

5-2020

Effects of Lasalocid and Energy Supplementation on Forage Intake, Energy Metabolism, and Performance of Cattle Grazing Wheat Pasture

Autumn Tayler Pickett
University of Arkansas, Fayetteville

Follow this and additional works at: <https://scholarworks.uark.edu/etd>



Part of the [Animal Experimentation and Research Commons](#), [Animal Studies Commons](#), and the [Beef Science Commons](#)

Citation

Pickett, A. T. (2020). Effects of Lasalocid and Energy Supplementation on Forage Intake, Energy Metabolism, and Performance of Cattle Grazing Wheat Pasture. *Theses and Dissertations* Retrieved from <https://scholarworks.uark.edu/etd/3627>

This Thesis is brought to you for free and open access by ScholarWorks@UARK. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of ScholarWorks@UARK. For more information, please contact ccmiddle@uark.edu.

Effects of Lasalocid and Energy Supplementation on
Forage Intake, Energy Metabolism, and Performance
of Cattle Grazing Wheat Pasture

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Animal Science

by

Autumn Tayler Pickett
Southern Arkansas University
Bachelor of Science in Agricultural Sciences, 2018

May 2020
University of Arkansas

This thesis is approved for recommendation to the Graduate Council

Elizabeth Kegley, Ph.D.
Thesis Director

Stacey Gunter, Ph.D.
Committee Member

Paul Beck, Ph.D.
Committee Member

Abstract

Cattle grazing wheat pasture have the potential to gain BW exceptionally well, but excessive nitrogen intake results in increased excretion and increased greenhouse gas (GHG) emissions. Supplemental concentrates with the addition of an ionophore given to ruminants grazing wheat is a potential practice for producers to increase nitrogen efficiency while decreasing GHG emissions. Therefore, the objective of this experiment was to quantify the effects of energy (2.95 kg/d) and lasalocid (200 mg/hd/d) supplementation on nutrient intake, energy metabolism, respiratory gas fluxes, and performance of grazing cattle. Methane emissions were not affected ($P = 0.58$) by treatment, hence methane intensity ($P = 0.07$) and yield ($P < 0.01$) were reduced for supplemented cattle. Supplemented cattle had greater CO₂ emissions ($P = 0.04$) and O₂ consumption ($P = 0.03$). Average daily gain tended to be greater for supplemented cattle ($P = 0.09$) compared to Control (1.22 and 1.00 kg, respectively); but no effect ($P = 0.88$) was observed with the lasalocid. Fecal output was greater for supplemented cattle ($P < 0.01$), but forage intake was lower ($P < 0.01$) and nutrient intake was higher ($P < 0.01$) for supplemented cattle compared to Control. Supplemented cattle had lower forage intake with greater CO₂ emissions and O₂ consumption, but lasalocid did not affect any parameter measured. For Year 1 performance each kilogram of supplement increased ($P = 0.04$) ADG by 73 g/d; however, lasalocid did not increase ($P = 0.73$) ADG (avg ADG = 1.7 kg). Total BW gain by each steer (kg) was increased ($P = 0.04$) 4.7 kg for each kilogram of supplement fed daily and again, lasalocid did not increase ($P = 0.73$) performance (avg total BW gain = 109 kg). For Year 2 performance each kilogram of supplement increased ($P = 0.001$) ADG by 58 g/d; however, lasalocid did not increase ($P = 0.17$) ADG (avg ADG = 1.4 kg). Total BW gain by each steer (kg) was increased ($P = 0.001$) 3.7 kg for each kilogram of supplement fed daily and again, lasalocid did not increase ($P = 0.17$) performance (avg total BW gain = 88 kg).

Acknowledgements

It truly has been a privilege to work with the so many great people throughout the course of my master's project. First off, I would like to express my sincere gratitude to Dr. Stacey Gunter for giving me this opportunity and his continuous support of the project. His guidance helped me to be successful in my research and writing of this thesis. I could have not imagined having a better research advisor. I would also like to thank Dr. Elizabeth Kegley for acting as my academic advisor and pushing me to my fullest potential while I was in Arkansas. Also thank you for correcting all my abstracts and countless presentations throughout the year. Thank you, Dr. Paul Beck, for your expertise and guidance throughout my master's project. You all supported me greatly and were always willing to help with anything I needed.

Thank you to the USDA – ARS employees, especially thanks to the Range Crew and Tonya, for helping to set up everything I needed to make this project possible and assisting with my data collection and analysis. I don't know how this would have been possible without their assistance and patience with me. I would also like to personally thank Reagan Cauble for being patient enough to teach me lab techniques and statistics and keeping me sane throughout this entire experience, I don't think I would have made it through without your help. Thank you to all the other Graduate Students that helped in every aspect of my master's project as well.

I owe many thanks to my family and friends, your love, support, and impact on my life has shaped me into the person that I am today and for that I am grateful. One person I owe everything to is my mother, you may not be here anymore, but you made such an impact on my life and I am who I am because of you. I appreciate the constant encouragement as I have worked towards my degree. I could never thank everyone enough for everything they have done in my life.

Table of Contents

| | |
|--|----|
| Chapter 1: Literature Review | 1 |
| Introduction..... | 1 |
| Literature Review..... | 4 |
| Microbes and Microbial Fermentation..... | 4 |
| The Rumen and Rumen Microbes..... | 4 |
| Rumen Microbiome..... | 4 |
| Microbial Fermentation Products..... | 6 |
| Volatile Fatty Acids..... | 6 |
| Gas Composition..... | 7 |
| Ruminants and Greenhouse Gases..... | 7 |
| Methane Production..... | 8 |
| Mitigating Methane Production..... | 8 |
| Measuring Gas Emissions in Grazing Cattle..... | 9 |
| Open-Circuit Gas Quantification System..... | 10 |
| Estimating Forage Intake..... | 11 |
| Markers and Predicting Intake..... | 12 |
| External Markers..... | 12 |
| Ionophores and their Effects..... | 13 |
| Mode of Action..... | 14 |
| Ionophores on the Market..... | 14 |
| Effect on Performance and Intake..... | 15 |
| Effect on VFA Concentrations..... | 16 |
| Effect on Respiratory Gas Production..... | 16 |
| Supplementation of Grazing Cattle..... | 17 |
| Effect on Forage Intake..... | 18 |
| Effect on Animal Performance..... | 18 |
| Effect on Respiratory Gas Production..... | 19 |
| Summary of Literature Review..... | 21 |
| Chapter 2: Metabolism Experiment | 22 |
| Abstract..... | 22 |

| | |
|---|-----------|
| Study Site and Management..... | 23 |
| Pretrial Adaptation | 23 |
| Treatments and Experimental Design | 25 |
| Lab Analysis..... | 26 |
| Statistical Analysis | 27 |
| Results and Discussion..... | 28 |
| Forage and Supplement Chemical Composition | 28 |
| Methane Emissions..... | 29 |
| Carbon Dioxide Emissions and Oxygen Consumption | 32 |
| Forage Intake and Fecal Analysis..... | 34 |
| Performance..... | 36 |
| Chapter 3: Performance Trial | 44 |
| Abstract | 44 |
| Treatments and Experimental Design | 45 |
| Lab Analysis..... | 47 |
| Statistical Analysis | 47 |
| Results and Discussion..... | 47 |
| Forage and Chemical Composition | 47 |
| Performance..... | 48 |
| Implications..... | 55 |
| Literature Cited | 56 |

List of Tables

| | |
|---|----|
| Table 1: Chemical composition and digestibility of wheat forage masticate | 39 |
| Table 2: Chemical composition and digestibility of supplement..... | 40 |
| Table 3: Treatment effects on respiratory gas fluxes..... | 41 |
| Table 4: Intake estimates and digestibility parameters, DM basis..... | 42 |
| Table 5: Growth performance measures, metabolism trial..... | 43 |
| Table 6: Chemical composition, digestibility, and minerals of wheat pasture | 52 |
| Table 7: Year 1 performance and intake measures..... | 53 |
| Table 8: Year 2 performance and intake measures..... | 54 |

Chapter 1: Literature Review

Introduction

The livestock sector represents a significant source of greenhouse gas (**GHG**) emissions worldwide, generating carbon dioxide, methane, and nitrous oxide. These gases are made either directly, from enteric fermentation and manure management, or indirectly from feed production and conversion of forest into pastures. Methane is a very potent greenhouse gas that is a natural by-product of ruminal fermentation with a global warming potential 28 times that of CO₂ over a 100-yr time frame (IPCC, 2013). As much as 12% of the gross energy (**GE**) consumed by grazing cattle can be lost to the environment as methane (Johnson and Ward, 1996). Due to the negative effect that enteric methane emissions have on the efficiency of beef cattle and the environment, there is a need to develop strategies to reduce methane emissions without reducing net return to the beef cattle industry. Such data concerning wheat pasture grazing is lacking and leaves a large gap due to the large number of cattle that utilize these pastures every year.

Each year up to 7 million head of stocker cattle graze wheat each winter in Oklahoma and the southern Great Plains (Horn, 2006). The forage is high in crude protein and highly digestible and can support various classes of grazing livestock and cattle producers have used it for growing heifers, stocker cattle, and to some extent finishing cattle (Horn, 1983). These small-grain pastures are an important forage resource; however, forage mass often varies greatly over the grazing period and limits the forage intake and average daily gain (**ADG**) of the cattle. Wheat pastures are also known for excessive nitrogen (**N**) intake by the grazing ruminants resulting in the inefficient use of the forage. Feedstuffs in the rumen are hydrolyzed into glucose and other hexoses and pentoses, then monosaccharides are further metabolized causing the release of metabolic hydrogen. This hydrogen is then converted to dihydrogen through hydrogenase

activity transferred to the methanogenic archaea that use it to reduce CO₂ and other one-carbon compounds through the hydrogenotrophic pathway (Beauchemin et al. 2020). The rumen fermentation process results in the expulsion of greenhouse gases (mainly methane and nitrous oxide) from grazing ruminants as well as decreased performance for producers. The economic reality of growing cattle has stocker operators seeking opportunities to increase BW gain to help mitigate the economic volatility of the stocker phase, decrease greenhouse gas emissions, and increase the inefficiency in these cattle. Supplementation of these cattle is a possible management strategy that could be utilized by producers to obtain a more efficient beef cattle production system.

Supplemental concentrate has been introduced into production settings for a variety of reasons which include; an increase in ADG, stocking rate, and carcass grade; to extend forage during period of lower growth or bad weather, to enhance cattle management, reduce health or disease problems, supply additives, and possibly to increase profits to the cattle producers (Wagner et al., 1983). There has also been research that demonstrated that the introduction of highly digestible supplements that are low in protein augment ADG as well as increase the efficiency of nutrient utilization. With the increase in animal productivity and overall efficiency these supplements could also produce a positive impact on the greenhouse gas emissions from these grazing animals by altered ruminal metabolism or decreased methane intensity relative to unit of BW gain. Supplement for grazing cattle also allows the producer to introduce additives into the diet and may result in boosted performance or alter rumen metabolism.

One type of additive that are is currently in use is ionophores. There are different ionophores commercially available in the U.S., including lasalocid, the trade name is Bovatec, which was developed for growing and finishing cattle. This polyether ionophore was cleared by the Food

and Drug Administration in December 1984 and is reported to increase BW gain of cattle grazing pasture (Andersen and Horn, 1987). Ionophores are known to increase energy availability and N retention which increase feed efficiency, which in turn improves animal productivity. (Potter, et al., 1985; Russell et al., 1989). Since there is an increasing concern of global climate change and enteric methane emissions by domestic ruminants, ionophores are highly sought after in both production and environmental standpoints.

Wheat pastures in the Southern Plains are a unique production strategy where income can be reaped from the grazed forage followed by a grain harvest. Due to the large number of cattle that utilize these pastures, knowledge on how to optimize the cattle performance and utilize the forage efficiently is desired. The overall performance of these cattle is desirable, however N intake by these grazing animals is excessive. Supplemental grain that is highly digestible and low in protein enhances the BW gain of these cattle while also improving N utilization. When incorporating a supplementation program there is also the opportunity to supply feed additives to the grazing ruminants. Among these is the ionophore lasalocid, it alters ruminal fermentation and increases feed efficiency. This antibiotic is marketed to increase BW gains and also decrease the amount of methane emissions from cattle grazing pastures. With growing concerns of global climate change and the desire to maximize BW gains among cattle producers, implementing a supplementation program with the addition of lasalocid could positively impact both the production portion of agriculture and help to lessen the anthropogenic emissions by grazing ruminants. The objective of this experiment is to quantify the effects of supplemental energy and lasalocid supplementation on forage intake, energy metabolism, respiratory gas fluxes, and growth performance of cattle grazing winter-wheat pasture.

Literature Review

Microbes and Microbial Fermentation

The Rumen and Rumen Microbes

Microorganisms, bacteria, fungi, and protozoa inhabit the rumen and participate in a symbiotic relationship with the ruminant animal. This symbiotic relationship allows the organisms to live in association and both benefit from this relationship. Cattle provide a host environment for the microbes. Specifically, the rumen environment is moist because of host saliva and water consumption, is maintained at 39°C, an optimal temperature for enzyme activity, is primarily anaerobic, and provides continuous substrate availability (Millen et al., 2016). The microbes that are located in the rumen provide the enzymes that convert the low-quality, fibrous, plant material into useable energy for the ruminant (Cammack et al., 2018). This fibrous plant material contains a large number of glucose units joined together by Beta 1-4 bonds and is a structural component of primary cell walls of green plants. The mammals do not produce the enzyme, called cellulase, to break this bond to liberate the glucose, however, the microbes produce this enzyme as well as other enzymes that will break the Beta 1-4 bonds that make the monosaccharides unavailable to mammals without this symbiotic relationship. The microbes also allow for the synthesis of amino acids and vitamins absorbed in the small intestine for the health of the host (Millen et al., 2016). This symbiosis between the rumen microbiota and the host depends on the balance of the host environment and fermentation.

Rumen Microbiome

When examining the rumen microbiome, there are four main categories of microorganisms. The categories include bacteria, protozoa, and fungi. Bacteria is the predominant microbe, with numbers being approximately 10^{10} to 10^{11} cells/g of rumen contents (Church, 1988), account for

70 to 80% of the entire microbe population of the rumen (McAllister et al., 1994). The main targets for the bacteria include cellulose, hemicellulose, pectin, starch, and amino acids which are all predominantly found in forage-based diets (Puniya et al., 2015);(Stewart et al., 1997). The number of protozoa in the microbe population is very low, 0.01% or fewer of these microbial cells in the rumen, however it does account for nearly 50% of the biomass in the rumen (Williams et al., 1986; Sylvester et al., 2005). The protozoa have been documented to provide 62% of the cellulolytic activity (Coleman, 1985), but it has also been reported with a much lower activity level in other studies. Protozoa can engulf bacteria and feed particles and digest carbohydrates, proteins, and fats (Williams and Coleman, 1992). The final category of microbes are fungi and is reported to account for 5% to 20% of the biomass of the rumen. Fungi produce enzymes that are necessary for digestion of the plant fiber including cellulase, xylanase, and other hydrolases. Every category of microbe has a specific role and allows for the fermentation process of turning plant material into products for the ruminant animal.

Archaea, which is mostly comprised of methane-producing microbes, account for around 2% to 4% of the total microbiome mass. This group of microbes is very important however since they serve as an electron sink in the fermentation process (Cammack et al., 2018). The methanogens use intermediates of cellulolytic bacterial and anaerobic fungi fermentation to generate methane and adenine triphosphate (**ATP**). Henderson et al. (2015) reported that 77.7% of archaea were hydrogenotrophic methanogens, while 22.1% had the ability to grow with hydrogen plus methyl groups derived from methanol or methylamines. Methanogens able to form methane from acetate were extremely rare (<0.015%). Carbohydrates are the main dietary source of energy for ruminants and are hydrolyzed to glucose and other hexoses and pentoses, then monosaccharides are further metabolized to VFA, H₂, CH₄, and CO₂. During the metabolism of monosaccharides

metabolic hydrogen is released, and this is later converted to dihydrogen through hydrogenase activity. The dihydrogen is available in two forms, dissolved and gaseous, with dissolved being the only form being available to the microorganisms (Wang et al., 2014). Dihydrogen is then transferred to methanogenic archaea that use it to reduce CO₂ and other one-carbon compounds through the hydrogenotrophic pathway to CH₄ (Beauchemin et al. 2020). The differences in rumen microbial communities show variations in methane formation and the conversion of feed to animal products. Understanding the communities within the ruminant animal is key to understanding the transformation of plant material to both desirable and useful ruminant products.

Microbial Fermentation Products

Volatile Fatty Acids

The process of microbial digestion produces many different end products. The number one and most important product being volatile fatty acids (VFA). These VFA serve as the ruminant's main source of energy. There are three main VFA, which include acetate (two carbons), propionate (three carbon), and butyrate (four carbon). All of these are derived from monosaccharides metabolism and are absorbed through the walls of the rumen, and then they are transported by the blood to the liver. After they enter the liver they are converted to other sources of energy. The ratio of acetate: propionate: butyrate can vary from around 75:15:10 to 40:40:20. The ratios of propionate and butyrate can vary greatly based on the diet of the host ruminant (Bergman, 1990). According to one study that compared varying roughage levels and the concentrations of VFA in the rumen, the net production of propionate more than doubled on the low roughage diet with numerically lower production of acetate and butyrate (Sutton et al.,

2003). Higher concentrate diets result in a greater energy value compared to higher roughage diets.

Gas Composition

The composition of gases found in the rumen can vary from animal to animal and can even change from day to day. The change in amounts can be caused by shifts in the rumen ecology and the fermentation balance. The majority of the time the composition of gases includes over 67% carbon dioxide, 26% methane, 7% nitrogen, 0.5% oxygen, and 0.5% hydrogen (Kleiber et al., 1943). These gases escape the rumen in a process called eructation, which eradicates the gases and releases them into the environment and atmosphere. These gases are produced by the microorganism that inhabit the rumen environment and takes part in the microbial degradation of feedstuffs. Carbon dioxide production occurs when bacteria metabolize carbohydrates. Two of the main gases that the environmental sector is worried about is the production of methane and nitrous oxide (minor gas from rumen but major gas from feces compost) by ruminant animals due to the greater impact these gases have on the environment compared to carbon dioxide.

Ruminants and Greenhouse Gases

The agriculture sector represents a significant source of the greenhouse gas emissions in the world. These greenhouse gases include carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O). These gases can be produced directly by enteric fermentation or the decomposition of the manure and other organic material, or they can be produced indirectly from feed production and conversion of forests into pastures (Hristov et al., 2015). It is estimated that the livestock sector alone contributes about 13% of the total anthropogenic emissions (US EPA, 2016). Enteric fermentation and manure decomposition, the processes responsible for CH₄ and N₂O emissions, are the focal point for greenhouse gas emission mitigation for the livestock industries. While

N₂O is an important greenhouse gas, more emphasis is being put on mitigation strategies for CH₄ due to the larger percentage of emissions from the ruminant animals. Ruminants produce CH₄ as part of their normal digestive processes (direct emission), and it represents almost one third of the emissions from the agriculture economic sector.

Methane Production

Methane is an end product produced by hydrogen utilizing methanogenic archaea in the rumen. The methanogenic microbes are established early in life, even in pre-ruminant stages (Guzman et al., 2015). According to the Environmental Protection Agency (US EPA, 2016), methane was estimated to account for 10% of the total greenhouse gas emissions, and enteric fermentation accounts for 25% of the total methane emissions in the United States. Even if ruminants produce more carbon dioxide, which also contributes to greenhouse gases, methane is more efficient at trapping radiation. Methane has a comparative impact of 25 times greater than carbon dioxide over a 100-year period (US EPA, 2016). This production of methane also results in a 2% to 12% energy loss in the ruminant host (Johnson and Ward, 1996). Considering the animal inefficiency and the environmental impacts that methane production causes, methane mitigation strategies are highly desired.

Mitigating Methane Production

Since methane is a major concern with regards to the greenhouse gases and global warming there are research efforts underway to decrease ruminant animals' methane production. Many studies are trying different feeds and feed additives that may reduce the total amount of methane produced. One method is feeding small amounts of nitrate to cattle. This added nitrate would replace carbon dioxide as an electron acceptor, which in turn would result in the production of ammonia instead of methane. While this did reduce enteric methane emissions, there was an

issue with some toxicity, but this could be avoided with gradual acclimation of nitrate to the ruminants (Lee and Beauchemin, 2014). Another method that has been investigated is the addition of lipid sources to the diet such as tallow, sunflower oil, and whole sunflowers. All three lipid sources decreased methane emissions by an average of 17% when corrected for digestible energy intake (Beauchemin et al., 2007).

Both gross methane and methane yield are heritable traits (Pinares-Patiño et al., 2013); variation in methane yield can be attributed to direct host genetic influence independent of feed intake (Roehe et al., 2016). Thus, breeding an genetic selection may be used as a mitigation strategy. Diets can also affect the amount of methane production, forage based-diets are known to have greater methane emissions compared to concentrate-based diets per unit of BW gain. This increase can be associated with the increase in hydrogen availability caused by the increased pH, and in turn will be transformed into methane and released into the atmosphere. One method that the agriculture industry is starting to investigate is the use of ionophores to not only control methane emissions but also increase feed efficiency in the ruminant animal.

Measuring Gas Emissions in Grazing Cattle

With ruminants being labeled a source of enteric methane, which is a known anthropogenic greenhouse gas, systems are being developed to measure these methane emissions from grazing cattle. One method that is used regularly are respiration chambers. These chambers are used to collect all exhaled breath from the animal and measure the methane concentration. The chambers are known as the standard method for estimating these methane emissions however, they can be inaccurate simply because it can create an artificial environment for the animal. This can greatly affect the animal's behavior and emissions (McGinn et al., 2006). These chambers yield results that cannot be applied to grazing cattle.

A very common method used to measure this enteric methane is the SF₆ tracer technique. This technique is described as an inert tracer gas that is placed in the rumen (Jonker et al., 2016). Release rate of SF₆ through a permeation membrane is measured before the bolus containing the gas is put into the rumen. A capillary tube that is attached to a halter is placed on the animal's head and connected to an evacuated sampling canister. The CH₄ and SF₆ concentrations are determined by gas chromatography or other methods. This technique can also be accomplished by replacing the SF₆ with CO₂ as the tracer gas. The CH₄ to CO₂ ratio in the production of air is measured at regular intervals and combined with the calculated total daily CO₂ production of the ruminants. The calculations in this technique are the same as the SF₆ tracer but it is just a simple replacement of tracer gas to CO₂ (Storm et al., 2012).

Open-Circuit Gas Quantification System

Another way that researchers are collecting enteric methane emissions is using an open-circuit gas quantification system (**GQS**; C-Lock, Inc., Rapid City, SD). This machine consists of a head chamber that cattle grazing pasture can visit (3 to 8 min/visit; 3 to 6 visits/d). The animals are enticed to use this system by a small amount of bait the system drops when visited by the grazing animals. While the animal is consuming the feed the GQS captures the animal's breath cloud by exhausting air through the system. The breath is then analyzed for methane, carbon dioxide, and oxygen concentrations (Gunter and Beck, 2018). The measured concentrations that are collected from the system are uploaded to a server and processed further using algorithms to calculate total daily emissions and consumption of the desired gas for the study (Hristov et al., 2015). This method was more accurate than the use of tracers, which underestimate the CH₄ emissions by an average of 4% relative to the chamber technique (McGinn et al., 2006). According to Jonker et al., (2016) the use of the GQS did not differ from the gas concentrations found while using the

chambers, however, the use of the SF₆ yielded lower concentrations of CH₄. While the chamber and the GQS did not differ, the use of the GQS is a more accurate representation of grazing cattle emissions.

Estimating Forage Intake

When it comes to grazing research, being able to quantify dry matter intake (**DMI**) by animals is necessary for the estimation of nutrient consumption (Macon et al., 2014). However, it is very difficult to estimate DMI of grazing animals due to the fact that forage intake by the ruminants cannot be directly monitored or controlled. This difficulty is increased due to different factors that can change basic mechanisms such as selective grazing, herbage mass, sward structure and composition, climatic and environmental factors, and the complexities of the grazing process (Gunter, 2017). Techniques for estimating forage intake include the use of internal and external markers, ingestive behavior, disappearance of herbage mass, prediction from forage characteristics, and animal performance (Macon et al., 2014). All of these techniques have both their advantages and disadvantages, and it is important to note that all the techniques are just an estimation and not the true amount of forage intake. There are several direct methods used to estimate forage intake such as herbage mass changes, prediction from forage characteristics, or calculation of energy requirements for observed animal performance. However, these measurements are only adequate when estimating groups of animals or a pasture rather than the individual animal. Since some research has focused on individual animal performance on grazing forages, a more indirect approach is needed. The most commonly used being internal and external markers.

Markers and Predicting Intake

Due to the difficulty in making direct determinations of intake for individual animals in a group fed situation, a number of indirect methods have evolved and are being introduced into production and research situations. Markers are defined as an indigestible substance that are not secreted or absorbed by the animal, have passage rates similar to feeds, can be recovered completely after ingestion and allow for practical and precise chemical analysis (Velásquez et al., 2018). However, none of the substances in today's market meet all of the requirements for a suitable marker, but there are some that are adequate to use in research situations. The marker technique to estimate intake uses an external marker to estimate fecal output (**FO**) and an internal marker is used to estimate dry matter digestibility (**DMD**). These are then utilized to estimate intake by dividing FO by the indigestibility of the diet. There are some concerns with these markers, however with various issues arising from study to study. In grazing research there is a focus on how much forage intake is occurring from animal to animal so external markers are a desired means of evaluating individual animal intake.

External Markers

Several researchers have described the ideal marker and one of the main properties is that the daily dose of the marker can be recovered in the feces within 24 h. Two of the commonly used external markers are chromic oxide (Cr_2O_3) and titanium dioxide (TiO_2). Both the markers have this ability, and each has variable recovery rates. Studies with forage fed ruminants utilizing Cr_2O_3 are difficult due to the recovery rates evaluated in the feces of these animals. Fecal recovery (**FR**) rates have ranged from 0.80 to 1.23 g/g of DM (Velásquez et al., 2018). There are several studies that utilize this marker but there have also been some concerns with the use of this because of the carcinogenic effects of this chemical in livestock. Due to the carcinogenic

effects of Cr₂O₃, and that it is not legal to feed to livestock, the introduction of TiO₂ is being evaluated as an external marker in grazing livestock.

Since the majority of the research has focused on other external markers there is little research in the use of TiO₂ in predicting intake. This marker is an alternative to Cr₂O₃ in digestion studies for various ruminants. This marker can be legally added to the feed as a color additive in amounts that do not exceed 1% of the finished product, according to the Association of American Feed Control Officials (AAFCO) in 1996. Since FR rates are of such importance evaluating the usefulness of TiO₂ is needed. Just like Cr₂O₃ there are varying rates of FR for TiO₂. There seems to be greater variation in the recovery rates of this marker. The recovery rates varied from 0.90 to 1.02 g/g of DM in some studies, while others reported a greater recovery rate (Hafez et al., 1988;). Velásquez et al. (2017) strayed from the usual FR rates and reported recovery rate of 1.83 to 1.99 g/g of DM. Due to the lack of research with this external marker, more research is needed to determine the effectiveness of this marker.

Ionophores and their Effects

The rumen fermentation process reduces the efficiency of conversion of some feeds to useable products, therefore strategies to increase feed efficiency are highly sought after. Some of these strategies include heat treating, which alter protein structure, and coating the feed with inert ingredients to make them unavailable to the microbes (Callaway et al., 2003). These techniques would allow the nutrients to by-pass the ruminal fermentation process. Another method is introducing ionophores into the diet of ruminant animals. Ionophores, which were first used for controlling intestinal parasites in poultry, are now commonly used to improve the efficiency of fermentation while also decreasing the amount of methane released into the atmosphere by ruminant animals. Other benefits in ionophore supplementation include a decrease of dietary

protein deamination (less urinary ammonia excretion) and less lactic acid production in the rumen, resulting in lower incidence of acidosis and liver abscesses (Callaway, et al., 2003).

Ionophores are known to increase energy availability and nitrogen retention which increase feed efficiency, which in turn improves animal productivity. (Potter, et al., 1976a; Russell and Strobel, 1989).

Mode of Action

Ionophores are a type of non-medically important antibiotic used in ruminant animal production that alter ruminal fermentation. Normal fermentation ends with production of more acetate compared to propionate. Acetate is a less efficient VFA since it cannot directly be converted to glucose and ends with a net loss of two carbon dioxides. The loss of these carbons contributes to the production of methane and increases the amount of methane excreted into the atmosphere by the ruminant (Elanco Animal Health, 2015). Hook et al. (2009) indicated that the largest effect of ionophores on rumen microbiome is not a change in quantity or diversity of the methanogens but rather a shift from the gram-positive to gram-negative organisms. This shift in bacteria causes the ruminal fermentation to change from acetate to propionate. With a conversion of propionate to glucose there is no net loss of carbon and allows for a more efficient pathway in the rumen. The inhibition of acetogenic bacteria allows for an increase in energy status and an increase in feed efficiency, while also decreasing the amount of methane production in ruminant animals.

Ionophores on the Market

There are three main ionophores used in today's market. These include monensin (Rumensin), lasalocid (Bovatec), and laidlomycin propionate (Cattalyst). Monensin is the most widely used ionophore and is used most often in feedlot situations. This ionophore works to alter ruminant fermentation by decreasing methane production, the acetate to propionate ratio, and protein

degradation to ammonia (Russell and Houlihan, 2003). It also increases ruminal pH by decreasing VFA and lactate production (Domescik and Martin, 1999). Lasalocid is a polyether ionophore that is commonly used for increased rate of BW gain of grazing cattle (Andersen and Horn, 1987). While this ionophore is more known for its success in grazing cattle it can also be used in feedlot situations. Laidlomycin propionate is very similar in structure and function to the more widely used monensin but was more potent in its ability to alter the acetate: propionate ratio and decrease lactate accumulation (Domescik and Martin, 1999). This ionophore was specifically designed to be used in high-energy rations such as in feedlots. All ionophores have been used to increase feed efficiency while also decreasing the enteric methane.

Effect on Performance and Intake

The use ionophores is highly sought after mostly for their effect on ADG and feed efficiency in the ruminant animal. As a general rule, in forage settings the intake will be similar with greater BW gains or even an increase in forage intake. Several grazing studies with different forage qualities yielded different results for each quality of forage. These studies identified that poor-quality forage intake is depressed, medium quality intake is increased, and high-quality forages show a decrease in intake (Potter et al., 1976). Winter-wheat pastures being on the higher quality level, it is believed that intake will be decreased. This is thought to be caused by ionophores increasing metabolic efficiency and in turn would decrease the quantity of forage intake required to meet the animal's energy requirement (Ellis et al., 1983). According to Andersen and Horn, (1987) and Mir, (1994) the introduction of these ionophores do not change forage intake. Both studies found no change in the overall forage intake but, they did report a greater ADG in cattle that consumed ionophores. With no change being observed in forage intake but a greater ADG

being achieved in these cattle the use of ionophores seems to increase feed efficiency in these cattle.

Effect on VFA Concentrations

The addition of the ionophores and their impact on the ruminal fermentation process help to increase the feed efficiency. Studies associated with the use of ionophores in growing cattle reported changes in the ration of VFA in the rumen, increasing propionate and reducing molar percentages of butyric and acetic acid (Ellis et al., 2012). This shift would provide more energy from feed to the animal through increased overall glucose supply, increased production of propionate from the rumen increases hepatic gluconeogenic flux (Stocks and Allen, 2012). Bell et al. (2017) reported that the use of monensin (fed at a rate of 200 mg/d) decreased total VFA concentration, molar percentage of acetate (72.5 to 71.2%) and increased molar percentage of propionate (16.9 to 18.7%). This shift in acetate and propionate yielded a reduced acetate: propionate ratio from 4.34 to 3.85. Similar studies that have tested the effect of ionophores on cattle performance, while consuming forages, follow a similar trend even though some do not report any greater ADG for the animals.

Effect on Respiratory Gas Production

As mentioned earlier these antibiotics are marketed as decreasing methane production, which is becoming a greater concern as the years pass. In a normal rumen setting, the acetate levels are greater than that of propionate. The higher level of acetate in the rumen is considered less efficient and in fact causes a net carbon loss in the animal. This carbon is then emitted from the animal in the form of CH₄. When the rumen microbiome is fed ionophores, there is a shift where more propionate is being produced and nets no carbon loss (Domescik and Martin, 1999). This is thought to decrease the enteric methane produced by the ruminant animal. One study suggests

that the antibiotics act in the rumen by selecting for “succinate-forming *Bacteroides* and for *S. ruminantium*” which are considered propionate producers. This selection could lead to an increase in the formation of rumen propionate (Chin and Wolin, 1979). The increase in propionate would be associated with a decrease in methanogenic bacteria within the ruminant animal.

A study examining the efficacy of ionophores for mitigating enteric methane observed a decrease in enteric CH₄ by 27 to 30% for cattle receiving the concentrate diets. The supplementation of ionophores did not alter the total VFA concentrations but it did decrease the acetate: propionate ratio (Guan et al., 2006). Another study had different results, however Paisley and Horn (1988) conducted a study examining the effect of monensin and lasalocid and observed different effects for each one. The cattle receiving lasalocid showed a greater acetate concentration compared to the steers that were on the monensin treatment group. The steers receiving monensin had greater propionate ratio while the lasalocid steers experienced a greater butyrate amount. Although the shifts in VFA ratios were a little different between the ionophores the gas production was similar in the controls and the treatment steers.

Supplementation of Grazing Cattle

The use of a supplementation program for cattle consuming small grain diets such as wheat may be considered for various reasons. Some of these reasons include; an increase in ADG, stocking rate, carcass grade, to extend grass during short months or bad weather, to enhance cattle management, to reduce health or disease problems by supplying additives, and to possibly increase profits for cattle producers (Wagner et al., 1983).

Effect on Forage Intake

With cattle consuming forages, intake of available energy may not be adequate to meet the desired rates of animal performance. With grazing cattle, the amount of forage consumed depends on three factors: the availability of suitable forage, the physical and chemical composition of the forage, and the nutrient requirements of the animal (Minson, 1982). The introduction of this energy into the diet directly impacts the amount of voluntary forage intake. A data base study conducted by Moore et al. (1999) showed that intake was both decreased and increased by supplementation. This shift in forage intake was shown to change with the type of forage that was being grazed. Minson (1982) mentioned that the physical and chemical composition of the forage can directly affect the amount of forage consumed, and this effect can be observed from the data-base study by Moore et al. (1999). The majority of the increases in intake were associated with native forage, while the decreases were often observed when improved cool and warm season forages were offered to the cattle. Another study where four different supplements were being evaluated resulted in similar results. Steers that were fed an energy supplement decreased grazing time, intensity, and harvesting efficiency, they also experienced greater daily gain (Bodine and Purvis, 2003). This decrease in forage intake allows for greater stocking rates, extension of the available forage during short months or bad weather, and enhancement of cattle management.

Effect on Animal Performance

Growing cattle on winter wheat pasture is an important component of the beef cattle industry in the Southern Great Plains. With the concern of inadequate nutrition being provided, supplemental energy may be introduced into the diet to achieve the desired performance. Supplementation programs work to enhance the performance of the cattle as well as help the

producer to be more profitable. In several studies the level of performance for the cattle receiving supplementation are either greater or less than the expected values. The difference between the expected and observed performance depends on the associative effects of supplements upon the intake and energy concentration within the diet. Moore et al. (1999) examined the effects of supplementation on ADG and reported a wide variety of figures from less than 0.02 kg/d to greater than 0.40 kg/d increase in ADG for cattle fed a supplement. When looking specifically at grain-based supplements, the average increase in ADG ranged from 0.10 to > 0.40 kg/d. One study looking at energy and mineral supplementation reported a greater ADG for steers receiving supplementation with the ADG of the cattle ranging from 0.72 to 1.15 kg/d (Fieser et al., 2007). Wagner et al. (1983) again reported varying results from supplementation programs, on average cattle receiving supplement ranged from 1.60 to 3.00 kg ADG. Energy supplementation for cattle grazing small-grains pasture such as winter wheat, helps offset the large amounts of ruminally degradable N and obtains desired performance for the ruminants.

Effect on Respiratory Gas Production

One concern with the utilization of wheat pastures is the end products of the fermentation process. There is considerable variation among different diet types and the associative losses for the ruminant. High forage diets have greater waste product emission compared to other types of diets. Grazing ruminants experience high levels of rumen degradable protein within the diet, and therefore causing inefficient use of the forage along with greater amounts of carbon and nitrogen losses. These ruminants produce three of the most important greenhouse gases, which include CO₂, CH₄, and N₂O. The introduction of a supplementation program is a promising mitigation strategy for these greenhouse gases by manipulating the diet to improve the balance of nutrient inputs.

This inclusion of a supplement reduces emissions from grazing livestock. One study reported the addition of dried distiller grains (**DDG**), which are rich in fat, significantly decreased CH₄ at 12, 24, 36, and 48 h when compared to cattle receiving no supplement (Fonseca et al., 2017). The addition of this fat source reduces or eliminates protozoa as well as methanogenic bacteria in the rumen, and shifts the hydrogen sink through bio-hydrogenation to propionate production (Massé et al., 2014). Feeding high levels of low-fiber, starch-based energy supplements reduces forage intake and digestion so there is some concern with decreased performance for these cattle. However, it is thought that supplementation with high-fiber energy sources could increase ruminally available energy greatly altering the rumen environment. This excess of available energy may allow for a more suitable environment for fibrolytic microbes, and also enhance incorporation of rumen ammonia into microbial N by utilizing an alternate hydrogen sink. This would allow for consumption and use of forage nutrients to be sustained and utilized within the ruminant animal, therefore emitting less waste products. This would allow for less excreted nitrogen and degradable organic carbon, which would reduce CH₄ and N₂O emissions from grazing ruminants (Montes et al., 2013). Supplementation of these ruminants allows for an overall more efficient system within the animal, which would in turn reduce the environmental footprint of the livestock sector.

Summary of Literature Review

Cool-season annuals, such as wheat in the Southern Great Plains, provide an outstanding way for producers to grow cattle. Cattle grazing this forage have the potential to gain exceptionally well while grazing, however due to the excess N inclusion in the diet, efficiency of forage nutrient utilization is reduced. Grazing ruminants also have increased enteric methane production, therefore increasing emissions from the agriculture sector and increasing the overall greenhouse gases in the environment. Due to the inefficiency of forage nutrient utilization by these ruminants and the increase in greenhouse gases, the inclusion of supplemental concentrate is a possible solution for these problems.

Supplemental concentrates fed to ruminants grazing cool-season forages has been shown to increase performance and forage nutrient utilization and can be used to help extend the available forage for the cattle. When considering a supplementation program, the producer can also supply additives to these cattle such as ionophores. These ionophores work to alter rumen fermentation of these animals and create more efficient pathways in the rumen. They have been reported to increase performance while also decreasing greenhouse gas emissions from grazing livestock. With increasing concerns about climate change and a desire for increased animal production for producers, a way to impact both are sought after. The increased performance, decrease in anthropogenic greenhouse gases, and the overall increase in efficiency associated with animals fed an energy and ionophore based feed needs further investigation of their use and impact on the livestock sector.

Chapter 2: Metabolism Experiment

The metabolism experiment took place in fall of 2018 at the Southern Plains Experimental Range of the USDA, Agricultural Research Service located near Fort Supply, Oklahoma. Eighteen heifers were utilized in this study. The experiment started in October 2018 and consisted of two 21-d trials with 7 d in between the two blocks. The final BW were collected December 2018 when the study concluded.

Abstract

Cattle grazing wheat have the potential to gain BW exceptionally well, but excessive nitrogen intake results in increased excretion and increased greenhouse gas (GHG) emissions. Supplemental grain with the addition of an ionophore given to ruminants grazing wheat is a potential practice for producers to increase nitrogen efficiency while decreasing GHG emissions. Therefore, the objective of this experiment was to quantify the effects of energy and lasalocid supplementation on nutrient intake, energy metabolism, respiratory gas fluxes, and performance of grazing cattle. Heifers ($n = 18$) were used in a 49-d experiment that consisted of two 21-d Blocks with 7 d between them (initial BW = 311 ± 17.3 kg and 339 ± 16.7 kg, Block 1 and 2, respectively). Heifers were assigned randomly to one of three treatments: no supplement (CONTR, $n = 6$), 2.95 kg byproduct – fiber based control feed/d (ENR, $n = 6$), or 2.95 kg feed with lasalocid/d (LAS, $n = 6$). Cattle grazed an 8.1-ha paddock of winter wheat and had access to a GreenFeed system used to quantify the respiratory gas fluxes (CH_4 , CO_2 , and O_2). Cattle were weighed on d 0 and 22 of each block, on d 15 to 21 cattle were gathered every 12 h and fecal samples were collected. Data were analyzed using PROC MIXED in SAS (SAS Inst., Inc., Cary, NC). ADG tended to be greater for supplemented cattle ($P = 0.09$) compared to CONTR (1.22 and 1.00 kg, respectively); but no effect ($P = 0.88$) was observed for the LAS. Methane

emissions were not affected ($P = 0.58$) by treatment hence, methane intensity ($P = 0.07$) and yield ($P < 0.01$) were lower for supplemented cattle. Supplemented cattle had greater CO₂ emissions ($P = 0.04$) and O₂ consumption ($P = 0.03$). Fecal output was greater for supplemented cattle ($P < 0.01$). Forage intake was lower ($P < 0.01$) but total nutrient intake was greater ($P < 0.01$) for supplemented cattle compared to CONTR. Supplemented cattle had lower forage intake with greater CO₂ emissions and O₂ consumption, but the LAS did not affect any parameter measured.

Study Site and Management

The pasture used for this experiment consisted of 41-ha of level soils (0 to 1% slope) interspace with areas of heavier textured soils and has no well-defined drainage patterns. The pasture is located along the flood plain of the Beaver River and consists of Lincoln soils (loamy coarse sands). A center pivot irrigation system is located in the middle of the pasture which was utilized to maintain appropriate soil moisture throughout the experiment to promote forage growth. To help maintain the forage production 22 kg/ha of nitrogen was added to the field in late August and again in early September when the field was seeded. Wheat was planted in early September at a rate of 135 kg/ha. The 18 heifers were allowed to graze an 8.1-ha paddock, constructed from an electric fence, within the 41-ha pasture. To ensure that the heifers always had adequate forage the herbage mass was monitored and maintained above 1,000 kg/ha. Water was provided to the cattle by a trough filled by shallow wells (sulfates = 264 mg/L).

Pretrial Adaptation

To quantify the effects of the supplementation and LAS on respiratory gas fluxes an open-circuit gas quantification system (GreenFeed System [GQS; C-Lock, Inc., Rapid City, SD]) was used. According to Gunter and Beck (2018) a month of adaptation to the system is recommended

therefore, before the start of this study the cattle used in this experiment went through a pretrial adaptation period to the GQS. Briefly, one month prior to the study a group of 50 red Angus growing heifers, located at the Southern Plains Experimental Range in Fort Supply, Oklahoma, were gathered, weighed, and assigned a Radio Frequency Identification (**RFID**) tag. The individual RFID tag allowed the GQS to identify each heifer and record each time the animal visited the system. To help with the adaptation process the side panels attached to the head chamber were removed, and the heifers were gathered each morning for one week and placed in a smaller pen with the GQS. Due to the number of animals, after the cattle were observed using the machine daily, they were placed in another pasture to allow the remaining cattle the opportunity to use the GQS. During the last week before the start of the metabolism study all heifers were placed back in the pasture with the machine to observe the animals that were using the machine most frequently. The 18 cattle that were shown to use the system the greatest were selected for the metabolism experiment.

In addition to the adaptation to the GQS, the heifers were also exposed to the individual stanchions used for supplementation of the cattle during the study. Once a week the cattle were gathered, separated into groups of twelve, and were sorted into the individual pens where they received a small amount of alfalfa pellets. This process allowed for the animals to acclimate to the process of being individually fed and was used to lower the incidence of feed refusals when the study began. Cattle were also exposed to the use of an electric fence since the paddock used during the experiment was constructed from the same material. A small fence was built inside the corral, where the water was located for the cattle, so the heifers would have to walk past the fence. This brief exposure to the fence allowed them to experience the type of fence that would be used during the experiment to impart the boundaries.

Treatments and Experimental Design

Eighteen heifers were used to determine the effects of energy and lasalocid supplementation on forage intake, energy metabolism, and respiratory gas fluxes. The three treatments were applied in a randomized complete block design. Cattle were first divided into two groups where they were assigned to receive no supplement and acted as the control ($n = 6$), or they received a 14% CP byproduct fiber-based supplement at a rate of 2.95 kg/d ($n = 12$). The twelve heifers fed supplement were divided into two treatments, one receiving Purina 4-square Stocker/Grower 14 ($n = 6$) and the other receiving the 4-square Stocker/Grower 14 B60 ($n = 6$). Each morning the heifers were gathered and sorted and heifers that were assigned to receive supplement were put into their individual feeding stanchions where they received their respective supplement. All feed refusals were weighed and documented to be used to calculate daily supplement intake per heifer. After the end of Block 1 the cattle were rerandomized into the same treatments for Block 2 of the experiment.

All the cattle had ad libitum access to the wheat pasture and the GQS, where they could move freely in and out of the system. At each visit to the GQS the RFID ear tag assigned to each animal was recognized and when a feeding event was allowed, allotments of alfalfa pellets (32 ± 1.6 g of pellets/drop) were dropped at 24-second intervals with up to 8-drops/visit. There was a maximum of two visits/day with 10.5-hrs between each allowed visit, which evenly spaced out the feeding events throughout the day to control for circadian variation in greenhouse gas emission rates (Gunter and Bradford, 2015). To predict forage intake during this experiment the heifers received 128 g/d of a TiO_2 labelled wheat pelleted middling-based feed that contained 2% TiO_2 on a DM basis. The heifers were dosed daily using the GQS. The GQS was equipped with two feed bins, one contained the TiO_2 labelled feed and the other contained un-marked alfalfa

pellets. The GQS recognized each animal individually when they visited and on the first visit each day all received four drops of the TiO₂ marked pellet and the remaining four drops were the un-marked pellets. At the second visit the cattle received eight drops of only the un-marked alfalfa pellets. To ensure the GQS was collecting an accurate representation of the gas emissions from each animal it was calibrated once a week.

To determine performance during the relatively short experimental period of each block on d 0 and 22 of each block the heifers were gathered in the afternoon and placed in a pen without feed or water until the next morning (approximately 17 h). Cattle were then individually weighed, and this was used to calculate total BW gain and ADG. Sample collection occurred the last seven days of each block following a 14-d diet adaptation for each heifer. Samples of supplement and feces were collected from d 15 through 21, with supplement samples collected once daily and feces collected twice daily. Fecal samples were obtained by grab sampling from the rectum at 12 h intervals. On d 16 of each block, two ruminally fistulated steers (care described under the IACUC approved SOP for the Maintenance of Ruminally Cannulated Steers), that were adapted to grazing with the heifers, were used to obtain masticate samples representative of the heifers' diet. The rumen content of the steers was evacuated and then the steers were allowed to graze for ~30 minutes. After the grazing period, the samples were collected directly from the rumen and immediately frozen.

Lab Analysis

Fecal samples and supplement samples were placed in a forced-air oven at 60°C until dry and allowed to air-equilibrate. Masticate samples were composited for each block and lyophilized. All samples were then ground to pass a 2-mm screen through a Wiley Mill. From the individual fecal samples, a composite sample for each heifer and block were constructed by taking a small

sample (2 g) from the individual samples and mixing them to have a representative sample. Supplement samples were composited by block to allow for a characteristic example of the supplemental grain received.

Individual fecal, grain and masticate samples were analyzed for Ti concentration using x-ray fluorescence by the Delta Premium Soil Exploration Analyzer (Olympus Scientific Solutions America, Inc.; Waltham, MA). All composite samples were analyzed for neutral detergent fiber (**NDF**) and acid detergent fiber (**ADF**) according to Van Soest et al. (1991). Samples were also analyzed for nitrogen and calories via combustion calorimeter as described by the AOAC (1990). *In vitro* OM disappearance was determined according to Tilley and Terry (1963) as modified by White et al. (1981).

All samples were analyzed for indigestible ADF as described by Bohnert et al. (2002). Triplicate samples (0.5 g) of feces, supplement, and ruminal particulate were weighed into Ankom bags (F57; Ankom Co., Fairport, NY). The supplement and masticate samples were incubated for 16 h at 39°C in a solution containing 0.1% pepsin and 10% 1 N HCl using a DaisyII incubator.

Samples were then rinsed with warm (39°C) tap water and placed into four separate lingerie bags (33 samples and 2 blanks per bag). Samples were placed in four ruminally fistulated animals for 96 h in the rumen that were consuming low-quality forage ad libitum. The samples were then removed from the rumen, rinsed with warm (39°C) tap water until the rinse water was clear, and finally they were analyzed for ADF as described by Van Soest (1991).

Statistical Analysis

Outliers for CH₄ and CO₂ emissions and O₂ consumption were identified using the GLIMMIX procedure of SAS (SAS Inst., Inc., Cary, NC) by calculating a student residual. Any respiratory gas flux with a calculated student residual of greater than 3.0 or less than -3.0 were removed. The

removed values included all the quantified emissions from that visit to the GQS. All data were analyzed as a completely randomized block design using the MIXED procedure of SAS. The experimental unit was heifer with a fixed effect of treatment. For emissions/consumption and forage intake/fecal output the covariable of BW was included in the model. For GE of the fecal samples, ash content was used as a covariable in the analysis. Two contrasts were included: 1) supplemented vs. control cattle and 2) energy vs. ionophore cattle.

Results and Discussion

Forage and Supplement Chemical Composition

Chemical composition and digestibility of the wheat forage, for Block 1 and Block 2 is shown in Table 1. Two ruminally cannulated steers were used to collect masticate samples representative of the diet for the grazing cattle and samples were composited by block. *In vitro* DM and OM digestibility and calories were somewhat lower in Block 1 compared to Block 2. Ankom true digestibility of DM and OM were similar for both blocks. Between the two sampling dates the forage was in a state of rapid growth reaching higher levels of maturity causing an increase in the NDF and ADF concentrations for the forage. With the increase in maturity there was also a decrease in the nitrogen concentration within the forage which showed a concomitant decrease in the percentage of CP in the masticate samples.

For this experiment a control feed and one containing lasalocid were used. Chemical composition and digestibility of the two supplements are shown in Table 2. While the two feeds were supposed to be similar, other than the addition of the ionophore, there were numerical differences between them. The control feed had greater percentages of IVDMD and IVOMD compared to the feed containing the lasalocid. Ankom true digestibility of DM and OM and the calories were similar between the two batches. The NDF and ADF concentrations were lower for

the control feed. Numerical differences were also detected for nitrogen concentration, the ionophore feed had greater nitrogen, and therefore greater CP values, compared to the control feed. Even with these differences the feed did meet the guaranteed analysis on the feed label.

Methane Emissions

Enteric methane emissions were measured using the GQS that was placed in the pasture with the grazing cattle. The last 7 d of block were used to represent the individual animal methane emissions. Individual animal daily methane emission was expressed as methane production (g CH₄/animal per day). Along with daily emissions values, methane yield (g CH₄/ kg of DMI) and methane intensity (g CH₄/kg of BW and g CH₄/kg of ADG) were calculated. Daily methane emissions were not affected by treatment ($P = 0.58$) with an average value of 194 ± 7 g CH₄/animal per day. Thompson et al. (2019) reported a similar effect when supplementing cattle on wheat pasture with an energy supplement that included monensin. While their values of mean daily emissions were lower, 173 ± 12 g/d, there was no effect of treatment detected. Ebert et al. (2016) and Jiao et al. (2014) reported greater values of daily methane emission ranging from 272 to 351 g/d but they reported no treatment differences when including supplemental grain to grazing cattle.

While daily methane emissions were not affected by treatment, there were some differences observed for methane yield and intensity. No differences were observed for g CH₄/kg of BW between the treatments ($P = 0.31$). There was a tendency for the supplemented cattle ($P = 0.07$) to have lower methane intensity (173 g of CH₄/kg of BW gain) compared to the CONTR (245 g of CH₄/kg of BW gain), but no differences were detected between the ENR and LAS group ($P = 0.74$). This is in line with Beck et al. (2018), which reported that cattle with increasing levels of concentrate in the diet had lower emission intensity compared to cattle receiving no supplement.

This mitigation would be expected due to the increase ADG associated with the supplemented cattle but the mean total daily emissions remaining unchanged. Methane yield was affected by treatment ($P < 0.01$) which can be seen in the contrasts. Supplemented cattle had lower methane yield (37 g of CH₄/kg of DMI) compared to CONTR cattle (55 g of CH₄/kg of DMI) ($P < 0.01$), but the addition of LAS had no impact ($P = 0.50$). Methane yield estimates were greater for this experiment compared to previous experiments for cattle grazing high-quality forages (Grainger et al., 2010; Ebert et al., 2016) where values ranged from 18.1 to 27.2 g of CH₄/kg of DMI. The decrease in methane yield can be explained by the significant increase in daily DMI by addition of the supplement cattle diet but the lack of change in daily total methane emissions.

Carbohydrates are the main dietary source of energy for ruminants and are hydrolyzed to glucose and other hexoses and pentoses, then monosaccharides are further metabolized to VFA, H₂, CH₄, and CO₂. During the metabolism of monosaccharides metabolic hydrogen is released, and this is later converted to dihydrogen through hydrogenase activity. The dihydrogen is available in two forms, dissolved and gaseous, with dissolved being the only form being available to the microorganisms (Wang et al., 2014). Dihydrogen is then transferred to methanogenic archaea that use it to reduce CO₂ and other one-carbon compounds through the hydrogenotrophic pathway to CH₄ (Beauchemin et al. 2020). Methane represents the largest sink for hydrogen in the rumen. Redirecting the hydrogen away from methanogenesis to other fermentation end-products that can be utilized by the host-animal helps to decrease CH₄, emissions but could also benefit productivity via energy conservation.

Manipulating the diet of grazing cattle can be a highly effective CH₄ mitigation approach, but the efficiency depends on its effects on ruminal H₂ flow and concentration, the microbial community, fermentation pathways, residence time of feed in the rumen and interactions among

these factors. Strictly forage-based diets, like the wheat pastures utilized in this project, are associated with increased CH₄ due to the greater levels of rumen degradable protein within the diet causing inefficient use of forage nutrients along with greater amounts of carbon and nitrogen losses (Johnson and Ward, 1996). Supplementation of these cattle with a high-fiber energy source could increase ruminally available energy, therefore altering the rumen environment and allowing a more suitable environment for fibrolytic microbes and enhancing the incorporation of rumen ammonia into microbial nitrogen by utilizing an alternate hydrogen sink. This allows for consumption and use of forage nutrients to be sustained and utilized within the ruminant animal, producing reduced ammonia emissions and degradable organic carbon, which would reduce CH₄ emissions from the grazing cattle (Montes et al., 2013).

Ionophores are a type of non-medically important antibiotics used in ruminant animal production that alters ruminal fermentation and are proposed as a strategy for the mitigation of enteric CH₄ emissions. Hook et al. (2009) indicated that the largest effect of ionophores on rumen microbiome is not a change in quantity or diversity of the methanogens but rather a shift from gram-positive to gram-negative organisms. This shift in bacteria causes the ruminal fermentation to shift from acetate to propionate creating a more efficient pathway within the ruminant animal. Normal fermentation ends with the production of greater levels of acetate compared to propionate. Acetate is considered a less efficient VFA and results in the production of CO₂ and metabolic hydrogen, which is then converted to CH₄ and excreted into the atmosphere by respiration. Ionophores are also known to increase energy availability and nitrogen retention which increases feed efficiency, which in turn improves animal productivity (Potter et al., 1976b; Russell and Strobel, 1989).

This particular study showed that the addition of the ionophore had no impact on the CH₄ emissions. There have been a number of experiments with ionophores as a rumen modifier in various production systems, where CH₄ production was studied as a main objective either from mitigation or from an energy loss perspective. Some studies have shown a reduction in daily emissions by 19 ± 4 g/d (Appuhamy et al., 2013). While some studies showed success with the inclusion of these antibiotics, the overall effect of the ionophore inclusion appears to be inconsistent (Hristov et al., 2015).

Carbon Dioxide Emissions and Oxygen Consumption

Although methane was the main GHG of interest for this experiment, data related to CO₂ emissions and O₂ consumption were also available from the GQS and included in this analysis. There was a tendency for both the CO₂ emissions ($P = 0.09$) and O₂ consumption ($P = 0.08$) to be affected by treatment which can be further explained in the contrasts. Carbon dioxide emissions were affected by ENR and LAS ($P = 0.04$) with increased emissions being observed for the ENR and LAS (7,282 g/d per animal) compared with the CONTR (6,884 g/d per animal). Oxygen consumption followed a similar trend with supplemented cattle having greater consumption compared to CONTR ($P = 0.05$). Both CO₂ emissions ($P = 0.56$) and O₂ consumption ($P = 0.32$) were not affected by the inclusion of LAS into the diet. Using the emissions and consumptions measured for the individual animals the respiratory quotient (**RQ**; mol CO₂ / mol O₂) was also calculated. No treatment effects ($P = 0.84$) were observed for the RQ. No differences were observed for the supplemented cattle vs. control ($P = 0.56$) or the energy vs. ionophore ($P = 0.89$). The RQ is an indicator of metabolic fuel or substrate use in tissues. A ratio of 0.7 is indicative of mixed fat use, whereas a ratio of 1.0 indicates the exclusive use of carbohydrates.

The lack of difference in this experiment shows that all animals were utilizing the same substrate in the tissues.

Carbon dioxide is an additional GHG of concern when looking at it from an environmental standpoint and the emissions associated with the production of livestock. As discussed earlier, in the rumen polysaccharides (mainly cellulose, hemicellulose, and starch) are hydrolyzed to glucose and other hexoses and pentoses. Monosaccharides are then further metabolized into the VFA and some CO₂. Volatile fatty acids are then absorbed from the rumen and have various fates within the body to suit the needs of the ruminant animal. Oxidation of these compounds via the tricarboxylic acid (TCA) cycle eventually produces ATP through the electron transport chain, but this process also yields H₂O and CO₂. Ruminants have a glucose requirement for nervous tissue, red blood cells, and milk sugar production but very little or no glucose passes to the small intestine where absorption occurs, so there is not enough to meet a ruminant's entire requirement for glucose. Therefore, ruminants are always in a constant state of gluconeogenesis, meaning blood glucose is produced via the gluconeogenesis pathway. Propionate is the only VFA that can act as a direct precursor to this pathway, but this also requires large amounts of ATP. This ATP is produced via the electron transport chain.

Forage to concentrate ratios in the diets of ruminants can have a major impact on the metabolism of feedstuffs. Diets containing a higher proportion of concentrate have previously been shown to increase VFA disappearance from the rumen by absorption (Gäbel et al., 1991) and the net absorption rate *in vitro* (Uppal et al., 2003). Greater concentrate diets have also been shown to decrease the acetate:propionate ratios within the rumen. The supplemented cattle in this experiment were experiencing greater amounts of concentrate in their diet, compared to the control cattle that were consuming a high forage diet. This difference in diets could have shifted

the acetate:propionate ratio and possibly increased the VFA disappearance from the rumen by absorption. With the increase in VFA absorption conversion of these products into useable compounds within the animal is expected to increase. Propionate conversion into glucose would be expected to increase with the increased amounts of propionate experienced in supplemented cattle, therefore increasing the need for ATP needed for the pathway. This increased need of ATP would be met using the generated VFA via the TCA cycle and electron transport chain. With the conversion of these compounds the consumption of O₂ would be expected to increase and also increase CO₂ emissions from the supplemented cattle.

Forage Intake and Fecal Analysis

In order to predict forage intake cattle received a daily dose of TiO₂, which acted as an external marker, via the GQS. Fecal samples were then analyzed for Ti concentration and this information was used to calculate estimated forage intake according to Kartchner (1981). Fecal output was greater for supplemented cattle compared to the CONTR ($P < 0.01$; 1.35 and 0.97 kg DM/d for supplemented and CONTR, respectively), but the addition of LAS had no effect ($P = 0.34$).

Forage intake was affected by treatment ($P < 0.01$) which can be seen in the contrasts.

Supplemented cattle had lower forage intake ($P < 0.01$) compared to CONTR cattle, but LAS had no effect. Estimated forage intake values for the cattle were 3.44 and 2.61 kg/d DM for CONTR and supplemented cattle, respectively. While forage intake was lower for supplemented cattle, total intake was greater ($P < 0.01$). Total intake by supplemented cattle was estimated at 5.34 kg/d while control cattle were estimated at 3.44 kg/d DM. The addition of LAS again had no effect ($P = 0.26$) on total intake.

Fecal samples were also analyzed for total nitrogen, crude protein, and gross energy (GE). Total fecal nitrogen was not affected by treatment ($P = 0.20$) with the average amount being 2.3%.

Since CP was calculated from N content of the sample, fecal CP % followed a similar trend. No treatment effects were observed ($P = 0.20$) and both contrasts showed no differences (Supp. $P = 0.43$; LAS $P = 0.11$). Average fecal CP was 14.38%. Fecal GE was affected by treatment ($P = 0.02$). The contrasts show that the addition of an energy supplement increased the fecal GE values ($P = 0.007$), but the addition of LAS had no effect ($P = 0.65$). Values for fecal GE were 2,898, 3057, and 3033 cal/g for CONTR, ENR, and LAS treatment groups, respectively. Cattle that were supplemented had increased fecal GE compared to CONTR cattle. The explanation for this could have been the increase in dietary fat intake, causing greater excretion of fat. This would have greatly affected the GE of the fecal samples for supplemented cattle.

Wheat pastures are an important forage resource for stocker cattle in the southern Great Plains acting as a very nutrient dense forage. However, forage mass often varies greatly and the excessive nitrogen intake by the animals results in the inefficient use of the forage. The introduction of a highly digestible supplement low in protein augment ADG and increase nutrient utilization. Supplementation of cattle grazing wheat pasture is of interest to 1) provide a more balanced nutrient supply and feed additives such as ionophores or bloat preventive compounds, 2) substitute supplement for forage where it is desirable to increase stocking rate in relation to grazing management and/or marketing decisions, and 3) substitute supplement for forage under conditions of low forage standing crops (Horn et al., 2005).

Thompson et al. (2019) and Andersen et al. (1987) estimated voluntary forage intake and reported greater values of forage DMI that ranged from 4.13 to 8.93 kg/d. These values were greater than what was obtained from this trial but estimation of intake by ruminants is a complicated system to understand and monitor. Different influences can affect intake of grazing ruminants such as selective grazing, herbage mass, sward structure and composition, climatic

control and environmental factors, and the grazing process itself (Gunter, 2017). This trial utilized TiO₂ as an external marker to estimate fecal output and forage intake, which has had greater variation in the recovery rates (Hafez et al., 1988; Velásquez et al. 2018). This could have also caused the lower forage intake values obtained from this trial and more research is needed to determine the effectiveness of this marker.

While the forage intake values were lower for this experiment compared to previous research, the impact of the energy supplementation on forage intake was predictable. The addition of the energy supplement decreased forage intake for grazing cattle. Bodine and Purvis (2003) reported that steers fed an energy supplement decreased grazing time, intensity, and harvesting efficiency. Thompson et al. (2019) experienced a decrease in forage intake with increasing levels of supplementation for cattle grazing wheat pasture, but experienced an increase in total DMI with increasing supplement levels. This reflected the results that were obtained in this experiment.

While supplementation did affect intake the addition of the ionophore resulted in no differences, which is in line with previous research. Andersen and Horn (1987) reported that heifers grazing wheat pasture and receiving different levels of lasalocid in the diet experienced no differences in forage intake for the heifers. A project by Bell et al. (2017) measuring the effect of monensin on intake of hay showed that the addition of the ionophore had no effect on intake.

Performance

During the relatively short trial periods performance was assessed by obtaining a shrunk BW at the beginning and end of each Block. No treatment effects were observed for initial and final BW for Block 1 ($P = 0.83$; $P = 0.61$) or Block 2 ($P = 0.63$; $P = 0.58$). Average initial BW was 312 ± 8 and 340 ± 7 kg and average final BW was 330 ± 8 and 368 ± 7 kg for Block 1 and 2, respectively. Body weight change and ADG were calculated for each individual animal. A

tendency was observed in the contrasts with supplemented cattle having a greater BW change ($P = 0.09$) and ADG ($P = 0.09$) compared to the CONTR cattle. Supplemented cattle had an average BW change of 25.02 kg and ADG of 1.22 kg/d, while the CONTR cattle had an average BW change of 20.56 kg and ADG of 1.00 kg/d. The addition of LAS into the diet had no effect on BW change ($P = 0.88$) or ADG ($P = 0.86$). Average daily gain remains the most effective response variable for testing differences between treatments for grazing animals. Grazing researchers have long been concerned with errors in BW because of variances in gut fill. Aiken and Tabler (2004) reported that the use of unshrunk BW reduces the accuracy of the performance measures for grazing cattle during shorter periods. Therefore, a shrunk BW was used for this experiment to try and reduce these issues. But there is still some concern due to the unreliability of BW gain data over shorter periods because of the inconsistent ADG over unshrunk and shrunk BW.

Growing cattle on winter wheat is an important component of the beef cattle industry and supplemental energy can be introduced into the diet to achieve the desired performance. The difference between the expected performance and observed performance depends on the associative effects of supplements upon the intake and energy concentration within the diet. Supplementation for cattle grazing small-grains pasture helps to offset the large amounts of ruminally degradable N which in turn would increase performance. The effect of supplemental grain on performance is well documented in the literature but the values vary. Moore et al. (1999) examined the effects of supplementation on ADG and reported values ranging from 0.02 to 0.40 kg/d. Another study reported greater ADG with cattle gaining 0.72 to 1.15 kg/d for steers receiving concentrate supplement and mineral supplementation (Fieser et al., 2007). The results for our trial showed a tendency for an increase in animal performance for supplemented cattle

which is in line with previous research. The LAS was delivered at a rate of 66 mg/kg of supplement fed. This dosage of LAS is in line with the recommended amount since intakes of LAS in excess of 200 mg have not been shown to be more effective. The relatively short trial period experienced and low number of cattle used may have been the reason why more significant differences were not obtained in this experiment.

While the effect of supplemental concentrate is well documented and follows a similar trend in most research reports, the addition of the ionophore and its effect on performance is highly variable. The use of these antibiotics is highly sought after mostly for their effect on ADG in the ruminant animal. Ionophores work to alter ruminal fermentation and increase overall efficiency in grazing cattle. Andersen and Horn (1987) reported that cattle receiving lasalocid at a rate of 200 mg/d increased ADG by 0.11 kg/d. Oliveira et al. (2020) reported an increase in ADG values ranging from 0.16 to 0.12 kg/d for cattle receiving an ionophore in their diet. This experiment showed that the inclusion of the ionophore had no effect on grazing cattle performance. Due to the short trial period length and the small number of cattle per treatment, the performance of these cattle may not be an accurate representation of the effect of ionophores on growth performance. Performance will be better assessed in the next chapter that includes two years of performance data evaluating the effects of energy and lasalocid supplementation on stocker cattle performance

Table 1. Chemical composition and digestibility of wheat forage masticate

| | Block 1 | Block 2 |
|------------------|---------|---------|
| n ^a | 2 | 2 |
| IVDMD, % | 78.5 | 81.3 |
| IVOMD, % | 80.7 | 83.3 |
| IVDTD, % | 88.7 | 89.7 |
| IVOTD., % | 89.8 | 89.6 |
| NDF | 36.2 | 38.2 |
| ADF | 22.4 | 24.3 |
| Calories, cal/g | 4027.1 | 4268.3 |
| Nitrogen, % | 3.9 | 3.4 |
| Crude protein, % | 24.5 | 21.2 |

Two ruminally cannulated steers were used to collect masticate samples on one day of each block and composited by block.

Block 1 = 11/13/18

Block 2 = 12/11/18

^a n = number of different masticate samples included with each composite

Table 2: Chemical composition and digestibility of supplement

| | Block 1 | | Block 2 | |
|------------------|------------------|------------------|------------------|------------------|
| | LAS ^a | ENR ^b | LAS ^a | ENR ^b |
| n ^c | 6 | 6 | 5 | 6 |
| IVDMD, % | 74.9 | 79.0 | 74.8 | 77.6 |
| IVOMD, % | 75.1 | 78.0 | 74.9 | 76.7 |
| IVDTD, % | 84.9 | 83.9 | 82.9 | 85.3 |
| IVOTD., % | 83.3 | 81.9 | 80.8 | 83.6 |
| NDF | 35.5 | 29.5 | 35.6 | 28.1 |
| ADF | 12.1 | 8.6 | 11.3 | 9.0 |
| Calories, cal/g | 4160.5 | 4126.8 | 4102.4 | 4158.4 |
| Nitrogen, % | 2.8 | 2.7 | 2.8 | 2.60 |
| Crude protein, % | 17.3 | 16.7 | 17.5 | 16.3 |

During the data collection period each morning a grab sample of each supplement was obtained and composited by block to allow for analysis of the supplement

^a LAS = Purina 4-Square Stocker/Grower 14 B60 (Land O'Lakes Purina Feed, LLC; St. Paul, MN)

^b ENR = Purina 4-Square Stocker/Grower 14 (Land O'Lakes Purina Feed, LLC; St. Paul, MN)

^c n = number of samples included with each composite

Table 3: Treatment effects on respiratory gas fluxes

| | Treatments | | | SEM | P-value | |
|----------------------------|------------|------|------|--------|-----------|------|
| | CONTR | ENR | LAS | | Contrasts | |
| | | | | | Supp. | LAS |
| n | 12 | 12 | 12 | - | - | - |
| Methane | | | | | | |
| g CH ₄ /d | 190 | 193 | 201 | 16.78 | 0.47 | 0.50 |
| g CH ₄ / kg ADG | 245 | 165 | 180 | 34.83 | 0.07 | 0.74 |
| g CH ₄ / kg DMI | 55 | 36 | 38 | 2.78 | <0.01 | 0.50 |
| g CH ₄ / kg BW | 0.57 | 0.55 | 0.60 | 0.03 | 0.72 | 0.14 |
| Carbon Dioxide | | | | | | |
| g CO ₂ /d | 6884 | 7216 | 7348 | 202.69 | 0.04 | 0.56 |
| Oxygen | | | | | | |
| g O ₂ /d | 4537 | 4696 | 4820 | 149.58 | 0.05 | 0.34 |
| Respiratory Quotient | 1.10 | 1.11 | 1.11 | 0.01 | 0.56 | 0.89 |

a CONTR = control, no supplementation; ENR = supplementation with no ionophore; LAS = supplementation with the addition of an ionophore

b n = number of animals per treatment

c Contrasts: Supp = CONTR vs. ENR + LAS; LAS = ENR vs. LAS

Table 4: Intake estimates and digestibility parameters, DM basis

| | Treatments ^a | | | | P-value | |
|-------------------------------|-------------------------|-------|-------|------|------------------------|------|
| | CONTR | ENR | LAS | SEM | Contrasts ^c | |
| | | | | | Supp. | LAS |
| n ^b | 12 | 12 | 12 | - | - | - |
| Forage Intake, kg/d | 3.44 | 2.55 | 2.67 | 0.14 | <0.01 | 0.34 |
| Total Intake, kg/d | 3.44 | 5.26 | 5.41 | 0.18 | <0.01 | 0.26 |
| Fecal Output, kg/d | 0.97 | 1.37 | 1.32 | 0.14 | <0.01 | 0.30 |
| Fecal N, % | 2.33 | 2.34 | 2.23 | 0.27 | 0.43 | 0.11 |
| Fecal CP, % | 14.58 | 14.63 | 13.93 | 1.68 | 0.43 | 0.11 |
| Fecal GE ^d , cal/g | 2767 | 3201 | 3021 | 389 | <0.01 | 0.02 |

^a CONTR = control, no supplementation; ENR = supplementation with no ionophore; LAS = supplementation with the addition of the ionophore

^b n = number of animals per treatment

^c Contrasts: Supp = CONTR vs. ENR + LAS; LAS = ENR vs. LAS

^d Fecal GE model included ash content of the samples as a covariable

Table 5: Growth performance measures, metabolism trial

| | Treatments ^a | | | SEM | <i>P</i> -value | |
|-------------------|-------------------------|-------|-------|------|------------------------|------|
| | CON | ENE | ION | | Contrasts ^b | |
| | | | | | Supp. | B60 |
| Block 1 | | | | | | |
| n ^c | 6 | 6 | 6 | - | - | - |
| Initial BW, kg | 308 | 315 | 312 | 8 | 0.57 | 0.83 |
| Final BW, kg | 324 | 334 | 334 | 8 | 0.32 | 0.97 |
| Block 2 | | | | | | |
| n ^c | 6 | 6 | 6 | - | - | - |
| Initial BW, kg | 341 | 345 | 299 | 7 | 0.65 | 0.40 |
| Final BW, kg | 368 | 371 | 327 | 7 | 0.94 | 0.30 |
| Weight Change, kg | 20.56 | 24.80 | 25.25 | 5.00 | 0.09 | 0.88 |
| ADG, kg/d | 1.00 | 1.20 | 1.23 | 0.20 | 0.09 | 0.86 |

^a CONTR = control, no supplementation; ENR = supplementation with no ionophore; LAS = supplementation with the addition of an ionophore

^b n = number of animals per treatment

^c Contrasts: Supp = CONTR vs. ENR + LAS; LAS = ENR vs. LAS

Chapter 3: Performance Trial

The objective of the performance trial was to study a supplement high in digestible fiber at different levels of intake, with or without lasalocid, to examine the effect of energy and lasalocid supplementation on stocker cattle performance grazing winter-wheat pasture.

Abstract

Wheat pasture is unique to the Southern Plains where income can be reaped from the grazed forage followed by a grain harvest. The performance by grazing cattle is potentially excellent, but N intake is excessive, resulting in inefficient nutrient use. Supplemental energy, that is low in protein have been shown to augment ADG and improve N utilization. A total of 144 steers were used to examine a supplement high in digestible fiber at multiple level of intake with or without 66 mg/kg of lasalocid. Sixty-five (324 ± 7 kg) and 79 steers (239 ± 5 kg) for year one and year two, respectively, grazed 41-ha of irrigated wheat pasture for 64 d. The supplement (4-Square Stocker/Grower 14) was placed in one of two SmartFeed Plus feeders (C-Lock, Inc., Rapid City, SD); one feeder with the control feed and the other contained lasalocid. Each feeder was programmed to allow maximum intakes of, 1.2, 2.0, and 3.2 kg/d for year one and 1.36, 2.27, and 3.63 kg/d for year two, for steers at each level. Because steers had liberty to consume supplement at will and only limited by a maximum, actual supplement and LAS intakes were calculated for the grazing period and steer performances were regressed on supplement intakes. For year one, each kilogram of supplement increased ($P = 0.04$) ADG by 73 g/d; however, lasalocid did not increase ($P = 0.73$) ADG (avg ADG = 1.7 kg). Total BW gain by each steer (kg) was increased ($P = 0.04$) 4.7 kg for each kilogram of supplement fed daily and again, lasalocid did not increase ($P = 0.73$) performance (avg total BW gain = 109 kg). For year two, each kilogram of supplement increased ($P = 0.001$) ADG by 58 g/d; however, lasalocid did not

increase ($P = 0.17$) ADG (avg ADG = 1.4 kg). Total BW gain by each steer (kg) was increased ($P = 0.001$) 3.7 kg for each kilogram of supplement fed daily and again, lasalocid did not increase ($P = 0.17$) performance (avg total BW gain = 88 kg). Supplementation with a moderate CP feed increased the ADG of steers grazing winter-wheat pasture, but the addition of lasalocid showed no benefit in this experiment.

Treatments and Experimental Design

In year one a total of 66 steers grazed 41-ha of irrigated wheat pasture for 64 d with an average initial BW of 324 ± 7 kg. Treatments were assigned in a randomized complete block design. The steers were assigned to one of three groups: CONTR ($n = 24$), energy with lasalocid (LAS = 21), and energy with no lasalocid (ENR = 21). Within the LAS supplemented group there were three different levels of intake; the lowest level with steers receiving 1.2 kg/d ($n = 7$), the intermediate level of 2.0 kg/d ($n=7$), and finally the highest level receiving 3.2 kg/d ($n = 7$). The ENR group had the same intake levels offered; 1.2 kg/d ($n = 7$), 2.0 kg/d ($n = 7$), and 3.2 kg/d ($n = 7$). To assess performance a shrunk BW was obtained for each individual steer on d 0, 21, 42, and 64. Steers were gathered and held in a pen for 17 h without feed and water to obtain the individual shrunk BW.

For year two of performance a similar study to year one was performed with a few slight changes. Year two had a total of 79 steers that grazed for 64 d with an average initial BW of 239 ± 5 kg. The steers were assigned in a randomized complete block design to one of three treatment groups: CONTR ($n = 21$), LAS ($n = 28$), and ENR ($n = 30$). Within the two supplemented groups there were three levels of intake offered 1.36, 2.27, and 3.36 kg/d. The LAS supplemented group had a total of 28 steers with 9 on the lowest intake level, 10 at the intermediate, and 9 at the highest level of maximum intake. The ENR supplemented cattle had a

total of 30 steers with 11 on the lowest intake level, 9 at the intermediate level, and 10 on the highest level of intake. To assess performance a shrunk BW was obtained for each individual steer on d 0, 28 and 64.

Two weeks prior to the start of the study cattle were assigned an RFID tag which allowed the SmartFeed system to recognize each individual steer and feed them their respective supplement and intake levels. This pretrial adaptation allowed the steers to acclimate using the system. On d0 of the project cattle were gathered and placed in holding pen for 17 h to adjust for shrink BW and the initial trial BW were collected. After initial BW were taken, about every 21 d the cattle were gathered, and a shrunk BW was obtained. Along with BW, herbage mass and quality were also obtained every 21 d. This was accomplished by clipping forage at 40 paced transects and clipping the forage to the ground on two sides of a 61-cm rod placed between the drill rows in the pasture.

The ENR contained 14% CP (Purina 4-Square Stocker/Grower 14, Land O'Lakes Purina Feed, LLC; St. Paul, MN) and no ionophore, and the LAS supplement contained the ionophore lasalocid (Purina 4-Square Stocker/Grower 14 B60, Land O'Lakes Purina Feed, LLC; St. Paul, MN). The feeds were identical in every aspect of the guaranteed analysis except for the addition of the ionophore at a rate of 66 mg/kg. Feed was placed in two SmartFeed plus feeders with one containing the ENR and the other containing the LAS. The feeders were programmed to allow the maximum intake assigned to each steer. To ensure adequate supplement availability to the cattle the supplement level in the bin was kept with at least 22 kg in excess of what could possibly be consumed by steers in one day. Since the steers had liberty to consume supplement at will and only limited by a maximum, actual supplement intakes were calculated for the grazing period for each individual steer.

Lab Analysis

Lab analysis for this trial included determining the quality of the forage throughout the trial period. The forage was analyzed in composite groups from each day that the forage was clipped from the field. The composite groups were then analyzed for DM by drying at 105°C, ADF and NDF according to Van Soest et al. (1992), minerals using x-ray fluorescence by the Delta Premium Soil Exploration Analyzer (Olympus Scientific Solutions America, Inc.; Waltham, MA), N as described by the AOAC (1990), and finally CP was estimated by multiplying the nitrogen concentration by 6.25.

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS. The experimental unit was steer and the fixed effect for each model was treatment, with two contrasts being included. The two contrasts were the supplemented vs. control cattle and energy vs. ionophore cattle. The regression procedure of SAS was used to estimate performance measures. The model included the ADG and total BW gain regressed by daily supplement intake and daily supplement intake with or without the ionophore. Significance was declared at $P \leq 0.05$ and tendencies were defined between $0.05 < P \leq 0.10$.

Results and Discussion

Forage and Chemical Composition

Chemical composition, digestibility, and mineral analysis of the wheat forage that was available for grazing in year one and year two are shown in Table 6. All analysis measures followed a similar trend for both years. Dry matter and ash followed no particular trend throughout the experiment. The NDF and ADF concentration increased from the beginning of the experiment to the end. Nitrogen concentration decreased throughout the experiment, which caused a

concomitant decrease to CP concentration. In vitro DM and OM digestibility were shown to decrease. These increases in NDF and ADF and decreases in CP and digestibility can be associated with the maturity of the forage. Throughout the experiment the wheat forage was maturing shifting these digestibility and chemical composition values. Mineral analysis of the forage had a little greater variation within the numerical values but did show to have some trends for some of the minerals. Calcium, K, and S were shown to decrease throughout the experiment. The remaining minerals of Fe, Zn, Mn, and Ti had no particular trend. The changes in mineral concentration could also be attributed to the overall increase in forage maturity.

Performance

Year one performance measures are shown in Table 7. For BW, no differences were observed for d 0 ($P = 0.62$), d 21 ($P = 0.95$), d 42 ($P = 0.91$), or d 64 ($P = 0.95$). Total gain and ADG was calculated for each animal. For total BW gain no treatment effect was observed ($P = 0.14$), but when looking at the contrasts, supplemented cattle tended to have a greater total BW gain ($P = 0.08$). Average daily gain followed a similar trend with the supplemented cattle showing a greater ADG when compared to CONTR ($P = 0.08$). The addition of LAS however had no effect on total gain ($P = 0.32$) or ADG ($P = 0.34$) of grazing cattle. The regression procedure of SAS was used to obtain two parameter estimates. It was estimated that each kilogram of supplement consumed increased ADG by 73 g/d ($P = 0.04$) but the addition of LAS did not increase ADG ($P = 0.73$). Average ADG for this trial was 1.7 kg. Additionally, it was estimated that total BW gain was increased 4.7 kg for each kilogram of supplement fed ($P = 0.04$), but again the addition of LAS did not increase performance ($P = 0.73$). By design total intake and daily intake were affected by treatment ($P < 0.01$) with the supplemented cattle having the increased feed intake.

Year two performance measures are shown in Table 8. Since the cattle were randomly assigned to their treatments, and not stratified by BW, there were differences for initial BW ($P = 0.02$). Supplemented cattle ($P = 0.005$) had a greater average initial BW (244 kg) compared to the CONTR (226 kg). This trend was the same throughout the experiment with increased BW for the supplemented cattle at d 28 ($P = 0.002$) and d 64 ($P = 0.001$). Total gain was affected by treatment ($P = 0.007$), which can be further interpreted by the contrasts. Supplemented cattle had greater total gain with an average of 92 kg compared to the CONTR with an average of 82 kg ($P = 0.002$), but LAS had no effect ($P = 0.36$). Average daily gain followed a similar trend as total gain. Treatment affected ADG ($P = 0.007$) with supplemented cattle having greater ADG compared to the CONTR ($P = 0.003$). Average daily gain for supplemented and control cattle was 1.5 and 1.3 kg, respectively. The LAS again had no impact on ADG ($P = 0.34$). It was estimated that each kilogram of supplement consumed increased ADG by 58 g/d ($P = 0.0001$) but the addition of the ionophore did not increase ADG ($P = 0.17$). Average ADG for this trial was 1.4 kg. Additionally, it was estimated that total BW gain was increased 3.7 kg for each kilogram of supplement offered daily ($P = 0.0001$), but again the addition of LAS did not increase performance ($P = 0.17$). By design the total supplement intake and daily supplement intake were affected by treatment ($P < 0.01$). There was an interesting outcome when looking at the contrasts for this year of performance. Cattle that received the control feed compared to the feed that LAS had a tendency for greater total supplement intake ($P = 0.07$) and daily supplement intake ($P = 0.07$). It is not directly apparent why this happened, due to the lack of research reporting any issues with palatability of the ionophore lasalocid.

As discussed briefly in the previous chapter, the effect of supplemental concentrate on performance is well documented in the literature but the values vary. Fieser et al. (2007)

reviewed studies of cattle grazing wheat pasture supplemented with energy and monensin dating back to 1990. In those reports, supplement intake ranged from 0.40 to 2.28 kg/d with an average of 1.14 kg/d. Bodine and Purvis (2003) reported values of ADG around 0.24 to 0.73 kg/d for cattle receiving different types of supplementation. These cattle were grazing dormant winter range however, which could have possibly been the reason for the lower ADG compared to cattle grazing wheat pasture. Thompson et al. (2019) reported average daily gain ranged from 0.64 to 1.67 kg/d with a mean ADG of 1.07 kg/d. The difference between the expected performance and observed performance depends on the associative effects of supplements upon the intake and energy concentration within the diet. However, the overall impact of supplemental grain to grazing cattle tends to be very consistent throughout the research.

The use of ionophores was also examined in this research trial, but previous research with the inclusion of an ionophore is highly variable for grazing cattle. Oliveira et al. (2020) reported an increase in ADG values ranging from 0.16 to 0.12 kg/d for cattle receiving an ionophore in their diet. Another report showed similar values of ADG for cattle receiving lasalocid at a rate of 200 mg/d increased ADG by 0.11 kg/d. Other researchers have reported no differences in ADG for grazing cattle when the diet included an ionophore (Thompson et al., 2019; Mir and Mir, 1994). This trial showed that the addition of the ionophore had no effect on overall cattle performance which is in line with some previous research, but also contradicts some of the research. This just brings in to light the varying results obtained with the inclusion of the ionophore lasalocid for grazing cattle.

It is thought that supplying these grazing cattle with supplements helps to offset the large amounts of ruminally degradable N which in turn would increase forage utilization. This would result in greater energy intake for these ruminants. The energy is released when the feed is

completely oxidized to CO₂ and H₂O. This energy is then partitioned within the animal to various metabolic roles. Energy will first be used to satisfy the net energy of maintenance (NE_M). This portion includes the basal metabolism, daily activity, and thermal regulation. It is the minimum amount of energy required to keep the animal functioning and alive. Once the NE_M is met, the excess energy will be used in the net energy of production (NE_P) which is the energy in a certain product. This can include growth, milk, work, etc. This particular study showed that supplemented cattle had increased ADG compared to control cattle, this was most likely caused by the excess energy they were receiving. The excess energy within these animals would shift to the NE_P for growth which would have caused the change in performance measures. It would also allow these cattle to reach their genetic potential for growth. Overall, this experiment showed that the addition of a higher fiber, low protein energy supplement increased performance measures for grazing cattle, but the addition of the ionophore had no impact on any parameter measure.

Table 6. Chemical composition, digestibility, and minerals of wheat pasture

| | Year 1 | | | | Year 2 | | |
|------------------|-----------|-----------|----------|-----------|-----------|-----------|-----------|
| | 3/20/2018 | 4/10/2018 | 5/1/2018 | 5/23/2018 | 3/20/2019 | 4/17/2019 | 5/22/2019 |
| Date | | | | | | | |
| DM, % | 93.429 | 93.864 | 90.565 | 93.781 | 91.101 | 90.379 | 92.124 |
| Ash, % | 19.833 | 12.939 | 15.325 | 12.892 | 21.056 | 13.822 | 22.670 |
| NDF, % | 33.737 | 36.657 | 44.158 | 52.177 | 36.217 | 43.953 | 53.398 |
| ADF, % | 18.388 | 17.896 | 22.525 | 27.286 | 17.780 | 21.856 | 29.574 |
| Nitrogen, % | 3.258 | 2.690 | 2.241 | 1.591 | 2.778 | 2.219 | 1.455 |
| Crude Protein, % | 20.362 | 16.815 | 14.006 | 9.944 | 17.361 | 13.867 | 9.094 |
| IVDMD, % | 75.673 | 78.063 | 72.710 | 63.108 | 72.324 | 74.736 | 57.632 |
| IVOMD, % | 84.228 | 81.646 | 77.160 | 66.699 | 82.032 | 77.980 | 63.987 |
| Minerals | | | | | | | |
| Ca, % | 0.831 | 0.613 | 0.616 | 0.586 | 0.936 | 0.505 | 0.618 |
| K, % | 4.173 | 3.618 | 3.567 | 2.030 | 3.290 | 4.071 | 2.373 |
| S, % | 0.579 | 0.522 | 0.458 | 0.316 | 0.398 | 0.352 | 0.231 |
| Fe, % | 0.140 | 0.046 | 0.093 | 0.108 | 0.193 | 0.018 | 0.158 |
| Mn, % | 0.013 | 0.009 | 0.010 | 0.010 | 0.015 | 0.010 | 0.011 |
| Zn, % | 0.003 | 0.003 | 0.004 | 0.006 | 0.003 | 0.002 | 0.004 |
| Ti, % | 0.016 | 0.006 | 0.011 | 0.016 | 0.024 | 0.003 | 0.020 |

Throughout the performance trial forage samples were gathered and composited around every 21 days.

Table 7. Year 1 performance and intake measures

| | Treatments ^a | | | SEM | P-value Contrasts ^b | |
|------------------------|-------------------------|-------|-------|------|-----------------------------------|------|
| | CON | ENR | LAS | | Supp. | LAS |
| n ^c | 24 | 21 | 21 | - | - | - |
| Weight, kg | - | - | - | - | - | - |
| d 0 | 330 | 320 | 327 | 7.22 | 0.45 | 0.54 |
| d 21 | 368 | 365 | 369 | 7.48 | 0.94 | 0.76 |
| d 42 | 408 | 405 | 409 | 7.31 | 0.96 | 0.67 |
| d 64 | 439 | 436 | 439 | 7.44 | 0.87 | 0.78 |
| Total Gain | 109 | 116 | 112 | 2.38 | 0.08 | 0.32 |
| ADG | 1.70 | 1.80 | 1.75 | 0.03 | 0.08 | 0.34 |
| Intake | - | - | - | - | - | - |
| Total Intake/steer, kg | 0 | 66.34 | 64.89 | 4.79 | <0.01 | 0.82 |
| Daily Intake, kg/d | 0 | 1.04 | 1.01 | 0.07 | <0.01 | 0.82 |

^a CON = control, no supplementation; ENR = supplementation with no ionophore;

LAS = supplementation with the addition of an ionophore

^b Contrasts: Supp = CONTR vs. ENR + LAS; LAS = ENR vs. ION

^c n = number of animals per treatment

Table 8. Year 2 performance and intake measures

| | Treatments ^a | | | SEM | Contrasts ^b | |
|------------------------|-------------------------|-------|-------|------|------------------------|------|
| | CON | ENR | LAS | | Supp. | LAS |
| n ^c | 24 | 21 | 21 | - | - | - |
| Weight, kg | - | - | - | - | - | - |
| d 0 | 226 | 242 | 245 | 5.19 | 0.005 | 0.57 |
| d 28 | 254 | 275 | 279 | 6.10 | 0.002 | 0.59 |
| d 64 | 308 | 332 | 339 | 7.04 | 0.001 | 0.44 |
| Total Gain | 82 | 90 | 93 | 2.72 | 0.002 | 0.36 |
| ADG | 1.30 | 1.43 | 1.48 | 0.04 | 0.003 | 0.34 |
| Intake | - | - | - | - | - | - |
| Total Intake/steer, kg | 0 | 35.23 | 25.87 | 4.16 | <0.01 | 0.07 |
| Daily Intake, kg/d | 0 | 0.56 | 0.41 | 0.07 | <0.01 | 0.07 |

^a CON = control, no supplementation; ENR = supplementation with no ionophore;

LAS = supplementation with the addition of an ionophore

^b Contrasts: Supp = CONTR vs. ENR + LAS; LAS = ENR vs. ION

^c n = number of animals per treatment

Implications

Greenhouse gas emissions from livestock production are unavoidable due to the microbial activities occurring naturally in digestive tract. These emissions can be detrimental to the environment while also causing energy losses for the animal. These effects can be further exacerbated by cattle grazing small grains pasture, therefore mitigation strategies are wanted. This experiment utilized an energy supplement, with or without the addition of an ionophore, to assess its effects on forage intake, energy metabolism, respiratory gas fluxes, and performance. Results show that cattle receiving the supplement had lower forage intake but greater overall nutrient intakes. This caused them to have excess energy availability within the animal which produced an increase in performance for the supplemented cattle. Supplementation decreased methane yield and intensity without affecting overall daily emissions, but these supplemented cattle had greater CO₂ emissions and O₂ consumption. Overall, the introduction of a supplement into the diet of these grazing animals had a strong impact on performance and forage intake, but less of an impact on daily methane emissions. The addition of the ionophore into the diet had no impact on any parameter that was measured. Therefore, more research is needed to find a product that can be a positive impact for the environment, as well as, supply the producer with positive economic impacts.

Literature Cited

- Aiken, G. E., and S. F. Tabler. 2004. Technical note: Influence of fasting time on body weight shrinkage and average daily gain. *Professional Animal Scientist*. 20:524.
- Andersen, M. A. and G. W. Horn. 1987. Effect of lasalocid on weight gains, ruminal fermentation and forage intake of stocker cattle grazing winter wheat pasture. *Journal of Animal Science*. 65:865.
- AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA.
- Appuhamy, J. A., A. B. Strathe, S. Jayasundara, C. WagnerRiddle, J. Dijkstra, J. France, and E. Kebreab. 2013. Antimethanogenic effects of monensin in dairy and beef cattle: A meta-analysis. *Journal of Dairy Science*. 96:5161.
- Bailey E. A., E. C. Titgemeyer, K. C. Olson, D. W. Brake, M. L. Jones, and D. E. Anderson. 2012. Effects of supplemental energy and protein on forage digestion and urea kinetics in growing beef cattle. *Journal of Animal Science*. 90:3492.
- Beauchemin, K. A., S. M. Mcginn, and H. V. Petit. 2007. Methane abatement strategies for cattle: Lipid supplementation of diets. *Canadian Journal of Animal Science*. 87:431.
- Beauchemin, K. A., E. M. Ungerfeld, R. J. Eckard, and M. Wang. 2020. Review: Fifty years of research on rumen methanogenesis: Lessons learned and future challenges for mitigation. *Animal: an international journal of animal bioscience*. 14:2.
- Beck, M. R., L. R. Thompson, J. E. White, G. D. Williams, S. E. Place, C. A. Moffet, and R. R. Reuter. 2018. Whole cottonseed supplementation improves performance and reduces methane emission intensity of grazing beef steers. *The Professional Animal Scientist*. 34:339.
- Bell, N. L., R. C. Anderson, T. R. Callaway, M. O. Franco, J. E. Sawyer, and T. A. Wickersham. 2017. Effect of monensin inclusion on intake, digestion, and ruminal fermentation parameters by *Bos indicus* and *Bos taurus* steers consuming bermudagrass hay. *Journal of Animal Science*. 95:2736.
- Bergman E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological reviews*. 70:567.
- Bodine, T. N., and H. T. Purivs. 2003. Effects of supplemental energy and/or degradable intake protein on performance, grazing behavior, intake, digestibility, and fecal and blood indices by beef steers grazed on dormant native tallgrass prairie. *Journal of Animal Science*. 81:304.
- Bohnert D. W., C. S. Schauer, S. J. Falck, T. DelCurto. 2002. Influence of rumen protein degradability and supplementation frequency on steers consuming low-quality forage: II. Ruminal fermentation characteristics. *Journal of Animal Science*. 80:2978
- Callaway, T. R., T. S. Edrington, J. L. Rychlik, K. J. Genovese, T. L. Poole, Y. S. Jung, and F. J. Nisbet. 2003. Ionophores: their use as ruminant growth promotants and impact on food safety. *Current issues in intestinal microbiology*. 4:43.

- Cammack, K. M., K. J. Austin, W. R. Lamberson, G. C. Conant, and H. C. Cunningham. 2018. Ruminant Nutrition Symposium: Tiny but mighty: The role of the rumen microbes in livestock production. *Journal of Animal Science*. 96:752.
- Chin, M., and M. J. Wolin. 1979. Effect of Monensin and Lasalocid-Sodium on the Growth of Methanogenic and Rumen Saccharolytic Bacteria. *Journal of Applied Bacteriology*. 38:72.
- Church D. C. 1988. The ruminant animal: digestive physiology and nutrition.
- Coleman, G. 1985. The cellulase content of 15 species of entodiniomorphid protozoa, mixed bacteria and plant debris isolated from the ovine rumen. *Journal of Agricultural Sciences*. 104:349.
- Coulibaly N. D., D. J. Bernardo and G. W. Horn .1996. Energy supplementation strategies for wheat pasture stocker cattle under uncertain forage availability. *Journal of Applied Economics*. 28:172.
- Domesick E. J. and S. A. Martin. 1999. Effects of laidlomycin propionate and monensin on the in vitro mixed ruminal microorganism fermentation. *Journal of Animal Science*. 77:2305.
- Johnson E., M. Donald and G. Ward. 1996. Estimates of animal methane emissions. *Environmental monitoring and assessment*. 42:133.
- Ebert P., E. A. Bailey. A. L. Shreck, N. A. Cole, and J. S. Jennings. 2016. Effect of corn-based supplementation on gas emissions, performance, and energetic losses of steers grazing wheat pasture. *Journal of Animal Science*. 94:811.
- Ellis J. L., J. Dijkstra, A. Bannink, E. Kebreab, S. E. Hook, S. Archibeque, and J. France. 2012. Quantifying the effect of monensin dose on the rumen volatile fatty acid profile in high-grain-fed beef cattle. *Journal of Animal Science* Volume 90:2717–2726
- Ellis W. C., G. W. Horn, D. Delaney, and K. R. Pond. 1983. Effects of ionophores on grazed forage utilization and their economic value for cattle on wheat pastures. *National Wheat Pasture Symposium Proceedings*. 343.
- Fieser B. G., G. W. Horn, and J. T. Edwards. Effects of energy, mineral supplementation, or both, in combination with monensin on performance of steers grazing winter wheat pasture. *Journal of Animal Science*. 85:3470.
- Fonseca M., W. L. Crossland, A. B. Norris, A. K. Almeida, and L. O. Tedeschi. 2017. The potential benefit of corn dried distillers' grain (co)products (DDG) fed alone or in combination with ionophore and condensed tannin to mitigate methane emission in cattle. *Journal of Animal Science*. 95:282.
- Galyean, M. L. and S. A. Gunter. 2016. Predicting forage intake in extensive grazing systems. *Journal of Animal Science*. 94:26.
- Grainger, C., R. Williams, R. J. Eckard, and M. C. Hannah. 2010. A high dose of monensin does not reduce methane emissions of dairy cows offered pasture supplemented with grain. *Journal of Dairy Science*. 93:5300.

- Guan, H., K. M. Wittenberg, K. H. Ominski, and D. O. Krause. 2006. Efficacy of ionophores in cattle diets for mitigation of enteric methane. *Journal of Animal Science*. 84:1896
- Gunter, S.A. 2017. Predicting forage intake by grazing beef cattle. Florida Ruminant Nutrition Symposium, 28th Annual Meeting University of Florida, Institute of Food Agriculture Science, Gainesville, FL. 125.
- Gunter, S.A., and B.J. Bradford. 2015. Influence of sampling time on carbon dioxide and methane emissions by grazing cattle. *American Society of Animal Science Proceedings*. 66:201
- Gunter, S. A and M. R. Beck. 2018. Measuring the respiratory gas exchange by grazing cattle using an automated, open-circuit gas quantification system. *Translational Animal Science*. 2:11.
- Guzman, C. E., L. T. Bereza-Malcolm, B. D. Groef, and A. E. Franks. 2015. Presence of Selected Methanogens, Fibrolytic Bacteria, and Proteobacteria in the Gastrointestinal Tract of Neonatal Dairy Calves from Birth to 72 Hours. *Plos One*. 10:7.
- Hafez, S. W. Junge, and E. Kalm. 1988. Estimate of digestibility in dairy cows using an indicator method in comparison to the Hohenheimer feed value test. *Archiv für Tierernährung*. 38:929.
- Henderson, G., F. Cox, S. Ganesh, A. Jonker, W. Young, P. H. Janssen. 2015. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Scientific Reports*. 5:14567.
- Hristov, A.N., J. F. Giallongo, T. Frederick, H. Weeks, P. R. Zimmerman, M. T. Harper, R.A. Hristova, R.S. Zimmerman, and A.F. Branco. 2015. The use of an automated system (greenfeed) to monitor enteric methane and carbon dioxide emissions from ruminant animals. *Journal of Visualized Experiments*. 103:52904.
- Hook S. E., K. S. Northwood, A. D. Wright, and B. W. McBride. 2009. Long-term monensin supplementation does not significantly affect the quantity or diversity of methanogens in the rumen of the lactating dairy cow. *Applied Environmental Microbiology*. 75:374.
- Horn, F. P. 1983. Chemical Composition of Wheat Pasture. National Wheat Pasture Symposium Proceedings. 47-54
- Horn G. W. 2006. Growing cattle on winter wheat pasture: management and herd health considerations. *Veterinary Clinics Food Animal Practice*. 22:335.
- Horn G. W., P. A. Beck, J. G. Andrae, and S. I. Paisley. 2005. Designing supplements for stocker cattle grazing wheat pasture. *Journal of Animal Science*. 83:69.
- IPCC (Intergovernmental Panel on Climate Change.) 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the IPCC. Cambridge Univ. Press, Cambridge, UK.
- Jiao, H. P., A. J. Dale, A. F. Carson, S. Murray, A. W. Gordon, and C. P. Ferris. 2014. Effect of concentrate feed level on methane emissions from grazing dairy cows. *Journal of Dairy Science*. 97:7043.
- Johnson, D.E. and G. M. Ward. 1996. Estimates of animal methane emissions. *Environmental Monitoring Assessment*. 42:133.

- Jonker A., G. Molano, C. Antwi, and G. C. Waghorn. 2016. Enteric methane and carbon dioxide emissions measured using respiration chambers, the sulfur hexafluoride tracer technique, and a GreenFeed head-chamber system from beef heifers fed alfalfa silage at three allowances and four feeding frequencies. *Journal of Animal Science*. 94:4326
- Kartchner R. J. 1981. Effects of protein and energy supplementation of cows grazing native winter range forage on intake and digestibility. *Journal of Animal Science*. 51:432
- Kleiber, M., H. Cole, and S. Mead. 1943. Bloat in Cattle and Composition of Rumen Gases. *Journal of Dairy Science*. 26:929.
- Lee C. and K. A. Beauchemin. 2014. A review of feeding supplementary nitrate to ruminant animals: Nitrate toxicity, methane emissions, and production performance. *Canadian Journal of Animal Science*. 94:557.
- Macon B., L. E. Sollenberger, J. E. Moore, C. R. Staples, J. H. Fike, and K. M. Portier. 2014. Comparison of three techniques for estimating the forage intake of lactating dairy cows on pasture. *Journal of Animal Science*. 81:2357
- Massé, D. I., G. Jarret, C. Benchaar, and N. M. Saady. 2014. Effect of Corn Dried Distiller Grains with Solubles (DDGS) in Dairy Cow Diets on Manure Bioenergy Production Potential. *Animal: an international journal of animal bioscience*. 4:82.
- McAllister, T. A., H. D. Bae, G. A. Jones, and K. J. Cheng. 1994. Microbial attachment and feed digestion in the rumen. *Journal of Animal Science*. 72:3004.
- McGinn, S. M., K. A. Beauchemin, A. D. Iwaasa, and T. A. McAllister. 2006. Assessment of the Sulfur Hexafluoride (SF₆) Tracer Technique for Measuring Enteric Methane Emissions from Cattle. *Journal of Environmental Quality*. 35:1686
- Millen, D.D., M. De Beni Arrigoni, and R.D. Lauritano Pacheco, eds. 2016. Rumenology. Cham, Switzerland: Springer International Publishing.
- Minson, D. J. 1982. Effects of chemical and physical composition of herbage eaten upon intake. *AGRIS*. 167.
- Mir, P. S. and Z. Mir. 1994. Effect of live-yeast culture and lasalocid supplementation on performance of growing–finishing steers fed alfalfa-silage, corn-silage and high-grain diets sequentially. *Canadian Journal of Animal Science*. 74:563
- Montes F., R. Meinen, C. Dell, A. Rotz, A. N. Hristov, G. Waghorn, P. J. Gerber, B. Henderson, H P. S. Makkar, and J. Dijkstra. 2013. Special Topics: Mitigation of methane and nitrous oxide emissions from animal operations: II. A review of manure management mitigation options. *Journal of Animal Science*. 91:5070.
- Moore, J. E., M. H. Brant, W. E. Kunkle, and Hopkins D. I. 1999. Effects of supplementation on voluntary forage intake, diet digestibility, and animal performance. *Journal of Animal Science*. 77:122.
- Oliveira, R., P. Moriel, J. Vendramini, H. Silva, M. Vedovatto, J. Neiva, and D. Silva. 2020. Supplemental monensin affects growth, physiology, and coccidiosis infestation of early-weaned

- beef calves consuming warm-season perennial or cool-season annual grasses. *Applied Animal Science*. 36:108.
- Paisley, S. I., and G. W. Horn. 1988. Effect of Ionophores on Rumen Characteristics, Gas Production, and Occurrence of Bloat in Cattle Grazing Winter Wheat Pasture. *Animal Science Research Report*. p 141-146.
- Pinares-Patiño, C. S., S. M. Hickey, E. A. Young, K. G. Dodds, S. Maclean, G. Molano, and J. C. Mcewan. (2013). Heritability estimates of methane emissions from sheep. *Animal: an international journal of animal bioscience*. 2:316.
- Potter, E.L., C. O. Cooley, L. F. Richardson, A. P. Raun, R. P. and Rathmacher. 1976a. Effect of monensin on performance of cattle fed forage. *Journal of Animal Science*. 43:665.
- Potter, E.L., A. P. Raun, C. O. Cooley, R. P. Rathmacher, and L. F. Richardson. 1976b. Effect of monensin on carcass characteristics, carcass composition and efficiency of converting feed to carcass. *Journal of Animal Science*. 43:678.
- Potter E. L., M. I. Wray, R. D. Muller, H. P. Grueter, J. McAskill, and D. C. Young. 1985. Effect of Monensin and Tylosin on Average Daily Gain, Feed Efficiency and Liver Abscess Incidence in Feedlot Cattle. *Journal of Animal Science*. 61:1058.
- Puniya, A. K., R. Singh, and D. N. Kamra. 2015. Rumen microbiology: from evolution to revolution. New Delhi, India: Springer (India) Private Limited.
- Richards C. J., R. B. Pugh, and J. C. Waller. Influence of soybean hull supplementation on rumen fermentation and digestibility in steers consuming freshly clipped, endophyte-infected tall fescue. *Journal of Animal Science*. 84:678.
- Roehe, R., R. J. Dewhurst, C. Duthie, J. A. Rooke, N. Mckain, D. W. Ross, and R. J. Wallace. 2016. Bovine Host Genetic Variation Influences Rumen Microbial Methane Production with Best Selection Criterion for Low Methane Emitting and Efficiently Feed Converting Hosts Based on Metagenomic Gene Abundance. *PLOS Genetics*. 12:2.
- Russell J. B. and H. J. Strobel. 1989. Effect of ionophores on ruminal fermentation. *Applied Environmental Microbiology*. 55:1 – 6.
- Sartori, E.D., L. C. Canellas, and G. R. Pereira, F. G. Moojen, H. R. Carvalho, and J. O. Barcellos. 2017. Performance of beef heifers supplemented with sodium lasalocid. *Tropical Animal Health and Production*. 49:273
- Stewart, B. A., D. L. Grunes, A. C. Mathers, and F. P. Horn. 1981. Chemical Composition of Winter Wheat Forage Grown Where Grass Tetany and Bloat Occur. *Agronomy Journal*. 73:337.
- Stewart, C. S., H. J. Flint, and M. P. Bryant. 1997. The rumen bacteria. *The Rumen microbial ecosystem*. p 10 – 72
- Stocks S. E. and M. S. Allen. 2012. Hypophagic effects of propionate increase with elevated hepatic acetyl coenzyme A concentration for cows in the early postpartum period. *Journal of Dairy Science*. 95:3259 – 3268. <http://dx.doi.org/10.3168/jds.2011-4991>

- Storm, I. M., A. L. Hellwing, N. I. Nielsen, and J. Madsen. 2012. Methods for Measuring and Estimating Methane Emission from Ruminants. *Animal: an international journal of animal bioscience*. 2:160.
- Sutton, J., M. Dhanoa, S. Morant, J. France, D. Napper, and E. Schuller. 2003. Rates of Production of Acetate, Propionate, and Butyrate in the Rumen of Lactating Dairy Cows Given Normal and Low-Roughage Diets. *Journal of Dairy Science*. 86:3620.
- Sylvester, J. T., S. K. Karnati, Z. Yu, C. J. Newbold, and J. L. Firkins. 2005. Evaluation of a real-time PCR assay quantifying the ruminal pool size and duodenal flow of protozoal nitrogen. *Journal of Dairy Science*. 88:2083.
- Tilley, J.M.A., and R. A. Terry. 1963. A two stage technique for the in vitro digestion of forage crops. *Grass Forage Sci*. 18:104
- Thompson, L. R., M. R. Beck, S. A. Gunter, G. D. Williams, S. E. Place, and R. R. Reuter. 2019. An energy and monensin supplement reduces methane emission intensity of stocker cattle grazing winter wheat. *Applied Animal Science*. 35:433.
- Uppal, S. K., K. Wolf, S. S. Khahra, and H. Martens. 2003. Modulation of Na⁺ transport across isolated rumen epithelium by short-chain fatty acids in hay- and concentrate-fed sheep. *Journal of Animal Physiology and Animal Nutrition*. 87:380.
- U.S. EPA. 2016. Climate change indicators in the United States. Fourth edition. EPA 430-R-16-004.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*. 74: 3583
- Velásquez, A., G. D. Silva, D. Sousa, C. Oliveira, C. Martins, P. D. Santos, and R. Fukushima. 2018. Evaluating internal and external markers versus fecal sampling procedure interactions when estimating intake in dairy cows consuming a corn silage-based diet. *Journal of Dairy Science*. 101:5890.
- Wagner, D., R. Lowery, and M. Grigsby. 1983. Supplemental Feeding of Grain and Protein for Stocker Cattle on Small Grains Pastures. *National Wheat Pasture Symposium Proceedings*. p.327-342.
- Wang M., X. Z. Sun, P. H. Janssen, S. X. Tang, and Z. L. Tan. 2014. Responses of methane production and mentation pathways to the increased dissolved hydrogen concentration generated by eight substrates in in vitro ruminal cultures. *Animal Feed Science and Technology*. 194:1.
- White, L. M., G. P. Hartman, and J. W. Bergman. 1981. In vitro digestibility, crude protein, and phosphorus content of straw of winter wheat, spring wheat, barley, and oat cultivars in eastern montana. *Agronomy Journal*. 73:117.
- Williams, A. G. 1986. Rumen holotrich ciliate protozoa. *Microbiological Reviews*. 50:24
- Williams A. G., Coleman G. S. (1992). The Rumen Protozoa. New York: Springer-Verlag.