved by the Texas A&M University's IACUC committee. Each sample was filter sterilized, and 10 mL was added to 40 mL of a 4×concentrated bacterial growth media and inoculated with a respective bacterium (100 µL).
[2]Bacteria are *Clostridium aminophilum* (ATCC® 49906), *Clostridium sticklandii* (ATCC® 49905), and *Peptostreptococcus anaerobius* (ATCC® 27337) (ATCC, Manassas, VA).
An × indicates that at 24 h of incubation culture was clear or showed no growth and 'normal' indicates normal growth of bacteria.

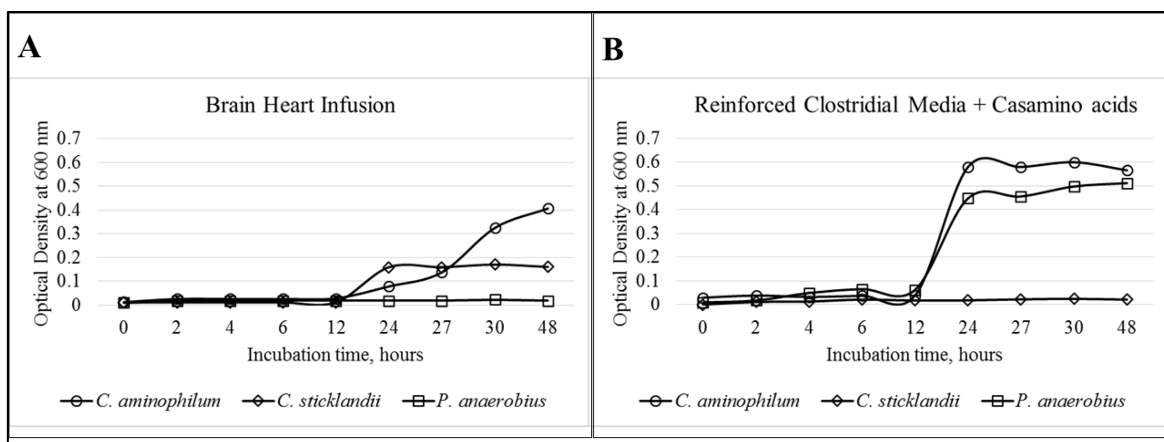

Figure 5.1. Comparison of growth curves of three hyper-ammonia-producing bacteria on A) Brain Heart Infusion broth and B) Reinforced Clostridial Media + Casamino acids. Bacteria are *Clostridium aminophilum* (ATCC® 49906), *Clostridium sticklandii* (ATCC® 49905), and *Peptostreptococcus anaerobius* (ATCC® 27337) (ATCC, Manassas, VA).

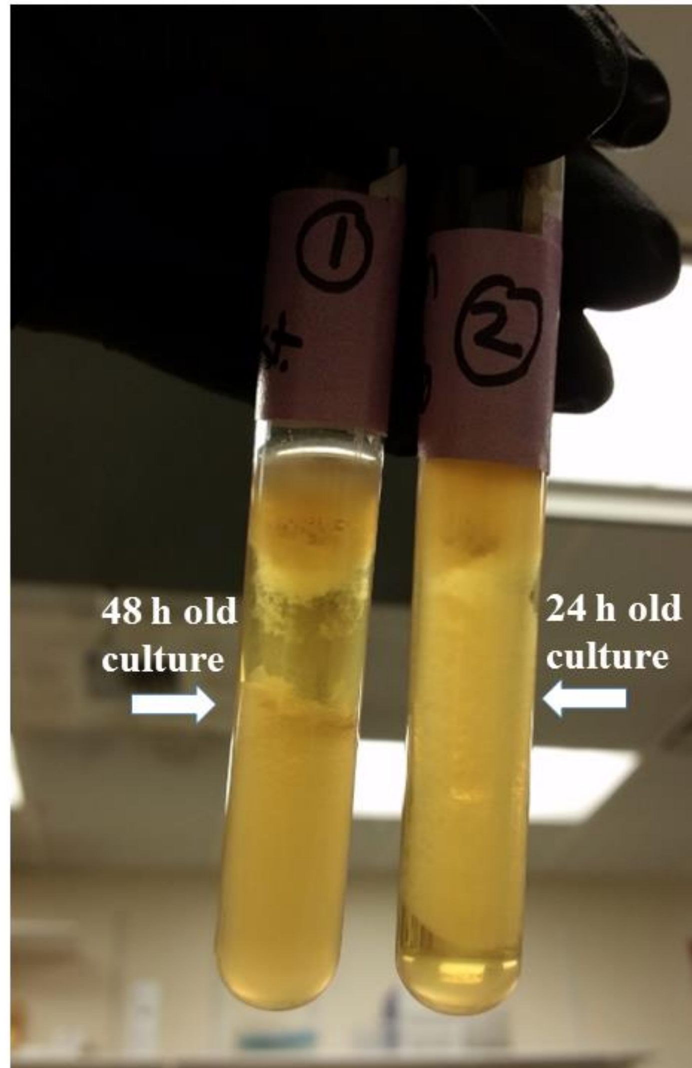

Figure 5.2. Biofilm formation of *Peptostreptococcus anaerobius* (ATCC® 27337) (ATCC, Manassas, VA) after 24 and 48 h of incubation in Reinforced Clostridial Media + Casamino acids broth at 39°C.

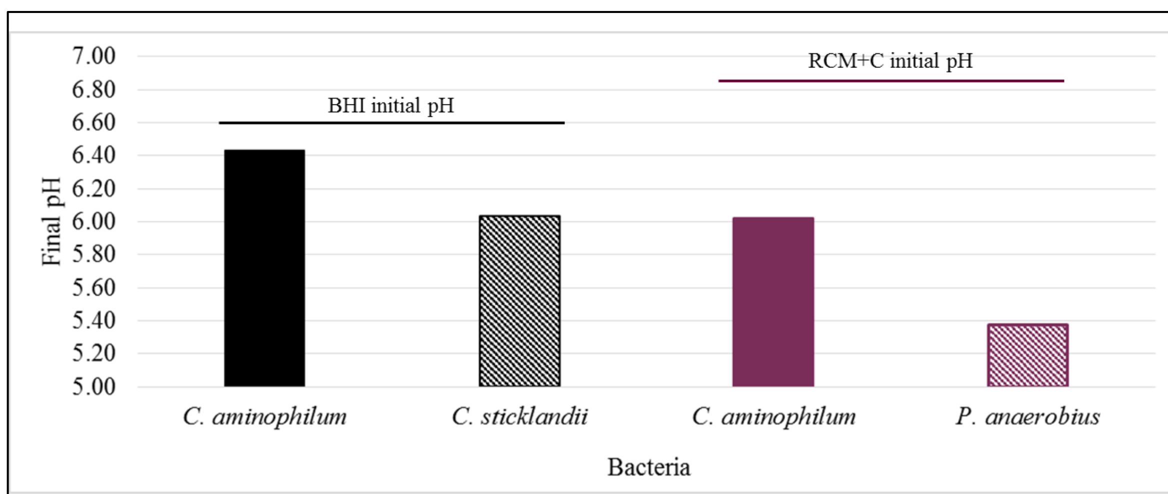

Figure 5.3. Comparison of media pH and final pH of bacterial broth cultures after 48 h of incubation at 39°C. BHI=Brain Heart Infusion broth media, pH = 6.68; RCM+C= Reinforced Clostridial Media + Casamino acids, pH=6.89.

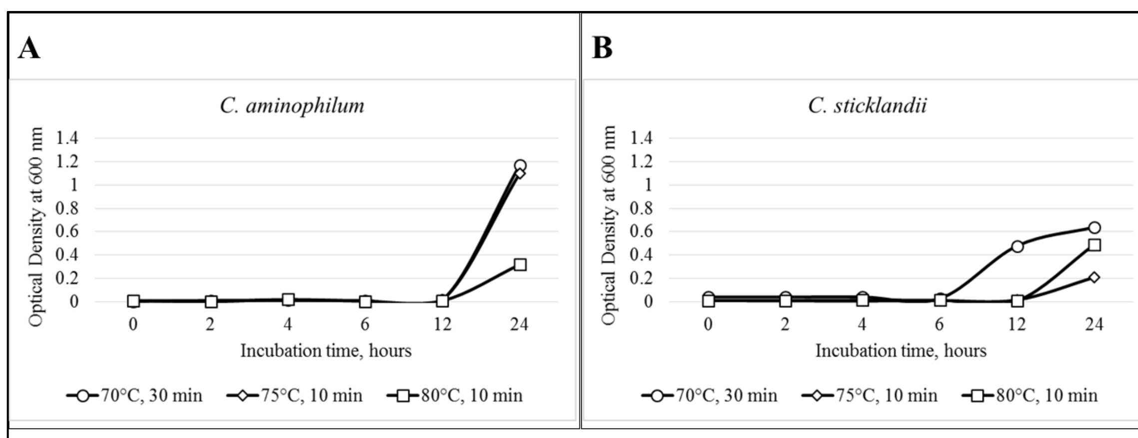

Figure 5.4. Comparison *Clostridium aminophilum* (ATCC® 49906) and *Clostridium sticklandii* (ATCC® 49905) growth after heat activation. Inoculum (100µL) was sub-sampled from cultures older than 72 h and inoculated into fresh media, then boiled at three temperatures for differing times.

CHAPTER VI

CONCLUSIONS AND FUTURE OUTLOOK

In summary, the use of antimicrobial feed additives to promote feed efficiency of beef cattle has been utilized for many years as an active management tool. However, increased regulation of their use for growth promotion has resulted in revitalized interest in alternative products. More research should focus on identifying and characterizing their efficacy in different sectors of the beef cattle industry to promote their use appropriately. Our studies highlight potential benefits of using ionophores, yeast, or bacteriophages to offset the habitual use of antibiotics in cattle production.

When cattle are fed moderate forage diets monensin reduces acetate to propionate ratio more than bambermycin without affecting total volatile fatty acids (**VFA**) which improves ruminal efficiency of nutrient utilization. However, long term feeding of monensin shows that there is some degree of microbial adaptation and therefore its feeding strategy should be investigated further to optimize its overall use in different feeding scenarios. We suggest to investigate the short and long term effects of pulse-feeding monensin on ruminal efficiency and animal performance.

Under thermo-neutral conditions, active dried yeast (**ADY**) supplemented in the diets of finishing steers was found to positively moderate ruminal pH of steers versus those who were not supplemented. However, this difference was no longer detected under heat stressed conditions. Supplementing ADY increased the energy available for maintenance under thermoneutral conditions but was not sufficient to overcome the energetic requirements of extreme heat stress. When steers were group fed and transitioned from a grower diet to a

finishing diet, we did not find any clear evidence that ruminal pH was stabilized above the acidosis thresholds more for ADY- than control-fed steers, likely due to the variation in eating patterns in group versus individual feeding. However, the ruminal pH of steers who consumed yeast during the dietary transition drifted less than the ruminal pH of control fed cattle, which may be considered more stable. Supplementing ADY did not result in any significant performance parameters versus control-fed steers. Dose administration of ADY and consistency of intake in group feeding scenarios is a challenge that will need to be addressed before consistent results will emerge and yeast products may be recommended for practical cattle feeding.

Bacteriophage therapy as a means of ruminal microbial control offer selectivity more than any other feed additive. Future work where phage may be used as a feed additive may look to high impact bacteria that cause easily measurable losses to truly understand efficacy. Ideal candidates are those implicated in ruminal acidosis and liver abscesses, as well as respiratory infections which may negatively impact feeding performance and growth efficiency.