

8-2019

## Technologies to Increase Animal Performance in Beef Production Systems

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Technologies to Increase Animal Performance in Beef Production Systems

A dissertation submitted in partial fulfillment  
of the requirements for the degree of  
Doctor of Philosophy in Animal Science

by

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## ABSTRACT

Three monensin levels during a stocker phase (0, 800, 1600 g/ton in a free choice mineral) and two levels during finishing (0 [U] or 37.5 mg/kg diet DM [M]) were used to determine the effects of monensin supplementation during a stocker and subsequent finishing phase on beef calves. Steers ( $n = 605$ ,  $BW = 278 \pm 27$  kg) were fed pearl millet hay with soybean hull and corn gluten feed supplement (0.5% BW daily [AF basis] Block 1) or grazed fall wheat (Block 2), spring wheat (Block 3), bermudagrass (Blocks 4 & 5) or wheat with mixed-grass baleage (Block 6). A subset of calves were transported 1,068 km to Canyon, TX (blocks 1, 2, and 4) or 636 km to Stillwater, OK (block 6) for finishing. Rumen fluid was collected on a subset of cattle ( $n = 30$ ) throughout the experiment to characterize rumen microbiota by next generation sequencing. There were no treatment  $\times$  diet interactions ( $P \geq 0.96$ ) during the stocker phase or location  $\times$  treatment interactions ( $P \geq 0.19$ ) during finishing, therefore data were pooled for statistical analysis. During the stocker phase, cattle consuming monensin had greater ( $P = 0.01$ ) final BW compared with the 0 treatment. Steers on 800 and 1600 had greater ADG ( $P = 0.02$ ) and total gain ( $P = 0.02$ ) than 0. Mineral intake decreased ( $P < 0.01$ ) as monensin level increased. Stocker  $\times$  feedlot treatment interactions were not observed for feedlot performance ( $P \geq 0.50$ ). Feedlot cattle consuming monensin had decreased ( $P < 0.01$ ) DMI and increased ( $P < 0.01$ ) G:F than those that did not. Cattle consuming monensin during finishing had increased ( $P = 0.03$ ) fat thickness. During the stocker phase, monensin treatments had decreased alpha community ( $P \leq 0.04$ ) compared to the 0 treatment. Cattle previously on the 0 and 1600 treatments during the stocker phase and were fed monensin at the feedlot had decreased alpha diversity ( $P = 0.04$ ) on feedlot d 14 compared to those that did not.

## TABLE OF CONTENTS

I.	Introduction.....	1
	Literature Cited.....	3
II.	Review of Literature.....	5
	Overview of the rumen microbiome.....	5
	Establishment of the rumen microbiome.....	5
	Rumen microbiome in forage-fed and grazing cattle.....	9
	Rumen microbiome of concentrate-fed cattle.....	14
	Ionophores and monensin overview.....	18
	Monensin mode of action.....	19
	Influence of monensin on the rumen microbiome using new technology.....	20
	Monensin effect on grazing cattle performance.....	25
	Monensin effect on finishing cattle performance.....	28
	Other dietary factors that alter the rumen microbiome.....	32
	Contributions of the rumen microbiome to production.....	34
	Conclusion.....	36
	Literature Cited.....	37
III.	EFFECT OF MONENSIN LEVEL ON MINERAL INTAKE AND PERFORMANCE OF GRAZING STEERS.....	43
	Abstract.....	44
	Introduction.....	45
	Materials and Methods.....	46
	Results and Discussion.....	51
	Implications.....	54
	Literature Cited.....	55
	Tables and Figures.....	57
IV.	EFFECT OF MONENSIN INTAKE DURING A STOCKER PHASE AND SUBSEQUENT FINISHING PHASE ON PERFORMANCE AND CARCASS CHARACTERISTICS OF FEEDLOT STEERS.....	61
	Abstract.....	62
	Introduction.....	63
	Materials and Methods.....	66
	Results and Discussion.....	67
	Conclusion.....	70
	Literature Cited.....	72
	Tables and Figures.....	73

V.	EFFECT OF MONENSIN INTAKE DURING A STOCKER PHASE AND SUBSEQUENT FINISHING PHASE ON RUMEN BACTERIAL DIVERSITY OF BEEF STEERS.....	77
	Abstract.....	78
	Introduction.....	79
	Materials and Methods.....	80
	Results and Discussion.....	83
	Conclusion.....	91
	Literature Cited.....	92
	Figures.....	94
VI.	Conclusions.....	101

## LIST OF TABLES AND FIGURES

Table 1: Nutrient composition of additional forages provided to grazing calves.....	57
Table 2: Forage mass, forage allowance and nutritive quality of pastures grazed by steers.....	58
Table 3: Effect of monensin supplementation on performance and mineral intake of grazing steers.....	59
Table 4: Ingredient composition of each diet fed at the Canyon location.....	73
Table 5: Ingredient composition of each diet fed at the Stillwater location.....	73
Table 6: Nutrient composition of final finisher diets for each treatment at each location.....	73
Table 7: Effect of monensin during the stocker phase and subsequent feedlot phase on feedlot performance of beef steers.....	74
Table 8: Effect of monensin during the stocker phase and subsequent feedlot phase on carcass characteristics of steers.....	75
Figure 1: Effect of monensin supplementation on free-choice mineral intake of beef steers over time.....	60
Figure 2: Effect of monensin treatment during a stocker phase and subsequent feedlot phase on ribeye area (cm <sup>2</sup> ) of steers.....	75
Figure 3: Effect of monensin treatment during a stocker phase and subsequent feedlot phase on yield grade of steers.....	76
Figure 4: Alpha diversity measures for rumen microbiome of stocker calves consuming hay and wheat diets.....	94
Figure 5: Shannon Index values for alpha diversity of feedlot steers.....	95
Figure 6: Observed OTUs for alpha diversity of feedlot steers.....	96
Figure 7: Bray-Curtis Index of beta diversity measure for calves throughout the stocker phase..	97
Figure 8: Bray-Curtis Index of beta diversity measure for cattle throughout the feedlot phase....	98
Figure 9: Relative abundance of the top 30 OTUs present in the rumen microbiome of stocker calves on each day of sample collection during the stocker phase.....	99
Figure 10: Relative abundance of the top 30 OTUs present in the rumen microbiome on each day of sample collection during the finishing phase.....	100

# CHAPTER I

## INTRODUCTION

Monensin is one of the most widely used ionophores in food animal production and is routinely fed to beef cattle due its consistent improvements in animal performance and health (Novilla, 2011; Duffield et al., 2012). Monensin alters fermentation in the rumen by inhibiting less efficient acetate producing ruminal bacteria, resulting in increased propionate production, improved feed efficiency and decreased methane production (Callaway et al., 2003). The rumen microbiota plays a key role in in the efficiency of digestion and the use of a fermentation modifier like monensin can enhance the effects of these microbes on the host animal (Morgavi et al., 2013).

Utilizing a stocker program can be a way to increase frame size and add value of calves on forages at a lower cost than that of a feedlot. To maximize the returns of a stocker operation, producers must put weight on calves as efficiently and economically as possible (Rhinehart and Poore, 2012). Supplementing ionophores like monensin to stocker calves have consistently shown to be an inexpensive management strategy to increase weight gain of forage-fed growing calves over a wide range of forage systems (Potter et al., 1976; Oliver, 1975; Boling et al., 1977; Males et al., 1979; Beck et al., 2016). Monensin provided free-choice mineral to stocker calves can be effective at improving animal performance (Fieser et al., 2007; Beck et al., 2014); however, mineral consumption can be reduced and may not deliver an adequate dose to have a gain response (Horn et al., 2005; Beck et al., 2014). Monensin level may need to be adjusted to achieve desired intakes of mineral to deliver the monensin effectively (Horn et al., 2005).

Monensin is widely used in the cattle finishing industry to improve feed efficiency, reduce metabolic disorders from variable feed intakes, and to prevent outbreaks of coccidiosis

(Duffield et al., 2012). A recent survey of 49 consulting feedlot nutritionists who service over 14,000,000 cattle annually found that 97.3% of the respondents use ionophores, of these 100% used monensin in their finishing diets (Samuelson et al., 2016). A meta-analysis by Duffield et al. (2012) concluded that including monensin in feedlot diets results in a 6.4% improvement in feed efficiency; however, it was reported that there were only a 2.5 to 3.5% improvement in trials conducted within the last 2 decades, suggesting that the efficacy of monensin may be declining. Due to the age of monensin feedlot research and the increased pressure from consumers to reduce antimicrobial feed additives in meat production, the use of monensin should be evaluated further in modern finishing systems.

The mode of action behind monensin is to eliminate certain bacterial species in the rumen to enrich the prevalence of more efficient species. The majority of this data is derived from pure culture-based bacterial isolation and estimations made from the production of various fermentation end products such as VFA and CH<sub>4</sub> (Callaway et al., 2003). Recent advancements in next-generation sequencing techniques provide opportunities to evaluate how monensin affects ruminal bacterial communities in a more comprehensive way (Kim et al., 2014).



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## **CHAPTER II**

### **REVIEW OF LITERATURE**

#### **Overview of the rumen microbiome**

The ruminant animal has a symbiotic relationship with its gastrointestinal microbes, and this relationship can play a vital role in both health and performance of the animal (Ley et al., 2008; Serikov and Finlay, 2009). Specifically, the rumen is an important site of fermentation, in which a complex anaerobic microbiota composed of bacteria, archaea, fungi, protozoa and viruses ferment dietary components into usable end-products for the animal (Rey et al., 2013). In adult animals, the rumen microbial biomass is largely composed of bacteria, approximately  $10^{10}$  to  $10^{11}$  cells per  $g^{-1}$  of ruminal fluid (Hungate, 1996). Conventional techniques of determining ruminal microorganisms involved culture-based isolation, which has provided valuable information on the diversity of the rumen microbiota. At least 200 species of bacteria have been identified using the culture based approach. In recent years, the diversity of the microbiota in humans and animals have been studied using a metaanalysis of 16S rRNA gene sequences from approximately 13,478 bacterial sequences. Using this genetic based analysis, the diversity of the rumen bacteria is estimated to be around 7,000 species, consisting of 5,271 operation taxonomic units, and represent 19 existing phyla (Chaucheyras-Durand and Ossa, 2014). The use of this technology has greatly enhanced our knowledge and capabilities of studying the microbiota, especially one that is as complex and diverse as the rumen.

#### **Establishment of the rumen microbiome**

The initial microbial community in newborn mammals is acquired from the surrounding metacommunity (Curtis and Sloan, 2004). This includes vaginal canal during birthing, contact with dam during suckling and grooming, environmental interaction, and via colostrum and milk

consumption (Rey et al., 2013). The establishment of the rumen microbiome early in life has shown to be critical to the hosts' metabolism, health, and immune function (Cammack et al., 2018). The neonate rumen is thought to be sterile at birth, but is initially colonized very early in life (Li et al., 2012; Jami et al., 2013). Although the rumen is undeveloped and non-functional in newborn calves, the mode of initial inoculation is thought to be attributed to swallowing of saliva and milk from the dam and other environmental interactions, as some ingesta is thought to pass the esophageal groove and enter the rumen (Hungate, 1966). Several experiments have investigated how the rumen microbiome develops in calves from birth to slaughter and this review will discuss them in more detail.

An experiment conducted by Rey et al. (2013) analyzed the change in rumen bacterial populations of dairy calves from birth to weaning at 83 days of age using 16S rRNA-based pyrosequencing. Calves were fed colostrum until d 3, and milk replacer with pelleted concentrate and hay from d 4 to 83. Rumen samples were collected daily from d 1 to 10, and then on d12, 15, 19, 22, 26, 29, 33, 36, 40, 43, 50, 62, and 83 of age. Bacterial communities were present at 2 d, with *Proteobacteria* (70%) and *Bacteroidetes* (14%) being the most prevalent genera and *Pasteurellanceae* (58%) being the dominant family. On d 3, the community shifted in which *Bacteroides* (21%), *Prevotella* (11%), *Fusobacterium* (5%), and *Streptococcus* (4%) were dominant genera, which continued until d 12. Following d 15, *Prevotella* became dominant (42%) with a majority of other genera decreasing or were no longer detected. This remained until the end of the experiment on d 83. The authors concluded that the shift in bacterial communities throughout the duration of the experiment was likely due to increasing feed intake, leading to the production of ruminal volatile fatty acids and enzymatic activities (Rey et al., 2013).

A similar study conducted by Meale et al. (2017) evaluated the effect of early weaning (6 week) versus late weaning (8 week) on the ruminal microbial populations of dairy calves. Bacteroidetes and Firmicutes were the most abundant phyla in pre- and post- weaned calves, respectively, regardless of weaning date. This research agrees with that of Rey et al. (2013), in which the Bacteroidetes phylum was dominated by the genus *Prevotella* in all calves older than 15 d of age, when consumption of solid feed was greater than 100 g/d. The dominance of *Prevotella* at this stage in life was no surprise to the authors, as this genus is comprised of species that are associated with the fermentation of non-structural carbohydrates and xylan (Meale et al., 2017). The  $\beta$ -diversity of the ruminal microbiome shifted more rapidly in early weaned calves, where a more gradual shift occurred for late weaned calves. This rapid shift compared to a more gradual shift may explain the greater decline in growth of the early weaned calves that was observed by the authors. They concluded that weaning later in life resulted in a more gradual shift in the microbial diversity in both rumen and fecal microbiota, and when observed with performance data, indicates a smoother transition during weaning for calves weaned at 8 weeks of age. The slower increase in solid feed consumption of late-weaned calves resulted in the development of a rumen microbiome that was better suited to perform the fermentative and metabolic functions before weaning, thus reducing the level of physiological stress associated with weaning, like what was observed in the early-weaned calves (Meale et al. 2017).

It appears that the rumen microbiome shifts with age and management of the host animal and tends to stabilize following weaning as the animal matures. An experiment conducted by Jami et al. (2013) evaluated the change in rumen microbiome of Holsteins from birth to two years of age, with sampling occurring at ages of 1 d, 3 d, 2 mo, 6 mo, and 2 yr. Calves were fed

colostrum from d 1 to 3, and then transitioned to consuming milk and a solid concentrate starter feed at the 2 mo of age sampling time. At maturity (6 mo and 2 yr of age) cattle consumed a diet of approximately 70% concentrate and 30% roughage. Surprisingly, the authors were able to detect a number of bacterial species that are abundant in mature rumens at only 1 d of age. These primarily consisted of Proteobacteria, Bacteroidetes, and Firmicutes. Even though the bacteria detected in the d 1 and 3 calves were similar in taxonomy, their abundance differences were quite distinct. A decrease in aerobic and facultative anaerobic bacterial species was observed as anaerobes such as *Prevotella* and *Ruminococcus* increased with maturity. Bacterial communities fluctuated early in life (1 d, 3 d, and 2 mo), but the within-group similarity increased with age (6 mo, 2 yr), suggesting a more diverse, but homogenous and specific community as the animals matured compared with a more heterogeneous, less diverse initial community observed in young calves (Jami et al., 2013).

Consistent findings suggest that the microbiota of the developing rumen (pre-weaning) is dominated by three main phylum (Bacteroidetes, Firmicutes, and Proteobacteria, although the relative abundance of each phyla can vary with different stages of development (Meale et al., 2017; Li et al., 2012; Jami et al., 2013). All of the research previously discussed involved dairy calves, as limited data is available on how the developing rumen microbiome changes in beef calves. It is difficult to make interpretations between the two, given the differences in production systems between dairy and beef cattle. It appears that the manipulation of the rumen microbiota early in life can persist into maturity; therefore, this is an area of production that warrants more research (Cammack et al, 2018).

## **Rumen microbiome in forage-fed and grazing cattle**

Once the rumen is developed and functional enough to handle solid feeds, calves begin to experience dietary shifts. Some of these dietary shifts are a result of environmental and seasonal changes that can alter the availability and quality of feedstuffs that require adaptation of the microbiome to allow continued, adequate performance of the host (Meale et al., 2017; Cammack et al., 2018). A prime example of this would be grazing cattle, which may experience a wide variety of dietary changes as forage type, quality or abundance may vary throughout the year. Fortunately, there is some data available on the rumen microbiome throughout several different pasture and forage-based systems that will be discussed.

***High-quality forage.*** An experiment conducted by Pitta et al. (2016) evaluated the rumen microbiome of calves grazing hard red winter wheat (*Triticum aestivum*) and how frothy bloat, a common metabolic disorder that occurs with grazing wheat, can affect the rumen microbiome. Bloat is described as a when an excess accumulation of gas in the rumen leads to increased intraruminal pressure with abdominal pressure, inhibiting the eructation mechanism and can disrupt the homeostatic environment within the rumen. Wheat is typically grazed in both the fall and spring, and this particular experiment occurred in the spring. The most abundant phyla observed by Pitta et al. (2016) for calves grazing wheat were Firmicutes (65%), then Actinobacteria (15%), Bacteroidetes (10%), and Proteobacteria (5-6%). When bloat occurred, bacterial phyla differed between bloated and non-bloated calves, in which Firmicutes and Proteobacteria were greater and Bacteroidetes and Actinobacteria were lower. At the genus level, wheat pasture frothy bloat did not create shifts in microbial populations but significant tradeoffs were observed. *Clostridium*, *Eubacterium*, and *Butyrivibrio* from the Firmicutes phyla were greater while *Ruminococcus* was lesser in bloated steers compared to non-bloated steers.

*Prevotella* from Bacteroidetes was reduced by half and *Bacteroides* and *Parabacteroides* increased for bloated calves. Furthermore, genera from Actinobacteria such as *Slackia*, *Atopobium*, *Eggerthella*, *Olsenella*, *Bifidobacterium*, *Collinsella*, *Gordonibacter*, and *Cryptobacterium* were decreased and *Geobacter*, *Desulfovibrio*, *Burkholderia*, *Pseudomonas*, *Shewanella*, *Vibrio*, *Pleobacter* and *Escherchia* of Proteobacteria were more abundant in bloated calves. The authors concluded that the effect of frothy bloat in stocker cattle grazing wheat pasture on the rumen microbiome is not associated with specific microorganisms, but rather due to changes in the community structure across all microbe domains (Pitta et al., 2016).

A different, similar experiment by Pitta et al. (2014) evaluated the dynamics in bacterial populations of both solid and liquid ruminal fractions of steers grazing maturing wheat forage of changing nutritive value. The wheat was still in a vegetative state for the first 28-d with a moderate CP value (21%); however forage quality diminished thereafter as the forage matured. The predominant phyla were Bacteroidetes (59-77%) and Firmicutes (20-33%) across both ruminal fractions. A shift towards Firmicutes was observed as the CP of the forage decreased with increasing maturity. This research demonstrates the influence of fermentation activity in the rumen as forage matures on changes with the microbiome, which could be explained by the increased fiber content and decreased digestion associated with mature forage (Pitta et al., 2014).

The previous paper discussed changes in the rumen microbiome with changes in forage nutritive quality. To demonstrate the opposite effect, Pitta et al. (2010) investigated the changes in ruminal bacterial diversity from steers fed low-quality forage to high-quality forage. Steers were fed bermudagrass (*Cynodon dactylon*) hay (11% CP and 68% NDF) for 34 days and then turned out to winter wheat (*Triticum aestivum*) pasture (20% CP and 44% NDF) and grazed for 28 d. Ruminal samples were obtained at the end of each forage feeding phase and analyzed using



a 16S-based pyrosequencing technique to characterize changes in bacterial communities between the different forage types. Based on these results, *Prevotella* and *Rikenella* were the predominant genera found in both diets. When cattle were transitioned to wheat, microbial communities shifted to *Prevotella* dominance (26% vs 52% for bermudagrass and wheat, respectively) and a decrease in *Rikenella* (21% vs 9%, for bermudagrass and wheat, respectively). The shift in *Prevotella* from bermudagrass hay to wheat is consistent with research on transitioning cows from forage to grain diets, in which increases in *Prevotella ruminicola* and *Prevotella bryantii* were observed using quantitative PCR techniques (Tajima et al., 2000). The similarities between the current paper of Pitta et al. (2010) and Tajima et al. (2000) on the shift of *Prevotella* is likely attributed to the introduction of highly fermentable carbohydrates and protein found in the wheat forage and grain compared to the hays that were used in both studies.

A different experiment investigated the change in ruminal bacterial communities when Holstein heifers were fed orchardgrass (*Dactylis glomerata*) as pasture or hay (Mohammed et al., 2014). Heifers sequentially grazed orchardgrass pasture (12% CP and 55% NDF) for 28 d, then fed orchardgrass hay (13.3% CP and 63% NDF) ad libitum for the next 28 d, then placed back on pasture (24% CP and 51% NDF) for the final 28 d. A different group of animals remained on pasture for the entire duration of the experiment. Ruminal samples were collected on the final 3 d of each 28 d period and bacterial community composition was determined using automated ribosomal intergenic spacer analysis and by pyrotag sequencing of 16S rRNA genes. The bacterial community was dominated by members of the phylum Firmicutes, which accounted for 58-86% of the sequences. The genus *Butyrivibrio* was the most abundant (20%), followed by *Prevotella* (2-14%) and *Ruminococcus* (2-7%). *Butyrivibrio* expressed the greatest diet-dependent shifts when heifers were switched from pasture to hay to pasture, in which

*Butyrivibrio* decreased during the change from pasture to hay, and increased when heifers were changed back from hay to pasture. Abundance of *Butyrivibrio* did not appear to change for heifers maintained on pasture for the entire duration. It is noteworthy that ruminal abundance of *Prevotella* was substantially lower (<20%) in this experiment compared to what is typically observed (40 – 60%) in cows fed forages and concentrates. Analysis of the community diversity and richness showed that community richness was not related ( $P > 0.05$ ) to dietary sequence or the diets (pasture vs hay). A Shannon index of community diversity was greater ( $P = 0.004$ ) in the hay versus pasture treatment community; however, the Simpson index did not differ ( $P > 0.05$ ) within the comparisons. This research demonstrates that changes in the rumen bacterial community composition can occur by feeding a hayed version of the same forage from adjacent paddocks (Mohammed et al., 2014).

The rumen bacterial communities can change throughout the year on the same pasture, as this is demonstrated by the work of Noel et al. (2017) in which cows continuously grazed ad libitum mixed ryegrass/clover pastures. Ruminal content samples were collected at five time points over a year's time, which occurred in May, August, November, February, and May of the following year. In months when forage was not adequate, pasture silage made from the same pasture type as the grazing pasture was supplemented at 30% of recommended intake. Supplemental silage was being provided during both May and August sampling dates. Diversity of rumen bacteria were determined using pyrosequencing of bacterial 16S rRNA genes. The bacterial community in this experiment was dominated by members of Firmicutes (82.1%) followed by Bacteroidetes (11.8%) and Fibrobacteres (2.4%). The largest group of genus level was that of undefined *Clostridiales* (22.9%) followed by undefined *Lachnospiraceae* (12.2%) and *Butyrivibrio* (10.2%). Most sequences (61%) could not be classified into the genus level.

The sequence analysis showed that bacterial communities remained constant among animals as only 0.13% and 0.59% of the total OTUs were unique to any one animal. An ANOSIM and Adonis analysis on the OTUs showed differences in bacterial communities across months. Surprisingly, a difference was detected between the months when animals were grazing pasture only and the months that supplemental silage was provided. This data suggests that the rumen microbial community adapts to changes in diet qualities throughout the year. The Noel et al. (2017) paper provides excellent information on how the microbiome changes with changing forage production and the authors conclude that this research shows the stability of the rumen microbes in pasture fed cows over time (Noel et al., 2017).

***Low-quality forage.*** When forage is of low-quality, protein is often the first limiting nutrient because the forage does not contain enough nitrogen to meet the requirements of the ruminal microbes or the animal (Köster et al., 1996); therefore, protein supplements are often provided to ruminants consuming forage of poor nutritive quality to improve animal performance (Hersom, 2007). The response of the rumen microbiome from this management strategy is demonstrated by Latham et al. (2018), in which steers were used in a Latin square experiments to test four different protein supplements (two levels of rumen undegradable intake protein (UIP) and two levels of highly rumen degradable intake protein (DIP) to provide 50 or 150 mg of N/kg of BW daily. These treatments were tested against a non-supplemented control. The basal diet consisted of rice straw (4.7% CP and 72.8% NDF) and was fed to exceed ad libitum intake. Rumen samples were collected at 0 and 4 h post- feeding and rumen bacterial taxa were sequenced using a Roche 454 platform based on the 16S rRNA gene. Across all samples, Bacteroidetes were considered the dominant phyla, followed by Firmicutes, which accounted for 65% and 28% of total bacterial abundance, respectively. When supplemented with protein,

Firmicutes had the greatest response, in which an 8.1% difference was observed between the control and the High UIP treatment. The families of *Prevotellaceae*, *Ruminococcaceae* and the genera *Prevotellaceae* decreased in abundance when protein with supplement compared with controls. The authors also noted that the greatest number of rare species was observed for the high DIP protein supplement, which suggests that the degradable protein available for microbial use provided greater diversity than the control or UIP treatments. With relatively low impacts on the abundance and diversity of the rumen microbes from supplementing steers consuming low-quality forage with additional protein, it was concluded that a dietary effect could have occurred due to the low digestion potential of the rice straw (Latham et al., 2018).

When evaluating literature on what can influence the microbiome of grazing cattle, it is not difficult to tell that we have barely scratched the surface of what we know or what we can study in this area. The data on bacterial communities of grazing cattle is very limited due to the complexity and diversity of the production systems associated with forage fed cattle and more research is needed to help further our understanding of the symbiotic relationship between grazing cattle and the contribution of their microbes.

### **Rumen microbiome of concentrate-fed cattle**

It was previously mentioned that cattle encounter many nutritional and dietary changes throughout the beef production system. An even more dramatic shift in dietary structure than weaning or changing forage types or quality would be the placement into a feedlot and the transition to a high-concentrate diet. Adaptation from a high-forage diet to a high-grain diet has shown to significantly alter both the rumen environment and bacterial population structure (Goad et al., 1998). To prevent metabolic disorders, this transition is often achieved by gradually

reducing the levels of roughage and replacing it with grain while adapting cattle to the new diets and management.

Work by Fernando et al. (2010) analyzed the rumen microbial population during this adaptation period. Cattle in this experiment were adapted to a high-grain diet using a step-up diet regimen in which animals were adapted every 7 d to diets containing grain to roughage ratios of 20:80, 40:60, and 60:40. A final diet consisted of 80:20 concentrate to fiber was fed for the final 7 d. Ruminant fluid was obtained from steers after 1 week of each diet step, in which the animal would start the next step of the diet. Microbial populations were distinguished using terminal restriction fragment length polymorph analysis, 16S rRNA gene libraries and quantitative real-time PCR. Results of analysis showed a shift in microbial population by diet 3 (60% corn, 40% hay) and this shift was more prominent by diet 4 (80% corn, 20% hay). No significant change in phyla was observed until diet 3, in which the ratio of Firmicutes:Bacteroidetes decreased (1.49 and 0.89 for high roughage and concentrate diets respectively). Steers on the high roughage diets had a greater abundance of bacteria in the phylum Fibrobacteres than animals consuming the high concentrate diet. Cattle on the final (80:20) diet displayed a greater number of the phylum Bacteroidetes than that of animals consuming the high-roughage diet. All of the bacteria within the phylum Fibrobacteres belonged to the genus *Fibrobacter*, whereas most of the bacteria within the phylum Bacteroidetes belonged to the genus *Prevotella*. The authors also reported a gradual decrease in cellulolytic bacteria including *Fibrobacter succinogenes* and *Butyrivibrio fibrisolvens* and an increase in amylolytic/lactate utilizing bacteria such as *Megasphaera elsdenii*, *Streptococcus bovis* and *Selenomonas ruminantium* when transitioning the rumen environment away from a high-roughage diet to a high-concentrate diet.

A different trial by Petri et al. (2013) also evaluated the change in the rumen microbiome of cattle being transitions to a feedlot, but also included an acidosis challenge. In this experiment, beef heifers were fed a high forage diet and then transitioned to a 60% forage 30% grain diet for 2 wk before being transitioned to an 81% grain 9% forage diet. A mineral supplement was included in all diets and made up for the remaining 10%. The acidosis challenge was achieved by restricting intake to 50% of ad libitum and pulse dosing ground barley grain into the rumen at 10 or 20% of ad libitum intake via the ruminal cannula. Rumen sampling occurred 4-h post-feeding and sampling days occurred during each of the diet phases. Using real-time PCR and pyrosequencing analysis of 16S rRNA, the overall core microbiome was found to consist of the phyla Bacteroidetes (32.8%), Firmicutes (43.2%), and Proteobacteria (14.3%). The microbiota differed between the high forage diet and the high grain diet, in which the high forage core microbiota consisted of Firmicutes (55%), then Bacteroidetes (26%) followed by Proteobacteria (5%) whereas the high grain diet resulted in core phylums of Bacteroidetes (40%), Firmicutes (37%) and Proteobacteria (18%). Real time qPCR was used to evaluate target bacteria of interest, including: *g. Ruminococcus*, *g. Prevotella*, *Selenomonas ruminantium*, *Fibrobacter succinogenes*, *Megasphaera elsdenii*, and *Streptococcus* spp. Of these, *Prevotella* was the most abundant during the acidotic challenge and the lowest in heifers fed the high-forage diet. *Selenomonas ruminantium* and *M. elsdenii* were detected to be the smallest proportion of the bacterial population for the forage diet. *Megasphaera elsdenii* was the most abundant 12 h following the acidotic challenge and quickly declined during recovery, whereas *S. ruminantium* increased during this time. The authors of this paper stated that this work provided evidence that *Streptococcus bovis* is a major contributor to ruminal acidosis; however, they do not elaborate on this statement. It could be inferred that, given the abundance of *S. bovis* increasing during the

acidotic event and the fact that *S. bovis* readily produces lactate, one could assume it could have contributed to acidosis.

A study on feedlot cattle by Petri et al. (2012) evaluated the effects of diets with or without roughage on rumen bacterial populations. The feedlot diets were barley grain-based and the diet with roughage contained 15% inclusion (DMB) barley silage as the roughage source and the diet without roughage contained 35% inclusion (DMB) of wheat dried distillers grains. After 14 d of diet adaptation rumen bacterial samples were collected 1 h before feeding and 3 h after feeding. Real-time PCR was used to evaluate the quantities of seven different bacterial species. A decrease was observed for the relative abundance of *Fibrobacter succinogenes* in cattle fed the diet without roughage compared to cattle that received diets with roughage. This would be feasible as *F. succinogenes* is a cellulolytic species and the low amount of substrate for it to adhere to in a diet with limited fiber would certainly explain a decrease in its numbers. Abundance of *Megasphaera elsdenii* tended to be greater for cattle fed the diet without roughage compared to the other treatment; however, *Ruminobacter amylophilus* tended to be greater for steers fed the diet that did receive roughage compared to the one that did not. This was explained in which *R. amylophilus* utilizes starch as an energy source, and the abundance of starch provided by the higher level of barley grain in the diet with roughage would lead to its increase in abundance. The authors concluded that removing roughage from the diet reduced cellulolytic bacterial species, as would be expected.

Available data that investigates the rumen microbiome of feedlot cattle that utilizes next generation sequencing technology for analysis seem to be heavily skewed towards research on adaptation-type diets in which cattle go from high-forage diets to high grain diets, or microorganisms associated with residual feed intake. Data on feedlot cattle is lacking in terms of

other aspects of production and how feedlot diets can influence the rumen bacterial community. If the rumen microbiome can be influenced by diet in a positive way, it may influence how we feed cattle in the future. Grain processing, byproduct feeds, feed additives, age of animal and pathogens associated with liver abscesses could be some areas of interest that would benefit with more research.

### **Ionophores and monensin overview**

Ionophores are compounds that facilitate specific ionic transport across biological membranes by forming lipid soluble, dynamically reversible complexes with cations (Novilla, 2018). In the livestock industry, ionophores play a significant role in improving the health and feed efficiency of animals that consume them (Novilla, 2011). The subclass of ionophores that are routinely used in livestock production are carboxylic ionophores, which has a selective toxicity to protozoan parasites and bacterial species, with margins of safety for the host animal when provided within the approved dosage ranges (Novilla, 2018). Currently there are three carboxylic ionophores available for ruminant animal production, these include: monensin (Rumensin; Elanco Animal Health, Greenfield IN), lasalocid (Bovatec; Zoetis, Parsippany, NJ), and laidlomycin (Cattlyst, Zoetis). Monensin was the first marketed ionophore of industry importance and is the longest-standing, most researched, and most widely used ionophore in beef cattle production (Novilla, 2011). A recent survey of 49 consulting feedlot nutritionists who service over 14,000,000 cattle annually found that 92.3% of nutritionists used ionophores in cattle receiving diets, of these, 77.3% used monensin. In finishing cattle diets, 97.3% of the respondents use ionophores, of these 100% used monensin in the finishing diets (Samuelson et al., 2016). Due to its high volume of data, and economic importance to the beef industry, monensin will be the sole focus of ionophores in this review.



## Monensin mode of action

A class of carboxylic polyether ionophore antibiotics called monensin were refined in the 1970s as an anticoccidial feed additive in poultry diets and were eventually found to have a similar effect on ruminants (Bergen and Bates, 1984). It is a biologically active compound produced by *Streptomyces cinnamonensis*, which when fed to cattle, has the ability to alter rumen fermentation (Boling et al., 1977). It does so through its antimicrobial properties by interfering with the normal transport of ions through the cell membrane, with gram-positive ruminal bacteria being the most sensitive to the presence of monensin (Cheng and Costerton, 1988). This can be explained by changes in pH and the sodium-potassium balance in the cell, leading to cell death from critical disturbances in cellular processes. This mechanism is thought to occur from the monensin molecule binding the sodium cation as a salt, thus losing a proton from the carboxyl group, moving the cation as a complex on the opposite side of the cell membrane. Once this occurs, the sodium cation is released and the carboxylate anion undergoes protonation (Lowicki and Huczynski, 2013). Monensin sensitivity and resistance of the bacteria is thought to be correlated with differences in the cell wall envelope. Selectively eliminating the less-efficient gram-positive species results in the selection of gram-negative bacteria that appear to be resistant to monensin due to the fact that they have more complex cellular wall construction that are not permeable to large antibiotic molecules and the complexes formed by them (Lowicki and Huczynski, 2013). Species like *Bacteroides* spp., *S. ruminantium* and *B. ruminicola* which produce propionate from succinate are more prevalent in the presence of monensin. The ionophore also selects against hydrogen and formate producing species (*R. albus*, *R. flavefaciens*, and *B. fibrosolvans*), thus decreasing the precursors for methane production (Demeyer and Van Nevel, 1988). Removing the selected bacterial species results in higher propionate to acetate

concentrations due to an increase in propionate-producing bacteria. Monensin in the rumen also results in greater protein availability because monensin inhibits proteolysis and deamination. It can also decrease the incidence of ruminal acidosis by maintaining a higher rumen pH from the removal of *streptococci* and *lactobacilli* spp., who are lactic acid producing bacteria (Russell and Houlihan, 2003).

### **Influence of monensin on the rumen microbiome using new technology**

Recently, the development and refinement of genetic based determination of microbial communities have enhanced the ability to determine how monensin alters rumen microbes in vivo. Kim et al. (2014a) used a pyrosequencing approach to investigate how 0 or 33 mg monensin/kg DM feed effected ruminal bacterial communities of cannulated steers consuming a diet of 60% DDGS, 15% corn, 10% corn silage and 15% supplement. Collectively, the most predominant phylum of bacteria present were Firmicutes (50-56% of total sequences), followed by Bacteroidetes (38-42% of total sequences). Proteobacteria (3-4%) and Actinobacteria (2-3%) accounted for the third and fourth largest phyla groups, respectively. Monensin reduced the relative abundance of gram-positive phylum Firmicutes compared to the control diet (49.8% vs 55.4%, respectively) and increased the gram-negative phylum Bacteroidetes compared to the control (41.6% vs 37.7%, respectively). At the genus level, *Succiniclasticum* (gram-negative), *Ruminococcus* (gram-positive), *Sharpea* (gram-positive), *Oscillibacter* (gram-negative), were at least two fold less abundant for cattle consuming monensin compared to the control. For bacterial diversity indices, monensin decreased the number of observed OTUs (733 vs 857, respectively) and Shannon index (6.92 vs 7.21, respectively) compared to the control, suggesting that feeding monensin to cattle can inhibit some rumen bacteria.

A different paper by Kim et al. (2014b) examined the effect of monensin alone or in combination with supplemental fat on ruminal bacterial communities of dairy cows. Three dietary treatments included a control diet of ground corn and alfalfa hay, the control diet plus 12g/909 kg DM monensin, and the control diet containing 12g/909 kg DM monensin and 4% added fat from a combination of distillers grains with solubles, roasted soybeans, and an animal-vegetable fat blend. Rumen samples were collected after a 3-week diet adaptation period and analyzed using 454 pyrosequencing analysis of 16S rRNA gene amplicons to determine bacterial communities. In total, Firmicutes (58%) and Bacteroidetes (32%) were the top 2 most abundant phyla, whereas TM7 (2%) and Actinobacteria (1%) followed as third and fourth most abundant, respectively. Including monensin in the diet slightly reduced the proportion of Firmicutes (gram-positive; approximately 51% vs 55%), and slightly increased the proportion of Bacteroidetes (gram-negative; approximately 40% vs 33%) compared to the control diet. Monensin reduced the abundance of gram-positive Actinobacteria and TM7; however, due to an overall low relative abundance, these 2 phyla may not play a significant role in rumen fermentation. At the genus level, monensin increased the relative abundance of the most predominant genus, Gram-negative *Prevotella* (19.0 vs 13.0%; monensin vs control treatment, respectively). Interestingly, of the top 9 genera, the proportion of Gram-negative *Syntrophococcus* decreased (0.2% vs 0.7%; monensin vs no monensin treatment, respectively) and the proportion of Gram-positive *Oribacterium* increased (0.7% vs 0.2%; monensin vs no monensin, respectively). This experiment did not observe large rumen microbial shifts from gram-positive bacteria to gram-negative bacteria in the presence of monensin as expected based on older research. Rather, the inhibition of gram-positive bacteria was minute and the authors hypothesized that factors other than the cell membrane

structure may influence the response to monensin, including abilities to resist adaptation and chemico-physical features of feed particles.

An experiment by Melchior et al. (2018) also investigated the effect of a dietary inclusion of monensin against a control on rumen microbial communities and methane production. Heifers were fed a diet consisting of 80% ground corn stalks, 10% corn silage, 7% wet distillers grains with solubles, and a vitamin and mineral supplement with or without 150 mg/day monensin. Diets were fed for approximately 53 days and rumen samples were obtained using a gastric tube on d 0, 18, and 53. Following collections, DNA from the samples were isolated using next-generation sequencing of the V1-V3 hypervariable region of the 16S rRNA bacterial gene to determine microbial communities. A subset of animals were placed in metabolism stanchions prior to each rumen collection period to determine gas exchange and nutrient balance. Gas samples that were obtained were analyzed for CO<sub>2</sub> and CH<sub>4</sub> production. In contrast with the previous paper, no differences were observed between treatments for total gram-positive or gram-negative relative abundance throughout the experiment. Likewise, the ratio of Firmicutes-to-Bacteroidetes did not differ between treatments on each of the sampling times. Monensin resulted in a reduction in phylum SR1 and several OTUs including: *Anaerofustis* (gram-positive), *Shuttleworthia* (gram-positive), Order Bacteroidales (gram-negative) and others totaling 14 unique bacterial species (9 on d 18 and 5 on d 53). Additionally, treatment had no effect on methane and carbon dioxide gas emissions or relative abundance of ruminal methanogenic archaea. It was concluded that monensin fed to heifers in a drylot did not contribute to overall shifts in Gram-positive and Gram-negative bacterial communities and may not be effective in reducing long-term methane production. Monensin did; however, result in finer microbial shifts which primarily occurred early in the experiment. Because of this, it was

stated that there may be a benefit from short periods of monensin supplementation, and a better understanding of the specific microbes that were altered is needed.

A more in-depth trial by Ogunade et al. (2018) used metagenomics and metabolomics analysis on rumen fluid to determine the effects of monensin on the functional attributes and metabolites of the rumen microbiota. Rumen-fistulated Holstein steers were randomly assigned to two treatments consisting of 0 or 200 mg/steer/d monensin. The treatment period lasted 39 d and steers had ad libitum access to red clover/orchard grass hay and a mineral mix was provided free choice. Treatments were applied by feeding a concentrate supplement of corn gluten meal, soy hull, and cracked corn fed at 4 kg/steer daily. Rumen samples were collected on the last day of the experiment at approximately 3, 6, and 9 h after feeding supplement. Collectively, sequences from these samples contained dominant phylotypes affiliated with Bacteroides ( $39.4 \pm 5.3\%$ ), Firmicutes ( $15.9 \pm 2.0\%$ ), Proteobacteria ( $1.9 \pm 0.9\%$ ), Actinobacteria ( $0.6 \pm 0.08\%$ ), and Euryarchaeota ( $1.0 \pm 0.9\%$ ). Statistically, no treatment effects were found at the phylum level; however, it was noted that the relative abundance of Gram-negative Bacteroides were numerically greater for steers consuming monensin ( $43.5 \pm 3.5\%$  vs  $35.3 \pm 3.1\%$ ). Using a linear discriminant analysis effect size (LEfSe) analysis, it was found that monensin increase relative abundance of genus *Mitsuokella* (Gram-negative), *Hallella* (Gram-negative), and *Propionispira* (Gram-negative) and reduced abundance of *Streptococcus* (Gram-positive), *Sphaerochaeta* (Gram-negative), *Burkholderia* (Gram-negative), *Lachnoanaerobaculum* (Gram-positive), *Terriglobus* (Gram-negative), *Fusobacterium* (Gram-negative), and *Methanobacterium* (Archaea). Genes obtained from the samples were aligned with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database using the basic local alignment search tool (BLAST) algorithm to obtain functional features of the genes. As determined by using KEGG orthology level 2, the

most predominant genes provided were that of those involved in nutrient metabolism.

Differences in function information between the two metagenomes of each treatment were analyzed using a Mann Whitney test with a significance level of  $P \leq 0.05$ . Based on this analysis, the relative abundance of genes associated with amino acid metabolism, lipid metabolism, and nucleotide metabolism were greater in the metagenome of steers consuming monensin compared to the control. A total of 245 metabolites were identified from the samples. From these, regression modeling was performed using orthogonal partial least squares discriminant analysis (OPLS-DA) to decrease dimension and identify group separation to determine significantly different metabolites. The OPLS-DA confirmed that the rumen metabolome can be altered by dietary monensin, which was validated using a permutation analysis ( $R^2 = 0.99$ ,  $Q^2 = 0.42$ ).

Metabolites were differentially expressed using a t-test and fold change (FC) of the peak intensities. Monensin increased concentrations of 24 total metabolites, including: linoleic acid, L-phenylalanine, hypoxanthine, oxysporidinone, L- $\beta$ -homomethionine, 2-methylgluric acid, isopongaflavone, catechol, pimelic acid, L-Histidine, tri-O-methylgenistein, oxysporidinone, formylindole, indole-3-carboxylic acid, and 8,15-DiHETE. Monensin also decreased proportions of acetate (52.3 vs 60.0 mM) compared to the control. This experiment demonstrates how monensin can change functional and metabolomic attributes of ruminal bacteria in forage-fed cattle. It further confirms that monensin affects rumen bacterial communities and alters ruminal fermentation, in which volatile fatty acid production is shifted in the favor of propionate, amino acid degradation is reduced, and decreasing the biohydrogenation of unsaturated fatty acids in the rumen. Additionally, all of the taxa that were enriched by the addition of monensin were Gram-negative species that play a role in propionate production. The current paper also concluded that both Gram-positive and Gram-negative species are suppressed by the presence of

monensin, which further supports the hypothesis that monensin sensitivity may be attributed to more than the cellular membrane structure of the bacteria as described by Kim et al. (2014b).

### **Monensin effect on grazing cattle performance**

Increased production of propionate and decreased acetate:propionate ratio, as well as increased DM and protein digestibility, gluconeogenesis, and glucose turnover are all benefits to ruminants consuming monensin (McCartor et al., 1979; Schelling, 1984). Because of the alteration to more efficient ruminal bacteria, the use of monensin has consistently shown to increase weight gain of grazing cattle. Multiple experiments by Potter et al. (1976) investigated the effects of 0, 50, 100, 200, 300, and 400 mg monensin/head/day on performance of calves grazing pastures or were fed green chop, both of which contained orchard grass, alfalfa, brome grass, and ladino clover. Monensin treatments from 100 to 300 mg/head/d improved live weight gain compared to other treatments. The 200 mg/head/d treatment had the apparent and calculated optimal gain response, in which ADG was improved by 18%. Similarly, a grazing trial by Oliver (1975) supplemented steers grazing coastal bermudagrass (*Cynodon dactylon*) with no corn, or 0.91 kg/head/d ground, pelleted corn containing 0, 25, 50, 100, or 200 mg monensin/head/d. The steers consuming monensin, regardless of level, had greater BW gain compared to those consuming the corn only supplement (0.73 vs 0.56 kg/d). Each monensin treatment differed from one another for BW gain, in which cattle consuming 100 mg/head/d monensin had the greatest BW gain (0.78 kg/d), followed by the 50 mg monensin treatment (0.73 kg/d), the 200 mg monensin treatment (0.71 kg/d), the 25 mg monensin treatment (0.70 kg/d), the corn only treatment (0.57 kg/d) and lastly, the forage only treatment (0.46 kg/d). Rouquette et al. (1980) also grazed steers on bermudagrass, with treatments being bermudagrass only, bermudagrass plus a 14% protein supplement fed at 0.91 kg/head/d, or bermudagrass plus 14% protein

supplement containing 200 mg monensin/head/d. Steers consuming the supplement containing monensin had a 45% improvement in gain compared with negative controls (0.45, 0.47, and 0.68 kg/d for negative controls, supplement, and supplement + monensin treatments, respectively).

An experiment by Males et al. (1979) found that supplementing steers grazing mature tall fescue (*Festuca arundinacea*) with 200 mg/head/d fed in a dry grain supplement gained faster than steers that received no monensin (0.64 vs 0.57 kg/d). Two performance experiments by Horn et al. (1981) used heifers grazing wheat pasture in a stocker program and received no supplement or were fed 0.91 kg/head daily of a pelleted supplement containing 0 or 100 mg of monensin. In Exp. 1, heifers receiving supplement had 0.09 kg greater BW gain compared to those who did not receive supplement and heifers receiving monensin had an additional 0.09 kg improvement in BW gain than those that received supplement without monensin. In Exp. 2, heifers consuming supplement containing monensin had greater BW gain compared to heifers not receiving supplement or supplement without monensin (ADG = 0.63 vs 0.55 and 0.56 kg/d for heifers consuming supplement with monensin vs no supplement and supplement without monensin, respectively). Vendramini et al. (2015) investigated the effects of two stocking rates (1.2 and 1.7 animal units/ha) and two monensin treatments (0 or 200 mg/d) on gain of heifers grazing bahiagrass (*Paspalum notatum*). Monensin had no effect on performance of heifers grazing warm season forage with limited supplementation.

Beck et al. (2014) investigated the effects of dried distillers grains plus solubles (DDGS) supplementation regime with or without monensin on performance of calves grazing warm season forages (primarily bermudagrass and crabgrass) or nontoxic endophyte-infected tall fescue. Treatments included no supplemental feed, 1.2 kg/head DDGS offered daily, 1.2 kg/head DDGS and 160 mg of monensin offered daily, 2.04 kg/head DDGS offered on alternate days,



and 2.04 kg/head DDGS and 320 mg of monensin offered on alternate days. Supplementation increased ADG by an average of 0.21 kg/d; however, the addition of monensin or timing of supplementation had no effect on performance of grazing calves. Fieser et al. (2007) conducted a 2-yr experiment to investigate different supplementation strategies and delivery methods of monensin on performance of steers grazing winter wheat pasture. In this study, treatments included a control with no supplementation, a free-choice nonmedicated mineral, a free-choice mineral containing 1,785 mg monensin/kg of mineral, a free-choice mineral containing 1,785 mg monensin/kg of mineral plus soybean hulls, and a soybean hull based energy supplement containing 165 mg of monensin/kg of supplement. The energy supplements were fed at a rate of 1.81 kg/steer on alternating days to achieve an average daily intake of 0.91 kg/steer. The addition of monensin increased daily BW gain during Yr 1 compared to the unmedicated mineral treatment; however, no differences were observed in Yr 2. Due to no differences in daily gain between the different delivery methods, it was also concluded that delivery method for energy, monensin, and mineral supplementation is not of importance for calves grazing winter wheat.

Including monensin in a supplement to grazing calves appears to have a variable effect on supplemental efficiency, or the added BW gain per weight of supplement fed. Beck et al. (2014) did not observe any differences in supplemental efficiency when monensin was included in a DDGS supplement fed to calves grazing multiple different forages. Horn et al. (1981) found that including monensin in a grain-based supplement increased daily gains per supplement fed during one experiment, but did not during a second experiment. Similarly, Fieser et al. (2007) reported that providing monensin in a mineral or soybean hull based supplement improved supplement conversion compared to a mineral without monensin to calves grazing wheat pasture; however, these results varied by year. These differences in the response of monensin provided in a

supplement to grazing calves may be explained by Horn et al. (2005), in which supplemental conversion of calves grazing wheat pasture and supplemented with monensin in a grain-based supplement was improved in four out of five trials. It was reported that the gain response may be directly related to variation in supplement intake. Cattle with lesser variability in supplement intake tended to consume the entire daily offered amount and had a better gain response than that of cattle with greater variation of intake. It was suggested that when providing monensin in a supplement to grazing calves, those who consume greater than 150 mg/d of monensin had greater weight gain; therefore, supplements containing monensin should be formulated to achieve desired mean herd intakes and minimize variability of supplement intake (Horn et al., 2005).

### **Monensin effect on finishing cattle performance**

The use of monensin fed to feedlot cattle has also been highly researched. Multiple experiments conducted by Raun et al. (1976) evaluated the effects of monensin level on performance and volatile fatty acids of finishing steers. In Exp. 1, steers were fed a 70% whole corn finishing diet containing 0, 100, 500, and 750 mg/day monensin top dressed on the ration. All cattle that received a monensin treatment gained equal or better than the cattle that did not. The treatment receiving 100 mg/d monensin had the largest gain response, where the control (0 mg/d) had the least. In Exp. 2 and 3 by Raun et al. (1976) finishing heifers and steers were used to determine the dose response of 0, 2.7, 5.5, 11, 22, 33, 44, and 88 ppm monensin on performance and volatile fatty acids. In Exp. 2, all monensin treatments had equal or greater gain response to the control except the 88 ppm treatment; therefore, it was dropped and not included in Exp. 3. Data between Exp. 2 and 3 were pooled, no experiment by monensin interactions were observed in the analysis of variance. Cattle consuming monensin treatments greater than 22 ppm

monensin had decreased feed intake than those that were on treatments less than 22 ppm monensin. The treatment containing 5.5 ppm or greater monensin had improved feed efficiency compared to the 0 and 2.7 ppm monensin treatments. Cattle receiving the 2.7 ppm treatment had decrease acetate and increased propionate and treatments of 33 ppm or greater monensin had increased proportions of propionate than other treatments. This paper was one of the first to determine the appropriate levels of monensin to feed to finishing cattle. It was concluded that 11 ppm (mg/kg) yielded the largest improvement in gain (5.2%), whereas 33 ppm (mg/kg) resulted in the greatest improvement in feed efficiency (17%). It was stated that these responses were, in part, due to the effect monensin had on ruminal fermentation and the resulting end products.

Another early paper by Boling et al. (1977) investigated the effects of monensin fed to finishing cattle on performance, VFA, and carcass traits. Steers were fed *ad libitum* corn silage and 4.54 kg of a ground corn supplement that provided 0, 100, 200, or 300 mg monensin/steer daily. Rumen fluid was obtained from a subset of animals on d 56 and cattle were fed for 157 d until harvest. Cattle consuming monensin had improved feed efficiency compared to the control treatment. No other differences were observed for performance. Proportions of acetate and butyrate decreased as monensin level increased, whereas propionate increased with increasing monensin. There were no differences observed for carcass characteristics among the treatment groups. In conclusion, monensin improved feed efficiency of finishing cattle in this experiment, which was most likely due to the shift in VFA production.

In feedlots, metabolic disorders like subacute acidosis can be costly and are often characterized by reduced and erratic feed intake (Fulton et al., 1979). Monensin may be used as a tool to help eliminate intake and intake variation of high concentrate diets as demonstrated by Stock et al. (1995), who evaluated the effects of monensin and monensin/tylosin on intake

variation and digestive deaths of finishing cattle. The experiment uses 2,904 steers fed as pens at 4 commercial feedlots and were fed diets containing approximately 82% grain (flaked corn and corn silage or flaked milo and high-moisture corn), 8% roughage, 4% fat and 6 % supplement. Dietary treatments included: no monensin/tylosin (control), 22 mg/kg of monensin and 11 mg/kg tylosin, or 33 mg/kg monensin and 11 mg/kg tylosin. Cattle consuming monensin/tylosin treatments regardless of monensin level had decreased dry matter intake, increased daily gain, and improved feed efficiency compared to the control. Variation of intake among treatments did not differ. An individual feeding study was also conducted using a Calan gate system. Steers were adapted over a 12 d period to a 100% concentrate diet consisting of primarily dry-rolled corn and fed for 100 d. Treatments for this experiment were the dietary addition of 0 or 27 mg/kg monensin. It was also noted that these steers were not implanted and did not receive tylosin at any time during the trial. Using individual animal intake, dry matter intake variation was decreased from d 8 to 70 by feeding monensin than the control. The magnitude of intake variation was 5-10 times greater for individually fed animal compared to the commercial feedlot pens. It was noted that when individually fed steer intakes were averaged and treated as a pen, the variance of intake was reduced and not different among treatments. It was concluded that monensin can reduce animal intake variation; however, pen means may hide intake variation among individual animals.

More recently, finishing diets have undergone many changes since the introduction of monensin, including the dramatic increase in the use of distillers byproducts. Due to these byproducts having relatively high protein, fat, and possibly S content, interactions with monensin may alter its effectiveness. This was investigated by Depenbusch et al. (2008) who used finishing heifers and a 3 × 2 factorial arrangement of treatments. Factors included diet with treatments

being: a steam-flaked corn based finishing diet with 0 or 25% DMB inclusion of corn wet distillers grains plus solubles (WDGS) replacing a portion of the corn. The second factor was feed additive and treatments were: no additive, 300 mg of monensin, or 300 mg of monensin plus 90 mg of tylosin daily. Monensin did not change live animal performance. Monensin alone decreased *longissimus* muscle (LM) area when compared to the treatment with no feed additives and tended to decrease LM area when compared to the monensin plus tylosin treatment. It was concluded that the addition of monensin or a combination of monensin and tylosin had minimal impacts on animal performance, indicating that they may not be as effective in steam-flaked corn finishing diets with or without 25% WDGS.

Another large change in finishing cattle diets in recent years would be the utilization of a Beta-adrenergic agonist ( $\beta$ AA), which are a class of feed additives which are fed to finishing cattle 20 to 40 d prior to harvest to improve daily gain and carcass weights. Thompson et al. (2016) evaluated the effects of different ionophores and a  $\beta$ AA on animal performance and carcass characteristics. Steers were fed a steam-flaked corn based finishing diet with wet corn gluten feed and feed additive treatments consisted of: a control with no additives, 12.1 mg/kg DM laidlomycin propionate (LP), 12.1 mg/kg DM LP plus the  $\beta$ AA ractopamine hydrochloride (RH) fed at 300mg/animal/d on final 32 d, or 36.4 mg/kg monensin plus 300 mg/animal/d RH on final 32 d. Cattle consuming LP alone had greater final BW and hot carcass weight compared to the control. All treatments containing feed additives had greater daily gain and improved feed efficiency compared to the control. Monensin with RH increased LM area compared to the control. These results suggest that utilizing feed additives may improve performance of cattle and that there does not appear to be a negative effect of combining monensin with a  $\beta$ AA like RH.

## Other dietary factors that alter the rumen microbiome

Fat may be a dietary component that can alter the rumen microbiome, as stated in a paper by Kim et al. (2014b) that was previously described. Briefly, Holstein cows were fed three dietary treatments of a ground corn/alfalfa hay control diet, the control diet plus 12g/909 kg DM monensin, and the control diet containing 12g/909 kg DM monensin and 4% added fat from a combination of distillers grains with solubles, roasted soybeans, and an animal-vegetable fat blend. Rumen samples were analyzed using 454 pyrosequencing analysis of 16S rRNA gene amplicons to determine bacterial communities. When fat was added, the proportion of Firmicutes increased (approximately 69% vs 51%) and Bacteroidetes decreased (approximately 23% vs 40%) when compared to the control plus monensin diet. Of 9 dominant genera, *Olsenella* and *Syntrophococcus* increased by  $\geq 2$ -fold when fat was included in the diet. Additionally, fat decreased Shannon diversity index when compared to the treatments that did not contain fat. In this experiment, dietary fat shifted microbial communities in favor of Gram-positive bacteria and inhibited Gram-negative bacteria. It also decreased alpha diversity, or the variation of species present.

Additional feed additive and growth-promoting technologies may also influence the microbiome. Thomas et al. (2017) investigate the effects of management type on the gut microbes of feedlot cattle. Treatments consisted of commercially raised steers who received a calf hood implant (36 mg zeranol) at 74 d of age, reimplanted (80 mg trenbolone acetate and 16 mg estradiol) during backgrounding at 235 d of age and received a final implant (200 mg of trenbolone acetate and 20 mg estradiol) during the finishing phase at 330 d of age. The commercial treatment were also fed 478 g/ton monensin and 96 g/ton tylosin and received a  $\beta$ AA (RH) during the final 31 d prior to harvest. The second treatment consisted of naturally raised

cattle, which never received a hormonal implant, ionophores,  $\beta$ AA, feed-grade or injectable antibiotics or antimicrobials. All cattle in this experiment were fed a final finishing diet containing 40% wet corn gluten feed, 48% dry-rolled corn, 7% grass hay and 5% vitamin and mineral supplement. Samples of digesta from the rumen, colon and cecum were collected immediately after slaughter and later analyzed using metagenome sequencing. Alpha diversity, or diversity within a community indicated that the natural cattle had a greater Shannon index (4.69 vs 4.28) for rumen samples compared to the commercial cattle, indicating that the natural cattle had a more diversified rumen microbial community. At the phylum level, the most abundant were Bacteroidetes, Firmicutes, and Proteobacteria, respectively. There was no significant differences between the distribution of Bacteroidetes and Firmicutes in the rumen of natural and conventional cattle; however, the Firmicutes to Bacteroidetes ratio was greater for natural cattle compared to conventional ( $0.27 \pm 0.10$  vs  $0.21 \pm 0.06$ ). At the genus level, *Prevotella* was the most dominant genus (39.8%) present in the rumen. Four genera belonging to *Bacteroidetes* – *Spirosoma*, *Dyadobacter*, *Leadbetterella*, and *Zunongwangia* were more abundant in the rumens of natural cattle compared to conventional. Gram-positive *Ruminococcus* and *Fecalibacterium* were reduced in the rumens of conventional steers than in the natural cattle, and partially replaced by Gram-negative members of the *Negativicutes*. Antimicrobial resistant genes in the GI tract of cattle from both treatments were predicted by BLAST searching assembled metagenome contigs against the ResFinder database to determine if antimicrobial technologies increase the risk of antibiotic resistant microorganisms. Antimicrobial resistant genes were present in both naturally and conventionally managed cattle, suggesting that resistance exists within microbial populations rather than induced by treatments. It was concluded that methods for conventionally raising cattle has an expected effect on the rumen

microbial populations, but no correlations between antimicrobial resistant genes in the GI tract microbiome and the administration of antimicrobial feed additives was observed.

### **Contribution of the rumen microbiome to production**

It is apparent many factors can influence changes in the rumen microbiome, but what is not so clear is how these changes can impact the host animal. Increasing knowledge of functional genes and their expression has helped link microbial function with animal productivity (Firkins and Yu, 2015). The rumen microbiome is highly dominated at the phylum level, as Firmicutes and Bacteroidetes account for over 90% of 16S rRNA gene sequences in most reported data sets (Kim et al., 2011; Creevey et al., 2014). It is also highly skewed at lower taxonomic levels, in which *Clostridia* and *Bacteroidia* are most abundant at the class level, *Bacteroidales* and *Clostridiales* are the most abundant at the order level, *Prevotellaceae*, *Lachnospiraceae*, and *Ruminococcaceae* are the most abundant at the family level, and *Prevotella* is predominant at the genus level in most of the studied rumen microbiomes (Firkins and Yu, 2015).

The ability to efficiently digest plant components is an important function of the ruminant animal. Three major cellulose degrading bacteria in the rumen have been identified, which include: *Ruminococcus albus* and *Ruminococcus flavefaciens* from the Gram-positive Firmicutes phylum and *Fibrobacter succinogenes* from the Gram-negative Fibrobacteres phylum (Hespell, 1981). Genome sequencing has revealed that each of these species has evolved a highly efficient and specific mode of plant cell wall destruction that is differential from the other two bacteria. It may imply that these bacterial species play complimentary roles in the degradation of fiber in the rumen (White et al. 2014).

Feed efficiency of cattle can be one of the most important factors that directly affects production and profitability. Paz et al. (2018) characterized the rumen microbiomes of two large



cohorts of cattle (steers and heifers) to identify specific bacterial members associated with feed efficiency traits. From their model for heifers, they determined that OTUs belonging in the families of Ruminococcaceae, Victivallaceae, and an unclassified OTU belonging to the order of Bacteroidales were associated with an increase in average daily feed intake (ADFI); whereas OTUs in the family Prevoellaceae and an unclassified OTU of the order Bacteroidales were associated with decreased ADFI. The model for steers determined that families of Bifidobacteriaceae, Lachnospiraceae, Paraprevotellaceae, and Veillonellaceae were associated with an increase in ADFI, whereas families of Lachnospiraceae, S24-7, Veillonellaceae, and an unclassified OTU of the Bacteroidales order were associated with a decrease in ADFI. Five of the six OTUs in the average daily gain (ADG) model were shared with the ADFI model in heifers, with the addition of the Prevotellaceae family, which was associated with increased ADG. The ADG model for steers only shared two OTUs with the ADFI model and consisted of OTUs including Lachnospiraceae, Prevotellaceae, Ruminococcaceae, S24-7, and Veillonellaceae families positively related to ADG. Families of Erysipelotrichaceae, Lachnospiraceae, Prevotellaceae, and an unclassified OTU from the order Bacteroidales were negatively associated with ADG. Five of the OTUs shared between the ADFI and ADG models for heifers were also found in the feed efficiency (G:F) model. There was an addition of the family Fibrobacteraceae with a negative coefficient for G:F. The G:F model for steers shared four OTUs with the ADFI model and four OTUs with the ADG model, with the addition of Lachnospiraceae, Prevotellaceae, and Spirochaetaceae families which were associated with decreased G:F. This experiment suggests that microbial communities can influence feed efficiency, and that approximately 20% of the variation in feed efficacy traits (ADFI, ADG, G:F) in beef cattle could be explained by the rumen microbiome.

Research by Shabat et al. (2016) suggest that microbe genes and species can be used to predict a cow's feed efficiency phenotype, and that a lower richness, or the count of species, was tightly linked to greater feed efficiency. It was also determined that *Megasphaera elsdenii* and *Coprococcus catus* were independently found to be enriched in the microbiomes of highly efficient animals. They concluded that a small number of species can have a large impact on the productivity of the host animal.

### **Conclusion**

The rumen microbiome is a very complex ecosystem that can influence both the health and production of the animal. A better understanding of this symbiotic relationship could help improve the production efficiency of the beef cattle industry, and contribute towards the goal of meeting the food demands of a growing population (Cammack, 2018). As for studying microbial communities in the ruminant, research is challenging. This review shows that diet type, diet quality, and many other contributing factors can greatly influence the rumen microbiome of cattle, and they represent only the beginning of understanding beef cattle production through microbiome and metagenomics research. With recent advances in technology and techniques, there is still a vast amount of information out there to be discovered in terms of the rumen microbiome and how it can be utilized to produce beef (Myer et al., 2017).

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### CHAPTER III

#### EFFECT OF MONENSIN DOSE IN A SELF-FED MINERAL SUPPLEMENT ON MINERAL INTAKE AND PERFORMANCE OF GROWING STEERS ON FORAGE-BASED DIETS

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## ABSTRACT

Beef cattle are commonly fed ionophores on pasture as they have shown to improve animal growth but often reduces consumption when offered in a free choice mineral supplement, leading to questionable dose levels of monensin for growth promotion. The objective of this research was to determine the effect of monensin (Rumensin, Elanco Animal Health, Greenfield, IN) intake in a free choice loose mineral on performance of grazing beef steers. Treatments consisted of offering ad libitum access to minerals (AMPT-A, ADM Animal Nutrition, Quincy, IL) with 0 g monensin/ton (0), 800 g monensin/ton (800), or 1,600 g monensin/ton (1600) with the hope of providing daily monensin intakes of 0, 100, and 200 mg monensin/steer. Steer calves ( $n = 605$ ,  $BW = 278 \pm 27$  kg) were fed pearl millet (*Pennisetum glaucum*) hay with soybean hull and corn gluten feed supplement (0.5% BW daily [AF basis] Block 1) or grazed fall wheat (*Triticum aestivum*) pasture (Block 2), spring wheat pasture (Block 3), summer bermudagrass (*Cynodon dactylon*; Blocks 4 & 5) or winter wheat with mixed-grass baleage (Block 6). Bodyweights were determined every 28 d and mineral consumption was measured weekly. There were no treatment  $\times$  diet interactions ( $P \geq 0.96$ ), therefore data was pooled for statistical analysis as a RCBD with pasture as the experimental unit using the Mixed procedure of SAS (SAS Inst. Inc. Cary, NC). Calves supplemented with monensin had greater ( $P = 0.01$ ) final BW compared with the 0 treatment. Steers on the 800 and 1600 treatments had linearly greater ADG ( $P = 0.02$ ) and total gain ( $P = 0.02$ ) than 0. Mineral intake decreased linearly ( $P < 0.01$ ) as monensin level increased, providing average monensin doses ranging from 0 to 170 mg/animal daily. Supplementation of monensin to stocker cattle improved growth performance compared to an unmedicated control. Adequate monensin for improved growth performance appeared to be provided by a dose of at least 109 mg/animal daily for calves grazing a broad range of forages.

**Key words:** Monensin, grazing steers, stocker cattle

## INTRODUCTION

The stocker cattle industry in the Southeast United States consists of purchasing calves in order to increase value by improving health and appearance, commingling calves into marketable groups, and to add BW using forage-based diets (Rhinehart and Poore, 2013). Utilizing pastures and forages has always been an important component to the stocker system, and producers can maximize the potential of their operation by putting weight on calves as efficiently and economically as possible (Rhinehart and Poore, 2013). Utilizing ionophores such as monensin have shown to be an inexpensive measure to increase weight gain of forage-fed growing calves compared to unmedicated supplements (Potter et al., 1976; Oliver, 1975; Boling et al., 1977; Males et al., 1979; Beck et al., 2016) or minerals (Fieser et al., 2007; Beck et al., 2014). The improved performance of calves consuming ionophores is largely attributed to changes in ruminal fermentation, in which an increase the production of propionate and decrease the acetate:propionate ratio, as well as increasing protein digestibility and gluconeogenesis are all benefits to ruminants consuming monensin (Fuller and Johnson, 1981; Schelling, 1984). Traditionally, 200 mg/head/d appears to be the level of monensin most widely used in supplementation research, as it has consistently shown to improve gain of forage-fed cattle (Males et al., 1979; Rouquette et al., 1980; Potter et al., 1986). A smaller number of studies suggest that a lower level of monensin (50-100 mg/d) may have performance benefit compared to higher level when provided to grazing cattle (Oliver, 1975; Boling et al., 1977). Monensin provided in free-choice mineral supplements has been proven to be effective in improving animal performance (Fieser et al., 2007; Beck et al., 2014); however, mineral consumption may

be reduced and does not always provide the full recommended dose (Horn et al., 2005; Beck et al., 2014).

The objectives of this experiment were to determine the effects of monensin supplementation on growth performance and mineral intake of forage-fed beef steers and if providing monensin impacts weight gain by reducing dose from low mineral intake. Our hypothesis was that supplementing grazing steers with monensin in the form of a loose mineral will improve growth performance and decrease mineral intake compared to a control.

## **MATERIALS AND METHODS**

All procedures in the following experiment conducted at the University of Arkansas Division of Agriculture Livestock & Forestry Research Station near Batesville, AR (35°50'N, 91°48'W; elevation 150 m) were approved by the University of Arkansas Institutional Animal Care and Use Committee (Protocol #17018).

### **Treatments and Cattle Blocks**

This research was replicated over a 14 mo period using multiple groups of cattle and multiple forage types at the University of Arkansas Livestock and Forestry Research Station located near Batesville, Arkansas. The area consisted of peridge silt loam soil, which is deep, well drained and of moderate fertility. Treatments consisted of offering calves free choice access to nonmedicated mineral (**0**; AMPT-A; ADM Animal Nutrition, Quincy, IL), or supplemented with monensin via mineral containing 800g/ton monensin (**800**; AMPT-A MON 800; ADM Animal Nutrition) or 1,600g/ton monensin (**1600**; AMPT-A RU; ADM Animal Nutrition). Target daily mineral intake was 113 g/steer in order to provide monensin intakes of 0, 100, and 200 mg monensin/steer/day.

**Block 1.** On October 10 and 24, 2016, crossbred beef steers ( $n = 167$ ) of similar age and bodyweight were obtained from the University of Arkansas Livestock and Forestry Research Station herd ( $n = 63$ ) or were supplied by a local cooperator ( $n = 104$ ) and transported to the University of Arkansas Livestock and Forestry Research Station in Batesville, AR. On December 7, 2016, approximately one-half of these calves ( $n = 84$ ,  $BW = 302 \pm 25$  kg) were allocated to 1 of 12, 0.45 ha pastures (7 calves/pasture) with little residual forage mass and were allowed ad libitum access to large round bales of pearl millet hay (*Pennisetum glaucum*, Table 1) fed in ring-style feeders and replaced as needed. Additionally, calves were supplemented each day with a soybean hull/corn gluten feed blend fed at 0.5% of BW (as-fed basis).

**Block 2.** The remaining steers ( $n = 83$ ,  $BW = 260 \pm 28$  kg) not used in block 1 were allowed to graze wheat forage (*Triticum aestivum*, Table 2) in 1 of 24 (3 or 4 calves/pasture) 1.6 ha dedicated wheat fields from December 7, 2016 to February 17, 2017 or March 1, 2017. Wheat pastures were established using similar methods described by Beck et al. (2019). Briefly, the fields were prepared using one of two methods. Approximately one-half of the fields were prepared using a conventional tilled method, in which fields were chisel plowed twice then disked twice with an offset disk and finished by disking an additional 2 times prior to planting to achieve a residual cover of <5% before planting. The remaining fields were established via a no-till method, in which they were subjected to a summer chemical fallow by applying 4.68 L/ha of glyphosate [N-(phosphonomethyl) glycine; Roundup Original Max, Monsanto Co., St. Louis, MO] twice during the summer prior to planting to prevent residual plant growth. Wheat seed was drilled into the previous crop residue. All pastures were planted at a rate of 101 kg/ha using a grain drill in 17.8 cm rows at a depth of approximately 2.5 cm on August 15, September 1, and

September 15, 2016. Each pasture was fertilized in September with 68 kg of N/ha as ammonium nitrate.

**Block 3.** A set of preconditioned crossbred steer ( $n = 240$ ,  $284 \pm 24$  kg) arrived from a local cattle supplier on February 22, 2017 and were placed on 1 of 30 (8 steers/pasture) 1.6 ha wheat pastures on March 3, 2017 to graze out spring wheat (Table 2) from the previous block. Pastures were established as described above and were fertilized with an additional 68 kg of N/ha as ammonium nitrate in February before the initiation of spring grazing. Calves were removed on April 6, April 21, and April 27, 2017 once forage matured and became limiting to animal performance, with TDN < 70% (NRC, 1996).

**Blocks 4 and 5.** On May 24 2017, crossbred steers ( $n = 126$ ) were obtained from the Livestock and Forestry Research Station herd (Block 4,  $n = 70$ ,  $237 \pm 25$  kg) or a local supplier (Block 5,  $n = 56$ ,  $335 \pm 20$  kg) and placed on 18 1.6 ha (7 steers/pasture) bermudagrass pastures (*Cynodon dactylon*, Table 2). Pastures were pre-established and fertilized on May 25, 2017 with 336 kg/ha of 19-19-19 to provide 64 kg of N, P, and K per ha. An additional 135 kg of urea/ha was applied on July 6, 2017 to provide 62 kg/ha of N. Cattle remained on pasture until August 17, 2017 for block 5 and September 14, 2017 for block 4.

**Block 6.** Steer calves ( $n = 72$ , BW =  $251 \pm 38$  kg) from the University herd were placed on 18 1.6 ha (4 steers/pasture) wheat pastures (Table 2.) on November 29, 2017 and grazed until February 14, 2018. Pastures were established using similar methods described for block 2 and were planted between September 1 and September 11, 2017. Due to poor forage availability, calves were also supplied with mixed grass baleage (Table 1) to extend the grazing period. Baleage was cut and wrapped at approximately 65% moisture using an inline bale wrapper. Baleage was offered ad libitum in ring style hay feeders and replaced as needed.

## **Cattle Management**

All steers in this experiment were implanted with 40 mg trenbolone acetate, 8 mg estradiol, 20 mg of tylosin (Component TE-G with Tylan, Elanco Animal Health, Greenfield, IN) on the day of placement onto pasture. Cattle were individually weighed, unshrunk, for two consecutive days at the initiation and termination of each experimental block. Additionally, steers were weighed full on 28 d intervals. Once in pasture, cattle had free choice allowance to well-sourced drinking water accessed in an automatic-fill, freeze-proof water tanks (Mirafount 3390; MIRACO Livestock Water Systems, Grinnell, IA). Mineral was offered in covered feeders (Ground Mineral Feeders, Sioux Steel Co., Sioux Falls, SD). Each mineral contained 14-16% Ca from  $\text{CaCO}_3$  and  $\text{CaHPO}_4$ , 4% P from  $\text{CaHPO}_4$ , 19-23% NaCl from salt, and 3% Mg from  $\text{MgCO}_3$ . They also contained vitamins (440,925 IU of Vitamin A/kg, 11,023 IU of Vitamin D<sub>3</sub>/kg; and 220 IU of Vitamin E/kg) and trace minerals (150 mg/kg of Co from  $\text{CoCO}_3$ , 1,200 mg/kg of Cu from  $\text{CuCl}_2$ , 200 mg/kg of I from ethylenediamine dihydriodide, 3,600 mg/kg of Mn from MnO, 25 mg/kg of Se from  $\text{Na}_2\text{SeO}_3$ , and 4,200 mg/kg of Zn from ZnO). Mineral was weighed each week by removing any remaining mineral from the feeder and placing into a container on a zeroed portable scale to determine weekly mineral intake. After it was weighed, the remaining mineral was returned to the feeder and the amount that was consumed was replenished.

## **Forage Sampling and Analysis**

Forage samples were collected at the beginning and end of each 28-d period. Forage mass was determined using a calibrated rising plate meter with 20 sampling points per pasture (Michell and Large, 1983). Calibration forage samples were obtained at each sampling by clipping all forage within a single 0.1m<sup>2</sup> frame in each pasture to a 2.5-cm stubble height.

Clipped calibration samples were dried to a constant weight at 50°C under forced air. These weights were used to relate forage mass (kg of DM/ha) to plate height using linear regression for forage mass prediction.

During the same time of forage mass sampling, additional samples within each pasture were collected to represent diets consumed by grazing calves. Multiple grab samples were obtained from all pastures to mimic forage selected by grazing steers (Gregorini et al., 2006). Samples were dried under forced air at 50°C, and then ground to pass a 2-mm screen (Thomas A. Wiley Laboratory Mill, Model 4, Thomas Scientific, Swedesboro, NJ) and analyzed for nutrient composition using near-infrared reflectance spectroscopy (Feed and Forage Analyzer model 6500, FOSS North America, Eden Prairie, NM). Total digestible nutrient (TDN) content was calculated based on species specific equations (wheat TDN, % =  $73.5 + 0.62 \times \% \text{CP} - 0.71 \times \% \text{ADF}$ ; bermudagrass TDN, % =  $111.8 + 0.95 \times \% \text{CP} - 0.36 \times \% \text{ADF} - 0.70 \times \% \text{NDF}$ ) that were developed using Arkansas forages (Davis et al., 2002).

### **Statistical Analysis**

Forage DM mass and nutritive quality data were analyzed as a repeated measures using the Mixed procedure of SAS (SAS Institute, Cary, NC). The model included treatment, sampling month, and the treatment  $\times$  month interaction. The repeated measure was month within each cattle block and the subject was pasture. Least square means were separated using the predicted differences option in SAS. There were no treatment  $\times$  forage type interactions ( $P \geq 0.96$ ) for performance data, therefore data was pooled for statistical analysis. Performance data including initial BW, final BW, total weight gain and ADG for the stocker phase were analyzed as a randomized complete block design using the Mixed procedure of SAS. Pasture was included as the experimental unit and block was included as the random effect. Contrasts used to separate



means were: (1) 0 vs monensin and (2) 800 vs 1600. Mineral intake and monensin consumption were analyzed as a randomized complete block design using the Mixed procedure of SAS (SAS Institute), as repeated measures. For the repeated measures analysis, the model included treatment, time, and the treatment  $\times$  time interaction. Compound symmetry was used as the covariance structure. Contrasts that were used to separate means include: (1) 0 versus 800 and 1600 and (2) 800 vs 1600. Due to a treatment  $\times$  time interaction ( $P < 0.01$ ) for mineral intake, treatment least square means were separated within time to using the predicted differences option of SAS. Significance was declared with  $P \leq 0.05$  and tendencies declared at  $P > 0.05$  and  $P \leq 0.10$ .

## **RESULTS AND DISCUSSION**

### **Forage Mass and Nutritive Content**

The mean CP (% DM basis), NDF (% DM basis), ADF (% DM basis), TDN (% DM basis), forage mass (kg of DM/ha) and forage allowance (kg of forage DM/kg of steer BW) are presented by month in Table 2. No differences among treatments were observed for CP (treatment  $P = 0.44$ ; treatment  $\times$  month  $P = 0.62$ ), NDF (treatment  $P = 0.56$ ; treatment  $\times$  month  $P = 0.57$ ), ADF (treatment  $P = 0.58$ ; treatment  $\times$  month  $P = 0.44$ ), or TDN (treatment  $P = 0.53$ ; treatment  $\times$  month  $P = 0.92$ ). In addition, no differences were observed among treatments for forage mass (treatment  $P = 0.46$ ; treatment  $\times$  month  $P = 0.83$ ) or forage allowance (treatment  $P = 0.22$ ; treatment  $\times$  month  $P = 0.75$ ) throughout the duration of each grazing period.

### **Animal Performance**

The effect of monensin level supplemented to grazing steers is presented in Table 3. The final BW of steers fed the monensin treatments did not differ ( $P = 0.59$ ) but were greater ( $P < 0.01$ ) than that of the control treatment. This response can be explained by differences in growth

rate among treatments, in which cattle consuming monensin gained 0.09 kg/d more ( $P < 0.01$ ) and gained a total of 7 kg more ( $P < 0.01$ ) during the grazing period compared to cattle that did not receive monensin. This gain response agrees with that of Horn et al. (1981), who reported that heifers who received 100 mg of monensin on wheat pasture also had a 0.09 kg improvement of daily gain compared to heifers who received an unmedicated supplement. It also agrees with that of Oliver (1975) who found that supplementing calves grazing bermudagrass with monensin levels ranging from 25 to 200 mg/head/d improved BW gain compared to a treatment without monensin. Likewise, Males et al. (1979) found that supplementing steers grazing mature tall fescue (*Festuca arundinacea*) with 200 mg/head/d fed in a dry grain supplement gained faster than steers that received no monensin. It appears that monensin can be effective at improving weight gain of calves consuming a wide range of forages. In the current experiment, monensin improved daily gain by 6.0% for calves consuming the hay and corn gluten/soybean hull supplement, 8.6% for calves grazing wheat pasture, and 9.6% for calves grazing bermudagrass compared to calves that did not have access to monensin. Additionally, monensin delivered in a free choice mineral may be effective at improving gain of grazing calves when compared to supplying unmedicated mineral. Fieser et al. (2007) reported that providing monensin in a free choice mineral improved ADG by 31% in yr 1 and 5.5% in yr 2 for calves grazing wheat pasture compared to a treatment consuming an unmedicated mineral. Similarly, Beck et al. (2014) found that supplementing monensin in a mineral increased ADG of calves on wheat pasture by 6.1% compared to a mineral without monensin.

No differences were observed for ADG ( $P = 0.96$ ) or total BW gain ( $P = 0.90$ ) between the two treatments containing monensin (target intakes of 100 and 200 mg). This disagrees with that of Oliver (1975), who found that grazing steers supplemented with 0, 25, 50, 100, or 200 mg

monensin/head/d had the greatest gain response at 100 mg/head/d followed by the 50 mg/head/d, suggesting a lower level may result in better growth performance compared to a higher level of monensin. This contrasts with Potter et al. (1976), who supplemented grazing calves with 0, 50, 100, 200, 300, and 400 mg monensin/head/day and found that the optimal gain response occurred for calves consuming 200 mg/head/d monensin. Based on data from previous research and the current experiment, the effect on monensin level on BW gain can be variable; however, regardless of monensin level, gain responses are likely for grazing calves supplemented with an appropriate amount of monensin compared to cattle that do not consume monensin.

### **Mineral Intake**

Actual monensin intakes based on mineral consumption calculated to be 0, 109, and 170 mg/head/d for the 0, 800, and 1600 treatments, respectively. Daily mineral consumption for the steers were 142, 124, and 96 g/d for the 0, 800, and 1600 treatments, respectively. Including monensin in the mineral decreased ( $P < 0.01$ ) overall consumption compared to the unmedicated mineral. This agrees with Fieser et al. (2007); who reported that including monensin in a mineral mixture decreased intake of the mixture by 63% in year 1 and 55% in year 2. A study by Beck et al. (2014) found that providing monensin in a free choice mineral decreased mineral intake compared to an unmedicated control, and that in general, intake of the unmedicated mineral was in excess of the targeted intake. In the current trial, cattle consuming the higher level of monensin (1600) had decreased ( $P < 0.01$ ) mineral consumption compared to the lower level treatment (800), which has the potential to be an economical advantage to producers, given similar gain responses with less required mineral. Beck et al. (2014) determined that supplementing monensin reduced the total cost per kg of gain compared to an unmedicated

mineral, and that suppling monensin in a free choice mineral reduced total cost per kg of gain compared to suppling monenin in a pressed protein block.

A treatment  $\times$  time interaction ( $P < 0.01$ ) was observed for mineral intake; therefore, treatment mean differences were evaluated within each week (Figure 1). No differences were observed for mineral intake for the first 3 weeks of the experiment. At week 4 and for the remainder of the study, cattle on the 0 treatment had the greatest ( $P < 0.01$ ) mineral consumption, whereas the cattle on the 1600 treatment had the least. The 800 treatment cattle had intermediary intakes between the other two treatments.

### **IMPLICATIONS**

These results indicate that monensin supplementation provided in a mineral can improve final BW and gain of steer calves in a variety of forage systems. Additionally, it confirms that monensin may be used as an intake limiter of free-choice loose mineral provided to calves on pasture and ensures this is not detrimental to response to monensin. It was also concluded that feeding a higher level of monensin did not have any additional benefits compared with the lower level, with the exception of reducing mineral consumption.

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## TABLES AND FIGURES

**Table 1.** Nutrient composition of additional forages provided to grazing calves.<sup>1</sup>

Item	Hay	Baleage
Dry matter, %	88.4	36.9
Crude protein, %	15.7	12.7
NDF, %	64.1	63.9
ADF, %	37.5	42.4

<sup>1</sup>Hay = pearl millet hay fed to block 1; Baleage = mixed-grass baleage fed to steers in block 6.

**Table 2.** Forage mass, forage allowance and nutritive quality of pastures grazed by steers.

Item	Sampling Time <sup>1</sup>				
	1	2	3	4	5
<b>Block 2 (Winter Wheat)</b>					
Forage Mass, kg of DM/ha	1,792	1,570	1,169	-	-
Forage Allowance, kg DM/kg BW	3.13	2.14	1.46	-	-
Crude Protein, %	27.3	22.1	24.3	-	-
NDF, %	38.2	49.2	49.1	-	-
ADF, %	18.2	25.3	27.0	-	-
TDN, %	82.0	74.2	72.3	-	-
<b>Block 3 (Spring Wheat)</b>					
Forage Mass, kg of DM/ha	1,462	1,832	2,250	-	-
Forage Allowance, kg DM/kg BW	2.33	2.56	3.03	-	-
Crude Protein, %	29.7	23.1	18.7	-	-
NDF, %	39.0	45.4	53.2	-	-
ADF, %	17.8	24.0	31.0	-	-
TDN, %	82.5	75.6	67.8	-	-
<b>Blocks 4 and 5 (Bermudagrass)</b>					
Forage Mass, kg of DM/ha	2,704	3,359	3,740	4,158	4,519
Forage Allowance, kg DM/kg BW	4.47	5.04	5.36	5.47	6.38
Crude Protein, %	20.8	15.2	20.8	16.7	11.0
NDF, %	49.6	61.1	59.3	64.3	70.9
ADF, %	27.5	34.2	29.5	32.1	38.9
TDN, %	71.7	64.2	69.5	66.6	59.0
<b>Block 6 (Winter Wheat)</b>					
Forage Mass, kg of DM/ha	1,179	506	-	-	-
Forage Allowance, kg DM/kg BW	2.14	0.66	-	-	-
Crude Protein, %	30.7	30.6	-	-	-
NDF, %	35.6	43.9	-	-	-
ADF, %	18.1	21.7	-	-	-
TDN, %	82.2	78.1	-	-	-

<sup>1</sup>Includes month in which pastures were sampled for each block. Block 2: November = 1, February = 3; Block 3: February = 1, April = 3; Blocks 4 and 5: May = 1, September = 5; Block 6: November = 1, February = 2.

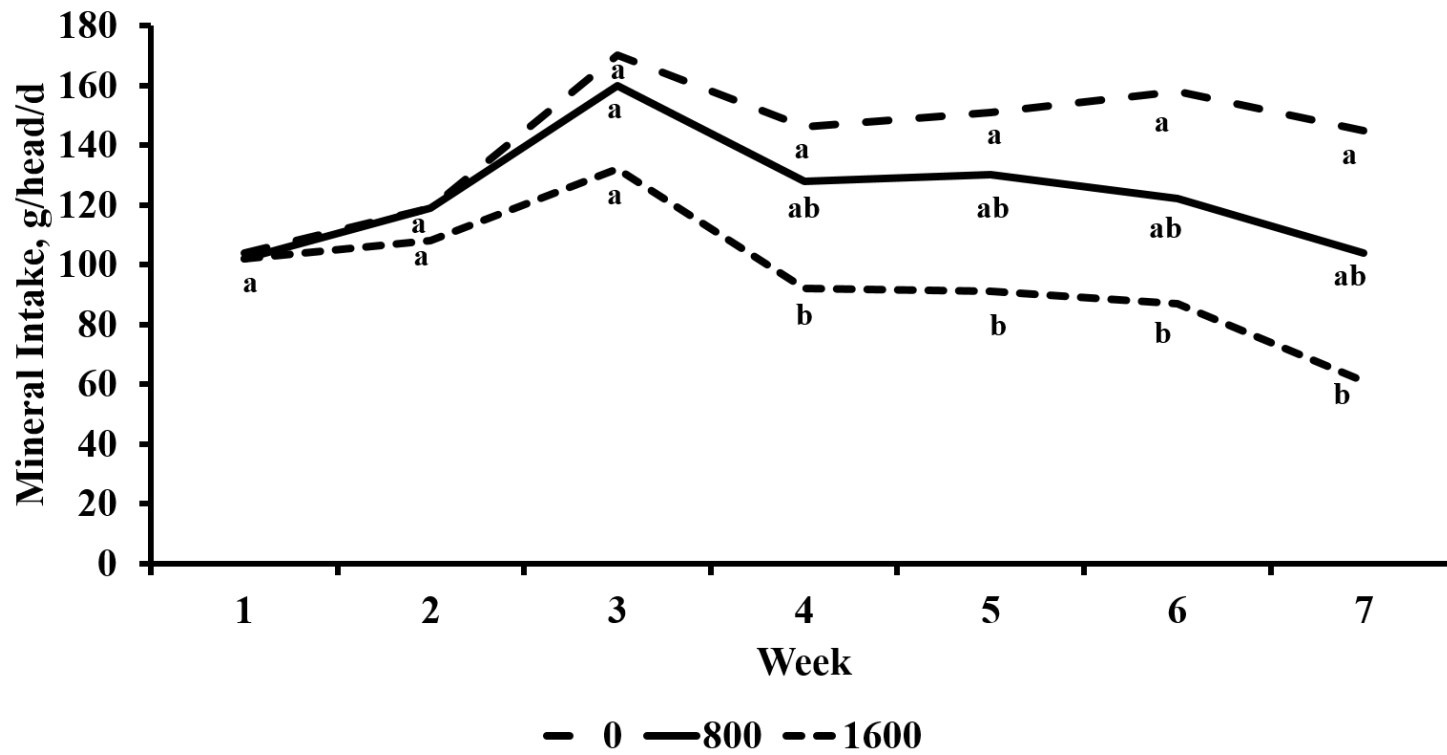


**Table 3.** Effect of monensin supplementation on performance and mineral intake of grazing steers.

Item	Treatment <sup>1</sup>			SEM	Contrast <sup>2</sup>	
	0	800	1600		1	2
Initial BW, kg	278	279	277	14.74	0.98	0.46
Final BW, kg	351	359	357	11.21	<0.01	0.59
ADG, kg/d	0.96	1.05	1.05	0.10	<0.01	0.96
Total gain, kg	73.0	80.4	80.1	8.19	<0.01	0.90
Mineral intake, g/head/d	141.9	123.7	96.4	6.62	<0.01	<0.01

<sup>1</sup>Steers given free choice access to nonmedicated mineral (0; AMPT-A; ADM Animal Nutrition, Quincy, IL), or supplemented with monensin via mineral containing 800g/ton monensin (800; AMPT-A MON 800; ADM Animal Nutrition) or 1,600g/ton monensin (1600; AMPT-A RU; ADM Animal Nutrition).

<sup>2</sup>Treatment least square means were separated using the contrasts: 1 (0 vs 800 and 1600) and 2 (800 vs 1600).



**Figure 1.** Effect of monensin supplementation on free-choice mineral intake of beef steers over time. Treatments without common superscript at each time point differ ( $P \leq 0.05$ ).

## CHAPTER IV

### EFFECT OF MONENSIN INTAKE DURING A STOCKER PHASE AND SUBSEQUENT FINISHING PHASE ON PERFORMANCE AND CARCASS CHARACTERISTICS OF FEEDLOT STEERS

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## ABSTRACT

Three experiments were conducted to determine the effects of monensin supplementation during the stocker and finishing phase on feedlot performance and carcass characteristics of beef steers. In Exp. 1 and 2, steers ( $n = 61$ ,  $BW = 345 \pm 20$  kg and  $n = 70$ ,  $BW = 298 \pm 26$  kg) were fed hay and supplement or grazed wheat pasture (Exp. 1) or bermudagrass (Exp. 2) and then transported 1,068 km to Canyon, TX for finishing. For Exp. 3 steers ( $n = 59$ ,  $BW = 322 \pm 31$  kg) grazed wheat pasture and then transported 636 km to Stillwater, OK for finishing. Treatments were arranged as a  $3 \times 2$  factorial with three monensin levels during the stocker phase (0, 800, 1600 g/ton fed in a free choice mineral) and two levels during finishing (0 [U] or 37.5 mg/kg diet DM [M]). There were no location  $\times$  treatment interactions ( $P \geq 0.19$ ) for performance or carcass measurements, therefore data were pooled across locations. Stocker  $\times$  feedlot treatment interactions were not observed for performance data ( $P \geq 0.50$ ), but were observed for carcass characteristics. Cattle consuming M in the feedlot had decreased ( $P < 0.01$ ) DMI and increased ( $P < 0.01$ ) G:F compared with U. Cattle on 1600U treatment had the greatest LM area whereas 0U had the least ( $103.4$  vs  $96.3$  cm<sup>2</sup>). Steers on the 800U and 1600U treatments had decreased YG and 800M had the greatest ( $2.31$  and  $2.22$  vs  $2.75$ ). Cattle consuming M during finishing had increased ( $P = 0.03$ ) fat thickness compared to U. Supplementation of monensin to stocker cattle may have minimal impact on subsequent feedlot performance. Providing monensin in feedlot diets still decreases DMI and improves feed efficiency of feedlot cattle.

**Key words:** monensin, feedlot, finishing

## INTRODUCTION

In the livestock industry, ionophores play a significant role in improving the health and feed efficiency of animals that consume them (Novilla, 2011). Specifically, monensin is a carboxylic polyether ionophore which has the ability to alter ruminal fermentation through its antimicrobial properties when fed to ruminants (Boling et al., 1977). This modification of ruminal fermentation results in increased production of propionate and decreased acetate:propionate ratio, as well as increased DM and protein digestibility, gluconeogenesis, and glucose turnover in ruminants consuming monensin (McCartor et al., 1979; Schelling, 1984). Feeding monensin has consistently shown to increase weight gain of grazing cattle (Potter et al., 1976; Oliver, 1975; Boling et al., 1977; Males et al., 1979) and improve feed efficiency of feedlot cattle (Raun et al., 1976; Boling et al., 1977; Duffield et al., 2012) compared to unmedicated controls. A meta-analysis by Duffield et al. (2012) of 40 peer-reviewed articles of the impact of monensin on growing and finishing cattle concluded that using monensin results in a 6.4% improvement in feed efficiency; however, it was reported that there were only a 2.5 to 3.5% improvement in trials conducted within the last 2 decades. This suggests that the efficacy of monensin may be declining, which may be related to cattle types and diet components changing. Additionally, current research on monensin is specific to single stages of beef cattle production; therefore, data is limited on whether monensin fed in early phases of production impact performance in later phases.

The objective of this experiment was to determine the effect of dose and monensin consumed during a stocker phase on performance and carcass characteristics of beef steers during a subsequent finishing phase. The hypothesis was that supplementing growing calves with

monensin during a stocker phase would diminish its effects later in production, once cattle were placed in a feedlot.

## MATERIALS AND METHODS

All procedures in the following experiment conducted at the University of Arkansas Division of Agriculture Livestock & Forestry Research Station near Batesville, AR (35°50'N, 91°48'W; elevation 150 m) were approved by the University of Arkansas Institutional Animal Care and Use Committee (Protocol #17018).

### *Cattle Management and Treatments*

Cattle used in this experiment were derived from the same cattle in the experiment described in the previous chapter. A subset of 63 animals from block 1 of the stocker trial (n = 42 from wheat pasture and n = 21 from hay dry lots) were transported 1,068 km to the West Texas A&M Research Feedlot located near Canyon, TX for finishing immediately following the termination of the grazing trial. A second group of steers (n = 70) from block 2 of the stocker phase were also transported to the same Canyon, TX feedlot for finishing once the stocker phase was complete. A third set of steers (n = 60) from block 6 of the grazing trial were transported 636 km to feedlot in Stillwater, OK for finishing.

Treatments for this experiment were arranged as a 3 × 2 factorial with factors consisting of level of monensin provided during the previous stocker phase, and whether or not monensin was provided in the feedlot diet following the stocker phase. Treatments during the stocker phase consisted of offering grazing calves a free choice loose mineral (AMPT-A, ADM Animal Nutrition) with 0 g monensin/ton (**0**), 800 g monensin/ton (**800**), or 1600 g monensin/ton (**1600**) designed to supply a daily dose of 0, 100, or 200 mg of monensin/d. Cattle were placed in feedlot pens by the three previous treatments and two finishing phase treatments in which

monensin was provide at 0 mg/kg diet DM (**U**) or 37.5 mg/kg diet DM (**M**) provided in the diet, for a total of 6 treatments.

**Canyon Location.** Cattle arrived at the Canyon feedlot on March 2, 2017 and September 14, 2017. During initial processing, steers received an implant consisting of 200 mg trenbolone acetate and 40 mg of estradiol (Revalor-XS; Merck Animal Health, Madison, NJ) and ivermectin (Noromectin, Norbrook Laboratories, Newry, UK). Cattle were blocked by pasture-off weights and allocated randomly, within previous stocker treatment to new treatment and pen (12 pens per turn, 5 to 7 animals per pen). Steers were housed in outdoor, uncovered 6.1 × 26.9 m soil surface pens providing at least 23 m<sup>2</sup> of pen space per steer. Feed bunks were observed daily at 0700h before feed delivery. The amount of feed offered was increased 0.31 kg of DM per steer if there was no residual feed remaining at the time of observation for 2 consecutive days. Steers were fed a starter ration for 10 d following arrival. After 10 d, the steers were transitioned by feeding a 50:50 blend of the starter ration and a step-up ration for 2 d, then the step-up ration for 10 d, followed by a 50:50 blend of the step-up and final finishing diet before feeding the finishing diet for the remainder of the experiment (Table 4). Following a confirmed diagnosis of coccidiosis, all pens of cattle from group 1 were administered amprolium (Corid 1.25% Crumbles; Merial LTD., Boehringer Ingelheim, Duluth, GA) by mixing in feed following the manufacturer's label recommendations. Data from two animals were not included in the dataset: an 800U died 16 d after feedlot arrival due to illness unrelated to treatment; and a 1600U animal diet on d 27 from an unknown cause. The steers were shipped for harvest by 2 weight blocks, in which heavy blocks of cattle shipped earlier than light blocks. Cattle at the Canyon feedlot were fed for 132 and 163 d for group 1 and 171 and 199 d for group 2.

***Stillwater Location.*** Steers arrived to the Stillwater feedlot on 15 February 2018 and received a vaccine for bovine respiratory disease (Titanium 5 + PH-M, Elanco Animal Health, Greenfield, IN), a vaccine for clostridial (Vision 7 with spur; Merck Animal Health), a dewormer (fenbendazole, Safe-Guard, Merck Animal Health) and an implant of 80 mg trenbolone acetate and 16 mg estradiol (Revalor-IS; Merck Animal Health) at initial processing. Additionally, cattle in Stillwater were reimplanted on d 85 with 200 mg trenbolone acetate and 20 mg estradiol (Revalor-200; Merck Animal Health). Steers were also blocked by pasture off-weights and randomly allocated within treatment to new treatment and pen (n = 12 pens, 2 per treatment). The cattle were transitioned to a finisher diet by feeding a starter diet for 9 consecutive days, and then then were stepped up every 5 d until the final finisher diet was fed throughout the remainder of the trial (Table 5.). Feed bunks were managed to contain trace amounts of remaining feed prior to feeding. Cattle were housed in partially covered pens. One animal from 0R was removed from the data on d 58 due to an injury. Cattle in Stillwater were fed for 175 d.

In total, steers (n = 190; initial BW =  $321 \pm 26$  kg) were fed for an average of 169 days. A total of 36 feedlot pens (6 per treatment) were used with 5-7 steers per pen. Individual animal weights were determined every 28 d. Finisher diets in this experiment included 10.2 mg/kg of tylosin (Tylan, Elanco Animal Health) and cattle were fed a targeted 250 mg/steer/d of ractopamine hydrochloride (Optaflexx; Elanco Animal Health) approximately 30 d prior to shipping for harvest.

***Carcass data collection.*** Once cattle were visually estimated to have 1.27 cm of backfat, steers from both feedlot locations were transported to a commercial abattoir (Tyson Fresh Meats, Amarillo, TX) for harvest. Carcass data were obtained by trained personnel from the West Texas A&M University Beef Carcass Research Center (Canyon, TX). Hot carcass weight (HCW) was



determined on the day of harvest and *longissimus* muscle (LM) area, subcutaneous fat thickness, percentage of KPH fat, and marbling score were determined after a 48 h period of refrigeration. These measurements were used to calculate USDA yield grade ( $2.50 + (2.5 \times \text{fat thickness}) + (0.2 \times \% \text{ KPH fat}) + (0.0038 \times \text{HCW}) - (0.32 \times \text{LM area})$ ). Dressing percent was calculated by dividing HCW by the final live BW.

**Statistical Analysis.** A preliminary analysis was conducted with a fixed effects model of feedlot location  $\times$  stocker treatment  $\times$  feedlot treatment. Interactions were nonsignificant for performance data ( $P \geq 0.19$ ), and for carcass measurements ( $P \geq 0.26$ ); therefore, data were pooled across locations. Data were analyzed with pen as the experimental unit using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included stocker treatment, feedlot treatment, and the stocker  $\times$  feedlot treatment interaction. Location, block within location, and the location  $\times$  block within location were considered random. Frequency distributions of carcasses that graded USDA quality grade of choice or greater (choice, premium choice, prime) or less (select, standard) were analyzed as binomial proportions using the GLIMMIX procedure of SAS. Mean differences were considered significant at  $P \leq 0.05$  and tendencies at  $P > 0.05$  and  $P \leq 0.10$ .

## RESULTS AND DISCUSSION

There were no stocker  $\times$  feedlot treatment interactions observed ( $P \geq 0.49$ ) for live animal performance; therefore, only main effects of each will be discussed. Data representing the effects of monensin supplementation during a stocker phase and a following finishing phase on feedlot performance is represented in table 3. A main effect of stocker treatment was observed ( $P < 0.01$ ) for initial BW, in which cattle previously on the 800 and 1600 treatments were 12.6 and 9.3 kg heavier ( $P < 0.01$ ), respectively, compared to 0 at the initiation of the finishing phase.

This can be explained by the improvement of growth rate from supplementing monensin that was observed during the stocker phase and discussed in the previous chapter, which resulted in heavier calves being placed into the feedlot. Interestingly, no differences were observed ( $P \geq 0.12$ ) for BW on d 28, 56, 84 or at the conclusion of the feedlot phase, suggesting that cattle not previously on monensin were able to compensate for BW early in the finishing phase.

The response in BW might be explained by ADG, in which a stocker treatment main effect tendency was observed ( $P = 0.09$ ), in which cattle previously on monensin treatments during the stocker phase gained approximately 0.4 kg less ( $P \leq 0.07$ ) during the first 27 d of finishing than cattle not previously supplemented with monensin. A main effect tendency of stocker phase treatment was also observed for ADG from d 56 to 83, in which cattle previously on the 1600 treatment had greater ( $P = 0.03$ ) ADG than that of the 0 treatment. No other differences were observed ( $P \geq 0.24$ ) for ADG or total gain during the finishing period ( $P \geq 0.54$ ) across all treatments.

An effect of feedlot treatment was observed ( $P < 0.01$ ) for DMI throughout the entire experiment, in which cattle on the M treatments consumed 0.6 kg of DM per day less than the U treatments. This agrees with that of Raun et al. (1976), who observed a decrease in DMI for cattle consuming finishing diets containing greater than 22 mg/kg of monensin. Likewise, Stock et al. (1995) reported that including monensin at 22 or 33 mg/kg in finishing diets decreased DMI compared to an unmedicated treatment. Stock et al. (1995) also reported that including monensin in a finishing diet reduced DMI variation of individually fed steers and suggests that monensin may decrease the severity of metabolic disorders by modulating changes in dry matter intake.

The comparable values for gain along with the reduction in intake for cattle consuming M resulted in a 6.9% improvement in feed efficiency for cattle on the M treatments compared to the U treatments (G:F; 0.170 vs 0.159 for M and U, respectively;  $P < 0.01$ ). The results agree with a meta-analysis over a total of 40 peer-reviewed articles and 24 trial reports conducted by Duffield et al. (2012), who reported that monensin, on average, improved feed efficiency of finishing cattle by 6.4%. In the current experiment, the improvements in feed efficiency by including monensin in the finishing diet appear to have occurred after d 28. No difference was observed ( $P = 0.86$ ) between feedlot treatments for the first 27 d, however the inclusion of monensin increased ( $P \leq 0.02$ ) G:F from d 28 through the end of the experiment. Boling et al. (1977) also observed an improved in feed efficiency by feeding monensin to finishing cattle. It was explained by a measured alteration in volatile fatty acid profiles, in which proportions of acetate and butyrate decreased as monensin level increased, whereas propionate increased with increasing monensin. In the current experiment, a stocker treatment main effect tendency was observed ( $P = 0.06$ ) for G:F in the first 27 d, in which cattle previously on the 0 treatment tended to have greater ( $P \leq 0.06$ ) G:F compared to cattle that were on the monensin treatments during the stocker phase, which may have contributed to the compensatory gain that was observed early in the finishing phase.

The results for the effect of monensin during a stocker and feedlot phase on carcass characteristics is presented in table 4. A stocker  $\times$  feedlot treatment interaction was observed ( $P = 0.03$ ) for LM area. Cattle on the 1600U had the greatest LM area compared to the 0U treatment, which had the least (103.4 vs 96.3 cm;  $P < 0.01$ ). The remaining treatments were intermediary, in which 800U, 0M, and 1600M treatments did not differ ( $P \geq 0.12$ ) from the 1600U but were greater ( $P \leq 0.04$ ) than the 0U treatment. The 800M treatment did not differ ( $P \leq$

0.10) from the 0U, 800U, 0M and 1600M treatments but had a decreased ( $P = 0.05$ ) LM area than the 1600U treatment. Depenbusch et al. (2008) reported that including monensin in finishing diets decreased LM area when compared to a treatment with no feed additives; however, in the current study it is difficult to directly relate the difference in LM area with monensin alone. A treatment interaction was also observed ( $P = 0.01$ ) for USDA yield grade. Steers on the 800M had a greater ( $P \leq 0.02$ ) yield grade compared to the 800U, 1600U, 0M, and 1600M treatments, but not different ( $P = 0.16$ ) from the 0U cattle. The 800U and 1600U treatments had decreased ( $P \leq 0.06$ ) yield grade compared to the 0U and 800M treatments, but did not differ ( $P \geq 0.13$ ) from the 0M and 1600M treatments. Limited data suggests that monensin influences yield grade, and in the current experiment it is difficult to interpret that monensin treatment had a direct effect on yield grade, and that other factors including fat and muscle composition may have influenced these results.

A main effect for stocker treatment was observed ( $P = 0.05$ ) for marbling score. Steers previously on the 1600 treatment during the stocker phase had decreased ( $P \leq 0.04$ ) marbling score when compared to the 0 and 800 stocker treatments. Additionally, a feedlot treatment main effect was observed ( $P = 0.03$ ), in which cattle consuming M in the feedlot had greater ( $P = 0.03$ ) fat thickness at harvest compared to the U cattle. It could be that including monensin in the diets altered energy supply during the middle phase of the growth curve, thus altering body composition via altering mature size. This can result in earlier maturing cattle and enhanced fattening at lighter slaughter weights (Owens et al., 1993).

## **CONCLUSION**

Cattle supplemented with monensin during a stocker phase may compensate to similar bodyweights early during finishing; therefore, minimal impacts of monensin supplied early in

production on performance later on is expected. The inclusion of monensin in finishing diets resulted in a reduction in intake and improvement in feed efficiency throughout much of the feedlot phase. Additionally, monensin may alter age of maturity and tissue deposition, resulting in cattle that are ready for market earlier than cattle not consuming monensin in the feedlot.

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## TABLES AND FIGURES

**Table 4.** Ingredient composition of each diet fed at the Canyon location.

Item, % DM	Starter	Step	Finisher
Steam-flaked corn	13.9	27.9	37.3
Corn gluten feed <sup>1</sup>	56.4	48.0	43.5
Corn stalks	19.1	11.5	4.3
Corn oil	-	1.9	3.8
Molasses	7.3	7.3	7.3
Supplement <sup>2</sup>	3.3	3.4	3.8

<sup>1</sup>Cargill Sweet Bran, Dalhart, TX.

<sup>2</sup>Formulated to meet or exceed the vitamin and mineral recommendations from the NRC (2016) and to provide 0 or 37.5 mg/kg or monensin (Rumensin; Elanco Animal Health, Greenfield, IN) and 10 mg/kg of tylosin (Tylan; Elanco Animal Health).

**Table 5.** Ingredient composition of each diet fed at the Stillwater location.

Item, % DM	Starter	Step 1	Step 2	Step 3	Step 4	Finisher
Dry-rolled corn	15.1	22.6	34.8	42.9	50.0	60.7
Corn gluten feed <sup>1</sup>	51.9	41.1	29.0	29.1	29.2	20.9
Prairie hay	28.0	31.1	31.0	22.8	15.6	8.2
Liquid supplement <sup>2</sup>	-	-	-	-	-	5.3
Dry supplement <sup>3</sup>	5.0	5.2	5.2	5.2	5.2	4.9

<sup>1</sup>Cargill Sweet Bran, Dalhart, TX.

<sup>2</sup>Liquid supplement, Westway Feed Products, New Orleans, LA.

<sup>3</sup>Formulated to meet or exceed the vitamin and mineral recommendations from the NRC (2016) and to provide 0 or 37.5 mg/kg or monensin (Rumensin; Elanco Animal Health, Greenfield, IN) and 10 mg/kg of tylosin (Tylan; Elanco Animal Health).

**Table 6.** Nutrient composition of final finisher diets for each treatment at each location.

Item, % DM	Canyon		Stillwater	
	Unmedicated	Monensin	Unmedicated	Monensin
Dry matter, %	71.3	70.6	77.5	77.1
Crude protein, %	14.3	14.5	12.7	13.1
TDN, %	86.7	88.1	84.8	86.9
NEm, Mcal/kg	2.1	2.2	2.1	2.2
NEg, Mcal/kg	1.5	1.5	1.4	1.5
Ca, %	1.00	0.93	0.45	0.43
P, %	0.58	0.58	0.45	0.46

**Table 7.** Effect of monensin during the stocker phase and subsequent feedlot phase on feedlot performance of beef steers.

Item	Stocker <sup>1</sup>					Feedlot <sup>1</sup>			
	0	800	1600	SEM	<i>P</i> -value <sup>2</sup>	Unmed	Mon	SEM	<i>P</i> -value <sup>2</sup>
Initial BW, kg	314	327	324	13.85	<0.01	321	322	13.82	0.78
d 28 BW, kg	370	373	370	16.46	0.79	369	373	16.38	0.31
d 56 BW, kg	435	437	433	15.53	0.64	436	434	15.45	0.54
d 84 BW, kg	504	510	514	26.04	0.12	508	511	25.96	0.46
Final BW, kg	636	644	638	11.50	0.67	638	640	10.97	0.74
ADG, kg/d									
d 0 to 27	1.95	1.57	1.62	0.51	0.09	1.76	1.66	0.51	0.49
d 28 to 55	2.39	2.39	2.34	0.07	0.74	2.35	2.40	0.06	0.47
d 56 to 83	2.70	2.86	3.13	0.53	0.09	2.80	2.99	0.52	0.24
d 0 to end	1.89	1.87	1.86	0.07	0.76	1.86	1.89	0.07	0.58
DMI, kg/d									
d 0 to 27	8.05	8.12	8.21	0.57	0.71	8.36	7.90	0.57	0.01
d 28 to 55	11.19	11.23	11.24	0.47	0.97	11.52	10.92	0.46	<0.01
d 56 to 83	12.29	12.52	12.23	0.44	0.44	12.76	11.92	0.43	<0.01
d 0 to end	11.28	11.41	11.31	0.33	0.80	11.64	11.03	0.32	<0.01
G:F, kg/kg									
d 0 to 27	0.236	0.190	0.198	0.048	0.06	0.207	0.209	0.048	0.86
d 28 to 55	0.215	0.214	0.209	0.013	0.70	0.205	0.221	0.013	0.02
d 56 to 83	0.227	0.234	0.259	0.047	0.08	0.225	0.256	0.047	0.01
d 0 to end	0.167	0.163	0.163	0.007	0.60	0.159	0.170	0.006	<0.01
Total BW gain, kg	322	317	315	11.13	0.64	316	319	10.64	0.67

<sup>1</sup>Steers given free choice access to nonmedicated mineral (**0**; AMPT-A; ADM Animal Nutrition, Quincy, IL), or supplemented with monensin via mineral containing 800g/ton monensin (**800**; AMPT-A MON 800; ADM Animal Nutrition) or 1,600g/ton monensin (**1600**; AMPT-A RU; ADM Animal Nutrition) during the stocker phase and with 0 (**Unmed**) or 37.5 (**Mon**) mg/animal daily of monensin supplied in a TMR during the feedlot phase.

<sup>2</sup>No stocker phase × feedlot phase interactions were observed ( $P \geq 0.15$ ).

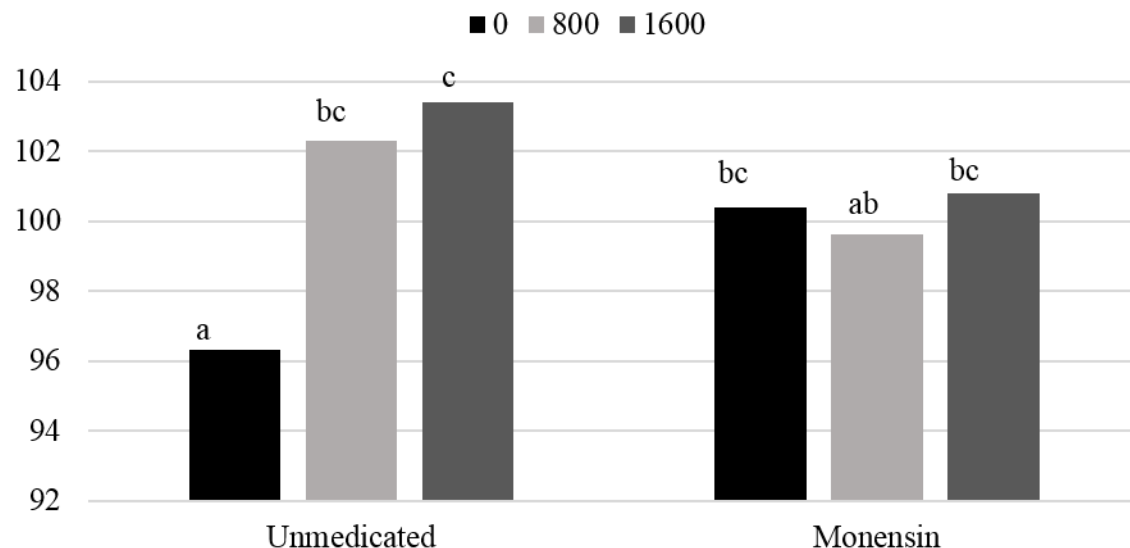


**Table 8.** Effect of monensin during the stocker phase and subsequent feedlot phase on carcass characteristics of steers.

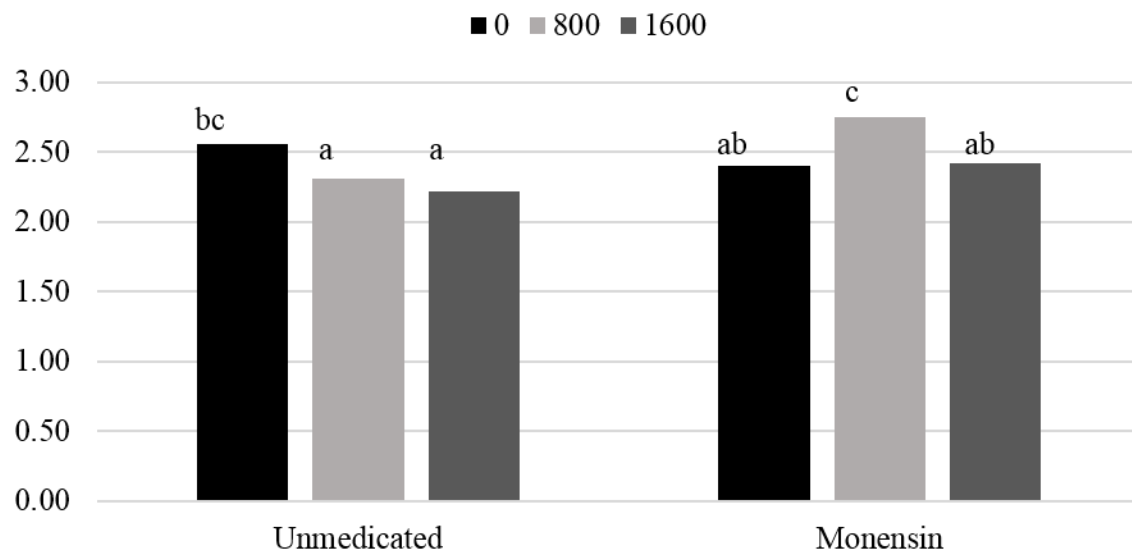
Item	Stocker <sup>1</sup>					Feedlot <sup>1</sup>			
	0	800	1600	SEM	P-value <sup>2</sup>	Unmed	Mon	SEM	P-value <sup>2</sup>
HCW, kg	391	401	393	7.45	0.13	393	396	7.13	0.50
Dressing %	61.26	61.91	61.40	0.34	0.06	61.48	61.57	0.32	0.71
Marbling score	445	447	423	17.46	0.05	434	442	16.99	0.37
Fat thickness, cm	1.21	1.31	1.22	0.05	0.17	1.19	1.30	0.05	0.03

<sup>1</sup>Steers given free choice access to nonmedicated mineral (**0**; AMPT-A; ADM Animal Nutrition, Quincy, IL), or supplemented with monensin via mineral containing 800g/ton monensin (**800**; AMPT-A MON 800; ADM Animal Nutrition) or 1,600g/ton monensin (**1600**; AMPT-A RU; ADM Animal Nutrition) during the stocker phase and with 0 (**Unmed**) or 37.5 (**Mon**) mg/animal daily of monensin supplied in a TMR during the feedlot phase.

<sup>2</sup>No stocker phase × feedlot phase interactions were observed ( $P \geq 0.19$ ).



**Figure 2.** Effect of monensin treatment during a stocker phase and subsequent feedlot phase on ribeye area (cm<sup>2</sup>) of steers. A stocker phase × feedlot phase interaction was observed ( $P = 0.03$ ), bars without a common superscript differ ( $P \leq 0.05$ ).



**Figure 3.** Effect of monensin treatment during a stocker phase and subsequent feedlot phase on yield grade of steers. A stocker phase  $\times$  feedlot phase interaction was observed ( $P = 0.01$ ), bars without a common superscript differ ( $P \leq 0.05$ ).

## CHAPTER V

### EFFECT OF MONENSIN INTAKE DURING A STOCKER PHASE AND SUBSEQUENT FINISHING PHASE ON RUMEN BACTERIAL DIVERSITY OF BEEF STEERS

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## ABSTRACT

Three monensin levels during a stocker phase (0, 800, 1600 g/ton fed in a free choice mineral) and two levels during finishing (0 [U] or 37.5 mg/kg diet DM [M]) were used to determine the effects of monensin supplementation during a stocker and subsequent finishing phase on rumen bacterial communities of beef steers. Thirty calves were fed pearl millet hay with soybean hull and corn gluten feed supplement or grazed wheat pasture during a stocker phase and then placed in a feedlot for finishing. Rumen fluid was collected throughout the experiment and microbiota were characterized by next generation sequencing. Alpha diversity measures were analyzed using the Wilcoxon Rank Sum Test. Beta diversity was measured using the Bray-Curtis distance matrices and analyzed using the ANOSIM command. The relative abundance of OTUs were analyzed using the MIXED procedure of SAS using repeated measures. During the stocker phase, monensin treatments of cattle consuming the hay diet had decreased alpha community (Shannon,  $P = 0.01$ ) compared to the 0 treatment. At the end of the stocker phase, cattle consuming the hay diet on the 1600 treatment had decreased (Shannon,  $P = 0.04$ ) alpha diversity compared to the control. On d 28 of the stocker phase, alpha diversity of calves grazing wheat pasture on the 1600 treatment decreased (Shannon,  $P = 0.02$ ) and tended to decrease (Shannon,  $P = 0.07$ ) for the 800 treatment compared to the 0 treatment. Cattle that were previously on the 0 and 1600 treatments during the stocker phase and were fed monensin at the feedlot had decreased alpha diversity (Shannon,  $P = 0.04$ ) on feedlot d 14 compared to those that did not. The most predominant abundance of OTUs for cattle consuming hay with supplement belonged to the genus *Prevotella* (20.1%), unclassified *Bacteroidetes* (13.8%), unclassified *Firmicutes* (9.9%), and unclassified *Lachnospiraceae* (8.6%). Cattle grazing wheat pasture had the most abundant OTUs belonging to the genus *Prevotella* (17.8%), unclassified

*Lachnospiraceae* (11.7%), unclassified *Bacteroidetes* (11.0%), and unclassified *Firmicutes* (8.9%). In the feedlot, the most abundant OTUs included *Prevotella* (22.2%), unclassified *Lachnospiraceae* (17.2%), unclassified *Clostridiales* (6.1%), unclassified *Bacteroidetes* (5.8%), and unclassified *Bacteroidales* (5.1%).

**Key words:** monensin, rumen, microbiome, bacteria

## INTRODUCTION

Monensin is a class of carboxylic polyether ionophore antibiotics which has the ability to modify rumen fermentation when fed to cattle (Schelling, 1984). It is effective through antimicrobial properties by interfering with the normal transport of ions through microbial cell membranes (Cheng and Costerton, 1988). Monensin can benefit ruminants by selectively eliminating more sensitive, less efficient ruminal bacteria species and enriching propionate producing bacteria that appear to be less sensitive (Lowicki and Huczynski, 2013). Decreasing selected bacterial species can result in more energetically favorable propionate to acetate concentrations, thus improving feed utilization efficiency (Boling et al., 1977). Additionally, monensin has been reported to decrease methane producing microorganisms and decrease the incidence of ruminal acidosis by maintaining a higher rumen pH from the reduction in lactic acid producing bacteria, both of which can result in improved animal performance (Russell and Houlihan, 2003). A majority of the assumptions about monensin altering rumen microbial communities are derived from traditional culture-based population estimates and the production of various fermentation end products such as VFA and CH<sub>4</sub> (Callaway et al., 2003). Recent advancements in next-generation sequencing techniques provide opportunities to evaluate how monensin affects ruminal bacterial communities in a more specific and complete manner (Kim et

al., 2014). Using such techniques have shown that monensin can alter the rumen microbiota of cattle (Kim et al., 2014a; Kim et al., 2014b; Ogunade et al., 2018; Melchior et al., 2018); however, data is limited on its effects over multiple stages of beef production.

The objective of this experiment was to evaluate the effects of monensin on the diversity of ruminal microbial communities of forage-fed beef calves throughout the duration of a stocker phase and subsequent feedlot phase. Our hypothesis was that monensin would decrease microbial diversity and relative abundance of gram-positive bacterial species in the rumen, regardless of production stage.

## **MATERIALS AND METHODS**

### **Animal Management**

Experimental procedures were approved by the University of Arkansas Animal Care and Use Committee (protocol #17018). Crossbred beef steers ( $n = 30$ , initial BW =  $285 \pm 29$  kg) were obtained from the University of Arkansas Livestock and Forestry Research Station herd and were stratified by BW and assigned randomly to pasture and treatment. Treatments were arranged as a  $3 \times 2$  factorial with factors consisting of monensin provided during the stocker phase, and if monensin was provided in the feedlot diet following the stocker phase. Treatments during the stocker phase consisted of offering grazing calves a free choice loose mineral (AMPT-A, ADM Animal Nutrition) with 0 g monensin/ton (**0**), 800 g monensin/ton (**800**), or 1600 g monensin/ton (**1600**) with the hope of providing monensin intakes of 0, 100, and 200 mg monensin/steer/day. When the stocker phase was complete, cattle were placed in a feedlot where monensin was provide at 0 mg/kg diet DM (**U**) or 37.5 mg/kg diet DM (**R**) in a TMR.

Calves in this experiment were a subset of blocks 1 and 2 from the experiment previously described; therefore, management of cattle has already been discussed in detail. Briefly, steers

were backgrounded and then half of these calves were placed into pastures and had free-choice access to pearl millet hay (*Pennisetum glaucum*) and were supplemented daily with a soybean hull/corn gluten feed blend. The second half of the calves were placed into pastures and allowed to graze wheat pasture (*Triticum aestivum*). After the stocker phase, steers were transported to the West Texas A&M Research Feedlot located near Canyon, TX for finishing. At the feedlot, cattle were transitioned to a high-concentrate finisher diet for the first 28-d by replacing portions of corn stalks with steam-flaked corn weekly. The final diet consisted of steam-flaked corn (37.3% of diet DM), corn gluten feed (43.5%), corn stalks (4.3%), corn oil (3.8%), molasses (7.3%), and a vitamin and mineral premix supplement (3.8%). Cattle remained on this diet for the remainder of the study until approximately 30-d prior to harvest, in which the beta-adrenergic agonists ractopamine hydrochloride (Optaflexx, Elanco Animal Health, Greenfield, IN) was included in the diet at 250 mg/head/d. Cattle were on feed for 132 and 164 d for the heavy and light blocks, respectively. Once cattle were visually determined to be market ready, cattle were transported to a commercial abattoir for harvest.

### **Sample Collection**

Ruminal fluid were collected immediately prior to the initiation of the stocker phase, on d 28, 56, and at the end of the stocker phase before leaving the Batesville Station. Samples were then collected approximately 12 hr following the arrival to the feedlot in West Texas. Cattle did not have access to feed but did have ad libitum access to water between arriving to the feedlot and collecting the d 0 feedlot sample. Rumen content samples were also obtained 14, 28, and 56 d after arrival to the feedlot and immediately before a diet change to include ractopamine hydrochloride (Optaflexx, Pre-Ba, 101 and 131 DOF), approximately 30 days before harvest. A final sample (Final, 131 and 161 DOF) was collected prior to shipping cattle to a commercial

abattoir for harvest. The samples were obtained via the mouth using a sterilized stomach tube into 50 mL conical tubes and immediately placed in an insulated receptacle that contained dry ice and kept until transported to the laboratory. After arrival, samples were stored at -20°C until analysis.

### **DNA Extraction, Library Preparation, and Sequencing**

The DNA from rumen samples were isolated using a commercial microbial DNA isolation kit (DNeasy PowerSoils Kit, Qiagen Inc., Germantown, MD) following the manufacturer's protocol. Following isolation, DNA concentrations were determined using a spectrophotometer (Nanodrop One/C, Fisher Scientific, Hanover Park, IL) and then diluted to 10 ng of DNA as required for library preparation. Samples were amplified by PCR using dual index primers, selected to amplify the V4 region of the 16S rRNA gene in bacteria. Successful amplification was checked by using agarose gel electrophoresis and then the samples were normalized using a normalization kit (SequalPrep<sup>tm</sup> Normalization Kit, Life Technologies, Grand Island, NY). Following normalization, 5 µL aliquots from each sample were pooled to create a library. The library was sequenced using a next generation sequencer (Illumina MiSeq® v2, San Diego, CA) at the University of Arkansas Biomass Research Center in Fayetteville.

Sequences were processed using the mothur program (version 1.39.1) following the MiSeq SOP as described by Kozich et al. (2013) and sequences longer than 275 base pairs were removed (Schloss et al., 2009). The sequences were aligned with the SILVA release 128 database (Quast et al., 2013). To remove excess noise and reduce sequencing error, sequences were removed if they contained more than 2 mismatches during the pre-cluster method (Huse et al., 2010) and any chimeras that were identified were also removed from the samples (Edgar et al., 2011). Operational taxonomic units (OTU) were classified at the genus level using the



Bayesian method (Cole et al., 2009) with a limit of 97% similarity. Sequences were subsampled at 3,000 reads and used to determine alpha and beta diversity measures.

### **Statistical Analysis**

Alpha diversity measures including the Shannon Diversity Index (Shannon and Weaver, 1949) and the number of Observed OTUs, were analyzed using the Wilcoxon Rank Sum Test in RStudio (RStudio Inc., Boston, MA). Beta diversity was measured using the Bray-Curtis distance matrices (Bray and Curtis, 1957) and analyzed in mothur (version 1.39.1) using the ANOSIM command (analysis of similarity). The relative abundance of OTUs that made up greater than or equal to 1% of total relative abundance at each sampling time were analyzed using the MIXED procedure of SAS using repeated measures. Animal was considered the experimental unit and sampling day was used as the repeated measure with steer as the subject. The model for the stocker phase included monensin treatment within each dietary forage, sampling day, and the interaction. The feedlot phase model included the previous stocker treatment, feedlot treatment, sampling day, stocker treatment  $\times$  feedlot treatment, stocker treatment  $\times$  day, feedlot treatment  $\times$  day and stocker treatment  $\times$  feedlot treatment  $\times$  day. Least square means were separated within time to using the predicted differences option of SAS. Significance was declared at  $P \leq 0.05$  and tendencies at  $0.05 < P \leq 0.10$ .

## **RESULTS AND DISCUSSION**

### **Alpha Diversity Measures**

Alpha (within community) diversity was evaluated using the Shannon index (Shannon and Weaver, 1949) and the number of observed OTUs for the rumen of steers throughout a stocker and feedlot phase. During the stocker phase (Figure 1), community differences of the rumens for cattle consuming hay and supplement did not differ on d 0 (Shannon,  $P \geq 0.14$ ;

observed OTUs,  $P \geq 0.30$ ). On d 28 of the stocker phase, cattle consuming monensin with the hay diet had decreased alpha community (Shannon,  $P = 0.01$ ; observed OTUs,  $P \leq 0.02$ ) compared to the 0 treatment, however the monensin treatments did not differ each other (Shannon,  $P = 1.0$ ; observed OTUs,  $P = 0.40$ ). Additionally, at the end of the stocker phase (d 85), cattle consuming the hay diet on the 1600 treatment had decreased (Shannon,  $P = 0.04$ ; observed OTUs,  $P = 0.01$ ) alpha diversity compared to the control and the 800 treatment tended to have a decreased (Shannon,  $P = 0.10$ ; observed OTUs,  $P = 0.04$ ) alpha diversity compared to the 0 treatment. The two monensin treatments did not differ (Shannon,  $P = 1.0$ ; observed OTUs,  $P = 0.83$ ) from each other on the final day of the stocker phase for cattle consuming a hay diet.

Alpha diversity did not differ (Shannon,  $P \geq 0.68$ ; observed OTUs,  $P \geq 0.40$ ) on d 0 of the stocker phase for calves grazing wheat pasture; however, on d 28 alpha diversity for the 1600 treatment decreased (Shannon,  $P = 0.02$ ; observed OTUs,  $P = 0.02$ ) and tended to decrease (Shannon,  $P = 0.07$ ; observed OTUs,  $P = 0.07$ ) for the 800 treatment compared to the 0 treatment. No differences (Shannon,  $P \geq 0.40$ ; observed OTUs,  $P \geq 0.10$ ) in alpha diversity were observed at the end of the stocker phase for calves grazing wheat pasture.

In the feedlot, alpha diversity (Figure 2; Figure 3) did not differ (Shannon,  $P \geq 0.14$ ; observed OTUs,  $P \geq 0.53$ ) on d 0; however, including monensin in the feedlot diet decreased alpha diversity on d 14 for cattle previously on the 0 treatment (Shannon,  $P = 0.04$ ; observed OTUs,  $P = 0.11$ ) and cattle previously on the 1600 treatment (Shannon,  $P = 0.04$ ; observed OTUs,  $P = 0.02$ ). Interestingly, the opposite occurred on d 28, in which cattle consuming monensin in the feedlot tended to have greater alpha diversity for cattle previously on the 0 treatment (Shannon,  $P = 0.10$ ; observed OTUs,  $P = 0.06$ ) and the 1600 treatment (Shannon,  $P = 0.10$ ; observed OTUs,  $P = 0.04$ ). On d 56, cattle previously on the 0 treatment that consumed

monensin in the feedlot tended to have a greater ( $P = 0.06$ ) Shannon index compared to cattle that did not have monensin. No differences ( $P \geq 0.11$ ) were observed among treatments for Shannon index throughout the remainder of the finishing phase, including the pre beta-agonist and final samples. Providing monensin in the feedlot diet decreased the number of OTUs for cattle previously on 800 treatment ( $P = 0.02$ ) and 1600 ( $P = 0.04$ ) and tended to decrease for the 0 treatment ( $P = 0.06$ ) on the pre beta-agonist sample compared to cattle that did not receive monensin in the feedlot. Additionally, cattle consuming monensin in the feedlot that previously did not during stocker phase had decreased ( $P = 0.01$ ) number of OTUs on the final sampling date compared to cattle that did not receive monensin at either phases of production.

Alpha diversity represents the diversity within each community and a greater alpha diversity would indicate a greater variety of bacterial species would indicate a greater variety and distribution of species present. In the current experiment, the effect of monensin on alpha diversity early within each phase agrees with that of Kim et al. (2014), in which monensin decreased Shannon index values. The mode of action of monensin is to selectively eliminate certain species of ruminal microbes (Schelling, 1984); therefore, decreasing the variety of bacterial species in the rumen would be expected. Limited data exists on how monensin impacts microbial communities over time; however, in the current study, it appears that these effects primarily occur early within each production phase and may be more consistent on lower quality diets.

### **Beta Diversity Measures**

Beta diversity, the diversity in microbial communities between different environments, differed (Bray-Curtis, ANOSIM  $P < 0.01$ ) in community structure and membership on d 0 of the stocker phase (Figure 4) between the 0 and 800 treatments; however, no other differences ( $P \geq$

0.10) in beta diversity was observed during the stocker phase. In the feedlot (Figure 5), community structure and membership differed between the 1600R and 1600U treatments on d 14 ( $P = 0.02$ ), d 28 ( $P = 0.02$ ) and tended to differ ( $P = 0.08$ ) immediately prior to including a beta-agonist. Beta diversity also differed ( $P < 0.01$ ) between the 800R and 800U treatments prior to the beta-agonist. No other differences ( $P \geq 0.13$ ) in community membership and structure was observed in this experiment.

### **OTU Distribution**

The most abundant OTUs at the phylum level in the rumen of stocker calves belonged to *Bacteroidetes* (43.1%) followed by *Firmicutes* (42.2%) and *Actinobacteria* (2.7%). In the feedlot, the most abundant phyla included those that belonged to *Firmicutes* (44.9%), *Bacteroidetes* (37.2%), *Proteobacteria* (6.1%) and *Actinobacteria* (5.9%). The most predominant abundance of OTUs for cattle consuming hay with supplement (Figure 6) belonged to the genus *Prevotella* (20.1%), unclassified *Bacteroidetes* (13.8%), unclassified *Firmicutes* (9.9%), and unclassified *Lachnospiraceae* (8.6%). Similarly, cattle grazing wheat pasture (Figure 6) also had the most abundant OTUs belonging to the genus *Prevotella* (17.8%), unclassified *Lachnospiraceae* (11.7%), unclassified *Bacteroidetes* (11.0%), and unclassified *Firmicutes* (8.9%). In the feedlot (Figure 7), the most abundant OTUs at the genus level included *Prevotella* (22.2%), unclassified *Lachnospiraceae* (17.2%), unclassified *Clostridiales* (6.1%), unclassified *Bacteroidetes* (5.8%), and unclassified *Bacteroidales* (5.1%).

### ***Stocker cattle consuming hay and supplement***

A treatment main effect was observed ( $P = 0.05$ ) for relative abundance of OTUs belonging to the genus *Prevotella*, in which cattle on the 1600 treatment had greater ( $P = 0.01$ ) abundance of *Prevotella* compared to the 0 treatment. A treatment  $\times$  time interaction was

observed ( $P = 0.04$ ) for relative abundance of unspecified bacteria. The 0 treatment had greater ( $P < 0.01$ ) relative abundance of unspecified bacteria compared to the 800 and 1600 treatments on d 28 of the stocker phase and greater ( $P = 0.01$ ) abundance than the 800 treatment on d 85. A treatment  $\times$  time interaction was also observed ( $P < 0.01$ ) for relative abundance of *Paraprevotella*, in which cattle on the 800 and 1600 treatments had greater ( $P \leq 0.01$ ) relative abundance of *Paraprevotella* on d 28 compared to the control. Additionally, a treatment main effect trend was observed ( $P = 0.09$ ) as cattle on the 0 treatment tended to have greater relative abundance of the OTU *Saccharofermentans* compared to the 800 treatment.

### ***Stocker cattle grazing wheat pasture***

A treatment  $\times$  time interaction was observed ( $P = 0.04$ ) for OTUs belonging to *Bacteroidetes*, in which relative abundance was greater on d 28 ( $P = 0.02$ ) and d 85 ( $P = 0.05$ ) for the 800 treatment compared to the 1600 treatment. A main effect for treatment was observed ( $P = 0.02$ ) for relative abundance of *Ruminococcaceae*, in which cattle on the 0 treatment had a greater ( $P < 0.01$ ) abundance than 800 and tended to have a greater ( $P = 0.09$ ) abundance than the 1600 treatment. Additionally, steers on the 800 treatment had decreased ( $P = 0.02$ ) relative abundance compared to the 0 treatment and tended to have decreased ( $P = 0.06$ ) presence compared to the 1600 treatment of *Clostridiales*. A treatment  $\times$  time interaction was observed ( $P = 0.03$ ) for abundance of OTUs belonging to unspecified bacteria. Cattle on the 0 treatment had decreased ( $P \leq 0.04$ ) relative abundance of unspecified bacteria on d 0 compared to the other treatments. On d 28, steers receiving the 1600 treatment had decreased ( $P = 0.02$ ) unspecified bacteria compared to the 0 treatment and tended to have decreased ( $P = 0.06$ ) unspecified bacteria compared to the 800 treatment; however, no differences ( $P \geq 0.49$ ) were observed for this OTU on d 85. A treatment  $\times$  time interaction ( $P < 0.01$ ) also occurred for the relative

abundance of *Coriobacteriaceae*. On d 28, steers on the 800 treatment had a greater ( $P < 0.01$ ) presence than the other two treatments. On d 85, cattle belonging to the 0 treatment had decreased ( $P \leq 0.05$ ) relative abundance of *Coriobacteriaceae* compared to the 800 and 1600 treatments, and the 1600 treatment tended to have a greater ( $P = 0.10$ ) abundance of this OTU compared to the 800 treatment. Additionally, a treatment  $\times$  time interaction ( $P = 0.02$ ) occurred for the abundance of the OTU *Succiniclasticum*. On d 0, the 800 treatment had decreased ( $P < 0.01$ ) abundance compared to the other treatments and tended to have decreased ( $P = 0.07$ ) abundance compared to the 1600 treatment on d 28, but no differences ( $P \geq 0.13$ ) occurred on d 85 for relative abundance of *Succiniclasticum*.

### ***Feedlot Cattle***

In the feedlot, cattle consuming monensin had decreased ( $P = 0.01$ ) relative abundance of the OTU genus *Prevotella* compared to cattle that did not. A feedlot treatment  $\times$  day interaction was observed ( $P < 0.01$ ) for the relative abundance of *Firmicutes*, in which cattle consuming monensin in the feedlot tended to have decreased ( $P = 0.09$ ) abundance on d 14 and had decreased ( $P < 0.01$ ) abundance of *Firmicutes* on d 28 of the finishing phase. This agrees with that of Kim et al. (2014a) and Kim et al. (2014b), who also reported that including monensin in cattle diets decreased the abundance of *Firmicutes*. A stocker treatment  $\times$  time interaction ( $P = 0.02$ ) occurred for the relative abundance of *Clostridiales*. Interestingly, cattle previously on the 1600 treatment had greater ( $P = 0.03$ ) abundance of *Clostridiales* on d 56 compared to the 0 treatment, and had lower ( $P = 0.05$ ) abundance compared to the 0 treatment on the sampling date prior to starting a beta-agonist, and a lower ( $P \leq 0.02$ ) abundance of *Clostridiales* than cattle previously on the 0 or 800 treatments at the final sampling time. A feedlot treatment  $\times$  time interaction also occurred for the relative abundance of *Clostridiales*, as cattle consuming

monensin in the feedlot had decreased abundance on d 28 ( $P = 0.03$ ) and prior to starting the beta-agonist ( $P < 0.01$ ). Ogunade et al (2018) also reported that monensin reduces the relative abundance of multiple species within the *Clostridium* genus. A stocker treatment  $\times$  time interaction was observed ( $P < 0.01$ ) for relative abundance of *Ruminococcaceae*. Cattle on the 0 treatment during the stocker phase had greater abundance of *Ruminococcaceae* compared to the 1600 treatment on d 14 ( $P < 0.01$ ), d 28 ( $P = 0.04$ ), prior to the beta-agonist ( $P = 0.01$ ), and tended to be greater at the end of the finishing phase ( $P = 0.10$ ). Additionally, cattle previously on the 800 treatment had greater ( $P < 0.01$ ) relative abundance of *Ruminococcaceae* compared to the 1600 treatment prior to the beta-agonist and greater ( $P \leq 0.04$ ) abundance than the other two treatments at the final sampling time. A feedlot treatment  $\times$  time interaction ( $P < 0.01$ ) also occurred for relative abundance of *Ruminococcaceae* as steers not receiving monensin in the feedlot diet had greater presence of *Ruminococcaceae* compared to cattle that did on d 14 ( $P < 0.01$ ), prior to the beta-agonist ( $P = 0.04$ ), and at the end of finishing ( $P < 0.01$ ). A stocker treatment main effect was observed ( $P = 0.03$ ) for relative abundance of the OTU belonging to *Bacteroidales*, in which cattle that were supplemented with monensin during the stocker phase had decreased ( $P \leq 0.03$ ) abundance of *Bacteroidales* during the finishing phase compared to cattle previously on the 0 treatment. A stocker  $\times$  feedlot treatment interaction ( $P = 0.03$ ) occurred for the relative abundance of *Gammaproteobacteria*, in which cattle consuming the 0R treatment had decreased abundance compared to the 1600R ( $P = 0.05$ ) and the 1600U ( $P = 0.02$ ) treatments. Steers receiving the 0U feedlot treatment had a lower ( $P = 0.04$ ) abundance of the OTU *Gammaproteobacteria* compared to the 1600U treatment, and the 800U treatment had a lower ( $P < 0.01$ ) abundance compared to the 800R, 1600R, or 1600U treatments. A stocker treatment main effect ( $P = 0.04$ ) occurred for the presence of the OTU *Olsenella*, in which cattle

previously on the 800 treatment had greater ( $P = 0.01$ ) abundance during finishing compared to the 0 treatment and tended to have greater ( $P = 0.07$ ) abundance during finishing compared to the 1600 treatment. Additionally, a feedlot treatment  $\times$  time interaction was observed for the relative abundance of *Olsenella*. Feeding monensin in the feedlot increased ( $P = 0.04$ ) the abundance of *Olsenella* on d 14, and then decreased ( $P < 0.01$ ) the abundance on d 28.

It appears that the microbial shifts in the rumen of cattle fed monensin occurred early, as a majority of the effects monensin had on these communities occurred on d 28 or less, regardless of production phase. This agrees with that of Melchior et al. (2018), who found that the most numerous shifts in rumen gram-positive and gram-negative bacterial species in response to monensin occurred on d 18 of the experiment and were minimal thereafter. Additional data on the effects of monensin on the rumen microbiome over time is limited; however, these studies suggest that there may be a benefit from short periods of monensin supplementation and additional research is needed.

The dogma of the effect monensin has on rumen bacteria is that monensin targets gram-positive bacterial species and inhibits them more than gram-negative bacteria. The mode of action that monensin has on bacteria is thought to be correlated with differences in the microbial cell wall structure (Cheng and Costerton, 1988). Gram-positive bacteria lack the complex cellular wall construction that gram-negatives have. These are not permeable to large antibiotic molecules and the complexes formed by them which allow gram-negative bacteria to be less sensitive to the presence of monensin (Lowicki and Huczynski, 2013). The current study partially disagrees with this principle based on how monensin effected the relative abundance of certain classes of bacteria. During the stocker phase, monensin did inhibit the abundance of gram-positive *Saccharofermentans*, *Ruminococcacea*, and *Clostridiales* and enriched the



presence of gram-negative *Prevotella* and *Paraprevotella*; however, at one point it also enriched the abundance of gram-positive *Coriobacteriaceae*. Additionally, feeding monensin in a feedlot diet inhibited gram-positive *Firmicutes*, *Clostridiales*, and *Ruminococcaceae* but also inhibited gram-negative *Prevotella* and enriched gram-positive *Olsenella*. This is supported by that of Kim et al. (2014a), Kim et al. (2014b), Ogunade et al. (2018) and Melchior et al. (2018) who also reported mixed effects of monensin on gram-positive and gram-negative rumen bacteria in cattle. The conclusion from this data is that the dogma from culture-based techniques that suggest monensin inhibits gram-positive bacteria but not gram-negative bacteria is not the case. Monensin may inhibit numerous minor species rather than gram-positive bacteria as a whole. It also suggests that monensin sensitivity may not be directly related to microbial cell wall structure and that other factors including dietary chemophysical features and specific species ability to adapt to monensin may influence its efficacy (Kim et al. 2014b).

## **CONCLUSION**

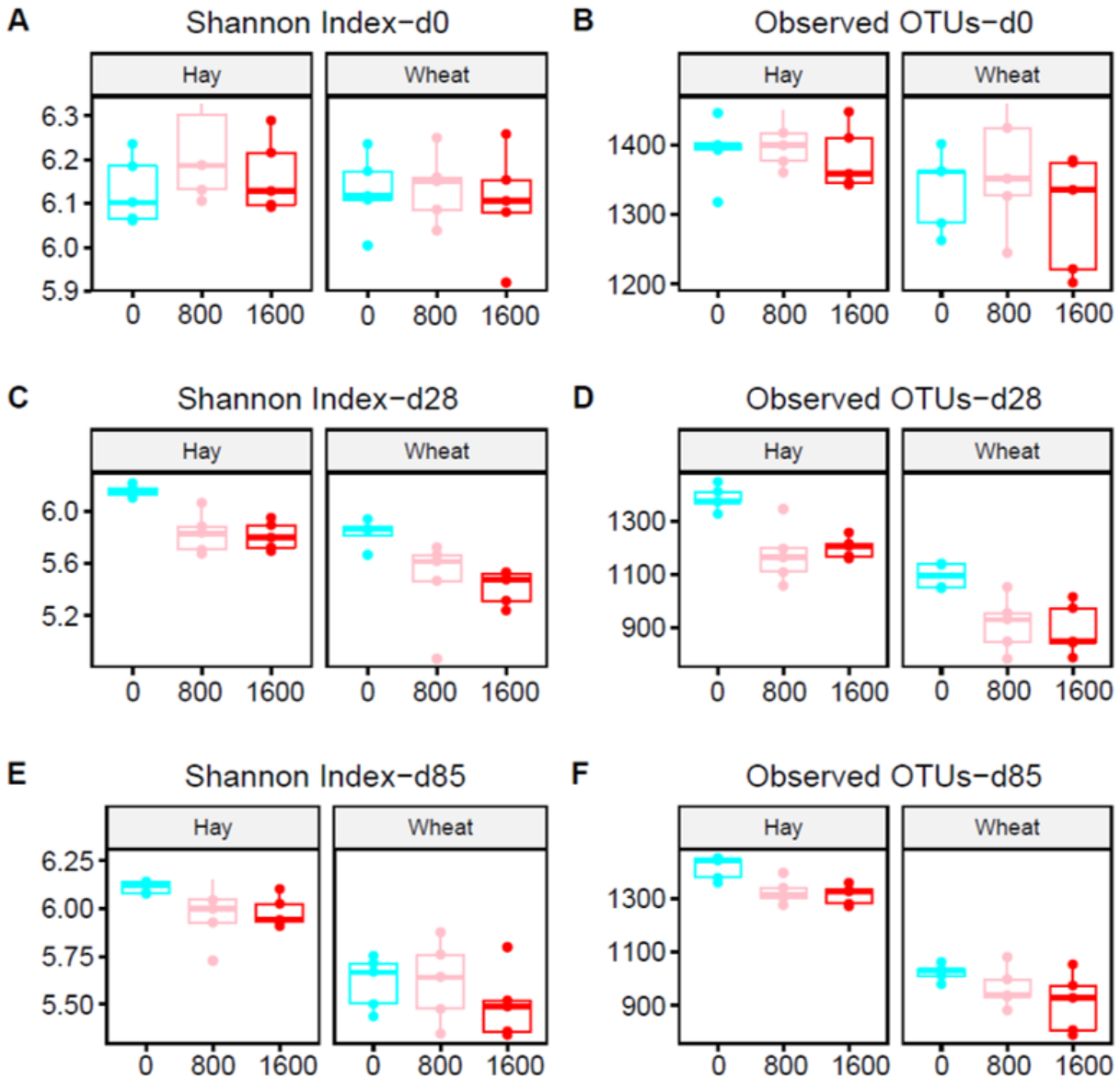
Supplementing monensin was successful in altering rumen bacterial communities of beef calves during multiple stages of production. Inconsistencies of these changes that was observed between the different production stages suggest that dietary factors may influence the effect monensin has on rumen bacteria. Additionally, the greatest effect of monensin appears to occur early and additional research may be beneficial in determining the ideal strategy for monensin supplementation. It was also determined that monensin targets specific species, rather than just gram-positive bacteria as previously reported.

## LITERATURE CITED

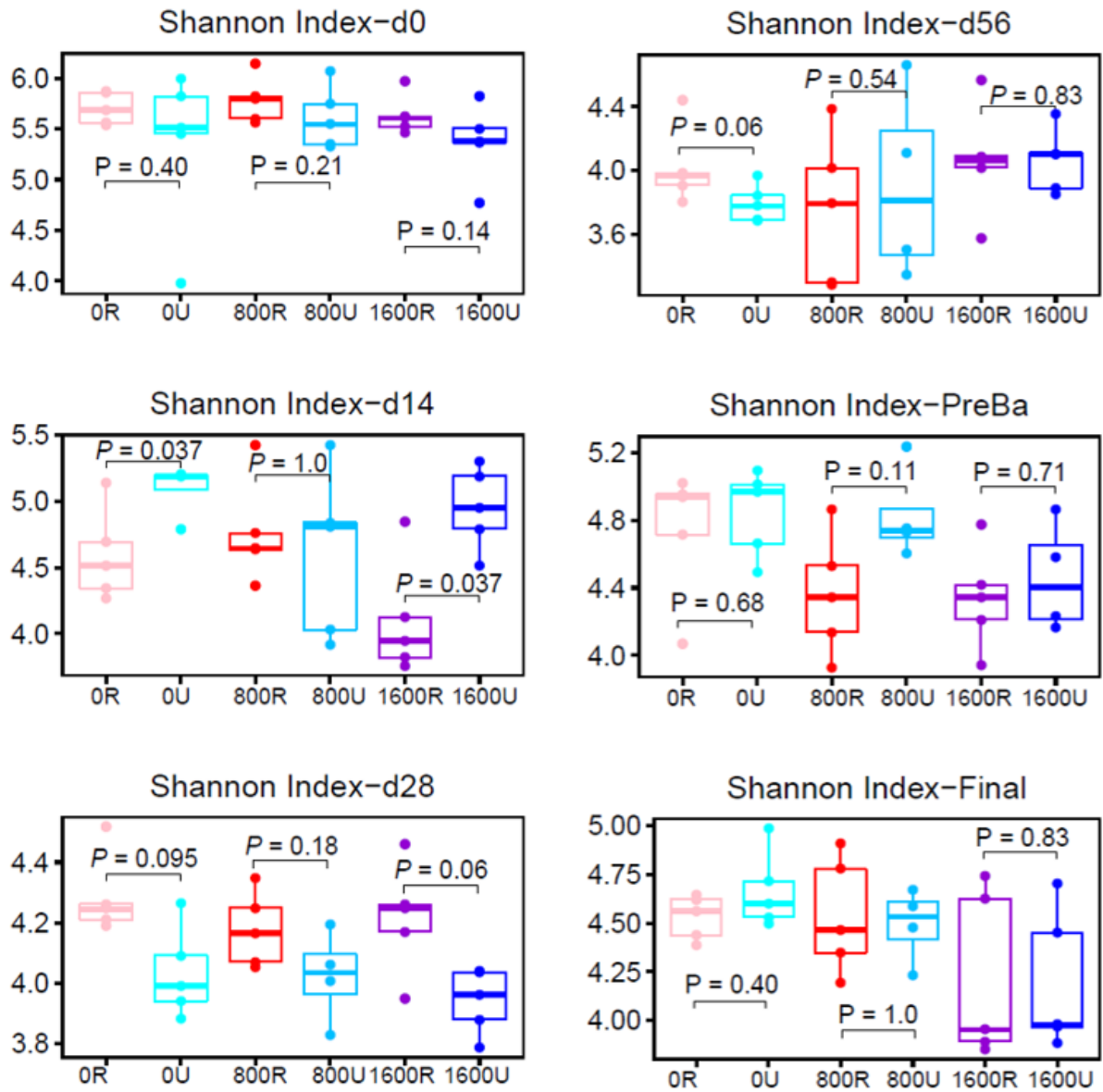
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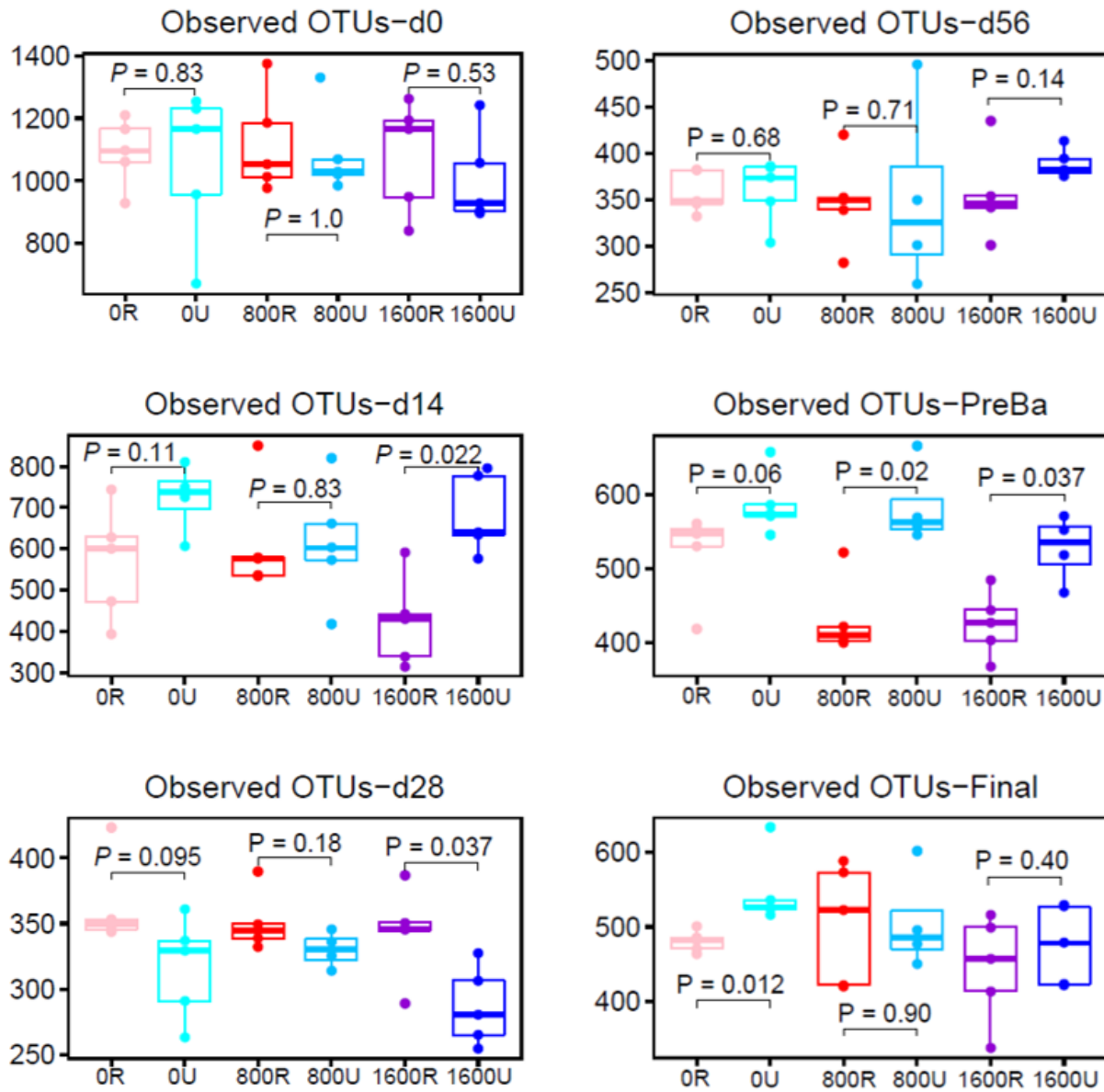
## FIGURES



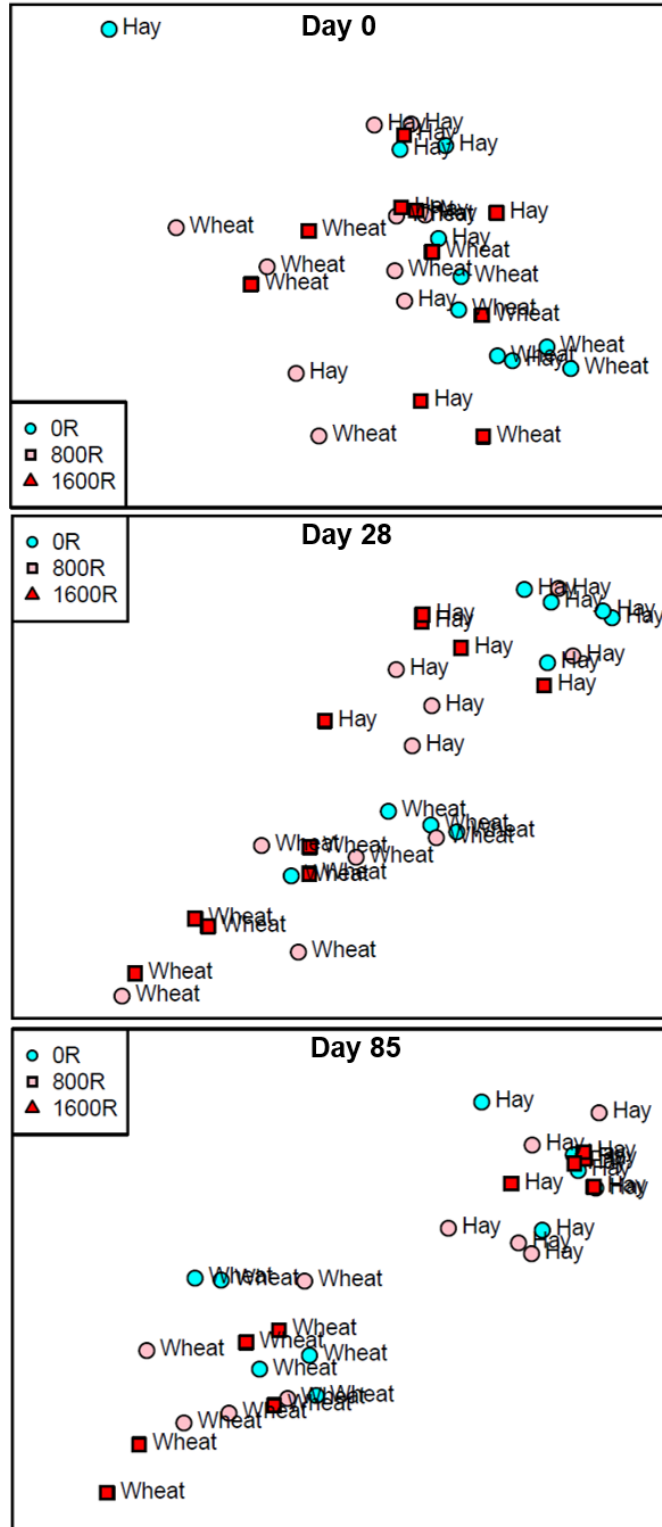
**Figure 4.** Alpha diversity measures for rumen microbiome of stocker calves consuming hay and wheat diets. Treatment (x-axis) includes steers given free choice access to nonmedicated mineral (0), or supplemented with monensin via mineral containing 800g/ton monensin (800) or 1,600g/ton monensin (1600).



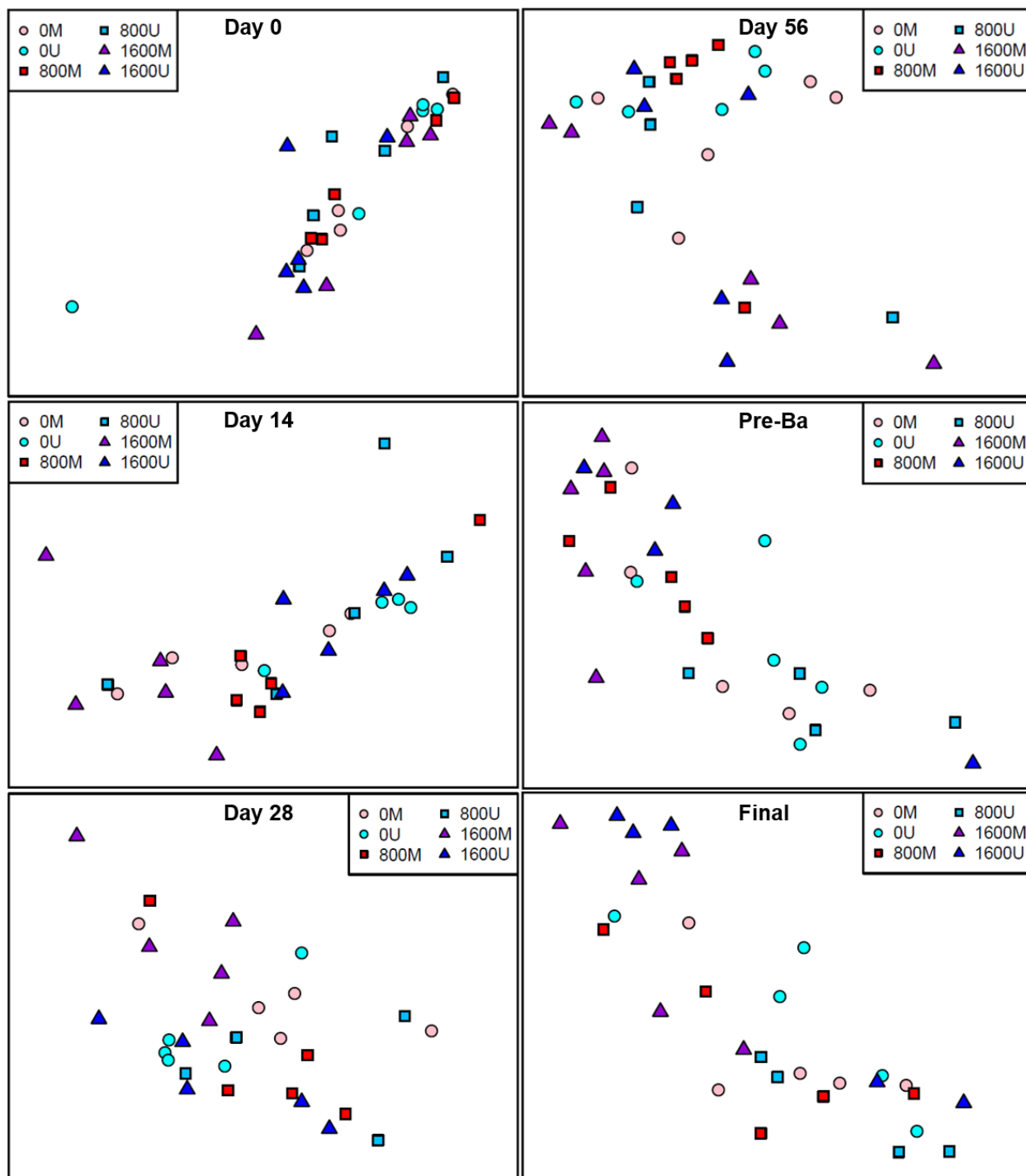
**Figure 5.** Shannon Index values (y-axis) for alpha diversity of feedlot steers with treatments (x-axis) including steers given free choice access to nonmedicated mineral (0), or supplemented with monensin via mineral containing 800g/ton monensin (800) or 1,600g/ton monensin (1600) during the stocker phase and with 0 (U) or 37.5 (M) mg/animal daily of monensin supplied in the diet during the feedlot phase.



**Figure 6.** Observed OTUs (y-axis) for alpha diversity of feedlot steers with treatments (x-axis) including steers given free choice access to nonmedicated mineral (0), or supplemented with monensin via mineral containing 800g/ton monensin (800) or 1,600g/ton monensin (1600) during the stocker phase and with 0 (U) or 37.5 (M) mg/animal daily of monensin supplied in the diet during the feedlot phase.

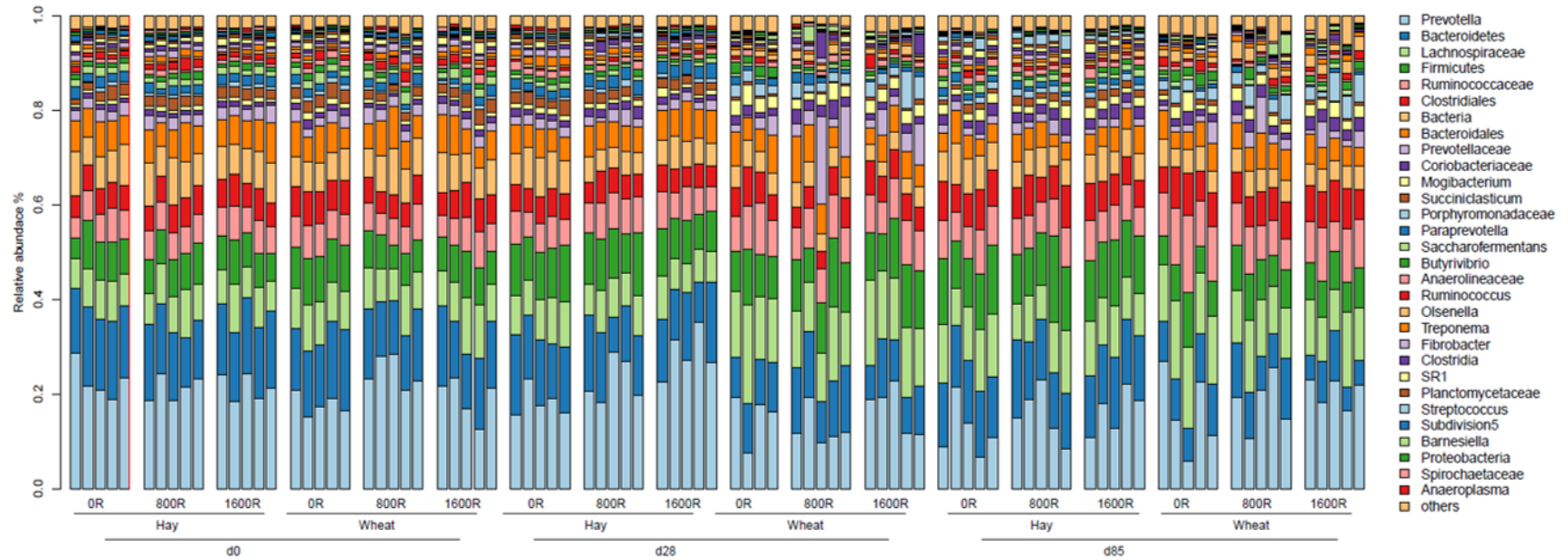


**Figure 7.** Bray-Curtis Index (PC1: 25.15%; PC2: 6.18%) of beta diversity measure for calves throughout the stocker phase.

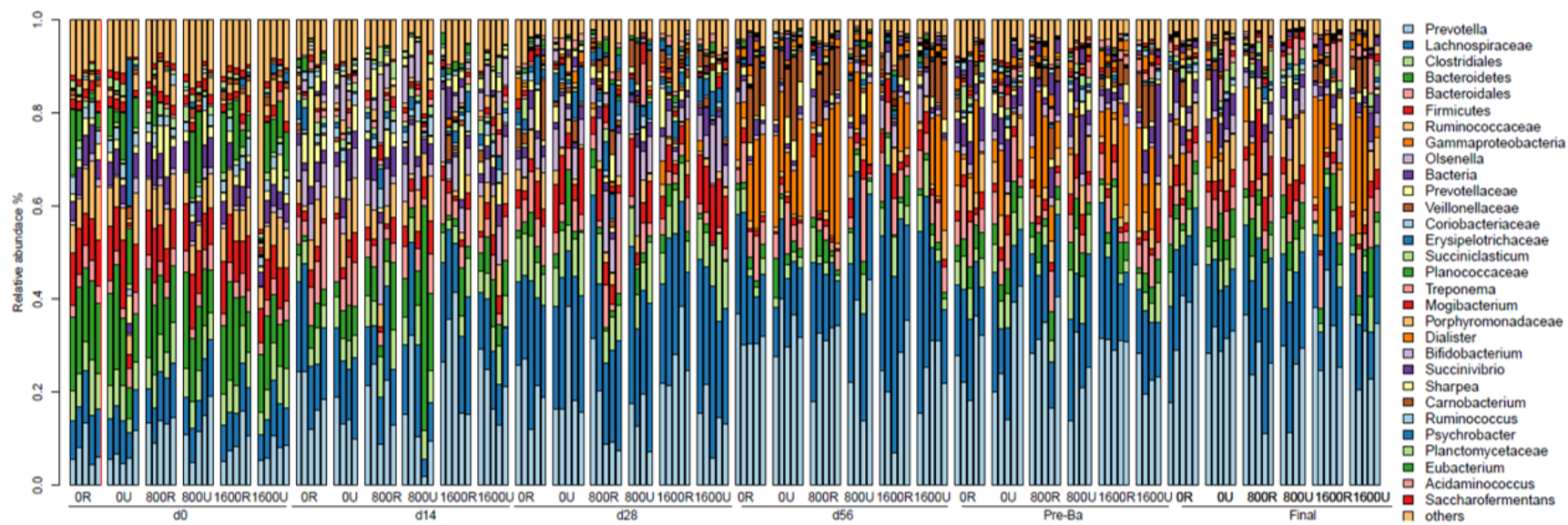


**Figure 8.** Bray-Curtis Index (PC1: 25.15%; PC2: 6.18%) of beta diversity measure for cattle throughout the feedlot phase.





**Figure 9.** Relative abundance of the top 30 OTUs present in the rumen microbiome of stocker calves on each day of sample collection during the stocker phase.



**Figure 10.** Relative abundance of the top 30 OTUs present in the rumen microbiome on each day of sample collection during the finishing phase.

## **CHAPTER VI**

### **CONCLUSION**

In stocker cattle, supplementing monensin in a free-choice mineral increased BW gain of calves compared to an unmedicated control on a variety of forage systems. Mineral consumption was reducing by including monensin; however, targeted intakes were still achieved and a gain response was observed. No differences were observed between the low and high dose of monensin for animal performance, with the exception of mineral intake. Decreasing mineral intake with similar gain responses by feeding a higher level might be an economical advantage that producers should consider. Feeding cattle monensin in the feedlot resulted in decreased feed intake and improved feed efficiency compared to cattle that did not. Feedlot diets containing monensin also resulted in greater fat thickness at finishing, suggesting that cattle consuming monensin were ready for market sooner and may have reduced the number of days required on feed; however, this was not measured in this experiment. It did not appear that previous supplementation of monensin altered performance in later stages of production. The use of monensin did alter rumen microbial communities in all stages of production, but not in a way that is described by early research. This suggests that further research is needed on the rumen microbiome using next-generation sequencing techniques.