

EFFECT OF MONENSIN AND FORAGE SOURCE ON DIGESTION AND ENERGY
METABOLISM IN LIMIT-FED BEEF COWS

A Thesis

by

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ABSTRACT

As beef demand increases and available land resources are diminished, innovative approaches to livestock production are required to meet global demand for food. One such strategy is the intensification of cow-calf systems, which allow for greater dietary control during times of limited forage availability. Monensin, an ionophore feed additive, may have value in intensified cow-calf operations by increasing feed utilization and energy efficiency. Furthermore, forage source may affect digestion and ruminal fermentation of limit-fed diets. Three experiments were designed to determine the effect of monensin on energy and nitrogen balance in nutrient-restricted bred heifers and to evaluate the effect of differing forage sources in a limit-fed total mixed ration (TMR) as well as consequences of limit feeding on voluntary intake and ruminal fill. Monensin had no effect on intake ($P > 0.94$) or digestion ($P > 0.52$) in limit-fed bred heifers. There were also no differences ($P > 0.16$) observed in fecal, urinary, methane, or heat energy losses due to monensin inclusion, and thus, monensin also had no effect ($P = 0.36$) on RE. Nitrogen balance did not differ ($P > 0.13$) between control and monensin heifers. In assessing the inclusion of varying forage sources in a limit-fed TMR, DE intake was greater ($P < 0.03$) for bermudagrass than alfalfa with milo stalks being intermediate. Dry matter digestion (DMD) was greater ($P < 0.02$) for wheat straw and bermudagrass than milo stalks, and there was a tendency ($P = 0.06$) for alfalfa DMD to be lower than wheat straw DMD. Organic matter, NDF, and ADF digestion were greater ($P < 0.02$) for wheat straw than alfalfa or milo stalks. Ruminal DM fill was not

different ($P = 0.18$) between treatments and averaged 4.90 kg; however, liquid fill was greater ($P < 0.02$) for alfalfa and milo stalk treatments than bermudagrass with a tendency ($P = 0.06$) for wheat straw to also be greater than bermudagrass. Ruminal solid passage rate was greatest ($P < 0.01$) for steers consuming wheat straw diets and not different between bermudagrass, alfalfa, and milo stalk diets. Dry matter intake and ruminal DM fill following feed restriction remained lower ($P < 0.04$) than pre-trial levels, while ruminal liquid fill returned to pre-trial levels by d 10 of refeeding. Results of these experiments suggest that adding monensin to limit-fed, corn stalk-based diets has little effect on the energy and nitrogen balance of confined heifers. Additionally, there does not seem to be a clear advantage of feeding one forage over another when considering limit-fed TMR, and voluntary intake and ruminal fill are not restricted following a prolonged period of limit feeding.

DEDICATION

I dedicate this thesis in memory of Betty Louise Waite, affectionately known by all in our family as “Meemaw”. She was always one of my biggest cheerleaders and made it a priority to be present at all of my football games, basketball games, school programs, stock shows, rifle meets, and many, many other events while I was growing up. Even into college, she and Peepaw traveled five hours to Oklahoma to support me at the end of my internship at the Noble Foundation! I’ll never forget what an impression that made on my mentors at Noble, and I just said, “Yep, that’s my Meemaw and Peepaw!” Whether she knew it or not, Meemaw taught me how to be a more compassionate, outgoing, and strong person. She was kind, and loving, and made a friend in everyone who had the pleasure of knowing her. She radiated Southern charm with a smile on her face and joy in her heart at all times. She was a very strong and independent woman with her own opinions and ideas. She loved adventure, learning new things, and seeing new places. Her family meant everything to her, and she shined bright with the light of Jesus. One of my goals in life is to be half the woman my Meemaw was. She is one of my biggest inspirations, a major reason for my success thus far, and I’m so thankful she was my grandma.

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Contributors

This work was supervised by a thesis committee consisting of Dr. Tryon Wickersham (advisor), and Dr. Gordon Carstens of the Department of Animal Science and Dr. Jason Sawyer (co-advisor) of the Department of Nutrition and Food Science.

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All other work conducted for the thesis was completed by the student, under the advisement of Dr. Tryon Wickersham of the Department of Animal Science and Dr. Jason Sawyer of the Department of Nutrition and Food Science.

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CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Demand for food will increase in the coming years as the global population increases. It is estimated that an additional 2.3 billion people will be living in the world in less than 35 years (UN DESA, 2015). Furthermore, economic growth in many developing countries has led to an increase in meat consumption; as disposable income rises in traditionally poor families, the additional money is largely spent on food, specifically meat and milk products (Delgado, 2003; Meade et al., 2011; WTO, 2014). Therefore, an increasing demand for livestock products is expected over the course of the next several decades. To meet this increase in demand, farmers and ranchers worldwide will be asked to maximize production with diminishing resources.

Advancements in technology and production efficiency will be fundamental to achieving the goal of feeding an expanding and wealthier global population. To further complicate matters, increasing land values accompanied by urban expansion and recent instances of prolonged drought have led to a continual decrease in land resources for cattle producers (Eng, 2013). Reduced pasture availability for grazing impacts the maintenance of a grazing cow-calf population, the financial stability of grazing operations, and ultimately the world supply of beef.

As demand increases and land resources are diminished, innovative approaches to livestock production are required to meet global demand for food. One such strategy

is the intensification of cow-calf systems. One manifestation of this type of management system involves limit feeding beef cows and their calves in drylots, similar to traditional feedyards. Intensified cow-calf systems give producers the flexibility to meet cow nutritional requirements during times of limited forage availability rather than liquidating the cow herd (Eng, 2014). Furthermore, limit feeding decreases energy requirements and improves feed efficiency (Freetly and Nienaber, 1998; Trubenbach, 2014) in beef cattle, providing a practical feeding strategy in intensified systems. Whether implemented as year-round or partial confinement, there is potential for drylot systems to supplement traditional grazing operations when forage resources are limited or cost-prohibitive.

Monensin, an ionophore feed additive, may have value in intensified cow-calf operations by increasing feed utilization and energy efficiency. Many have reported on its efficacy in feedlot and grazing animals (Dinius et al., 1976; Potter et al., 1976; Raun et al., 1976; Thornton and Owens, 1981), although questions about adaptation (Mbanzamihigo et al., 1996) and extent of impact on energy metabolism (Goodrich et al., 1984) still remain. If monensin improves beef cow performance, or reduces feed required in intensive management systems, drylots become a more viable option for increasing beef production while increasing the cost effectiveness of the system.

Bioenergetics

A simple experiment conducted by Antoine Lavoisier in the 1770's led to the complex theory of bioenergetics in livestock species. Using a guinea pig and a chamber of ice, Lavoisier was able to prove that living things give off energy in the form of heat.

He concluded that the guinea pig was able to inhale oxygen, convert it to heat energy through the process of combustion, and, in turn, melt the ice surrounding the guinea pig (Kleiber, 1961). This fundamental concept inspired subsequent studies that have been used to construct the energy system that is now used in livestock nutrition.

Armsby and Fries (1915) were among the first to describe the pattern of energy expenditure in cattle. Energy intake is defined as gross energy (GE; Figure A1), and is simply the heat of combustion of a particular diet. Nutritional energetics follows the first law of thermodynamics, therefore, gross energy that enters the body as ingested feed has one of the following fates: it may exit the body in the feces, in the urine, as a gas, as heat, be used in the production of milk, or be retained in body tissues. Therefore, GE alone is not sufficient to describe the amount of energy available to be utilized by the animal. Instead, GE is further partitioned into more discrete representations of energy usage. Digestible energy (DE) is defined as GE intake less energy contained in the feces (FE), or $DE = GE - FE$. Fecal energy makes up the largest fraction of energy loss in ruminant animals and varies greatly depending on the diet (National Academies of Sciences, Engineering, and Medicine, 2016). High fiber diets typically result in greater FE loss and have lower DE values than high-concentrate diets. Metabolizable energy (ME) is DE minus gaseous energy (GASE) and urinary energy (UE). Gaseous energy primarily includes methane (CH_4), and to a lesser extent CO_2 and H_2 , produced from fermentation and released through respiration and eructation. The most precise estimation of energy that remains in body tissues and is available for biosynthetic processes is retained energy (RE), also referred to as net energy (NE). Retained energy is

ME minus energy released from heat production (HE) and can be calculated as $RE = GE - (FE + GASE + UE + HE)$, or $RE = ME - HE$. The RE fraction represents the remaining chemical energy from the diet that is available for synthetic pathways, such as tissue growth, fetal development, or lactation.

Gaseous and heat energy expenditures can be somewhat difficult to capture without proper equipment. Calorimetry experiments are most commonly used to determine GASE and HE. Two calorimetry methods exist: direct and indirect calorimetry. Direct calorimetry involves direct measurement of heat production whereas indirect calorimetry measures gaseous energy exchanges that can be used to calculate heat production using an empirical equation. Indirect calorimetry is the more commonly preferred method of measurement due to the more simplistic nature of the design and operation. Indirect calorimeters measure oxygen (O_2) consumption, CO_2 production, and CH_4 production via an open-circuit air system. Simply put, intake air from the surrounding environment infiltrates an airtight box structure where the animal has placed its head. As the animal eats and maintains normal activity within the box, respired air is collected. Samples of both intake and respired air are taken at consistent time intervals for the duration of the experiment and are analyzed for O_2 , CO_2 , and CH_4 . Fluctuations in gas concentrations between beginning and ending air quality represent metabolic contributions imposed by the animal (Nienaber and Maddy, 1985). Calorimeter gas measurements and urinary nitrogen data from metabolism trials are then used in equations developed by Brouwer (1965) to predict GASE and HE. Calorimetry

experiments offer accurate estimates of GASE and HE losses that can be used to calculate RE.

Efficiency of metabolizable energy utilization

Early ruminant nutritionists initially quantified NE using a single value until Lofgreen (1963a) proposed an alternative method of expressing NE. Lofgreen (1963a) defended and encouraged the adoption of the two NE terms because they more accurately described energy supplied by feedstuffs and the specific requirements of cattle. Lofgreen further clarified that feeds have a greater value for NE_m than NE_g , therefore, the separate terminology allows for much more precise ration formulation and prediction of animal performance.

Net energy required for maintenance is equivalent to the animal's fasting heat production (FHP), which is heat energy associated with only the most basic processes for maintaining life functions, plus the heat of activity (Lofgreen, 1963b). Dietary NE_m values are determined by the amount of a particular feedstuff at which the animal neither gains nor loses energy. Alternatively, NE_g is the energy necessary for an additional pound of gain above maintenance energy levels.

The incremental change between FHP and energy equilibrium ($RE = 0$; Figure A2) is the partial efficiency of ME utilization for maintenance (k_m). Energy gains above maintenance are represented by k_r , which is the partial efficiency of ME utilization for growth (Garrett and Johnson, 1983). Linear regressions of feed intakes above or below energy equilibrium have different slopes, and thus are unequal in their contribution to NE. Metabolizable energy is more efficiently used towards maintenance than growth

requirements, as Lofgreen mentioned (1963a). This difference in efficiency results from differences in heat increments associated with digestion and absorption of food for maintenance and that related to product formation (Ferrell and Oltjen, 2008), such that ruminants fed above maintenance have a greater proportion of heat energy losses than those fed at or below maintenance. Additionally, increasing intake invariably increases the weight of visceral organs, such as the liver and digestive tract. As a result, heat production increases due to the increase in metabolic activity and energy required to maintain greater organ mass (Garrett and Johnson, 1983). This method of quantifying NE provides significant advantages to ruminant nutritionists by allocating feed energy into fractions that are more representative of the final role they play in metabolism.

Advantages of limit feeding

Feed management becomes particularly important when discussing the practicality of feeding cattle in confinement. Feed costs make up the largest fraction of livestock production expenses and have substantially increased since the turn of the century (USDA, 2014). One potential obstacle for intensified beef cow systems is the procurement, processing, and delivery of feed ingredients to cattle at cost effective levels. A primary purpose for limit feeding cattle is to mitigate the risk of herd liquidation whenever forage availability is scarce; therefore, the feeding strategy used in intensified systems must be a cheaper alternative than grazing cattle. One method to reduce cost is by decreasing maintenance energy requirements.

Freetly and Nienaber (1998) showed that limit-fed cows adapted to new levels of energy intake and utilized nutrients more efficiently than cows on a higher plane of

nutrition. In their study, mature, dry cows were either continuously fed at maintenance for 224 days, or fed 35% below maintenance for the first 112 days and 35% above maintenance for the last 112 days. Measurements of body weight, heat production, retained energy, and retained nitrogen all indicated that limit-fed cows reached a new, lower maintenance equilibrium by the end of the restricted feeding phase, allowing for improved feed efficiency upon transition to a higher energy diet. There was no difference in net energy retention and heat production between the two treatments, and limit-fed cows retained more nitrogen than controls. Ultimately, cattle adapted to a lower level of feed intake without any detrimental effects on energy metabolism, suggesting that limit feeding may be a useful feeding strategy when forage availability is limited.

Camacho et al. (2014) performed a similar experiment and also found energetic benefits to restricting intake in cows early in gestation followed by realimentation during late gestation. Control cows were fed at 100% of NRC estimated maintenance requirements while restricted cows only received 60% of their predicted maintenance requirements. Body weight decreased for nutrient restricted cows from days 30 to 140 of gestation. Furthermore, rumen and liver weights were decreased by nutrient restriction. Despite this, gravid uterus weight remained consistent between treatments and was not affected by plane of nutrition. Interestingly, when nutrient restricted cows were transitioned to the control diet after day 140 of gestation and fed at maintenance until day 254 of gestation, no differences were observed in organ weight between control and restricted cows at day 254. This finding suggests that after 110 days of restricted intake, the metabolic activity of limit-fed cows had adapted to a lower level of energy intake,

decreasing the maintenance requirement and allowing more energy to be utilized for growth.

Effect of limit feeding on visceral organ mass

Maintenance energy requirements are a direct function of fasting metabolism (NRC, 2000). Fasting metabolism is measured as fasting heat production (FHP) and includes heat energy losses associated with essential metabolic processes, physical activity, and regulation of body temperature (NRC, 2000). Basal energy expenditures and FHP are predominately associated with the metabolic activity of visceral organs, such as the gastrointestinal tract, liver, and heart (Ferrell and Jenkins, 1985). Therefore, much research has been conducted evaluating the relationship between organ size, metabolic activity, and maintenance energy requirements in ruminants (Ferrell, 1988; Burrin et al., 1990; Fluharty and McClure, 1997; Meyer et al., 2010).

Metabolic activity of the gastrointestinal tract (GIT) and liver account for nearly 50% of the total energy expenditure in ruminants (Ferrell, 1988); therefore, organ size and activity have a large effect on the energy required for maintenance, and perhaps, can be manipulated by feed intake. Ferrell (1988) showed that FHP increased as lambs were fed to gain weight more rapidly. When harvested, lambs fed at a higher rate of gain had greater visceral organ mass (VOM), suggesting a positive correlation between intake, VOM, and FHP. Meyer et al. (2010) examined the effect of nutrient restriction on VOM and found that VOM increased with day of gestation and limit-fed cows had lighter GIT weights than cows on a higher plane of nutrition. Limiting intake to 68% of NRC requirements decreased ruminal and liver weights by 26% and 34%, respectively. When

restricted cows were refed to the same level as controls, there were no differences observed in organ weights. Others (Burrin et al., 1990; Fluharty and McClure, 1997) have reported similar effects on organ size and presented evidence (Burrin et al., 1990) proposing that reduced liver size results in decreased metabolic activity, subsequently reducing energy expenditures, allowing limit-fed animals to be more efficient with their available nutrients. Therefore, a natural conclusion is that one mechanism whereby limit feeding decreases maintenance requirements is a reduction in VOM and associated metabolic activity.

With ruminal size decreased by restricted intake, it is worth considering what effect, if any, limit feeding has on subsequent *ad libitum* ruminal fill and digestion kinetics. Short-term increases in ruminal distension caused linear reductions in voluntary DM intake (Allen, 1996), as would be expected. Conversely, inert fill used to increase distension over a longer time period resulted in an increase in voluntary intake (Allen, 1996). This response to long-term distension may have occurred due to a physiologic adaptation to reticulorumen stimulation, which allowed for greater intake. If this same logic is implemented in the reverse situation (i.e. decreased distension over a long period of time), is it possible that voluntary DM intake would be limited? Research concerning the long-term effects of limit feeding on subsequent voluntary intake and ruminal fill is sparse and bears further investigation.

Comparison of forages in limit-fed diets

Common limit-fed diets often include crop residues, low-quality hays, or byproducts, such as distillers' grains (Jenkins, 2014); availability and cost per unit of

energy supplied are primary determinants of which feedstuffs are the most economical in a limit-feeding program. Selected feed ingredients may be grazed, fed separately, or delivered to the cattle in the form of a total mixed ration (TMR), depending on the management system in place. Baber et al. (2016) concluded that there were no negative effects on intake, nutrient digestion, or ruminal fermentation in cattle that received forage and concentrate portions separately compared to those that were fed a TMR in a limit-fed system.

Forage characteristics, such as fiber content and particle size, have significant effects on digestion and ruminal fermentation, and these effects vary among forage species (National Academies of Sciences, Engineering, and Medicine, 2016). Additionally, when feed ingredients are combined in mixed diets, interactions between the forage and grain fractions of the ration may cause unexpected increases or decreases in digestion and utilization of nutrients, otherwise known as associative effects (Dixon and Stockdale, 1999). Limited research has been conducted on the efficiency with which varying forages are utilized in limit-fed TMR.

Summary of limit feeding

Ultimately, the strategic restriction of feed for beef cows may be a useful feeding strategy for increasing efficiency of feed utilization in intensified cow-calf operations. Future research in this area should focus on additional management practices that can supplement limit feeding to further improve animal efficiency and reduce production costs during times of limited forage availability.

Agricultural contributions to atmospheric methane

Global surface temperature has risen rapidly since 1975, approximating a 0.2°C increase every 10 years (Hansen et al., 2006). This rise in temperature may be due to human activities, including those related to agriculture. Although the energy sector is responsible for the largest majority of U.S. greenhouse gas emissions at 83.6%, agriculture ranks second with 8.4% of the total (EPA, 2016). In its annual inventory of greenhouse gases, the EPA also reported that greenhouse gases, predominantly CH₄, and nitrous oxide (N₂O), are released from several different agricultural sources; these include rice production, manure management, soil management, burning of forage residues, and enteric fermentation. In fact, the second largest anthropogenic source of atmospheric CH₄ is of agricultural origins; in 2014, enteric fermentation of domestic livestock species contributed 23% of the total U.S. CH₄ emissions, second only to natural gas systems. Furthermore, of all livestock species, beef cattle account for 71% of the CH₄ from fermentative processes (EPA, 2016). It is then understandable that livestock producers are taking appropriate steps to mitigate CH₄ losses from cattle as they represent a significant source of CH₄ to the atmosphere in relation to other agricultural sources.

Increased awareness of agriculture's suspected contribution to climate change has caused many consumers to become increasingly critical of modern food production. Additionally, state governments have issued legislation that incentivizes or requires businesses to decrease greenhouse gas emissions. In fact, California and other West Coast states consider carbon and CH₄ emissions to be such a detriment to the

environment that factories and farms in those states are taxed on the amount of greenhouse gasses they release into the atmosphere (California, 2016). With the looming threat of increased regulation and in an effort to be more environmentally conscious, livestock producers are reevaluating their production methods and searching for ways to mitigate CH₄ energy losses.

Methane production in cattle

Due to its consequence on environmental quality and the energetic loss it represents in agricultural systems, it is essential to understand the biological mechanisms by which CH₄ is synthesized within the ruminant. Formation of CH₄ in the rumen is a dynamic process that is influenced by many factors; it is dependent on the composition of rumen microbes as well as characteristics of the feed, such as level of intake, type of carbohydrate, and level of processing. The rumen microbiome is made up of many diverse strains of protozoa, bacteria, and fungi, and differs between individuals.

Rumen methanogens are specialized bacteria that utilize only a few selective substrates in the production of CH₄. They are proficient at scavenging hydrogen (H₂) and carbon dioxide (CO₂) produced by other rumen microbes during fermentation and using those compounds to synthesize CH₄ (Baker, 1999). In this way, methanogens serve as a hydrogen sink and remove excess H₂ that would hinder fermentation. While this is an invaluable asset to the rumen environment and health of the animal, the resulting release of CH₄ to the atmosphere via eructation or respiration is a disadvantage both to the global environment and to animal productivity. Johnson and Johnson (1995) estimated that anywhere between 2 and 12% of ingested feed energy is lost as CH₄ in beef cattle.

This percentage is not insignificant and represents a real cost to producers. In 2012, beef cattle producers spent 8.9 billion dollars on feed costs alone (USDA, 2014). It is evident that strategies need to be developed and (or) implemented to minimize CH₄, thus increasing feed utilization and profit margins. Reducing methanogenic substrates or even altering the population of methanogenic bacteria in the rumen affects energy partitioning; the energy once used to produce CH₄ can instead be used towards maintenance or other productive processes. This is a more efficient use of feed resources and would improve returns to the producer while also satisfying environmentally conscious consumers.

Activity and prevalence of rumen methanogens are influenced by altering diet quantity and quality. Van Nevel and Demeyer (1996) summarized the effects of feeding level, feeding frequency, carbohydrate source, and feed processing on the extent of methanogenesis in ruminant livestock species. They reported that increased intake, decreased frequency of feeding, increased proportion of soluble carbohydrates, and increased processing of feedstuffs decrease the quantity of CH₄ produced. Manipulating these dietary factors changes the composition of substrates entering the rumen, and subsequently, the products of microbial fermentation.

Alterations in pH, microbial populations, and other aspects of the rumen environment can trigger a shift in fermentation patterns, which may divert H₂ away from CH₄ synthesis and towards other anabolic processes. A prime example of this is the inverse relationship between methanogenesis and propionate production. Fermentation of carbohydrates in the rumen yields an excess of free H₂. Commonly, the excess H₂ can

be used in one of two pathways: the production of CH₄ or the production of the volatile fatty acid (VFA), propionate. As previously mentioned, CH₄ is an energetic loss to the animal and a pollutant to the environment. In contrast, propionate is a primary precursor to glucose (Nagaraja et al., 1997) and can be used to synthesize body tissues or provide energy. Numerous studies (Wolin, 1960; Czerkawski, 1969; Johnson et al., 1993; Russell, 1998) have described the competition observed between the two pathways. Ultimately, propionate is a much more efficient and desirable end product of fermentation than CH₄. Therefore, it would be beneficial to discover methods that either create an unfavorable environment for methanogenic bacteria or directly inhibit methanogenesis to produce more propionate and less CH₄.

Ionophore introduction

Ionophores, such as monensin and lasalocid, are a family of antimicrobial feed additives that are used in ruminant diets to increase feed efficiency. Originally developed as coccidiostats in poultry, ionophores alter ruminal fermentation end products and enhance feed efficiency in ruminant livestock species (Dinius et al., 1976; Richardson et al., 1976). Since FDA approval in 1975, ionophores have been widely used in feedlot diets to increase growth and improve the feed to gain ratio in beef cattle. Furthermore, ionophores are known for their ability to mitigate CH₄ production.

Ionophores make up 30% of the total yearly antibiotic use in food-producing animals, second only to tetracyclines, which account for 42% (FDA, 2014). Aside from increasing ADG, ionophores may also be used to prevent bloat and acidosis in cattle (Bergen and Bates, 1984; Goodrich et al., 1984; Nagaraja et al., 1997; Callaway et al.,

2003). Due to the extensive use in food animals, ionophores are scrutinized for their perceived contribution to antimicrobial resistance. However, the FDA has labeled ionophores as “not currently medically important” due to their nonexistent use in human medicine and the lack of research proving their ability to produce antibiotic resistant strains of bacteria (Russell and Houlihan, 2003).

In 2013, the FDA announced the implementation of a regulation known as the Veterinary Feed Directive (VFD) to govern over-the-counter use of “medically important” antibiotics in food-producing animals (FDA, 2014). Per the VFD, antibiotics that are used in both human and livestock medicine are not to be included in livestock feed unless prescribed by a veterinarian. The directive takes full effect in 2017 and is designed to reduce the occurrence of antibiotic resistance. As a “not medically important” antibiotic, ionophores are not covered under the VFD and may be used at the discretion of the livestock producer.

Monensin mode of action

Monensin, perhaps the most common ionophore, is the active ingredient in the feed additive RumensinTM (Elanco, Greenfield, IN), which is commonly included in feedlot rations. Rumensin is also the only ionophore approved for use in mature beef cows. It is necessary to clarify the ruminal mode of action of monensin to understand its potential impact on feed efficiency and energy utilization. Monensin, discovered in 1967 (Agtarap et al., 1967), is a polyether antibiotic compound biosynthesized by the bacterium *Streptomyces cinnamonensis*.

Ionophores, or “ion bearers”, are widely recognized for their ability to transport alkali metal cations across cell membranes. Oxygen atoms on the interior of the monensin chain structure (Figure A3) provide suitable binding sites for positively charged cations, resulting in a lipophilic complex capable of permeating the lipid bilayer of cell membranes. Bergen and Bates (1984) describe monensin as a cation-proton antiporter, meaning it transports cations, such as Na^+ , and protons (H^+) simultaneously in opposite directions across biological membranes. As a mobile carrier, monensin has an affinity for sodium (Na^+) ten times greater than that of any other cation. Ion exchange facilitated by monensin transporters is more efficient than transport by other molecules (Pressman, 1976). Increased membrane activity depletes the H^+ gradient and, subsequently, intracellular ATP. Therefore, cells that rely on substrate level phosphorylation for energy are unable to keep up with the increased demand for ATP and undergo cell death. Conversely, cells that produce energy via oxidative phosphorylation are able to compensate for the increase in ATP requirements and maintain their normal function (Bergen and Bates, 1984).

Survivability of rumen bacteria in the presence of monensin is largely determined by the structure of their cell membrane (Russell and Strobel, 1989), more specifically, their classification as either a gram-positive or gram-negative bacteria (Bergen and Bates, 1984). Gram-positive cell walls are characterized by a plasma membrane and a thick peptidoglycan layer; gram-negative bacteria possess a two-layered cell wall with a plasma membrane, thin peptidoglycan layer, and outer membrane. The single layer

structure of the cell membrane in gram-positive bacteria makes them more susceptible to ionophores than gram-negative bacteria (Russell and Strobel, 1989).

However, Bergen and Bates (1984) believe the persistence of gram-negative species stems from physiological features other than cell wall structure. Rumen bacteria are primarily obligate anaerobes (Hungate, 1975) and, thus, rely on substrate level phosphorylation for energy metabolism due to the toxic effects of oxygen. As mentioned previously, cells dependent on substrate level phosphorylation are more likely to be negatively affected by monensin. Nonetheless, some cells attain energy through an alternative reduction reaction where fumarate acts as the terminal electron acceptor rather than oxygen allowing them to sustain metabolic functions in the presence of ionophores. This reduction reaction is catalyzed by fumarate reductase, which converts fumarate into succinate, a metabolic precursor to the VFA propionate. Fumarate reduction results in a net gain of ATP and provides energy to the cell. Fittingly, fumarate reductase is most prevalent in gram-negative bacteria, which provides an additional advantage that these species have over their gram-positive counterparts (Bergen and Bates, 1984; Hellemond and Tielens, 1994).

Russell and Strobel (1989) disputed the conclusions made by Bergen and Bates and provided evidence of a ruminal bacteria species that is sensitive to monensin despite its ability to synthesize succinate. Even so, neither theory of cell membrane structure or energy-producing pathway applies perfectly to all bacteria. Although the relationship between ion exchange and subsequent changes in ruminal fermentation and metabolism

due to ionophore feeding is not well defined, it can be inferred that altering the flux of ions is a crucial step in improving animal production and efficiency.

Effect of monensin on ruminal acetate to propionate ratio

Modifications induced by monensin at the cellular level are responsible for the many significant changes in ruminal fermentation patterns. Enhancement of propionate production is a crucial first step in the process as it facilitates the occurrence of other biological processes in the rumen. Propionate has been recognized for its gluconeogenic properties and has been estimated to account for 25-60% of the glucose produced in ruminants (Bergman et al., 1966; Wiltrout and Satter, 1972; Bergman, 1990). As the only gluconeogenic VFA, increasing propionate production may lead to increased tissue synthesis in cattle.

Richardson et al. (1976) were among the first to provide evidence for the use of monensin as a growth promotant. Researchers in this study analyzed the effects of monensin both *in vitro* and *in vivo*. In the *in vitro* experiment, significant increases in propionate concentration were observed when rumen fluid from concentrate-fed cattle, forage-fed cattle, and sheep were treated with monensin. Additionally, the percentage of acetic and butyric acids decreased as monensin levels increased from 0.1 to 25 ppm. These responses remained true when monensin was fed *in vivo* to cattle consuming either a concentrate ration or *ad libitum* forage. Dinius et al. (1976) drew similar conclusions noting that the acetate:propionate ratio decreased with each increment of monensin inclusion. These results have been frequently replicated (Perry et al., 1976; Potter et al., 1976; Moseley et al., 1977; Van Nevel and Demeyer, 1977; Thornton and

Owens, 1981) with forage and high grain diets and at varying levels of monensin inclusion. In all experiments, there was no significant change in total VFA concentration.

Relatively few explanations have been presented that clarify the exact mechanism by which monensin shifts the pattern of VFA production in the rumen. It is understood that a significant change in the rumen microbiome must occur, and the most likely organisms to be affected are gram-positive bacteria. For instance, the bacterial species *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Butyrivibrio fibrisolvens* are all known to be susceptible to monensin due to their gram-positive cell wall structure (Chen and Wolin, 1979). Interestingly, each of these organisms are producers of acetate, butyrate, and H₂ (Chen and Wolin, 1979; Russell and Houlihan, 2003). In contrast, the ruminal bacterial family *Prevotella*, formerly *Bacteroides*, and *Selenomonas ruminantium* are gram-negative in their cell wall structure and are resistant to monensin (Chen and Wolin, 1979). These bacteria are prominent succinate and propionate producers; in fact, *S. ruminantium* plays a critical role in the conversion of succinate to propionate and is responsible for the majority of propionate synthesis in the rumen when glucose concentrations are low (Hungate, 1975; Chen and Wolin, 1979; Stewart et al., 1997). Therefore, the microbial response to monensin results in a shift in fermentation end products and provides evidence for the decrease in the acetate to propionate ratio.

Effect of monensin on methanogenesis

Aside from VFA production, monensin also has a major impact on methanogenesis in ruminants. Several studies (Dinius et al., 1976; Thornton and Owens,

1981; Wedegaertner and Johnson, 1983) have observed a significant decrease in CH₄ production with monensin inclusion. Schelling (1984) summarized the results of several monensin trials and reported that CH₄ was reduced anywhere from 4 to 31%. It appears methanogenesis is inversely related to propionate production. Thornton and Owens (1981) observed a 16 and 24% decrease in CH₄ production on low and high roughage diets, respectively, which followed the pattern of increase in propionate from 38 to 66% on low and high roughage diets, respectively.

However, research has shown that monensin does not interact directly with rumen methanogens to inhibit CH₄ production. In an *in vitro* study conducted with ovine rumen contents, Van Nevel and Demeyer (1977) observed the inhibitory effect of monensin on rumen bacteria. When CO₂ and H₂ were used as substrates, no change in CH₄ production was detected. Additions of monensin to incubation with formate as a substrate caused a noticeable reduction in methanogenesis, suggesting monensin inhibits rumen bacteria that catabolize formate into CO₂ and H₂ rather than hindering rumen methanogens directly. Such formate-producing species include *R. albus*, *R. flavefaciens*, and *B. fibrisolvens*, all of which are gram-positive bacteria and are known to be inhibited by monensin (Chen and Wolin, 1979). Thus, removal of these species from the fermentation process results in less CH₄ formation. Furthermore, the increase in propionate production by gram-negative bacteria serves as a “hydrogen sink”, shifting the flow of H₂ ions away from CH₄ synthesis and towards propionate synthesis (Slyter and Wolin, 1976). Together, these metabolic responses to monensin act to decrease CH₄ production.

Effect of monensin on ruminal protein degradation

Monensin has additional ruminal effects on protein metabolism (Dinius et al., 1976; Van Nevel and Demeyer, 1977; Poos et al., 1979). In the presence of monensin, a greater amount of protein escapes ruminal degradation. As a result, rumen ammonia (NH₃) levels are decreased, possibly through direct inhibition of proteolytic or deaminative enzymes produced by rumen protozoa. Monensin has decreased protozoal population by 10 to 64% depending on dietary protein source (Richardson et al., 1978; Poos et al., 1979). However, others (Dinius et al., 1976) have not witnessed a significant change in rumen protozoal numbers due to monensin inclusion. Russell et al. (1988) attempted to identify alternative ways in which monensin hinders protein metabolism by isolating monensin-sensitive, NH₃-producing bacteria. Isolations resulted in the identification of two bacteria species, *Peptostreptococcus* and *Clostridium*, both of which have even greater NH₃-producing activity than *B. ruminicola*, an important contributor of NH₃ in the rumen. *Peptostreptococcus* and *Clostridium* species are also gram-positive bacteria that are inhibited in the presence of monensin, thus inhibition of these NH₃-producing bacteria may further explain the decrease in NH₃ that is observed when monensin is included in ruminant diets.

Inhibition of ruminal protein digestion by monensin further impacts other characteristics of protein degradation. For instance, fewer peptides are broken down into amino acids in the rumen, resulting in fewer substrates for microbial crude protein (MCP) synthesis (Tamminga, 1979). Evidence for this was provided by Hanson and Klopfenstein (1979), who suggested that monensin in fact inhibits MCP synthesis.

Further, Poos et al. (1979) found that bacterial N flow decreased by more than 30% when monensin was supplemented at 200 mg/d in a concentrate diet. Since MCP makes up the majority of the protein that will be metabolized in the small intestine (National Academies of Sciences, Engineering, and Medicine, 2016), a major consequence of limiting MCP production is the risk that the animal is no longer able to meet its requirement for protein.

An increase in escape protein makes up for the reduction in MCP synthesis. The protein-sparing effect of monensin allows a larger proportion of dietary intake protein to escape rumen degradation and enter the small intestine where it is digested into amino acids and small peptides that are absorbed and used to meet the animal's protein requirements (National Academies of Sciences, Engineering, and Medicine, 2016). Research (Bergen and Bates, 1984) showed anywhere from a 22 to 55% increase in escape protein in animals fed monensin compared to their control counterparts. Less rumen NH_3 production coupled with greater amounts of bypass protein should theoretically result in less nitrogen loss, more amino acids that are available for absorption in the small intestine, and thus, a greater amount of nitrogen retained in body tissues. Although no statistical differences were detected, Dinius et al. (1976) concluded that cattle on a forage diet receiving monensin tended to have less nitrogen excreted in urine and feces and more retained nitrogen. Monensin has been shown to increase retained nitrogen by 3.7% in sheep (Joyner et al., 1979), indicating that nitrogen was spared and utilized more effectively towards protein synthesis. Additionally, Orskov et al. (1979) and Byers (1980) each reported instances of improved nitrogen retention with

increased propionate concentrations induced by monensin inclusion. Even so, in two *in vivo* lamb trials, Poos et al. (1979) found either no difference or a decrease in nitrogen retention with monensin.

Effect of monensin on energy metabolism

Perhaps the capstone achievement of monensin is its ability to improve energy balance. All previously mentioned effects work in harmony to culminate in this important biological response. Improvements in energy partitioning by ruminants consuming monensin have been well documented (Potter et al., 1976; Raun et al., 1976; Richardson et al., 1976; Joyner et al., 1979; Byers, 1980; Goodrich et al., 1984), and conclusions are fairly consistent across studies. The energy preserving effect of monensin has several implications on animal performance that are reached through a complex sequence of events.

Raun et al. (1976) discovered that monensin significantly decreased DMI up to 13% in feedlot cattle receiving *ad libitum* access to high-grain rations; however, limit-fed cattle had no change in DMI (Raun et al., 1976). In a study using *ad libitum* fed cattle on an average quality forage, little effect on DMI was observed at monensin doses below 300 mg/d (Potter et al., 1976). While monensin did not impact intake in limit-fed animals, those receiving monensin had a 17% increase in ADG (Raun et al., 1976). Potter et al. (1976) observed a similar increase of 17% in ADG in grazing cattle fed monensin. Further, *ad libitum* fed cattle tended to have greater ADG at levels of monensin up to 44 ppm, but beyond this point ADG decreased compared to controls (Raun et al., 1976). Ultimately, decreased feed consumption and improved ADG led to

increased feed efficiency at all levels of monensin inclusion with both limit-fed and *ad libitum* feeding strategies (Raun et al., 1976). Potter et al. (1976) observed that the greatest improvement in feed utilization occurred at 200 mg/d of monensin, and Raun et al. (1976) maximized feed to gain ratios at 33 ppm. It is presumed that greater feed to gain conversions in monensin-fed cattle is the result of increased energy availability and utilization.

The effects of monensin on energy balance have been studied less extensively. Joyner et al. (1979) conducted an indirect calorimetry study in sheep and reported positive effects on energy balance. Fecal, urinary, and CH₄ energy were decreased 7, 16, and 30%, respectively, when monensin was included at 20 ppm. Consequently, the authors detected an 8% increase in the ME concentration of the diet. Although heat production increased due to monensin, RE improved by 15% compared to controls. However, it should be noted that these results were based on a relatively short feeding period; therefore, it is possible that rumen adaptation may occur and decrease the extent of monensin efficacy.

In a long-term feeding trial (Byers, 1980), feeder calves received either *ad libitum* or limited amounts of a corn silage ration. Monensin was included in the diet at 200 mg daily for more than 200 days, and energy retention was improved by 6% with no sign of adaptation. Monensin increased the NE_m value of the diet, resulting in a 5.4% reduction in the amount of DM necessary for maintenance, but had little effect on the NE_g value. Thus, the greatest response to monensin was observed in cattle that were fed closest to their maintenance requirements.

The most widely accepted theory by which monensin enhances energy utilization hinges on the gluconeogenic properties of propionic acid produced during rumen fermentation. Potter et al. (1976), Raun et al. (1976), and Richardson et al. (1976) all discussed the possible ways in which increases in propionate improves beef cattle performance. Richardson et al. (1976) concluded that gross retained energy might be increased as much as 5.6% during fermentation alone due to more feed energy being converted to metabolizable end products, such as propionate. Calculations revealed that 5 to 10% increases in propionate concentration could increase ME by 3 to 6% in feedlot cattle (Raun et al., 1976). Potter et al. (1976) observed a 40% increase in propionate proportions in forage-fed cattle treated with monensin and associated it with the significant increases reported for plasma glucose. All authors have acknowledged previous research supporting the efficient use of propionate as a substrate in the gluconeogenesis pathway.

As an odd-chain VFA, propionate is the only VFA that can be utilized in the tricarboxylic acid (TCA) cycle and result in a net synthesis of glucose (Bergman, 1990). Ruminant propionic acid accounts for roughly 45 to 62% of the glucose carbon synthesized by ruminants. There are three prominent pathways by which glucose is generated from propionate, and as much as 70% of the propionate used in gluconeogenesis is first converted to lactate, and subsequently glucose (Leng et al., 1967). Thus, monensin increases the amount of carbon directed towards propionate synthesis, which provides more energy to the animal than either acetate or butyrate and serves as a potential source of energy savings.

Summary of monensin

Monensin is an effective feed additive for improving feed efficiency in ruminant species. Modifications in DM intake, ruminal acetate to propionate ratio, methanogenesis, and energy utilization are the primary mechanisms by which monensin acts in the body. However, some of these effects have shown to be short-lived (Mbanzamihigo et al., 1996; Joyner et al., 1979; Poos et al., 1979) due to a possible adaptation to monensin, although results are inconsistent across the literature.

Overall summary

Managing beef cows in drylot settings is a novel idea that still requires much investigation. However, limit feeding and ionophore use both seem to have potential to add value in intensified cow-calf systems. Intensified operations must prioritize minimizing feed costs and maximizing feed utilization to maintain cows as cheaply as possible and provide a practical alternative to grazing or delivering hay to cattle when forage availability is inadequate. Further research examining the effect of forage quality on digestibility and ruminal characteristics of limit-fed diets would provide greater insight into the optimal forage source that maximizes feed utilization. Additionally, the energetic consequences of feeding monensin to nutrient-restricted beef cows have yet to be fully elucidated. Greater knowledge on the effect of monensin on energy and nitrogen balance in limit-fed cows would be useful in determining the feasibility of incorporating ionophores into the feeding program of intensified cow-calf systems.

CHAPTER II

EFFECT OF FEEDING MONENSIN TO BRED HEIFERS FED IN A DRYLOT ON NUTRIENT AND ENERGY BALANCE

Synopsis

Including monensin in limit-fed diets of beef cows in drylots may significantly improve diet digestion and energy balance by altering ruminal fermentation end products and increasing feed efficiency. Sixteen pregnant MARC III (1/4 Angus, 1/4 Hereford, 1/4 Red Poll, 1/4 Pinzgauer) composite heifers were used in a 161-d completely randomized design. Heifers were randomly assigned to 1 of 2 treatments, 150 mg monensin per d (MON) or no monensin (CON), with 8 heifers in each treatment group. Heifers were limit-fed a corn stalk-based diet at 100% of ME_m requirements. Effects of monensin on energy and nitrogen balance were determined via total fecal and urine collections and open-circuit respiration calorimetry. Total fecal and urine collection occurred on d 14, 42, and 161 of monensin feeding, and calorimetry measurements were made on d 0, 3, 14, 28, 42, and 161 of monensin feeding.

Dry matter intake was not different ($P = 0.94$) for CON and MON heifers and, by design, increased ($P < 0.01$) from d 14 to d 161 of the trial to account for increasing fetal growth requirements. Dry matter, OM, NDF, and ADF digestibilities did not differ ($P > 0.52$) between treatments. No differences ($P = 0.91$) in GE intake were observed between CON and MON heifers, and DE and ME intakes were also not affected ($P > 0.58$) by monensin inclusion. Fecal, methane, urinary and heat energy losses made up

50.75%, 6.42%, 4.36%, and 50.36% of the GE intake, respectively, and were not different ($P > 0.16$) for MON and CON heifers; therefore, monensin also had no effect ($P = 0.36$) on RE. Nitrogen intake and excretion was not different ($P > 0.13$) between treatment groups, therefore retained nitrogen for MON heifers was not different ($P = 0.43$) from CON heifers (1.42 vs. -1.32 ± 2.34 g/d, respectively).

Introduction

As global population rises and land resources are diminished, innovative approaches to livestock production are required to meet global demand for food. One such strategy is the intensification of cow-calf systems, which involves limit feeding beef cows in drylots, similar to traditional feedlots. Intensified cow-calf systems give producers the flexibility to continue feeding cattle during times of limited forage availability rather than liquidating the cow herd (Eng, 2014). Furthermore, limit feeding has been reported to decrease energy requirements and improve feed utilization (Freetly and Nienaber, 1998; Trubenbach, 2014) in beef cattle, and thus, provides a reasonable feeding strategy in intensified systems.

Monensin is commonly included in traditional feedlot rations; however, its efficacy in limit-fed beef cows has not been studied extensively. Research conducted in beef steers has shown that monensin decreases the ruminal acetate to propionate ratio (Dinius et al., 1976; Potter et al., 1976; Richardson et al., 1976; Thornton and Owens, 1981), reduces methanogenesis (Dinius et al., 1976; Thornton and Owens, 1981; Schelling, 1984; Wedegaertner and Johnson, 1983), and improves feed efficiency by decreasing DMI and increasing ADG (Potter et al., 1976; Raun et al., 1976). Less is

known about the effect of monensin on energy metabolism and nitrogen balance in beef cows receiving limit-fed diets. Objectives of the current study were to determine if feeding monensin would increase retained energy and nitrogen balance of bred heifers receiving limit-fed corn stalk-based diets.

Materials and methods

This research was conducted according to experimental protocols approved by the Institutional Animal Care and Use Committee at the U. S. Meat Animal Research Center.

Sixteen pregnant MARC III (initial BW 482 ± 30.7 kg) were used in a 161-d completely randomized design to determine the effects of feeding monensin on energy balance and nutrient utilization when heifers were limit-fed a corn stalk-based diet. Heifers were randomly assigned to 1 of 2 treatments, 150 mg of monensin per d (**MON**) or no monensin (**CON**), with eight heifers in each treatment group. Heifers were housed in a semi-enclosed barn open to the south that was fitted with a Calan gate feeding system (American Calan, Northwood, NJ). Heifers had continuous access to fresh water and were fed individually. Diets were fed once daily at 0800 and consisted of corn stalks, corn silage, and wet distillers' grains with solubles (Tables B1 and B2). Monensin was delivered as a pelleted supplement that was top-dressed at 3% of the daily ration. Pellets of the same formulation, but not containing monensin were fed to CON at the same rate. Heifer intake was limited (below *ad libitum* consumption) to achieve 100% of estimated ME_m requirements, with daily feed amounts recalculated for the first, second, and third trimester of gestation to account for fetal growth requirements. Prior to the start

of the trial, heifers were adapted to close human contact, metabolism stanchions, fecal bags and harnesses, and headbox calorimeters to facilitate collection procedures.

Heifers were weighed individually and moved into individual metabolism stanchions (87 cm × 214 cm) in an enclosed, climate-controlled barn at the beginning of each collection period. Total fecal and urine collections were conducted over 96 h and occurred on d 14, 42, and 161 of feeding monensin. Total urine was collected by inserting a 24 french Foley catheter with a 75-mL balloon (Bardex, Murray Hill, NJ) into each heifer's bladder. Tygon tubing was connected to the Foley catheter and terminated into a plastic carboy (18L) that contained .36 M HCl. Canvas bags were placed on the heifers to collect total feces. Daily fecal and urine samples were weighed and a 3% subsample was collected and composited by heifer for each collection period. Feed refusals (when present) were collected and weighed each day and subsamples were composited by heifer.

Gas exchange was measured on d 0, 3, 14, 28, 42, and 161 of feeding monensin using portable respiration calorimeters (headboxes) designed for open-circuit calorimetry. Heifers received their daily feed allowance inside the calorimeters and had access to fresh water throughout the 24 h gas collection period. Individual oxygen consumption, carbon dioxide production, and methane production were collected over 24 h on d 2 of each collection period. Concentration of gases were determined as described by Nienaber and Maddy (1985) and heat production was calculated according to Brouwer (1965).

At the end of the collection period, heifers were weighed and returned to their pens.

Laboratory analysis

Diet, orts, and fecal samples were dried in a forced-air oven for 96 h at 55°C, allowed to air-equilibrate, and then weighed for determination of partial DM. Diet, orts, and fecal samples were then ground through a 1-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ) and dried at 105°C for 24 h for determination of DM. Organic matter was determined as the loss in dry weight upon combustion in a muffle furnace for 8 h at 450°C. Analysis for NDF and ADF was performed sequentially using an Ankom Fiber Analyzer with sodium sulfite omitted and without correction for residual ash (Ankom Technology Corp., Macedon, NY).

Energy values for diet, ort, and fecal samples were determined by direct calorimetry using a Parr 6300 Calorimeter (Parr Instrument Co., Moline, IL). To analyze urinary energy, cotton rounds were weighed and placed into bomb calorimeter crucibles. Standards were created using the average energy content of the cotton rounds. Four mL of urine were added to the crucible and differences in energy content were attributed to the urine. The difference of the urine and standard was divided by the mL of urine added to determine calories per mL of urine.

Diet, fecal, and urine samples were sent to a commercial laboratory (SDK Labs, Hutchinson, KS) for analysis of CP. Diet samples were also analyzed for monensin (Elanco Laboratory, Greenfield, IN).

Calculations

Total orts for each collection period were calculated by taking the weighted average of the feedbox and calorimeter orts:

$$\text{Total orts, g/d} = (\text{feedbox orts} \times 0.75) + (\text{calorimeter orts} \times 0.25)$$

Digestibility of DM, OM, NDF, ADF were calculated using:

$$\text{Digestibility, \%} = \left(\frac{\text{Intake} - \text{Fecal}}{\text{Intake}} \right) \times 100$$

where:

$$\text{Intake} = \text{DMI (kg/d)} \times \text{dietary nutrient concentration (\% DM)}$$

$$\text{Fecal} = \text{Fecal production (kg DM/d)} \times \text{fecal nutrient concentration (\% DM)}$$

Energy losses were calculated using the following equations presented by Blaxter (1965):

$$\text{CH}_4, \text{ mcal} = (\text{CH}_4 \times 9.45) \div 1000$$

$$\text{Heat production, mcal} = (3.866 \times \text{O}_2 + 1.200 \times \text{CO}_2 - 0.518 \times \text{CH}_4 - 1.431 \times \text{N}) \div 1000$$

where:

$$\text{CH}_4 = \text{Methane production (L/d)}$$

$$\text{O}_2 = \text{Oxygen consumption (L/d)}$$

$$\text{CO}_2 = \text{Carbon dioxide production (L/d)}$$

$$\text{N} = \text{Urinary nitrogen excretion (g/d)}$$

Digestible energy intake (DEI), metabolizable energy intake (MEI), and retained energy (RE) were calculated using the following equations:

$$\text{DEI, Mcal} = \text{GEI} - \text{FE}$$

$$\text{MEI, Mcal} = \text{DEI} - \text{CH}_4 - \text{UE}$$

$$\text{RE, Mcal} = \text{GE} - \text{FE} - \text{CH}_4 - \text{UE} - \text{HP}$$

where:

$$\text{GEI} = \text{DMI (g/d)} \times \text{dietary energy (Mcal/g DM)}$$

$$\text{FE} = \text{Fecal production (kg DM/d)} \times \text{fecal energy (Mcal/kg DM)}$$

$$\text{UE} = \text{Urine production (kg/d)} \times \text{urinary energy (Mcal/kg)}$$

$$\text{HP} = \text{Heat production (Mcal)}$$

Retained nitrogen was calculated using:

$$\text{Retained N, g} = \text{Intake N} - \text{Fecal N} - \text{Urinary N}$$

Statistical analysis

Intake, digestion, methane, energy balance, and nitrogen balance data were analyzed using the PROC MIXED procedure in SAS 9.3 (SAS Inst. Inc., Cary, NC). Model fixed effects included treatment, day, and treatment \times day. Day was a repeated term with heifer as the subject. Treatment means were calculated using the LSMEANS option, and the pdiff function was used to separate treatment means. Responses were analyzed using initial BW as a covariate, but were not significant, and thus, were not included in the results.

Results

One heifer in the CON group died due to factors unrelated to treatment before the third collection period, thus, only 7 CON heifers were sampled on d 161 of monensin feeding.

No treatment \times day interactions were detected for any of the response variables tested. No differences ($P = 0.94$) in DMI (Table B3) were observed between CON and MON heifers. By design, DMI increased ($P < 0.01$) from d 14 to d 161 to account for fetal growth requirements. Dry matter, OM, NDF, and ADF digestibilities (Table B3) did not differ ($P \geq 0.52$) between treatments. Digestibility of OM was greatest ($P < 0.03$) on d 161, while NDF and ADF digestibilities were the lowest ($P < 0.02$) on d 42 and not different ($P \geq 0.12$) on d 14 and 161. Body weight (Table B4) tended to differ ($P = 0.08$) between CON and MON heifers, however, when expressed as change in BW from initial weight on d 0, no differences ($P = 0.29$) were found between treatments. Both BW and BW change were greater ($P < 0.01$) on d 161 than all other days.

Methane production (Table B5) was not different ($P = 0.40$) between treatments when expressed as liters of methane produced each day. Additionally, methane production was least ($P < 0.02$) on d 0 and greatest ($P < 0.01$) on d 161. Nonetheless, MON heifers produced 7% less ($P = 0.03$) methane than CON heifers when expressed as liters of methane produced per kg of metabolic body weight (MBW).

Gross energy intake (Table B6) did not differ ($P = 0.91$) between treatments and increased ($P < 0.01$) from d 14 to d 161 to account for fetal growth requirements. Digestible and metabolizable energy intakes were not different ($P \geq 0.58$) between treatments, and the ME:DE was not affected ($P = 0.22$) by monensin inclusion. Heat production was not different between treatments, and monensin also had no effect ($P = 0.36$) on RE (Table B6). Digestible energy intake, ME intake, RE, and ME:DE all increased ($P < 0.01$) from d 14 to d 161. Fecal, methane, urinary and heat energy losses

(Table B7) made up 50.75%, 6.42%, 4.36%, and 50.36% of the GE intake, respectively, and did not differ ($P \geq 0.16$) for MON and CON heifers.

Nitrogen intake and excretion (Table B8) was not different ($P \geq 0.13$) between treatment groups, and thus retained nitrogen for MON heifers was not different ($P = 0.43$) from CON heifers. However, fecal and urinary nitrogen excretion as a percent of total nitrogen excretion (Table B9) was less ($P < 0.04$) for heifers fed monensin than control fed heifers.

Discussion

Limit-fed heifers in the present study did not differ in DMI due to monensin inclusion in the diet. Monensin is commonly reported to reduce DMI (Perry et al., 1976; Raun et al., 1976; Joyner et al., 1979; Byers, 1980; Goodrich et al., 1984), although the extent of this response may be dependent on diet composition and concentration of monensin in the diet. However, the depression in DMI due to monensin seems to be most noticeable in cattle receiving greater amounts of readily fermentable carbohydrates than forage-based diets (Bergen and Bates, 1984). Additionally, Raun et al. (1976) noted that DMI is not affected by monensin when dietary intake is restricted. Thus, DMI in this study was most likely not affected by monensin due to limited intake of a high-forage diet.

Dry matter, OM, NDF, and ADF digestion did not differ with added monensin. When monensin was evaluated *in situ*, Dinius et al. (1976) also found no differences in DM or carbohydrate digestibility with or without monensin. However, Wedegaertner

and Johnson (1983) reported that monensin improved DM and NDF digestion in cattle consuming a cracked corn and corn silage diet fed above maintenance.

There was a tendency for BW to increase with monensin inclusion in the present study; however, when BW was calculated as the change from BW on d 0, the difference was no longer significant. Therefore, the changes observed in BW were not likely induced by feeding monensin, but rather differed enough on d 0 to cause a treatment effect. Furthermore, both BW and BW change data did not differ for d 0, 3, 14, 28, and 42, but increased on d 161. Day 161 of monensin feeding was approximately the beginning of the third trimester of gestation; therefore, conceptus and fetal tissue growth likely account for the increase in BW from d 42 to d 161.

Total liters of CH₄ produced each day were not different between treatments. This disputes the work of others who have reported that feeding monensin decreases the amount of methane produced by 16% when fed to growing steers in a lower forage diet and 24% when fed in a higher roughage diet (Thornton and Owens, 1981). Additionally, the concentration of methane production *in vivo* (Joyner et al., 1979) and *in vitro* (Dinius et al., 1976) has also been reported to be decreased when feeding monensin. However, in *Bos indicus* and *Bos taurus* steers consuming *ad libitum* bermudagrass hay, there were no differences in CH₄-producing activity when monensin was included either 0 or 200 mg/d (Bell, 2015). When expressed as liters per kg MBW, methane production was reduced by 7% in heifers receiving monensin compared to their control counterparts. Wedegaertner and Johnson (1983) observed a 26% reduction in methane per kg MBW between monensin and control steers receiving a cracked corn and corn silage diet fed

above maintenance. Furthermore, in an *in vitro* study, Dinius et al. (1976) proposed a ruminal adaptation to monensin after d 9 of feeding when methane concentrations for monensin treated cultures were no longer different from controls. Bell (2015) made only one observation of methane production in cattle 42 d after feeding monensin and found no differences, suggesting that any changes in methane production due to monensin did not persist beyond 42 d of monensin inclusion. Although liters of methane production per kg of MBW for CON and MON heifers in the present study were numerically similar on d 42 and 161, there is no evidence to suggest an adaptation to monensin, as there was no treatment by day interaction.

Gross energy intake was not different between treatment groups, and no differences were observed in DE or ME intake. In a study with lambs, Joyner et al. (1979) reported a 2.8% increase in DE and an 8.1% increase in ME due to dietary monensin inclusion of 20 ppm. Fecal, CH₄, and urinary energy losses as a percent of GE intake were not different between treatments, which supports why there were also no differences found in DE and ME intakes. No differences in heat production were noted for CON and MON heifers, and ultimately, RE did not change due to monensin and was slightly negative for both treatment groups throughout the experiment. Although Joyner et al. (1979) reported a 15% increase in RE due to monensin, the decrease in methane production in the present study was small, and thus it was not substantial enough to alter RE. Boardman (2015) also observed no change in HP or RE when monensin was included at 200 mg/d in beef cow diets fed at 80% or 120% of NRC (2000) requirements. Likewise, Thornton and Owens (1981) noted no difference in urinary

energy loss, ME values, or HP by feeding monensin. Nonetheless, when averaged across 3 concentrations of roughage inclusion (low, medium, and high), the inclusion of 200 mg/d of monensin did increase the ME of the diets by 5.2%. It was concluded that the increase in ME was because of decreased CH₄ energy loss and the tendency for monensin to decrease energy loss in the feces.

Both CON and MON heifers maintained their BW through d 42 of feeding monensin and had a large increase in BW from d 42 to 161 despite having a negative energy balance for the duration of the trial. Day 161 corresponded to the third trimester of gestation when the majority of fetal growth takes place, which may account for some of the observed increase in BW. Conceptus and tissue weights were predicted using the NRC model (2000) and were subtracted from BW (Table B4). Body weight gain remained positive after accounting for changes due to fetal growth, which contradicts the RE data. It is difficult to know what caused this response, although potential explanations include differences in intake during collection periods, changes in maintenance requirements due to cold stress, effects of limit feeding, or a combination of these factors. For instance, intake during the collection periods may have been lower than intake between collections, which would have caused RE to be negative just for the days on which RE was measured. Furthermore, the experimental diets were not formulated to account for the growth of young heifers, and the heifers were likely outside of their thermoneutral zone for a majority of the study, which likely increased their maintenance energy requirements and may account for the negative RE. Lastly, previous limit-feeding trials (Trubenbach, 2014; Boardman, 2015; Baber et al., 2016)

have observed increases in BW following a period of nutrient restriction, which is hypothesized to be a result of metabolic adaptation to limited energy intake.

Nitrogen consumption for CON and MON heifers was not different. Fecal and urinary nitrogen excretion also did not differ between treatments so that retained nitrogen was not affected by monensin inclusion. This response was not expected, as monensin is known for having a protein sparing effect in the rumen, leading to greater protein absorption in the small intestine and increased nitrogen retention (Russell and Strobel, 1989). Joyner et al. (1979), reported improved nitrogen digestibility with monensin as urinary nitrogen excretion was reduced and retained nitrogen was increased. Likewise, Dinius et al. (1976) observed a tendency for monensin fed steers to have greater nitrogen retention than control fed steers.

Results of this experiment indicate that adding monensin to limit-fed, corn stalk-based diets has little effect on the energy and nitrogen balance of confined heifers. Dry matter intake and nutrient digestibilities were not affected by monensin. Furthermore, fecal, CH₄, urinary, and heat energy losses were not reduced by monensin, as has been reported previously, thus no changes were observed in DE and ME intake or RE. Retained nitrogen was also not different between treatments. Many of the energy saving effects of monensin were not observed in this study and further research should be conducted to determine the efficacy of including monensin in limit-fed diets.

CHAPTER III
EFFECT OF FORAGE SOURCE ON DIGESTION AND RUMINAL
FERMENTATION IN LIMIT-FED STEERS

Synopsis

Physical and chemical forage characteristics influence diet digestion and rumen fermentation (National Academies of Sciences, Engineering, and Medicine, 2016). Therefore, selection of the forage component of diets offered to limit-fed cows may play a role in determining animal performance. To test this, seven steers were used in a replicated 4 × 4 Latin square with treatments consisting of: 1) wheat straw (WS), 2) bermudagrass (BG), 3) alfalfa (AL), or 4) milo stalks (MS). Response variables that were measured included intake, digestion, ruminal fill, rumen solid passage, and ruminal pH and VFA concentrations. Steers were limit-fed to provide 80% of NRC predicted NE_m requirements (NRC, 2000). Feeding periods were 14 d, with 7 d for adaption to treatments and 7 d for collection. Measurements of intake and digestion were made from observations made on d 8 through d 12, ruminal fluid was collected on d 13, and rumen evacuations were performed on d 14. Prior to limit feeding, steers were fed *ad libitum* bermudagrass for 14 d and rumen evacuations were conducted on d 14 to evaluate ruminal fill. Following the limit-feeding period, steers were realimented to *ad libitum* bermudagrass and rumen evacuations were performed on d 3, 6, 10, and 13 of refeeding to determine the effect of limit feeding on subsequent *ad libitum* intake and ruminal fill.

Gross energy intake was least ($P < 0.01$) for the AL (14.96 Mcal/d) treatment and highest ($P < 0.01$) for the WS and MS treatments (15.50 and 16.42 Mcal/d, respectively), with BG being intermediate (15.66 Mcal/d). Steers receiving WS had the greatest ($P < 0.01$) DE intake (11.97 Mcal/d). Organic matter digestion of the diet containing WS (75.7%) was greater ($P < 0.05$) than AL and MS (71.1 and 69.4%, respectively) and not different ($P = 0.27$) from BG (73.4%). Ruminal pH was greater ($P < 0.01$) for AL and MS than BG (6.51 and 6.51 vs. 6.40 ± 0.03 , respectively). Wheat straw had a lesser ($P < 0.03$) acetate to propionate ratio than BG and AL, but was not different ($P > 0.36$) from MS. Ruminal solid passage rate was greatest ($P < 0.01$) for steers consuming WS as the roughage portion of the TMR. Dry matter intake and ruminal DM fill following feed restriction remained lesser ($P < 0.04$) than pre-trial levels, while ruminal liquid fill returned to pre-trial levels by d 10 of refeeding.

Introduction

Increasing land values accompanied by urban expansion and recent instances of prolonged drought have led to a decrease in forage availability for cow-calf production (Eng, 2013). Intensified beef cow systems are emerging as an innovative solution to feeding cattle during times of limited forage availability and may provide a feasible alternative to liquidating the cowherd. Feed management is particularly important when discussing the practicality of feeding cattle in confinement. Feed costs must be minimized in order to realize the advantages provided through intensification. Previous research has shown that limit feeding can reduce the amount of feed that is needed to satisfy the maintenance energy requirements of beef cows (Freetly and Nienaber, 1998;

Trubenbach, 2014). There have been other benefits associated with the strategic restriction of feed in ruminants, such as increased diet digestion and feed utilization (Murphy et al., 1994). However, minimal research is available on the efficiency with which varying forages are utilized in limit-fed TMR. Additionally, research concerning the long-term effects of limit feeding on subsequent voluntary intake and ruminal fill is sparse and bears further investigation.

Objectives of the first experiment were to determine differences in intake, digestion, ruminal pH, and ruminal passage rate in steers receiving a limit-fed TMR with differing forage sources. A second experiment was conducted to evaluate the effect of restricting intake on ruminal capacity when cattle are returned to an *ad libitum* forage diet.

Materials and methods

This research was conducted according to experimental protocols approved by the Institutional Animal Care and Use Committee at Texas A&M University.

Experiment 1: Intake, digestion, ruminal fermentation, and ruminal fill

Seven steers (BW 508 ± 7.8 kg) were used in a replicated 4×4 Latin square design to evaluate the effects of offering different forages in a limit-fed total mixed ration on intake, digestion, ruminal fill, rumen solid passage, ruminal pH, and VFA concentrations. Treatments consisted of: 1) wheat straw (**WS**), 2) bermudagrass (**BG**), 3) alfalfa (**AL**), or 4) milo stalks (**MS**) included as the forage component of the diet. Each diet consisted of one of the forages (35%), cracked corn (29%), dried distillers' grains (27%), and mineral/vitamin premix (9%; Table C1). The TMR was limit-fed to provide

80% of NRC predicted NE_m requirements (NRC, 2000). Steers were initially adapted to housing and feeding protocols for 14 d; steers were housed in individual stalls in an enclosed, climate controlled barn, and had continuous access to fresh water.

Feeding periods were 14 d, with 7 d for adaption to treatments and 7 d for collection. Measurements of intake and digestion were made from observations made on d 8 through d 12. Hay, supplement, and orts were collected on d 8 through d 11 to correspond with fecal samples collected on d 9 through d 12. Fecal production was estimated using titanium dioxide as an external marker. Titanium dioxide (10 g/d) was hand mixed into the diet prior to feeding on d 5 through d 12. A fecal sample was collected prior to initiation of feeding titanium to determine baseline titanium levels. On d 9 through d 12 fecal grab samples were collected every 8 h, with sample time advancing 2 h each day so that 12 samples were obtained over a 4 d collection period. Fecal samples collected during the feeding of titanium were composited by steer and frozen at -20°C . Prior to analysis, each sample was thawed, thoroughly mixed, and a representative subsample was retained. On d 13, a suction strainer (Raun and Burroughs, 1962; 19 mm diam. And 1.5 mm mesh) was used to collect ruminal fluid samples before feeding (0 h) and at 2, 4, 8, 12, 16, and 20 h after feeding. A portable pH meter with a combined electrode (VWR SympHony, Radnor, PA) was used to measure the pH of each sample at the time of sampling. Subsamples of rumen fluid were prepared and frozen at -20°C for subsequent determinations of VFA concentrations. Before freezing, 8 mL of rumen fluid was combined with 2 mL of 25% *m*-phosphoric acid for VFA analysis. Rumen evacuations were performed on d 14 to determine ruminal fill and solid

passage rate. Total weight of the ruminal contents were determined by manually emptying the rumen of each animal prior to feeding (0 h) and 2 h after feeding. Ruminal contents were collected into barrels and at each evacuation time three samples were collected per steer. Ruminal contents were returned immediately following sampling.

Experiment 2: Responses in *ad libitum* ruminal fill

Prior to the beginning of the limit-fed experimental periods (Experiment 1, above), steers received *ad libitum* access to bermudagrass hay for 14 d. Dry matter intake was determined on d 9 through 13 of this period to establish baseline levels of intake. On d 14, rumen evacuations were performed to determine ruminal fill. Total weight of the ruminal contents were determined by manually emptying the rumen of each animal prior to feeding (0 h) and 4 h after feeding. Rumen contents were collected into barrels and at each evacuation time three samples were collected per steer. Rumen contents were returned immediately following sampling. At completion of the Experiment 1, steers were returned to *ad libitum* access to bermudagrass hay, and rumen evacuations were performed on d 3, 6, 10, and 13 of refeeding to determine the effect of limit feeding on subsequent ruminal fill.

Laboratory analysis

Hay, concentrate, fecal, and ruminal content samples were dried in a forced-air oven for 96 h at 55°C and allowed to air-equilibrate then weighed for determination of partial DM. Hay and concentrate samples were composited within period. Fecal samples were composited within steer for each period. Rumen samples were composited by steer within hour for each period. Hay, concentrate, and fecal samples were then ground

through a 1-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ) and dried at 105°C for 24 h for determination of DM. Organic matter was determined as the loss in dry weight upon combustion in a muffle furnace for 8 h at 450°C. Analysis for NDF and ADF was performed sequentially using an Ankom Fiber Analyzer with sodium sulfite omitted and without correction for residual ash (Ankom Technology Corp., Macedon, NY). For determination of acid detergent insoluble ash (ADIA), a sample was subjected only to the ADF protocol and was subsequently combusted in a muffle furnace for a minimum of 8 h at 450° C. Energy values were determined by direct calorimetry using a Parr 6300 Calorimeter (Parr Instrument Co., Moline, IL). Diet and fecal samples were sent to a commercial laboratory (SDK Labs, Hutchinson, KS) for analysis of crude protein, starch, and titanium. Ruminal fluid samples were thawed and centrifuged at 20,000 × g for 20 min at room temperature. Volatile fatty acid concentrations were measured using a gas chromatograph with methods described by Vanzant and Cochran (1994).

Calculations

Fecal production was calculated using:

$$\text{Fecal production, kg DM/d} = \frac{\text{Ti dosed (mg/d)} + \text{diet Ti (mg/d)}}{\text{Fecal Ti concentration (mg/kg DM)}}$$

where:

$$\text{Ti dosed} = \text{Ti (5,980 mg) per 10 g TiO}_2$$

$$\text{Diet Ti} = \text{DMI (kg)} \times \text{Diet Ti concentration (mg/kg DM)}$$

$$\text{Fecal Ti concentration} = \text{Fecal Ti concentration (mg/kg)} \div \text{Fecal DM (\%)}$$

Digestibility of DM, OM, NDF, ADF were calculated using:

$$\text{Digestibility, \%} = \left(\frac{\text{Intake} - \text{Fecal}}{\text{Intake}} \right) \times 100$$

where:

$$\text{Intake} = \text{DMI (kg/d)} \times \text{dietary nutrient concentration (\% DM)}$$

$$\text{Fecal} = \text{Fecal production (kg DM/d)} \times \text{fecal nutrient concentration (\% DM)}$$

Digestible energy intake (DEI) was calculated using:

$$\text{DEI, Mcal} = \text{GEI} - \text{FE}$$

where:

$$\text{GEI} = \text{DMI (kg)} \times \text{Dietary energy concentration (Mcal/kg DM)}$$

$$\text{FE} = \text{Fecal production (kg DM/d)} \times \text{Fecal energy concentration (Mcal/kg DM)}$$

Total VFA concentration was calculated using:

$$\text{Total VFA, mM} = \text{Sum of all VFA (mM)}$$

Molar percentages of each VFA were calculated using:

$$\text{VFA}_x, \% = \text{Concentration}_x \div \text{Total VFA}$$

where:

$$\text{Concentration}_x = \text{Individual VFA concentration (mM)}$$

Ruminal DM fill was calculated using:

$$\text{Ruminal DM fill, kg} = \text{Total ruminal contents (kg)} \times \text{Ruminal DM concentration (\%)}$$

Ruminal solid passage rate was calculated using:

$$\text{Solid passage rate, \% / h} = 100 \times (\text{Diet ADIA} \div \text{Rumen ADIA}) \div 24 \text{ h}$$

where:

$$\text{Diet ADIA} = \text{DMI (kg)} \times \text{Dietary ADIA concentration (\%)}$$

$$\text{Rumen ADIA} = (\text{h 0 Ruminant DM (kg)} \times \text{h 0 Ruminant ADIA (\%)} + \text{h 2 Ruminant DM (kg)} \times \text{h 2 Ruminant ADIA (\%)}) \div 2$$

Statistical analysis

Intake, digestion, and passage rate during the limit-feeding period were analyzed using the PROC MIXED procedure in SAS 9.3 (SAS Inst. Inc., Cary, NC). Model fixed effects included treatment and period, and steer was included as a random effect.

Ruminal pH, VFA profile, and ruminal fill during the limit-feeding period were analyzed using the PROC MIXED procedure. Model fixed effects included steer, treatment, hour, and treatment \times hour. Hour was a repeated term with steer as the subject. Treatment means were calculated using the LSMEANS option, and the pdiff function was used to separate treatment means.

Intake during the *ad libitum* period was analyzed using the PROC MIXED procedure. Model fixed effects included day with steer as a random effect. Ruminal fill during the *ad libitum* period was analyzed using the PROC MIXED procedure. Model fixed effects included steer, day, hour, and day \times hour. Hour was a repeated term with steer as the subject.

Results

Experiment 1

One steer was unable to complete the first collection period due to factors unrelated to treatment. Thus, there were only 6 replications of the AL treatment.

By design, DM intake (Table C2) was different ($P < 0.01$) between treatments. Intakes were 3.83, 3.56, 3.43, and 3.87 kg DM/d for WS, BG, AL, and MS diets,

respectively. Steers receiving the WS diet had the greatest ($P < 0.01$) DE intake (11.97 Mcal/d). Digestible energy intake was greater ($P < 0.03$) for BG (11.07 Mcal/d) than AL (10.24 Mcal/d) with MS (10.94) being intermediate. Dry matter, OM, NDF, and ADF digestion were different ($P < 0.04$) among treatments. Dry matter digestion (DMD) was greater ($P < 0.02$) for the WS (72.4%) and BG diets (71.0%) than MS (65.5%). AL (67.6%) DMD was intermediate. Organic matter, NDF, and ADF digestion were greater ($P < 0.02$) for WS than AL or MS. Starch digestion (Table B2) was not different ($P > 0.20$) across treatments and averaged 97.9%.

There was no ($P = 0.72$) treatment \times time interaction for ruminal pH. Mean ruminal pH (Table C3) was greater ($P < 0.01$) in steers fed AL and MS than those fed BG (6.51 and 6.51 vs. 6.40 ± 0.03 , respectively); steers fed WS (6.46) had ruminal pH intermediate to those fed AL and BG. For all treatments, a nadir in pH occurred 4 to 8 h post feeding. Mean total ruminal VFA concentration had a tendency ($P = 0.06$) to differ between treatments with MS having a lower ($P < 0.04$) total concentration of VFA than BG or AL (63.38 mM vs. 69.10 and 67.97 mM, respectively). Steers receiving WS had a lower ($P < 0.02$) molar acetate percentage than steers receiving AL and MS. Furthermore, WS steers had a greater ($P < 0.03$) molar propionate percentage than BG and AL steers, resulting in a lower ($P < 0.03$) acetate to propionate ratio (2.98 vs. 3.23 and 3.30 ± 0.08 , respectively). Propionate proportion in steers receiving MS was not different ($P > 0.16$) from steers consuming WS or BG and tended to be greater ($P = 0.07$) than AL fed steers. Milo stalk diets did not result in an acetate to propionate ratio different ($P > 0.11$) from WS, BG, or AL diets.

Ruminal DM fill (Table C4) was not different ($P = 0.18$) between treatments and averaged 4.90 kg; however, liquid fill was greater ($P < 0.02$) for AL (48.58 kg) and MS (48.85 kg) treatments than BG (42.69 kg) with a tendency ($P = 0.06$) for WS (47.16 kg) to also be greater than BG. Ruminal solid passage rate was greatest ($P < 0.01$) for steers consuming WS (3.57 %/hr) diets and not different between BG, AL, and MS diets (1.29, 0.70, and 1.30 %/hr, respectively).

Experiment 2

Intake and ruminal fill were averaged across treatments during the final limit-feeding period and used as d 0 values for experiment 2. There were no differences ($P > 0.13$; Table C5) in observed DMI between d 3, 6, 10, and 13 when bermudagrass hay was offered *ad libitum* and averaged 6.45 kg DM/d. Additionally, DMI during the *ad libitum* feeding of bermudagrass hay following 56 d on limit-fed rations remained lower ($P < 0.04$) than the benchmark *ad libitum* bermudagrass hay feeding period. Ruminal DM fill after limit feeding remained lower ($P < 0.01$) on d 3, 6, 10, and 13 (8.46, 9.17, 8.30, and 9.02 kg, respectively) than pre-trial *ad libitum* ruminal DM fill (10.57 kg). However, by d 10 and 13 of refeeding, ruminal liquid fill was not different ($P > 0.42$) from pre-trial ruminal liquid fill (74.97 and 78.79 kg vs. 77.01 kg, respectively).

Discussion

Based on estimated NE_m values, intake of each diet was fed such that steers received 80% of NRC predicted maintenance energy requirements (NRC, 2000). Thus, DM intake, by design, was different for each treatment, being least for AL (3.43 kg DM/d) and greatest for MS (3.87 kg DM/d). Steers consuming WS and BG had similar

digestibility coefficients for DM, OM, NDF, and ADF although WS had the greatest DE intake of any treatment. In a comparative study using cool- (C3) and warm-season (C4) forages, legumes tended to have greater DM digestibility than either C3 or C4 grasses (Reid et al., 1990). This was not observed in the current study as DM digestibility of the AL diet was similar to all other treatments. Organic matter, NDF, and ADF fractions were digested to a greater extent in steers receiving the WS diet compared to those receiving AL or MS diets. Conversely, Moore et al. (1990) found similarities between diet digestion when wheat straw or alfalfa was included at 35% of a mixed diet. However, the below average quality of the alfalfa hay used in the present trial could have accounted for some of the reduction in digestibility when compared to other forages. Reid et al. (1990) witnessed roughly an 11% decrease in fiber digestibility with alfalfa compared to C3 and C4 grasses, which was slightly greater than the 9% decrease in NDF digestion observed in the current trial between the AL diet and WS and BG diets. Although the MS diet had the numerically lowest DM digestibility, all other aspects of digestion were not statistically different from the BG diet, which is also a warm-season species.

Although ruminal pH was greater in steers fed AL and MS than BG, the magnitude of the difference was not great enough to have a biologically important impact on ruminal fermentation. Proportions of VFA were minimally altered by forage source as there was a less than 1 percentage unit reduction in the acetate to propionate ratio with the WS treatment compared to BG and AL. Ultimately, forage source did not have any biologically meaningful effects on ruminal pH and VFA profile of limit-fed

steers. Varga and Prigge (1982) reported no differences in ruminal fermentation characteristics between alfalfa and orchard grass hay. A study comparing early-cut ryegrass hay, late-cut ryegrass hay, and clover hay found that clover hay diets caused the greatest decrease in ruminal pH and increased total VFA concentrations without altering VFA proportions (Aitchison et al., 1986). Contrary to previous research, ruminal pH and VFA concentrations were statistically equal between the AL and WS treatments.

Ruminal DM fill was not affected by forage source. Ruminal liquid fill for the AL and MS treatments were similar to WS and greater than the BG treatment. Previous research has shown that legume diets have decreased ruminal fill compared to grass hay diets when fed *ad libitum* (Aitchison et al., 1986), however, this result was not observed in the present study, which most likely can be attributed to the small differences in DM intake due to limit feeding.

Ruminal solid passage rate for the BG, AL, and MS diets were much lower than solid passage rate for the WS treatment. In a meta-analysis of rumen solid passage rate in sheep and cattle, Evans (1981) observed a positive correlation between dietary roughage and solid turnover rates. All treatments in the current study were formulated to contain equal ratios of concentrate and roughage; WS diets, however, had the greatest proportion of chemical fiber among the four different forage species, which may explain the dramatic increase in solid passage rate. Furthermore, Reid et al. (1990) also found that C4 grasses, such as bermudagrass and milo stalks, tended to have slower passage rates than either C3 grasses, such as wheat straw, or legumes, like alfalfa. Surprisingly, the high passage rate of WS did not negatively affect diet digestion, which is not usually

observed as digestion and passage rate are competing processes (National Academies, of Sciences, Engineering, and Medicine, 2016).

Experiment 2 examined the response in ruminal fill upon *ad libitum* intake following a prolonged period of feed restriction. Upon completion of experiment 1, steers received *ad libitum* access to bermudagrass hay. Dry matter intakes on d 3, 6, 10, and 13 of refeeding were significantly greater than DM intake during experiment 1, but consistently remained lower than pre-trial intakes (Figure C1). It should be noted that, although steers were housed in an air-conditioned barn, pre-trial intakes were measured at the beginning of June and *ad libitum* realimentation occurred at the end of August when weather conditions are hot and humid, thus environmental factors may have affected feed consumption (National Academies of Sciences, Engineering, and Medicine, 2016). Ruminal DM fill followed a similar pattern to DM intake; in contrast, liquid fill steadily increased during refeeding and was not different from pre-trial levels by d 10 of realimentation (Figures C2 and C3).

Results of these experiments do not indicate a clear advantage of feeding one forage over another when considering limit-fed TMR. Wheat straw diets resulted in the greatest DE intake, which may increase feed utilization. However, steers consuming WS also had the greatest solid passage rate. The added effects of restricted intake and fast rates of passage on a limit-fed WS diet could cause the cattle to exhibit more instances of hungry behavior. Cattle consuming BG, AL, and MS all had similar responses in diet digestion and passage rates, and forage source did not negatively impact ruminal fermentation patterns. Additionally, following an extended period of limit feeding,

voluntary intake and ruminal fill were nearly doubled within the first 3 days of realimentation and were approaching pre-trial intake and fill after 6 days of refeeding. Further research evaluating the effect of forage type and quality on the metabolism of limit-fed TMR should be conducted. However, it seems that feeding decisions concerning roughage inclusion for limit-fed beef cows can be made based on economics without significant impacts on animal performance.

CHAPTER IV

SUMMARY

Results of these experiments indicate that monensin may not have the same effects in limit-fed, high-roughage diets as have previously been reported in feedlot cattle. There were no improvements observed in energy intake or diet digestion due to monensin inclusion. Although methane production per kg of MBW was reduced by monensin, fecal, CH₄, urine, and heat energy losses as a percentage of GE intake were not affected, and thus did not translate to greater RE. In addition, steers consuming WS as the forage portion of a limit-fed TMR had the greatest DE intake and fastest rate of passage. Digestion of the WS diet was not different from BG, and WS ruminal fermentation patterns did not differ from the other 3 diets. bermudagrass, AL, and MS diets were all comparable in diet digestion, ruminal pH, VFA profile, and passage rates. Furthermore, limit feeding does not appear to hinder subsequent voluntary intake and ruminal fill.

Monensin's value in limit feeding programs bears further investigation. Beef cows receiving high-concentrate diets may benefit more from monensin inclusion compared to the heifers consuming high-roughage diets in the present study. Further research evaluating the effect of forage type and quality on the metabolism of limit-fed TMR should be conducted. However, it seems that feeding decisions concerning limit-fed beef cows can be made based on economics without significant impacts on animal performance.

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APPENDIX A
CHAPTER I FIGURES

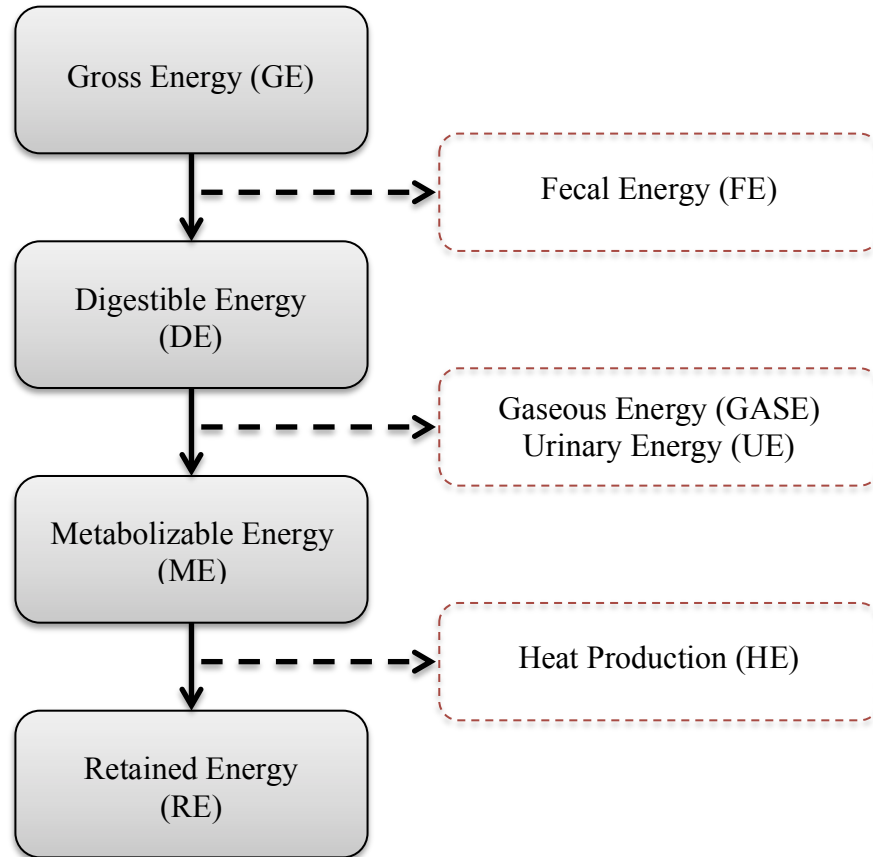


Figure A1. Outline of energy partitioning in beef cattle (NRC, 2016).

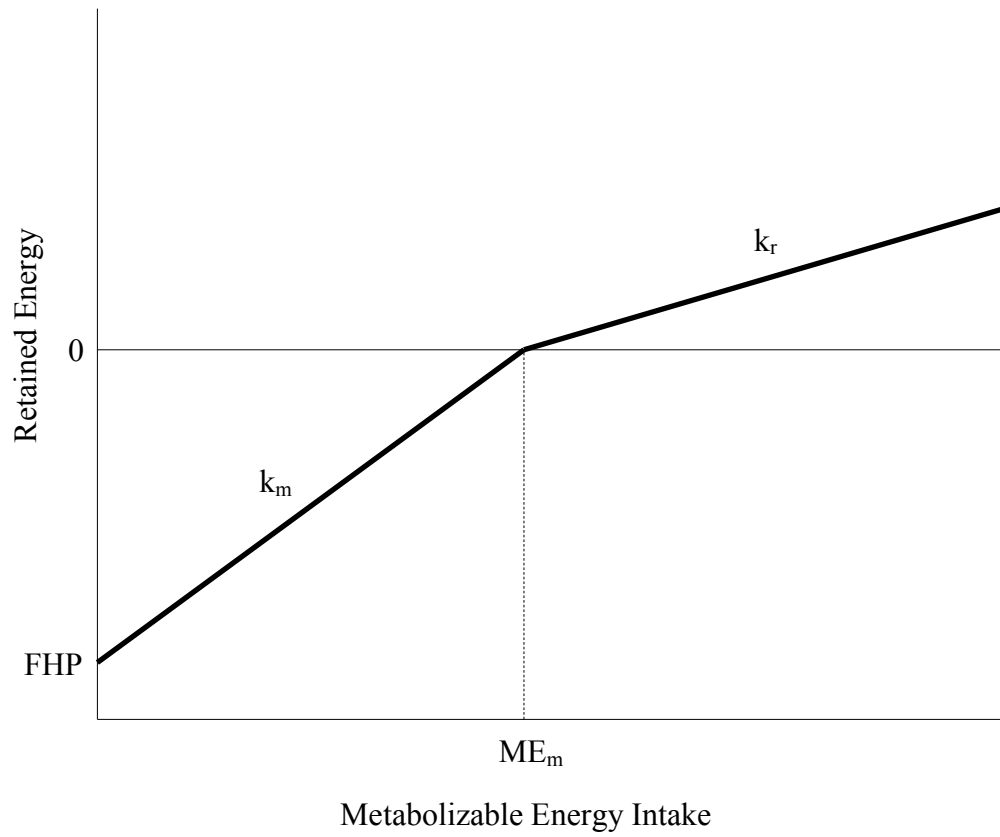


Figure A2. Partial efficiency of ME utilization for maintenance and growth (Garrett and Johnson, 1983)

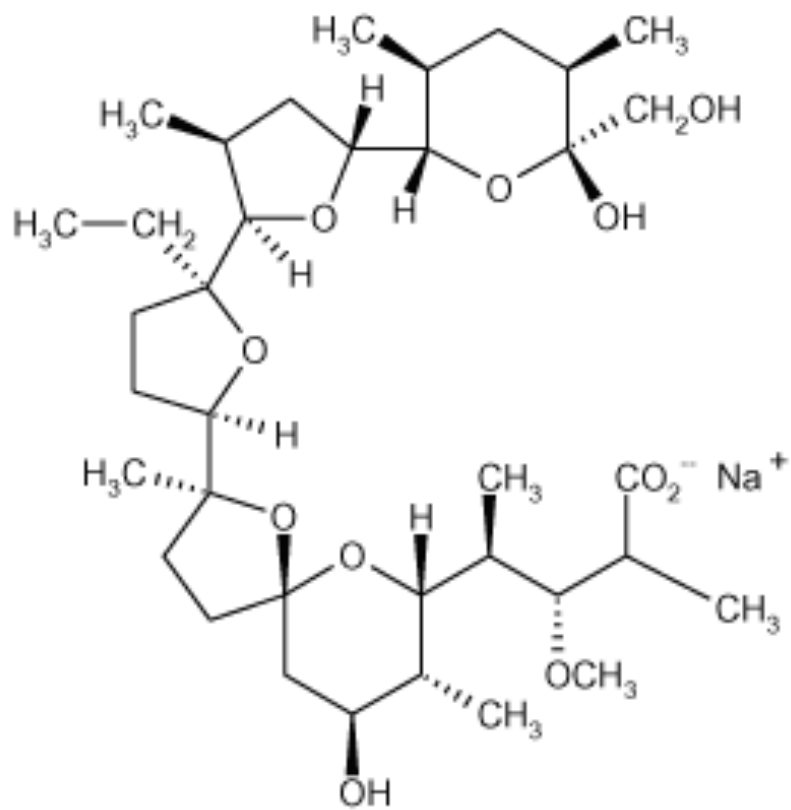


Figure A3. Chemical structure of monensin

APPENDIX B

CHAPTER II TABLES

Table B1. Ingredient composition of limit-fed corn stalk-based diets with or without 150 mg/d of monensin

Ingredient	Percent of diet (DM basis)	
	Control	Monensin
Corn stalks	80	80
Corn silage	10	10
Wet distillers' grains with solubles	7	7
Pellet supplement without monensin	3	0
Pellet supplement with monensin	0	3

Table B2. Nutrient composition of limit-fed corn stalk-based diets with or without 150 mg/d of monensin

Component	Control	Monensin
As fed basis		
DM, %	71.89	71.89
DM basis		
OM, %	83.19	83.19
NDF, %	66.81	66.81
ADF, %	40.78	40.78
CP, %	8.63	8.63
Monensin, mg	0	150

Table B3. Diet digestibility of limit-fed heifers receiving corn stalk-based diets with or without 150 mg/d of monensin

Item	Days on study			SEM ²	Probability ¹	
	14	42	161		Treatment	Day
DM intake, g/d						
Control	6,010 ^a	5,302 ^b	8,219 ^c	170.9	0.94	<0.01
Monensin	5,945 ^a	5,585 ^b	8,039 ^c	161.2		
Digestibility, %						
DM						
Control	42.7	41.1	44.4	2.16	0.52	0.77
Monensin	44.8	43.8	43.0	2.02		
OM						
Control	49.4 ^a	48.6 ^a	54.8 ^b	1.88	0.75	0.04
Monensin	50.4 ^a	50.9 ^a	53.0 ^b	1.76		
NDF						
Control	44.4 ^a	36.3 ^b	49.2 ^a	2.61	0.67	<0.01
Monensin	44.7 ^a	39.8 ^b	47.9 ^a	2.44		
ADF						
Control	36.3 ^a	25.7 ^b	43.5 ^a	3.69	0.79	<0.01
Monensin	36.9 ^a	29.9 ^b	40.9 ^a	3.45		

¹No interactions present ($P > 0.17$).

²Pooled standard error of least squares means (control $n = 7$; monensin $n = 8$).

^{a,b,c}Within a row, means across treatments without a common superscript differ between days ($P < 0.05$).

Table B4. Body weight measurements of limit-fed heifers receiving corn stalk-based diets with or without 150 mg/d of monensin

Item	Days on study						SEM ²	Probability ¹	
	0	3	14	28	42	161		Treatment	Day
BW, kg									
Control	470 ^a	468 ^a	468 ^a	461 ^a	463 ^a	544 ^b	11.7	0.08	<0.01
Monensin	495 ^a	494 ^a	495 ^a	493 ^a	494 ^a	578 ^b	11.6		
BW change, kg									
Control	0 ^a	-2.8 ^a	-2.6 ^a	-9.4 ^a	-7.7 ^a	73.5 ^b	4.80	0.29	<0.01
Monensin	0 ^a	-0.5 ^a	0.8 ^a	-1.8 ^a	-1.0 ^a	83.2 ^b	4.60		
BW – fetus, kg									
Control	469 ^a	466 ^a	466 ^a	459 ^a	460 ^a	525 ^b	11.7	0.08	<0.01
Monensin	493 ^a	493 ^a	494 ^a	491 ^a	491 ^a	559 ^b	11.6		
BW – fetus change, kg									
Control	0 ^a	-2.9 ^a	-3.0 ^a	-10.3 ^a	-9.1 ^a	55.7 ^b	4.80	0.29	<0.01
Monensin	0 ^a	-0.5 ^a	0.4 ^a	-2.6 ^a	-2.4 ^a	65.4 ^b	4.60		

¹No interactions present ($P > 0.90$).

²Pooled standard error of least squares means (control $n = 7$; monensin $n = 8$).

^{a,b}Within a row, means across treatments without a common superscript differ between days ($P < 0.01$).

Table B5. Methane production of limit-fed heifers receiving corn stalk-based diets with or without 150 mg/d of monensin

Item	Days on study						SEM ²	Probability ¹	
	0	3	14	28	42	161		Treatment	Day
CH ₄ , L/d									
Control	134.4 ^a	160.8 ^{bc}	169.8 ^c	158.3 ^b	161.7 ^{bc}	185.9 ^d	7.61	0.40	<0.01
Monensin	136.3 ^a	148.8 ^{bc}	159.3 ^c	146.8 ^b	163.7 ^{bc}	187.4 ^d	7.17		
CH ₄ , L/kg BW ^{0.75}									
Control	1.3 ^a	1.6 ^b	1.7 ^b	1.6 ^b	1.6 ^b	1.7 ^b	0.07	0.03	<0.01
Monensin	1.3 ^a	1.4 ^b	1.5 ^b	1.4 ^b	1.6 ^b	1.6 ^b	0.06		

¹No interactions present ($P > 0.69$).

²Pooled standard error of least squares means (control $n = 7$; monensin $n = 8$).

^{a,b,c,d}Within a row, means across treatments without a common superscript differ between days ($P < 0.05$).

Table B6. Energy partitioning by limit-fed heifers receiving corn stalk-based diets with or without 150 mg/d of monensin

Item	Days on study			SEM ²	Probability ¹	
	14	42	161		Treatment	Day
Energy Intake, Mcal/d						
GE						
Control	23.80 ^a	20.71 ^b	32.54 ^c	0.64	0.91	<0.01
Monensin	23.48 ^a	21.84 ^b	31.95 ^c	0.61		
DE						
Control	11.08 ^a	9.86 ^a	17.12 ^b	0.48	0.75	<0.01
Monensin	10.96 ^a	10.97 ^a	16.53 ^b	0.45		
ME						
Control	8.36 ^a	7.36 ^b	14.03 ^c	0.47	0.58	<0.01
Monensin	8.41 ^a	8.55 ^b	13.42 ^c	0.44		
ME:DE						
Control	0.75 ^a	0.74 ^a	0.82 ^b	0.02	0.22	<0.01
Monensin	0.77 ^a	0.77 ^a	0.81 ^b	0.01		
RE						
Control	-3.22 ^a	-3.64 ^a	-0.67 ^b	0.69	0.36	<0.01
Monensin	-3.90 ^a	-3.40 ^a	-1.78 ^b	0.65		

¹No interactions present ($P > 0.12$).

²Pooled standard error of least squares means (control $n = 7$; monensin $n = 8$).

^{a,b,c}Within a row, means across treatments without a common superscript differ between days ($P < 0.05$).

Table B7. Energy losses as a percent of gross energy intake by limit-fed heifers receiving corn stalk-based diets with or without 150 mg/d of monensin

Item	Days on study			SEM ²	Probability ¹	
	14	42	161		Treatment	Day
Energy loss, % of GE intake						
Fecal						
Control	53.5 ^a	52.4 ^a	47.4 ^b	1.59	0.60	<0.01
Monensin	53.2 ^a	49.8 ^a	48.2 ^b	1.49		
CH ₄						
Control	6.7 ^a	7.4 ^b	5.4 ^c	0.30	0.57	<0.01
Monensin	6.4 ^a	7.1 ^b	5.5 ^c	0.28		
Urine						
Control	4.7	4.7	4.0	0.31	0.26	0.28
Monensin	4.5	4.1	4.2	0.29		
Heat						
Control	48.9 ^a	53.2 ^b	45.3 ^c	1.77	0.16	<0.01
Monensin	52.4 ^a	54.7 ^b	47.7 ^c	1.66		

¹No interactions present ($P > 0.44$).

²Pooled standard error of least squares means (control $n = 7$; monensin $n = 8$).

^{a,b,c}Within a row, means across treatments without a common superscript differ between days ($P < 0.05$).

Table B8. Nitrogen intake and excretion of limit-fed heifers receiving corn stalk-based diets with or without 150 mg/d of monensin

Item	Days on study			SEM ²	Probability ¹	
	14	42	161		Treatment	Day
N intake, g/d						
Control	82.2 ^a	92.4 ^b	103.0 ^b	3.97	0.94	<0.01
Monensin	81.2 ^a	97.3 ^b	99.9 ^b	3.72		
N excretion, g/d						
Feces						
Control	50.6 ^a	43.5 ^a	76.5 ^b	3.97	0.13	<0.01
Monensin	44.9 ^a	40.1 ^a	71.5 ^b	3.71		
Urine						
Control	30.4 ^a	34.7 ^a	45.1 ^b	2.80	0.16	<0.01
Monensin	33.5 ^a	33.5 ^a	50.6 ^b	2.65		
Total						
Control	81.0 ^a	78.3 ^a	121.8 ^b	4.58	0.46	<0.01
Monensin	78.4 ^a	73.6 ^a	122.1 ^b	4.30		
Apparent N digested, g/d						
Control	31.6 ^a	48.9 ^b	25.9 ^a	6.11	0.17	<0.01
Monensin	36.3 ^a	57.2 ^b	28.4 ^a	5.78		
N retained, g/d						
Control	1.2 ^a	14.2 ^b	-19.3 ^c	5.89	0.43	<0.01
Monensin	2.9 ^a	23.7 ^b	-22.2 ^c	5.59		

¹No interactions present ($P > 0.58$).

²Pooled standard error of least squares means (control $n = 7$; monensin $n = 8$).

^{a,b,c}Within a row, means across treatments without a common superscript differ between days ($P < 0.05$).

Table B9. Nitrogen losses as a percent of nitrogen intake and excretion of limit-fed heifers receiving corn stalk-based diets with or without 150 mg/d of monensin

Item	Days on study			SEM ²	Probability ¹	
	14	42	161		Treatment	Day
N excretion, % of total N excretion						
Feces						
Control	62.3	55.8	62.9	2.44	0.04	0.13
Monensin	57.4	54.7	58.2	2.29		
Urine						
Control	37.7	44.3	37.1	2.44	0.04	0.13
Monensin	42.6	45.3	41.8	2.29		
N excretion, % of N intake						
Feces						
Control	61.5 ^a	47.2 ^b	76.1 ^c	5.23	0.18	<0.01
Monensin	55.3 ^a	41.4 ^b	75.1 ^c	4.95		
Urine						
Control	37.2 ^a	37.7 ^a	44.1 ^b	2.83	0.15	<0.01
Monensin	41.3 ^a	34.7 ^a	51.4 ^b	2.66		
Apparent N digested, % of N intake						
Control	38.5 ^a	52.8 ^b	23.9 ^c	4.88	0.09	<0.01
Monensin	44.7 ^a	58.6 ^b	27.3 ^c	4.62		
N retained, % of N intake						
Control	6.2 ^a	16.1 ^b	28.2 ^c	4.82	0.50	<0.01
Monensin	6.6 ^a	24.0 ^b	29.5 ^c	4.54		

¹No interactions present ($P > 0.25$).

²Pooled standard error of least squares means (control $n = 7$; monensin $n = 8$).

^{a,b,c}Within a row, means across treatments without a common superscript differ between days ($P < 0.05$).

APPENDIX C

CHAPTER III TABLES AND FIGURES

TABLES

Table C1. Nutrient composition of limit-fed TMR

Component	WS ¹	BG	AL	MS	Concentrate
As fed basis					
DM, %	86.28	85.66	80.26	85.83	81.77
DM basis					
OM, %	91.78	92.21	89.85	87.42	93.55
NDF, %	86.66	76.97	62.24	81.61	40.69
ADF, %	56.39	41.31	43.94	48.09	7.32
CP, %	3.31	9.42	16.97	5.72	21.41
Starch, %	0.30	1.97	1.50	0.37	28.35
GE, mcal/kg	4.13	4.38	4.30	3.98	4.40
Mean particle size, cm	1.57	1.55	0.89	1.50	0.38

¹WS = wheat straw; BG = bermudagrass; AL = alfalfa; MS = milo stalks.

Table C2. Intake and diet digestion in steers receiving a limit-fed total mixed ration

Item	Treatment ¹				SEM ²	Probability
	WS	BG	AL	MS		Trt
DM intake, kg/d	3.83 ^a	3.56 ^b	3.43 ^c	3.87 ^d	0.00	<0.01
Energy intake, Mcal/d						
GE	16.50 ^a	15.66 ^b	14.96 ^c	16.42 ^a	0.04	<0.01
DE	11.97 ^a	11.07 ^b	10.24 ^c	10.94 ^{bc}	0.25	<0.01
Digestibility, %						
DM	72.4 ^a	71.0 ^a	67.6 ^{ab}	65.5 ^b	1.70	0.03
OM	76.1 ^a	73.8 ^{ab}	71.7 ^b	70.0 ^b	1.47	0.04
NDF	72.4 ^a	69.3 ^{ab}	60.6 ^c	63.7 ^{bc}	2.41	0.01
ADF	60.5 ^a	51.8 ^{ab}	39.3 ^c	41.7 ^{bc}	3.76	<0.01
Starch	98.4	98.0	97.7	97.6	0.53	0.60

¹WS = wheat straw; BG = bermudagrass; AL = alfalfa; MS = milo stalks.

²Pooled standard error of least squares means ($n = 6$).

^{a,b,c}Within a row, means without a common superscript differ ($P < 0.05$).

Table C3. Ruminal pH and volatile fatty acid concentration of steers receiving limit-fed diets

Item	Treatment ¹				SEM ²	Probability		
	WS	BG	AL	MS		Trt	h	T × h
Ruminal pH	6.46 ^{ab}	6.40 ^b	6.51 ^a	6.51 ^a	0.03	0.03	<0.01	0.72
Total VFA, mM	67.28	69.10	67.97	63.38	1.55	0.06	<0.01	0.90
Molar percent								
Acetate	60.88 ^b	61.42 ^{ab}	62.47 ^a	62.38 ^a	0.45	0.05	<0.01	0.12
Propionate	20.73 ^a	19.51 ^b	19.23 ^b	20.26 ^{ab}	0.40	0.05	<0.01	0.17
Butyrate	11.74	12.63	11.84	11.83	0.34	0.19	<0.01	0.26
Isobutyrate	1.46	1.43	1.49	1.27	0.10	0.36	<0.01	0.34
Isovalerate	3.40	3.25	3.37	2.88	0.21	0.23	<0.01	0.45
Valerate	1.81	1.74	1.60	1.38	0.13	0.10	<0.01	0.06
Acetate:Propionate	2.98 ^b	3.23 ^a	3.30 ^a	3.13 ^{ab}	0.08	0.04	<0.01	0.06

¹WS = wheat straw; BG = bermudagrass; AL = alfalfa; MS = milo stalks.

²Pooled standard error of least squares means ($n = 6$).

^{a,b,c}Within a row, means without a common superscript differ ($P < 0.05$).

Table C4. Ruminal fill and solid passage rate in steers receiving a limit-fed total mixed ration

Item	Treatment ¹				SEM ²	Probability		
	WS	BG	AL	MS		Trt	h	T × h
Ruminal fill, kg								
DM	4.67	4.49	5.15	5.28	0.30	0.18	<0.01	0.44
Liquid	47.16 ^{ab}	42.69 ^b	48.58 ^a	48.85 ^a	1.70	0.05	<0.01	0.95
Ruminal solid passage rate, %/hr	3.57 ^a	1.29 ^b	0.70 ^b	1.30 ^b	0.61	0.01		

¹WS = wheat straw; BG = bermudagrass; AL = alfalfa; MS = milo stalks.

²Pooled standard error of least squares means ($n = 6$).

^{a,b}Within a row, means without a common superscript differ ($P < 0.05$).

Table C5. Dry matter intake and ruminal fill of steers during refeeding of *ad libitum* bermudagrass hay after restricted intake of a TMR

Item	Day of refeeding						SEM ³	Probability
	Pre-trial ¹	0	3	6	10	13		Day
DM intake, kg/d	7.28 ^a	3.76 ^c	6.19 ^b	6.61 ^b	6.31 ^b	6.69 ^b	0.30	0.03
Ruminal fill, kg								
DM	10.57 ^a	4.59 ^d	8.46 ^{bc}	9.17 ^b	8.30 ^c	9.02 ^{bc}	0.31	<0.01
Liquid	77.01 ^a	45.99 ^d	65.26 ^c	71.11 ^b	74.97 ^{ab}	78.79 ^a	1.68	<0.01

¹*Ad libitum* DM intake and ruminal fill prior to restricted intake.

²DM intake and ruminal fill at end of restricted feeding period.

³Pooled standard error of least squares means ($n = 7$).

^{a,b,c,d}Within a row, means without a common superscript differ ($P < 0.05$).

FIGURES

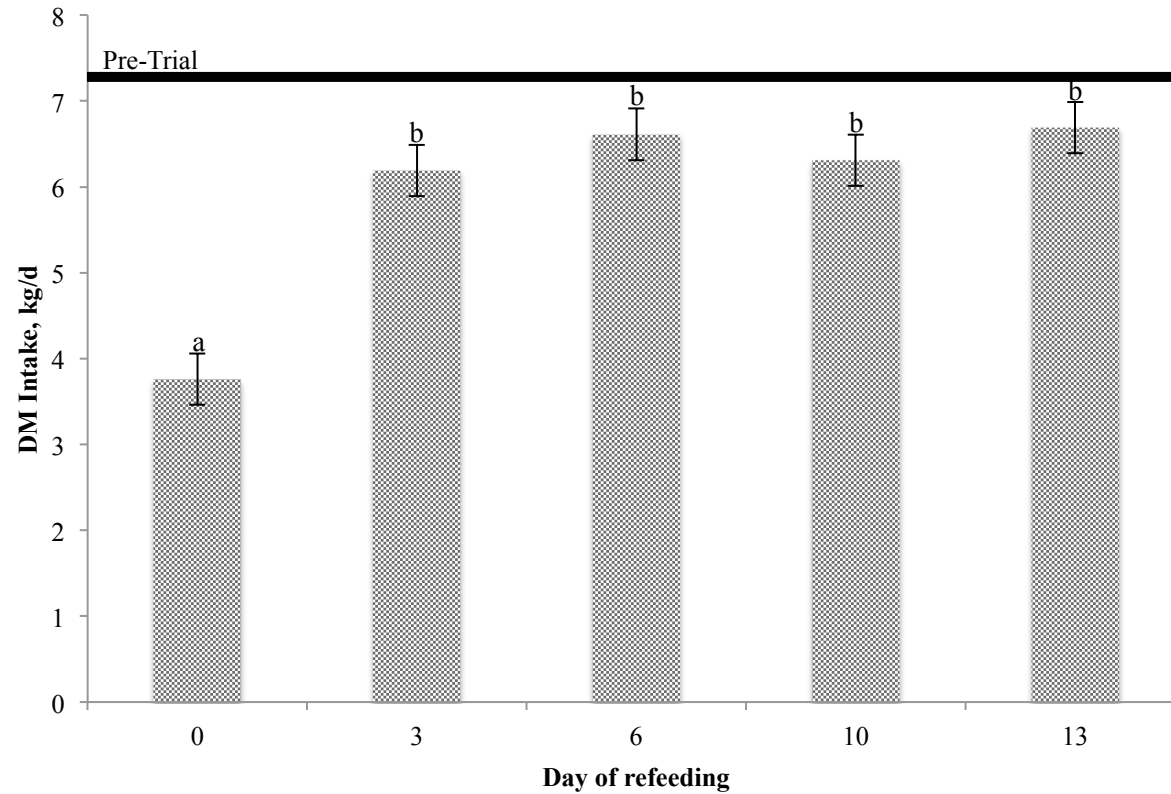


Figure C1. Dry matter intake of steers during refeeding of *ad libitum* bermudagrass hay after restricted intake. Pre-trial = *ad libitum* DM intake prior to restricted intake. Day 0 = limit-fed DM intake averaged across all treatments. Means without a common superscript differ ($P < 0.05$)

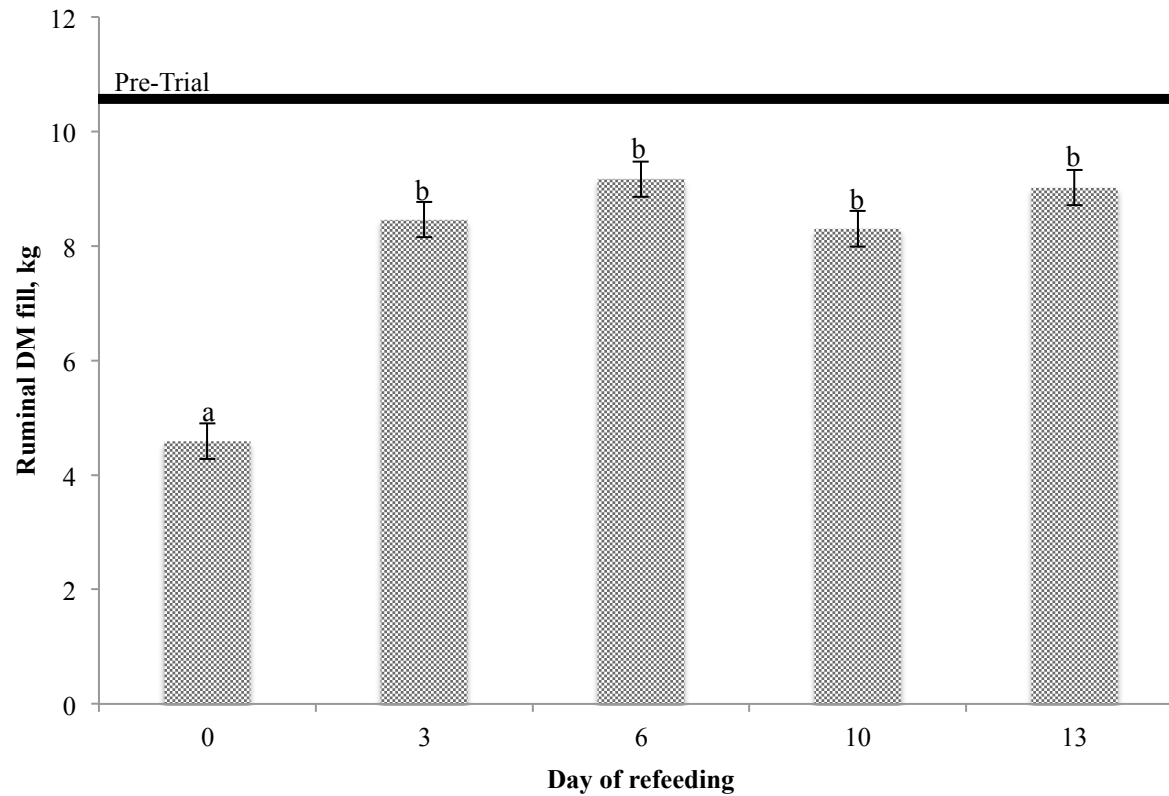


Figure C2. Ruminal DM fill of steers during refeeding of *ad libitum* bermudagrass hay after restricted intake. Pre-trial = *ad libitum* ruminal DM fill prior to restricted intake. Day 0 = limit-fed ruminal DM fill averaged across all treatments. Means without a common superscript differ ($P < 0.05$).

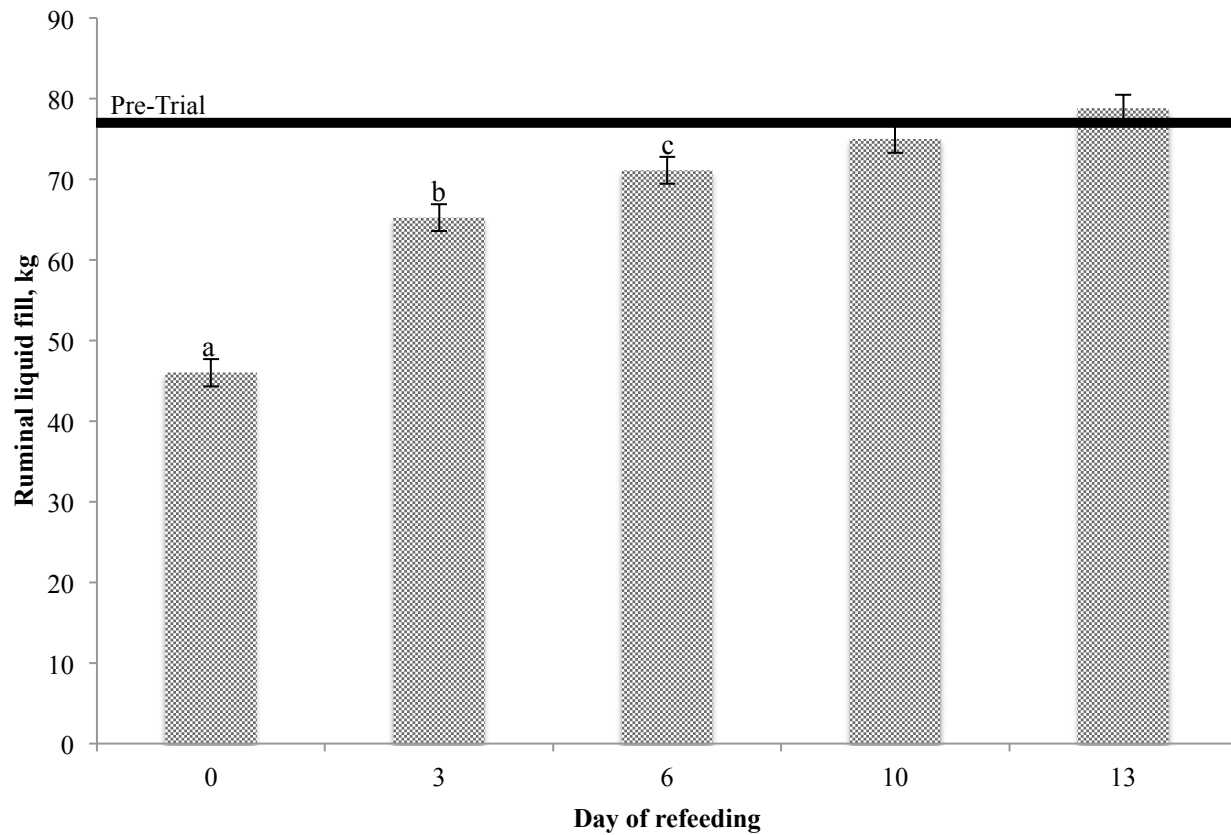


Figure C3. Ruminal liquid fill of steers during refeeding of *ad libitum* bermudagrass hay after restricted intake. Pre-trial = *ad libitum* ruminal liquid fill prior to restricted intake. Day 0 = limit-fed ruminal liquid fill average across all treatments. Means without a common superscript differ ($P < 0.05$).