

QUANTIFYING ENTERIC METHANE EMISSIONS BY  
STOCKER CATTLE GRAZING WINTER WHEAT

By

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QUANTIFYING ENTERIC METHANE EMISSIONS BY  
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Abstract: The carbon footprint of the beef industry has become an important topic for the general public, and therefore, stakeholders in the industry. Enteric methane is a major contributor to carbon footprint and is a significant energy loss to the animal. Therefore, any mitigation of enteric methane would help the animal be more energetically efficient and possibly improve performance. A production system that has garnered limited interest in the literature, in terms of enteric methane emissions, is winter wheat grazing in the Southern Great Plains. This is an economically important production system as 6-8 million cattle are brought into the region each winter to graze. Cattle are grazed on wheat from late fall to spring before grain harvesting in early summer. A popular supplement for producers in this system is a monensin-containing energy supplement that has been shown to increase animal gains. The objective of the experiment was to examine the effect that energy in conjunction with monensin have on the enteric methane emissions and performance of stocker cattle grazing winter wheat. Models were selected using mallows Cp using baseline CH<sub>4</sub>, total supplement intake, forage intake, initial body weight, sex, and monensin dose. Average daily gain (kg/d) had a positive relationship with total supplement intake and DMI ( $P = 0.016$ ). Daily methane production (g/d) had a positive linear relationship with initial body weight and DMI of forage, with heifers producing less methane than steers ( $P < 0.001$ ). Supplement intake reduced CH<sub>4</sub> emission intensity (g CH<sub>4</sub>/kg live weight gain;  $P = 0.028$ ). Methane yield (g CH<sub>4</sub>/ kg of intake) decreased with increasing DMI and decreasing body weight, and heifers yielded less CH<sub>4</sub> than steers ( $P < 0.01$ ). Based on these results, energy supplemented was determined to improve the sustainability of the stocker cattle sector.

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## NOMENCLATURE

3NOP	3-Nitroxypropanol
ADG	Average Daily Gain
BUN	Blood Urea Nitrogen
CO <sub>2</sub>	Carbon Dioxide
CoM	Methyl-Coenzyme M Reductase
DDGS	Dry Distillers Grains Plus Solubles
DM	Dry Matter
DMI	Dry Matter Intake
DMP	Daily Methane Production
EI	Emission Intensity (kg gain/g CH <sub>4</sub> produced)
GE	Gross Energy
GEM	GreenFeed System
GHG	Greenhouse Gases
GRG	Oklahoma Greengold
GT CO <sub>2</sub> eq	Gigatonnes Carbon Dioxide Equivalents
Ha	Hectare
HB	Head-box emission measurement system
iADF	Indigestible Acid Detergent Fiber
LCA	Life Cycle Assessment

MY	Methane Yield
NH <sub>3</sub>	Ammonia
PUN	Plasma Urea Nitrogen
RC	Respiration Chamber
RFI	Residual Feed Intake
RFID	Radio Frequency Identification System
SF <sub>6</sub>	Sulfur Hexafluoride
TMR	Total Mixed Ration
TSI	Total Supplement Intake
VFA	Volatile Fatty Acids
WDGS	Wet Distillers Grains Plus Solubles



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## CHAPTER I

### REVIEW OF LITERATURE

#### **Sustainability: A Brief Overview**

Sustainability is a complex issue that can be described as a “wicked problem” (Kebreab, 2012). A “wicked problem” is one that has no solution, but can only be managed (Rittel and Webber, 1973). Sustainable beef production certainly fits this description and explains why stakeholders have such differing ideas about what sustainability means. The NRC (2010) identified four goals to help define sustainable agriculture: 1) satisfy human food, feed, and fiber needs, and contribute to biofuel needs, 2) enhance environmental quality and the resource base, 3) sustain the economic viability of agriculture, and 4) enhance the quality of life for farmers, farm workers, and society as a whole. These four goals fall within the three major aspects of sustainability, which include environmental, social, and economic considerations. Anything that finds the nexus between these will help move the sustainability of the industry forward (NRC, 2010).

Douglass (1984) described three schools of thought that align with the three different pillars of sustainability: 1) food security, 2) environmental stewardship, and 3) societal focus. Most animal scientists understand the importance of research oriented

around food security and may be skeptical of practices outside mainstream agriculture (Thompson, 2007). Food security means improving agriculture productivity and food waste to meet the demands of a growing population (Kebreab, 2012). Food security has been a goal of animal scientists for many years. Armsby (1910) in the president's annual address in the American Society of Animal Nutrition described the diminishing food supply facing the future population. He suggested that improving technology and the efficiency of production was necessary to avoid a dwindling food supply.

Those that focus on environmental stewardship believe that natural ecology must be maintained before agriculture can be sustainable (Kebreab, 2012). This belief maintains that there is a finite supply, availability, and quality of resources, and that resource depletion and/or environmental damage are not acceptable (Kebreab, 2012). The societal aspect of sustainability focuses on preservation of natural resources, promoting rural cultures, and fostering self-reliance. It does not view agriculture as a primary entity, but rather it is embedded in a larger system with other sub-systems that all rely on the same limited resource base. This aspect claims that all members of society are stakeholders in sustainable agriculture with varying degrees of involvement (Kebreab, 2012). These schools of thought help to describe the complexity of the sustainability question and why there is such variability in how sustainability is defined by stakeholders.

### **Climate Change**

Agriculture productivity is largely dependent on climate (Adams, 1998). Climate is defined as the statistical description in terms of the mean and variability of

meteorological measurements (i.e. temperature, rainfall, etc.) over a period of time, typically 30 years (IPCC, 2013). The Earth's climate is powered by solar radiation. Over the past centuries the Earth's temperature has remained relatively stable, meaning that outgoing radiation was balanced with incoming solar radiation (IPCC, 2013). Of the incoming solar radiation, approximately 50% is absorbed by the earth's surface, 30% is reflected back to space by gases, aerosols, clouds, and the earth's surface, and the remaining 20% is absorbed in the atmosphere. The longwave radiation emitted from the earth's surface is absorbed by atmospheric constituents known as greenhouse gases (**GHG**) which reemits the radiation in all directions (IPCC, 2013). This process is commonly referred to as the greenhouse effect which is necessary to maintain livable surface temperatures (Place and Mitloehner, 2010; IPCC, 2013). The ability of GHG to impact surface temperatures has been established for over 100 years (Arrhenius, 1896). Since the Industrial Revolution, anthropogenic (human-caused) GHG emissions have increased and will continue to increase with increased fossil fuel combustion (Place and Mitloehner, 2012). Transportation accounted for 26.3% of the total U.S. GHG emissions in 2014 and is the largest end-use sector producing energy-related carbon dioxide emissions (EPA, 2016a). Agriculture accounted for 9.1% of total U.S. GHG emissions in 2014. Although soil management, such as fertilizer application, is the largest agriculture GHG contributor, enteric fermentation receives considerable attention from the general public (EPA, 2016a).

The rise in atmospheric GHG concentrations are projected to result in increasing climate variability and surface temperatures. In recent decades, temperatures on the Earth's surface, in the troposphere, and the oceans have all increased (Walsh et al., 2014).

This has caused variability in local weather patterns, such as an increase in the number of dry day's and increased heavy precipitation events (Walsh et al., 2014). In agriculture, there has been changes in growing season length (Walsh et al., 2014). The growing season will extend an estimated 24 days by 2050, but will increase heat stress, increase surface water losses, and overwintering insect populations (Hatfield et al., 2014). With increased carbon in the atmosphere, future projects indicate changes in crop yield, as well as potential changes in where crops are grown. Agriculture has thus far proven to be adaptable to the changing climate, as evident by continued growth and efficiency, but will need continue to search for new ways to adapt (Hatfield et al., 2014).

## **Enteric Methane**

### *Methanogenesis*

Methane production by ruminant animals occurs primarily in the rumen with a minor amount coming from fermentation in the hindgut (Patra, 2012). Methane emitted via hindgut fermentation accounts for only 10-15% of emitted methane (Huhtanen et. al., 2015). The rumen is an anaerobic environment for microbial fermentation of fibrous feeds (Krehbiel, 2014). Various species of bacteria, protozoa, fungi, and methanogenic archaea live in the rumen and they have a symbiotic relationship with the host animal by providing fermentation products. These products provide energy to the host primarily through short-chain volatile fatty acids and microbial cell protein, with carbon dioxide and hydrogen being byproducts of the fermentation process (Krehbiel, 2014).

Removal of H<sub>2</sub> is important for ruminal health and is accomplished by either VFA production, biohydrogenation, or conversion to CH<sub>4</sub>. Enteric CH<sub>4</sub> is produced by

methanogenic archaea, commonly referred to as methanogens. Different species of methanogens utilize different pathways to produce CH<sub>4</sub>. The most common pathway utilized in the production of CH<sub>4</sub> is the hydrogenotrophic pathway, which uses CO<sub>2</sub> and H<sub>2</sub> as substrates (Beauchemin et. al., 2008; Place and Mitloehner, 2010). The removal of H<sub>2</sub> serves a crucial role in rumen health as hydrogen can be toxic to certain bacteria and ruminal efficiency (Beauchemin et. al., 2009). Methanogenesis promotes more complete oxidation of fermented substrates and greater energy recovery by microbes (Patra, 2012). Under anaerobic conditions, fermentation of glucose from plant polymers occurs via the Embden-Meyerhof-Parnas pathway and gives off reduced co-factors, like NADH (Moss et al., 2000). These co-factors need to be re-oxidized to complete the fermentation process. Carbon dioxide acts as an acceptor in the absence of oxygen, although other compounds present in the rumen can also be utilized (Moss et al., 2000; **Equation 1**).



Methanogens utilize H<sub>2</sub> to produce CH<sub>4</sub> and H<sub>2</sub>O thereby preventing it from accumulating in the rumen. Hydrogen accumulation blocks the derivation of energy during fermentation by limiting the ability of the microbial populations to oxidize the co-factors responsible for electron transfer in the rumen (Beauchemin et. al., 2009).

Enteric CH<sub>4</sub> emissions are proportional to dry matter intake but can be influenced by a number of factors including type of carbohydrate, forage processing, dietary lipids, and manipulation of the rumen microbiome (Beauchemin et. al., 2009; Johnson and Johnson, 1995). Methane emissions represent a loss of approximately 2-12% of dietary gross energy (**GE**) intake (Johnson et. al., 1993). This inefficiency in the ruminant system

has made the inhibition of CH<sub>4</sub> production a thoroughly researched topic by ruminant nutritionists (Martinez-Fernandez et. al., 2014). If fermentation can be shifted or mitigating compounds added to the diet and methane production is decreased, then more energy may be available for improved production (McAllister and Newbold, 2008).

### *Methane Emissions*

The presence of methane in the atmosphere has been known since the 1940s and increasing atmospheric concentrations have been recorded since the 1980s (Migeotte, 1948; Rodhe, 1990). Methane is a potent greenhouse gas with a global warming potential (**GWP**) 25 times that of CO<sub>2</sub> (EPA, 2016a). The GWP can rise to 36 if the conversion of CH<sub>4</sub> to CO<sub>2</sub> through chemical transformations of CH<sub>4</sub> by indirect radiative forcing is considered (EPA, 2016a; EPA, 2016b). The rising concentration of methane in the atmosphere is correlated with rising anthropogenic methane emissions, with agriculture being a significant contributor (Moss et al., 2000). Recent reports have concluded that enteric CH<sub>4</sub> in the United States, predominantly from ruminant livestock, is responsible for 22.5% and 2.4% of U. S. CH<sub>4</sub> and GHG emissions, respectively (EPA, 2016a).

Methane production is highly variable between regions of the world. North America's emissions intensity (**EI**; CO<sub>2</sub> eq./kg CW) is only 11 kg CO<sub>2</sub> eq./kg CW in contrast to 24 kg CO<sub>2</sub> eq./kg CW in Latin America and the Caribbean. Sub-Saharan Africa and South Asia have the greatest EI at 41 and 49 CO<sub>2</sub> eq./kg CW, respectively (Gerber et al., 2013). The increased emissions in developing countries are due to low feed digestibility, poorer animal husbandry, low slaughter weights, and greater age at slaughter (Gerber et al., 2013). Developed countries feed more concentrate than their



developing counterparts which increases diet digestibility (Gerber et al., 2013). This leads to lower enteric and manure emissions over the lifespan of the animal by reducing days on feed and emitting less enteric methane per unit of feed consumed (Gerber et al., 2013). In developing countries, it is common to see lower growth rates and slaughter weights. These limitations on performance lead to an increase in emissions per kg of meat produced. European countries dilute the footprint of their beef sector as approximately 80% of their beef is produced from dairy animals (Gerber et al., 2013). In order to improve estimates of the beef industries carbon footprint and provide guidelines to policy makers for mitigation, we need to accurately quantify the emission rates on different diets and production systems.

### **Methane Measurement Systems**

Accurate and precise measurement techniques are critical to determine emission rates and the efficacy of mitigation strategies. There are several established techniques for measuring ruminant emissions (Hristov et al., 2015). Three of the most common systems are respiration chambers (**RC**), sulfur hexafluoride tracer gas technique (**SF<sub>6</sub>**) and head-box chambers (**HB**). Respiration chambers are considered the “gold standard” for measuring emissions (Hristov et al., 2015). The GreenFeed System (**GEM**; C-Lock Inc., Rapid City, SD) is a relatively new technology which utilizes spot measurements to estimate emission rates. These systems are not the only techniques used to measure emissions from ruminants, but are more common, and will be the main focus of this section.

#### *Respiration Chambers*

Respiration Chambers are the “gold standard” when it comes to the measurement of emissions from ruminants (Hristov et al., 2015). There are two types of respiration chambers, open and closed-circuit (Storm et al., 2012). The open circuit system is the more popular of the two systems (Storm et al., 2012). It consists of a pump to draw air from outside the system into the system, whereas as closed-circuit chambers have oxygen metered into the system and carbon dioxide is absorbed and weighed (Storm et al., 2012; Turner and Thornton, 1966). The respiration chamber technique is based on the first law of thermodynamics and involves the volumetric measurements of gases leaving the chamber (Krebeab et al., 2006). The chambers are typically made of steel with an air conditioning system to provide environmental control (Krebeab et al., 2006). The animal is placed in the respiration chamber and methane emissions are determined by the difference in concentration between inspired and expired air (Johnson and Johnson, 1995). This system is advantageous as it allows for accurate measurements of emissions from both ruminal and hindgut fermentation (Johnson and Johnson, 1995). The design may also allow for the measurement of total tract digestibility and determine the net energy yielded from known qualities of feeds (Hill et al., 2016).

This system does have limitations in its application. The main criticism is that the animal is often fed at maintenance, measurements are made over short periods, and the eating and behavior of the animal does not reflect that of animals in their production environment (Storm et al., 2012). The artificial environment inside the chamber alters the animals’ behavior which alters dry matter intake (**DMI**). With DMI being a main driver of methane production, any alteration changes total emissions and gross energy loss (Storm et al., 2012). Another criticism of this system is the large amount of cost and

labor. Cost can limit the capacity of the system and restrict the number of animals which can be examined experimentally (Storm et al., 2012). The cost and space limitations have led to the use of head-boxes in place of full body respiration chambers (Hill et al., 2016).

### *Head-boxes*

A ventilated head-box system uses the same principals as the whole body respiration chamber to measure gaseous emissions (Johnson and Johnson, 1995). Similar to the whole body chamber, animals are trained to enter the hood where analyzers record gas composition, pressure, and air flow (Kelly et al., 1994). A sleeve is placed around the animal's neck and closed to minimize the amount of air leakage (Johnson and Johnson, 1995). A slight negative pressure is maintained inside the hood to prevent gases leaving the system through the hood opening (Kebreab et al., 2006). The box is big enough to allow the animal to move its head unrestricted and allows for feed and water to be provided. This system allows for short measurement times and can detect slight changes in CH<sub>4</sub> concentration (Kebreab et al., 2006). The main advantage of this system over RC is decreased cost, but it still requires a restrained and trained animal (Johnson and Johnson, 1995). The disadvantage of this system is that hindgut emissions cannot be measured and labor costs are still high (Kebreab et al., 2006). Another negative, similar to RC is that it cannot be used to measure emissions on pasture (Kebreab et al., 2006; Hill et al., 2016). In order to measure gaseous emissions of grazing livestock other techniques are employed that do not restrict the animals in a box, but instead allow them to graze freely in their natural environment.

*SF*<sub>6</sub>

The most common method for measuring CH<sub>4</sub> emissions of grazing animals is the sulfur hexafluoride technique. Other gas tracers, such as labelled CH<sub>4</sub>, have been used but SF<sub>6</sub> is the most common (Vlaming, 2007). This method utilizes SF<sub>6</sub> to account for gas dilution as it exits the cow's mouth and mixes with ambient air (Johnson et al., 1994). It is based on the assumption that the SF<sub>6</sub> emission rate is equal to the CH<sub>4</sub> emission rate (Johnson et al., 1994). Prior to the experiment initiation, an SF<sub>6</sub> permeation tube is calibrated to determine the release rate of the gas. The tube is then placed in the rumen and air samples are taken from the mouth and nose using a stainless steel collection vessel and a capillary tube attached to a collection canister (Johnson et al., 1994). The gaseous concentration is determined using gas chromatography and CH<sub>4</sub> emission rate is calculated using the ratio of CH<sub>4</sub>/SF<sub>6</sub> multiplied by the release rate, with a correction factor applied for background SF<sub>6</sub> concentration (Johnson et al., 1994).

The major advantage of this technique is that it allows emission estimates from grazing animals (Kebreab et al., 2006). There are inconsistencies in the published literature on the accuracy of the SF<sub>6</sub> compared to respiration chamber or head-box technique. McGinn et al. (2006) found that SF<sub>6</sub> underestimated CH<sub>4</sub> emissions by 4%. This difference was not significant and they attributed it to post-ruminal CH<sub>4</sub> emissions (McGinn et al., 2006). Others have found differences of ≥10%, although discrepancies are neither consistent nor predictable (McAllister and Newbold, 2008; Lauback et al, 2014). Inconsistencies with the system are due to some limitations that have been found over the past two decades. One limitation is the permeation rate of the SF<sub>6</sub> tubes in the rumen (Storm et al., 2012). The SF<sub>6</sub> technique relies on maintaining a constant release

rate from the permeation tubes. Permeation rates pre- and post-experiment of the permeation tube can display a curvilinear release rate in the lab. This changing release rate may result in a decrease in rumen release rate by 6 to 11% (Storm et al., 2012; Vlaming, 2007). Studies have also shown that permeation tubes with a higher release rate estimate higher CH<sub>4</sub> emission rates and it is therefore recommended that only tubes with similar release rates be used (Vlaming, 2007; Pinares-Patino et al., 2008). Both within and between animal variability is another major limitation with this technique (Storm et al., 2012). A study comparing SF<sub>6</sub> to RC found high CV's with the SF<sub>6</sub> compared to the RC (Pinares-Patino et al., 2011). The within animal CV of the RC was 4.7%, 13.5% with the SF<sub>6</sub>, and 11.7% with the SF<sub>6</sub> within the chamber. The between animal CV for SF<sub>6</sub> was twice the CV for the RC (Pinares-Patino et al., 2011). To overcome the variability, more animals are needed on trial, but the moderate cost allows researchers to increase the sample size. This can be cost and labor prohibitive when using the RC or HB (Storm et al., 2012; Hill et al., 2016).

A further limitation of SF<sub>6</sub> technique is that it relies on 24-hour mass-sampling over the course of 5-7 days (Pinares-Patino et al., 2012). This does not allow for an estimate of the diurnal variation in methane emissions that is possible with the RC or HB systems. Lastly, a unique disadvantage of this system is the use of SF<sub>6</sub>. This gas is a highly potent GHG with a 100 year GWP of 22,800 (Vlaming, 2007).

### *GreenFeed*

The Greenfeed system is a new technology for quantifying emissions from ruminant animals and, like the SF<sub>6</sub>, it is able to estimate methane emissions by grazing

animals. The GEM is used to monitor CH<sub>4</sub>, CO<sub>2</sub>, and O<sub>2</sub> mass fluxes from the breath of ruminant animals (Hristov et al., 2015). The system consists of a portable head-box system that dispenses bait feed from an automatic feeder when an animal visits. An RFID system reads the animals tag and determines whether it is allowed to receive the bait feed or not. This is based on researcher specifications to keep the animal in the chamber long enough to obtain an accurate CH<sub>4</sub> estimate, and to get animals to visit equally throughout the day.

Like the SF<sub>6</sub>, the GEM is based on the use of a tracer gas (propane) but in a head chamber type system that estimates the daily emissions based on spot samples over the course of the experiment (Hristov et al., 2015). Its ability to quantify emissions over longer periods is valuable for grazing cattle in part because of the natural variability of nutritive quality of the forage over the growing season (Velazco et al. 2015). Unlike the previous systems, the GEM is non-intrusive, less expensive, and allows the animals to undergo normal feeding and behavior (Hristov et al., 2015).

Shortcomings for the GEM include unrepresentative sampling and the use of bait feed (Hristov et al., 2015). The bait feed attracts the animals into the headbox so that eructation events can be measured. This bait feed, however, can represent up to 5% of the animal's dry matter intake during a measurement event (Hristov et al. 2015), and because of this it should be considered in the overall analysis so that emission intensity per unit of DMI can be accurately estimated (Hristov et al., 2015). According to Hill et al. (2016) all spot sampling measurement systems, such as the GEM, result in highly variable data sets. This can be accounted for if enough data is collected from a large number of animals which results in a greater uniformity in sample frequency throughout the 24-h

measurement day and a representative flux can then be calculated (Gunter and Bradford, 2015).

Visits to the GEM can be classified as either useful or non-useful visits. A useful visit is when sampling occurs in an uninterrupted 3-5 min period (Velazco et. al., 2015). According to Velazco et. al. (2015) a measure of methane production rate should only be generated when an animal's head is continuously in the hood for 3 minutes to obtain enough eructation events for an accurate estimation of daily methane production (**DMP**).

Literature comparing GEM to other CH<sub>4</sub> measurement systems has been inconsistent, but generally shows similar estimates for DMP (Hammond et al., 2016a). Hammond et al. (2015) found a similar DMP estimate from GEM and RC on growing heifers in two different experiments ( $198 \pm 20.4$  and  $208 \pm 31.5$  for GEM;  $215 \pm 22.3$  and  $209 \pm 30.9$  for RC for experiments 1 and 2, respectively). These results were corroborated by Velazco et al. (2015), who reported no difference between systems. There are some experiments that were not able to detect treatment differences while using the GEM that other systems detected. Hammond et al. (2015) could not detect significant treatment effects on methane emissions that were evident with the RC and SF<sub>6</sub> systems. They attributed this to small sample sizes and the timing of measurements obtained.

Timing of measurements is an important consideration for estimating CH<sub>4</sub> emissions. Methane emissions are not equal throughout the day. There is a diurnal pattern which is affected by diet, amount of feed consumed, and feeding patterns (Hristov et al., 2015; Jonker et al. 2014). Rates of CH<sub>4</sub> emissions are highest during and immediately following a meal, and lowest before the first meal or grazing bout of the day (Laubach et

al., 2013). For this reason, it is important to consider timing of GEM visitation as a potential source of bias (Hammond et al., 2016). The GEM only obtains estimates when animals voluntarily visit the system, and Gunter and Bradford (2015) suggest weighting data according to the incidence and timing of visits in order to improve accuracy of estimates.

## **Methane Mitigation**

An abundance of CH<sub>4</sub> mitigation strategies have been studied with varying levels of success. These methods are described by Knapp et al. (2014) to fall into three categories: 1) dietary strategies, 2) rumen modifiers, 3) increasing animal production through genetics and other management approaches. There have been a number of summary papers discussing potential mitigation options that have been studied (Boadi et al., 2004; Hristov et al., 2013; Kebreab et al., 2006). This is of particular significance now as there is a rising concern about the impact of the beef cattle industry on the environment. Public perception falls under the society pillar of sustainability and therefore improving methane mitigation would improve all three pillars: economic, environmental, and societal. When examining mitigation options it is important to likelihood of farmer implementation. A farmer would not implement something that was not cost effective. The magnitude of CH<sub>4</sub> mitigation and consumer acceptance must also be considered for any potential mitigation tool (Hristov et al., 2013).

## **Dietary Strategies**

### *Type of Carbohydrate*



Type of carbohydrate consumed is an important factor for methane yield (Johnson and Johnson, 1995). Feeding more digestible carbohydrates can result in greater dry matter intake (**DMI**) and lower CH<sub>4</sub> yield per unit of feed consumed. Similar results occur when feeding readily fermentable carbohydrates in high concentrate diets. This decreases intake and results in lower CH<sub>4</sub> yield per unit of feed consumed. Carbohydrates influence CH<sub>4</sub> production through changes in ruminal pH due to alterations in the microbial population which can change ruminal end products (VFA) (Johnson and Johnson, 1995; Moe and Tyrrell, 1979). Roughage based diets, which contains more cellulose, hemicellulose, and lignin, take longer to ferment and favor acetate production (Moe and Tyrrell, 1979). Concentrate based diets are digested faster and favor propionate production (Johnson and Johnson, 1995). The shift to propionate production favors a decrease in methane production as propionate acts as a H sink thereby reducing metabolic H, whereas acetate results in a net gain of metabolic H (Knapp et al., 2014). Part of this shift in the acetate:propionate ratio is explained by the effect of pH alone (Russell, 1998). High concentrate diets have a lower ruminal pH; 6.2 vs. 6.9 for those on grass diets (Russell, 1998). The high starch diets are the primary diets used in most modern feedlots and a 25% addition of non-structural carbohydrates can decrease CH<sub>4</sub> by 20% (Moss et al., 2000). While this option can reduce enteric emissions, increased concentrate feeds would be coupled with increased fertilizer use and emissions from machinery (Boadi et al., 2004). Therefore, consideration needs to be made in order to balance the reduction in CH<sub>4</sub> with the increases in GHG from other sources (Boadi et al., 2004).

Aside from feeding a high starch diet, improving pasture quality can improve dietary digestibility and decrease CH<sub>4</sub> emissions. In a meta-analysis, Archimede et al.

(2011) looked at dietary characteristics of forages and legumes and their impact on CH<sub>4</sub> production. It was determined that tropical grasses produced more CH<sub>4</sub> than temperate grasses and that tropical legumes produced less CH<sub>4</sub> than temperate legumes (Archimede et al., 2011). This is due to the carbohydrate composition of the tropical grasses, or C<sub>4</sub> grasses. Tropical grasses are lower in quality due to an increased lignin content compared to C<sub>3</sub> grasses, which is less digestible in the rumen and results in an increased rumen retention time (Wilson, 1994; Archimede et al., 2011).

### *Level of Intake*

The quantity of feed that an animal consumes is considered a major driver of CH<sub>4</sub> production, with increasing DMI comes increasing CH<sub>4</sub>. It is interesting to note that the percentage of dietary GE lost as CH<sub>4</sub> decreases by 1.6% per unit of intake over maintenance (Johnson et al., 1993). This may be a consequence of reduced residence time in the rumen (Pinares-Patino et al., 2003), although it is likely influenced by diet type and time required to chew and reduce the particle size for passage (Ulyatt et al., 1986). The increased passage rate associated with high levels of intake decreases microbial access to organic matter which reduces the extent and rate of ruminal dietary fermentation (Mathison et al., 1998). When microbial access to the substrate is decreased, there is a corresponding decrease in CH<sub>4</sub> production (Mathison et al., 1998). A high rate of passage also favors increased propionate production which, as mentioned previously, removes H<sub>2</sub> from the rumen (Boadi et al., 2004). Janssen (2010) hypothesized that the outflow of rumen liquid rather than solid is responsible for the regulation of methanogenesis. A high liquid passage rate may reduce archaeal populations, resulting in the accumulation of metabolic H<sub>2</sub> and a reduction in CH<sub>4</sub> (Janssen, 2010).

The effect that level of intake has on CH<sub>4</sub> emissions is dependent on the diet type. It has been shown that increasing level of intake on forage diets, compared with concentrate diets, displays a proportionally lower impact on passage rate (Galyean and Owens, 1991). In contrast, concentrate diets can have a wide range of CH<sub>4</sub> production depending on level of intake. Mathison et al. (1998) found that feeding concentrate diets at maintenance levels lost 9.2% of GE as CH<sub>4</sub>, but when feeding at 1.75 times maintenance the loss was dropped to 5.3%. When feeding concentrates at higher levels, there was an increase in passage rate, but a drop in pH as well. This drop in pH decreases the protozoal numbers which has a close association with methanogenic archaea due to the amount of H<sub>2</sub> they release (Boadi et al., 2004).

### *Feed Processing*

Feed processing is an effective CH<sub>4</sub> mitigation strategy through its effects on digestibility, energy losses, and passage rate (Hristov et al., 2013). This is true for both forage and grain processing. Grinding or pelleting forages can significantly decrease methane production (Blaxter, 1989). This is partially explained by the increased rate of passage that occurs with processed forages (Johnson and Johnson, 1995). As discussed earlier, when the rate of passage is increased the acetate to propionate ratio is decreased favoring a decrease in CH<sub>4</sub> production. Le-Liboux and Peyraud (1999) found that grinding alfalfa reduced total digestibility of organic matter and cell-wall constituents, but had no effect on digestibility of starch.

Processing grains increases total tract digestibility, increases feed efficiency, and has a similar response on CH<sub>4</sub> production as processing forages (Firkins et al., 2001).

The increased digestibility and feed efficiency leads to increased animal performance and less days on feed, which decreases the amount of CH<sub>4</sub> emitted per unit of product produced (Hristov et al., 2013). Grain processing can improve carbon footprint/emission intensity through increased performance and decreased days on feed, but also directly by improving digestibility, decreasing intake, and increase rate of passage (Boadi et al., 2004; Hristov et al., 2013). Hales et al. (2012) found that cattle on a steam flaked corn diet produced 18% less methane as a % of GE intake than cattle on a dry rolled corn diet. Similar results were found when comparing raw and processed maize at varying levels of protein degradability (Pattanaik et al., 2003). With the exception of high protein availability, processing maize decreased CH<sub>4</sub> production for medium and low protein availability by 1.0 g d<sup>-1</sup> and 1.8 g d<sup>-1</sup> respectively (Pattanaik et al., 2003). Although grain processing may have a negative impact on NDF digestibility (Firkins et al., 2001), the significant impact on CH<sub>4</sub> production makes it a viable methane mitigation option for producers (Hristov et al., 2013).

### *Lipid Supplementation*

Supplemental fat has long been studied by ruminant nutritionists because of its impacts on rumen activity and animal performance. From a nutritional perspective, fats can be categorized based on their impacts on ruminal activity and fiber digestion (Jenkins, 1997). Calcium salts of fatty acids and hydrogenated fats are specifically designed not to alter rumen activity and digestion (Jenkins, 1997). A group of fats including unaltered extracts from plant and animal sources cause abnormal rumen fermentation, and therefore are the compounds that result in decreased CH<sub>4</sub> production (Jenkins, 1997). These include animal based tallow and grease, oils from plants (i.e.

soybean oil and cottonseeds), and high fat byproducts such as distillers' grains (Jenkins, 1997). There are two potential modes of action as for how supplemental lipids reduce methanogenesis. The first is that lipid particles coat the fiber in the diet and reduce microbial attachment and digestibility (Hristov et al., 2013). The second mode of action is unsaturated lipids acting as a H<sub>2</sub> sink (Hristov et al., 2013). Fats in the rumen are known to undergo biohydrogenation and when H<sub>2</sub> molecules are saturating fats, they are removed from the metabolic H<sub>2</sub> pool and will not go toward the production of methane (Czerkaswski and Clapperton, 1984). This mechanism is thought to play a small role and it has been suggested that only 1 to 2% of metabolic H<sub>2</sub> goes to biohydrogenation (Johnson and Johnson, 1995; Jenkins et al., 2008).

In a meta-analysis conducted by Grainger and Beauchemin (2011), CH<sub>4</sub> production is reduced by increasing levels of dietary fat, displaying a significant linear and curvilinear response, for diets containing up to 13.0% fat. In a review paper by Patra (2012), it is suggested that fat content of the diet should not exceed 6-7% of dietary DM. This is due to its ability to decrease DM digestibility and intake (Patra, 2012). Feeding supplemental fats have decreased CH<sub>4</sub> emissions over long periods of time (Grainger and Beauchemin, 2011; Patra, 2012). Grainger et al. (2009) found that over a 12-week period, whole cottonseed supplementation had a persistent reduction in CH<sub>4</sub> emissions.

Using fats to replace a portion of grain in the diet has been shown to decrease CH<sub>4</sub> production. In a study by McGinn et al. (2009), dried maize DDGS replaced a portion of barley grain and resulted in a decrease in CH<sub>4</sub> production from 23.8 to 19.9 g/kg DMI. Hales et al. (2013) reported a similar response when including WDGS in the diet. They saw a 11% decrease in CH<sub>4</sub> production as compared to the control. However, the feeding

of high-fat by-products may cause a shift in GHG emissions from CH<sub>4</sub> to N. Hales et al. (2012) reported an increase in total N excretion by 18% and also an increase in urinary N by 35% when including 30% WDGS in the diet. This increased excretion is due to higher N content of the diet. By increasing urinary N output, the amount of N that is available for rapid volatilization in the form of ammonia or nitrous oxide is increased and must be considered when considering this mitigation option (Hristov et al., 2014; Place, 2016). Fat supplementation can have a negative impact on DMI and animal production. If production for the herd is decreased to the point that replacement animals are needed to recapture that lost product, it may counter out any beneficial CH<sub>4</sub> mitigation that fat supplementation may provide (Hristov et al., 2013).

## **Inhibitors**

### *Ionophores*

Ionophores are commonly used in today's beef industry for their impacts on animal health and efficiency (Byers and Schelling, 1980; Callaway et al., 2003). These compounds are classified as anti-microbials that facilitate the transport of ions across cell membranes (Place et al., 2011). This leads to a disruption of the chemi-osmotic gradient of the cell, which often lead to a decreased ATP-production efficiency of the cell (Place et al., 2011). Ionophores selectively inhibit gram positive over gram negative bacteria which favors propionate production (Appuhamy et al., 2013). Some ionophores have also been shown to reduce the amount of protozoa which, in addition to shifting the acetate:propionate ratio, can reduce the amount of CH<sub>4</sub> produced, particularly in intensive systems (Appuhamy et al., 2013). Monensin is the most studied ionophore and its impact

on CH<sub>4</sub> production has been inconsistent (Appuhamy et al., 2013; Hristov et al., 2013). Past studies have shown conflicting results in terms of efficacy and duration of CH<sub>4</sub> mitigation (Hristov et al., 2013). Grainger et al. (2010) found that monensin did not affect CH<sub>4</sub> production in either grazing or chamber experiments, and concluded that monensin may not be a viable mitigation strategy for grazing dairy cows. However, in a 6-month trial on dairy cows consuming a 60:40 forage-to-concentrate TMR Odongo et al. (2007) reported a sustained reduction of 7% in DMP. Potential explanations for these differences could be different diets or level of monensin dose (Appuhamy et al., 2013; Hristov et al., 2013; Grainger et al., 2008). Appuhamy et al. (2013) conducted a meta-analysis on the anti-methanogenic effects of monensin and found that differences between studies could be explained when adjusted for DMI differences or monensin dose.

### *Defaunation*

There is a known association and cross-feeding between protozoa and methanogenic archaea (Vogels et al., 1980). Archaea associate with protozoa because protozoa produce large amounts of H<sub>2</sub> which the archaea use to produce CH<sub>4</sub>. Defaunation is the removal of protozoa from the rumen as a means of CH<sub>4</sub> mitigation. This is accomplished by dietary agents, chemical agents, or isolation at birth, and has been shown to reduce ruminal CH<sub>4</sub> production by 20 to 50% (Whitelaw et al., 1984; Itabashi et al., 1994; Van Nevel and Demeyer, 1996). Ruminal protozoa are not necessary for normal rumen functioning (Jounay and Ushida, 1999), but it has been shown that defaunation may depress fiber digestion (Itabashi, 2001). This method of CH<sub>4</sub> mitigation must be weighed against its possible impact on the efficiency of the animal (Boadi et al., 2004). Ciliate protozoa store energy in the form of carbohydrates and help supply

carbohydrates to the microbes (Puniya et al., 2015). This helps to maintain a stable rumen microbial community. Some species are important when animals are consuming high grain diets for maintenance of rumen health. *Entodinium* species engulf carbohydrates from the diet and help modulate rumen pH. When cattle are consuming grain diets, the ruminal pH is lower than that of forage diets, so any pH modulation provided by protozoa can help the rumen maintain normal function and therefore improve animal performance (Jounay and Ushida, 1999). Studies have shown that methane production has an inconsistent response to partial or complete defaunation (Hristov et al., 2013). Popova et al. (2011) found that with a 65% difference in ruminal protozoa populations there was no difference in CH<sub>4</sub> production. Itabashi et al. (1984) isolated goats at birth and found a 3.5% increase in methane production when fed a grain diet. In contrast, Whitelaw et al. (1984) found a decrease of 49.6% in CH<sub>4</sub> production by defaunated beef cattle. This is supported by studies conducted with sheep. Yanez-Ruiz et al. (2007) observed a 25.9% decrease in sheep feed a roughage and concentrate diet at a 1:1 ratio. Due to the inconsistent response and the beneficial effects of protozoa when feeding a high grain diet, defaunation is not a recommended CH<sub>4</sub> mitigation practice (Hristov et al., 2013).

### *3-Nitrooxypropanol*

A new feed additive (3-nitrooxypropanol; **3NOP**) has received considerable attention in recent years (Hristov et al., 2013). It acts by inhibiting Methyl-Coenzyme M reductase (**CoM**; Martinez-Fernandez et al., 2014), which catalyzes the last step of the reduction of methyl-coenzyme M to CH<sub>4</sub> in the methanogenesis pathway (Attwood and McSweeney, 2008). Early literature has shown 3NOP can reduce methane production and increase propionate concentration (Haisan et al., 2014). Haisan et al. (2014) found that



3NOP reduced methane yield by 40% for supplemented cattle, without reducing DMI. Martinez-Fernandez et al. (2014) found a similar response of 24% reduction in CH<sub>4</sub> per unit of DMI. However, research by Vyas et al. (2016) found that 3NOP had a tendency to reduce DMI and ADG of finishing beef cattle. Romero-Perez et al. (2014) found similar results for beef cattle fed a high forage diet. Other CH<sub>4</sub> inhibitors, such as bromochloromethane, 2-bromo-ethane sulfonate, and chloroform are limited due to toxicity, rumen adaptation, or environmental regulation (Hristov et al., 2013). To this point, there has not been any signs of animal toxicity issues from 3NOP in beef cattle, sheep, or dairy cattle (Martinez-Fernandez et al., 2014; Haisan et al., 2013; Reynolds et al., 2014; Romero-Perez et al., 2014).

3-Nitropoxypropanol's effects on the microbial community of cattle has had varying results. Lopes et al. (2016) found the composition of methanogenic archaea was not affected by 3NOP supplementation, but total methanogen counts tended to be lower. These results were similar to Romero-Perez et al. (2016), who found total methanogen counts were lower when cattle were fed 3NOP on a forage based diet. Romero-Perez et al. (2014) found no change in bacteria, protozoa, or methanogen numbers in beef cattle supplemented 3NOP and his results are corroborated by Haisan et al. (2014) and Martinez-Fernandez et al. (2014). These contrary findings necessitates additional research in order to clarify the impacts that 3NOP has on the rumen microbial population.

### **Improving Animal Performance**

Improving animal performance includes a large collection of management techniques with the broad goal of maximizing final product compared to total inputs of

the system. This includes using antibiotics, genetic selection, growth hormones, and probiotics (Knapp et al., 2014). By increasing animal productivity, the proportion of CH<sub>4</sub> produced per unit of product is decreased (Boadi et al., 2004).

### *Direct-Fed Microbials*

Direct-fed microbials are commonly used as supplements in animal production (Hristov et al., 2014). The mode of action has not been defined, but there has been promising in vitro results showing a potential CH<sub>4</sub> mitigation effect (Boadi et al., 2004). It is hypothesized that probiotics provide nutrients that stimulate the growth of ruminal bacteria resulting in increased bacterial population (Newbold et al., 1996), or that probiotics stimulate lactic acid utilizers resulting in a reduction of lactic acid and a more stable rumen environment (Boadi et al., 2004). Lactic acid producing bacteria and lactic acid utilizers have been inoculated together to promote a more desirable intestinal microflora, stabilize ruminal pH, and promote rumen health (Hristov et al., 2013). Inoculating with lactic acid bacteria requires careful management in scenarios that subacute rumen acidosis may occur (Hristov et al., 2013). Frumholtz et al. (1989) found that *Aspergillus oryzae* reduced CH<sub>4</sub> emissions by 50% in vitro, but increased the acetate:propionate ratio. They hypothesized that this was a result of decreased protozoal population. In contrast, Takahashi et al. (1997) observed an increase in CH<sub>4</sub> emissions by 18% DMP in sheep fed a probiotic preparation. For direct fed microbials to be a viable CH<sub>4</sub> mitigation option, there needs to be more research on specific strains that have consistent results, and there needs to be more in vivo studies to determine their efficacy in live animals (Boadi et al., 2004).

### *Genetic Selection*

There has been increased research looking at genetic selection for CH<sub>4</sub> emissions. Studies have found a difference between low and high-residual feed intake (**RFI**) animals and CH<sub>4</sub> emissions. Residual feed intake is defined as actual feed intake minus the expected feed intake (Koch et al., 1963). It is thought that low RFI animals will consume less without sacrificing performance (Herd et al. 1997). McDonnell et al. (2016) found that low-RFI animals actually produce more CH<sub>4</sub> than high-RFI animals, and this was believed to be due to an increase in ruminal organic matter digestibility. Fitzsimmons et al. (2013) in contrast, saw a reduction in CH<sub>4</sub> emissions from low-RFI animals when compared to their high-RFI counterparts. This inconsistency is prevalent throughout the literature, but Australia has implemented RFI into the traits used in their sire selection (Arthur et al., 2004). Alford et al. (2006) concluded that this would decrease their emissions by an estimated 3.1% over 25 years on a national scale. There is still a lack of information on the reliability of RFI rankings across diets and production settings (McDonnell et al., 2016). However, with the majority of CH<sub>4</sub> emissions from beef production in the United States coming from the grazing sector, selecting for low-RFI animals may decrease the carbon footprint of the beef system as a whole (McDonnell et al., 2016). There are, however, inconsistent results in the literature. Jones et al. (2011) could not detect a difference between RFI cattle when cattle were grazing lower quality pasture. This would make selecting for RFI unnecessary as 71% of the GHG produced by beef cattle comes from the cow-calf sector (Rotz et al., 2013).

### *Growth Hormones*

From 1977 to 2007 the U.S. Beef industry reduced its environmental footprint through improved reproduction and the use of growth hormones (Capper et al., 2011). These include  $\beta$ -agonist, steroidal implants, ionophores (in beef systems), and rBST (in dairy systems) (Capper and Hayes, 2015; Knapp et al., 2014). Stackhouse et al. (2012) completed an LCA comparing three different angus production systems in California: 1) Angus with no implants or  $\beta$ -agonist, 2) Angus with an estrogen/trenbolone acetate-based implant during the stocker phase, and 3) Angus with zilpaterol hydrochloride along with an implant. It was found that treatment with an implant and implant plus a  $\beta$ -agonist decreased the carbon footprint of the Angus production systems by 4% and 9%, respectively (Stackhouse et al., 2014). When comparing the  $\text{NH}_3$  emissions from these systems, they found that the  $\beta$ -agonist system reduced emissions by 6% and 14% when compared to the natural and implant systems, respectively (Stackhouse et al., 2012). They hypothesized that this was due to the physiological response to the  $\beta$ -agonist, which increases muscle mass via protein synthesis.

The value of growth hormones to the productivity of the beef industry was highlighted by Capper and Hayes (2012). They examined what would happen if growth promoting technologies were removed from the production system. Removing growth promoting technologies increased manure production by 10.1%, N excretion by 9.8%, and P excretion by 10.6% (Capper and Hayes, 2012). Growth promoting technologies were also seen to reduce the amount of land, water and fertilizer needed in the production system (Capper and Hayes, 2012).

### **Summary of Literature Review**

Sustainability is a broad and complex science even when viewing it from the perspective of beef production. This literature review focused only on one aspect of sustainable beef production (CH<sub>4</sub> mitigation options), attempted to highlight areas worth exploring, and those that have been exhausted. With increased public concern about the impact of the beef industry on GHG emissions, and the expected climate change that will occur, it is important for scientists and producers to find ways to mitigate GHG emissions. Being that CH<sub>4</sub> is a byproduct of ruminal fermentation, it is intuitive that management decisions have the potential to mitigate its production.

Quantifying the emissions of cattle has been difficult to accomplish, particularly in grazing settings. The SF<sub>6</sub> technique is one that is well established and is often the main method used in this environment (Hill et al., 2016). The extensive research with this system has allowed researchers to develop new technologies to improve on its shortcomings. The GEM system is one technology that allows cattle to be in a natural environment (like SF<sub>6</sub>) but does not restrict them (unlike SF<sub>6</sub>). Instead it brings the cattle in with a pelleted bait feed and utilizes spot measurements to estimate methane emissions. Velazco et al. (2015) has shown that the GF is able to detect treatment differences and therefore can be utilized to determine mitigation strategies, and may be useful for long term quantification of herd DMP.

Methane emissions can be mitigated in many ways, with a large influence by management decisions. It has been well established that cattle on a high quality diet have less emissions per unit of feed consumed than those on a low quality diet (Johnson and Johnson, 1995). If all cattle are on a high quality diets then how the diet is processed also plays a major role in emission rates, along with level of intake (Johnson and Johnson,

1995). In addition to high quality diets, anything that allows the animal to be more feed efficient will typically reduce the emission rate of the animal. This is due to the fact that the animal is likely to have a shorter life span or produce more product which dilutes the emissions rates.

The carbon footprint of the beef industry can be complex. When the amount of CH<sub>4</sub> emitted is decreased, every aspect of the system must be examined to determine if emissions are increasing from a different source, or compromising the efficiency of the system. Therefore, any applicable mitigation strategy is one that improves all three branches of sustainability, or at least does not negatively affect any one branch. This will help the beef industry advance into the future in a sustainable and profitable manner.

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## CHAPTER II

### SUPPLEMENTING ENERGY IN CONJUNCTION WITH MONENSIN IMPROVES SUSTAINABILITY OF STOCKER CATTLE GRAZING WINTER WHEAT

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**Abstract:** The objective of this study was to determine the effect of a monensin-containing energy supplement on CH<sub>4</sub> emissions and performance of stocker calves grazing winter wheat. Eight steers (BW = 261 ± 32.9 kg) and 8 heifers (BW = 239.97 ± 21.02 kg) were grazed on a 9-ha pasture with a GreenFeed CH<sub>4</sub> measurement system (GEM; C-Lock Inc., Rapid City, SD) after initial adaptation in a drylot. For 2 wk prior to treatments, baseline CH<sub>4</sub> emissions was measured for each animal. Calves were randomly assigned within sex to receive 0, 0.21, 0.43, 0.64, 0.86, or 1.07 kg/d of a supplement (primarily ground corn and wheat middlings with mineral supplements and 150 mg/kg monensin). The GEM bait feed was pelleted wheat middlings. Calves were fed 3 d per wk in individual stalls and orts were weighed; actual supplement intake was used for analysis. This resulted in a relatively uniform distribution of actual supplement intake in the range of 0.53 to 1.46 kg/d, when GEM bait intake was included. Forage

intake was estimated by bolusing TiO<sub>2</sub> for 14 days then collecting feces for 5 d, at the end of the experiment, using TiO<sub>2</sub> and indigestible acid detergent fiber as external and internal markers. Because several predictor variables were available, dependent variables of interest were subjected to backwards stepwise regression (PROC GLMSELECT in SAS) with baseline CH<sub>4</sub>, total supplement intake, forage intake, initial body weight, sex, and monensin dose in the model. Animal performance increased linearly with total supplement intake and forage intake ( $P = 0.02$ ;  $R^2 = 0.45$ ). Supplement intake quadratically reduced forage intake ( $P < 0.01$ ;  $R^2 = 0.47$ ). Methane production increased with increasing forage intake and initial body weight, but the heifers had a lower overall production than steers ( $P < 0.01$ ;  $R^2 = 0.74$ ). Supplement intake reduced CH<sub>4</sub> emission intensity (g CH<sub>4</sub>/kg live weight gain;  $P = 0.028$ ). Methane yield (g CH<sub>4</sub>/ kg of intake) decreased with increasing DMI and decreasing body weight, and heifers yielded less CH<sub>4</sub> than steers ( $P < 0.01$ ;  $R^2 = 0.837$ ). These results suggest that supplementing cattle grazing wheat pasture with an energy/monensin supplement improves sustainability by reducing emission intensity.

**Key Words:** Wheat Pasture, Grazing, Enteric Methane, GreenFeed, Energy Supplementation

## Introduction

Enteric methane is a major contributor to the carbon footprint of the beef industry and has received considerable attention from researchers and the public. Methane is a natural byproduct of ruminal fermentation and is a potent greenhouse gas (**GHG**), with a global warming potential 28 times that of carbon dioxide over a 100-yr period (IPCC, 2013). Global GHG emissions from agriculture is estimated to be 7.1 gigatonnes of CO<sub>2</sub>

equivalents, or 14.5% of total anthropogenic GHG production (FAO, 2013). Of these, 2.8 gigatonnes come from enteric methane production, with cattle being responsible for 77% (FAO, 2013).

Evaluating production systems of different regions provides insight into the efficiency of systems and appropriate mitigation options (Hill et al., 2016). Several publications have quantified regional GHG emissions of beef cattle from both modeling and empirical methods (Stackhouse et al., 2012; Pelletier et al., 2010; Ebert, 2016). Winter wheat grazing in the southern Great Plains has thus far garnered limited interest, in terms of CH<sub>4</sub> quantification (Ebert, 2016). Wheat can be grazed from November to March before grain harvesting in the early summer (Ebert, 2016). In January of 2017 there were 1.8 million head of cattle grazing small grain pasture in the Southern Great Plains (USDA, 2017). Supplemental energy (**ENE**) is commonly provided to cattle grazing winter wheat. A common energy supplement is ground corn or milo and may contain monensin. Providing supplemental energy to wheat grazing cattle has been shown to increase animal gains and profitability (Hogan, 1982; Horn and Paisley, 1999). Previous literature has demonstrated the benefit of increasing live weight gains as a tool for GHG mitigation (Herrero et al., 2016). Therefore, the objective of this study was to quantify CH<sub>4</sub> using the GreenFeed system (**GEM**; C-Lock Inc., Rapid City, SD) and calf performance at different levels of ENE supplementation.

## **Materials and Methods**

All procedures used in this experiment were in accordance with Oklahoma State University Animal Care and Use Committee (ACUP # AG-16-19).

## Animals and Treatments

Eight spring born crossbred Angus steers (BW=262 ± 33 kg) and eight heifers (BW=240 ± 21 kg) were selected from a group of 24 based on willingness to use the GreenFeed, and placed in a 9.15 ha wheat pasture. Acclimation to GEM occurred in a drylot at the Oklahoma State University Nutrition and Physiology Barn. Once placed in the pasture all calves were acclimated to individual feeding stanchions for two weeks prior to experiment initiation. Stanchions were 1.8 by 0.9 by 0.6 m, and were located in a barn adjacent to the pasture. Animals were allowed 30 minutes to consume ENE and anyorts were weighed. They were randomly assigned to one of the following supplement intake levels: 0, 0.5, 1.0, 1.5, 2.0, or 2.5 kg as fed. Two animals (1 steer and 1 heifer) were assigned to each of the first five supplement levels and six animals (3 steers and 3 heifers) were assigned to the 1.07 kg/d treatment. The ENE supplement formulation was a ground corn-based energy supplement containing monensin (34 mg/kg; **Table 1**). Cattle were fed in the stanchions three days per week at 0700 for 7 weeks and unshrunk body weights were recorded once per week on validated scales to determine animal performance.

## Pasture

All cattle were housed in a 9.15-ha wheat pasture located at the Oklahoma State University Wheat Barn (Stillwater, OK) from January 9 to February 26. Precipitation at this site was 12.09 cm for the months of January and February which was greater than the 30-year average of 7.62 cm (<http://mesonet.org>). A rising plate meter (Jenquip, Feilding, New Zealand) was used to determine forage mass of the pasture. Two sets of 30 plate

meter height readings were recorded on day 1 and every two weeks thereafter for the duration of the experiment. Readings were taken at random locations across the pasture to account for spatial variation (Reuter et al., 2012). For all sampling days, 10 additional plate meter heights were recorded, that encompassed the range of forage mass in the pasture, and hand clipped to ground level. All clippings were then weighed wet, dried in a 40°C oven, and weighed again to calculate DM content. A regression line was fit for each measurement day and applied to the corresponding plate meter readings to estimate forage mass in the pasture as described by Moffet et al. (2012) and Reuter et al. (2012).

### Emissions Measurements

Methane was quantified using the GEM spot measurement system. Spot measurements were averaged across the 49 d trial period to determine average daily methane production for each individual animal (**DMP**; Hristov et al., 2015). The bait feed consisted of pelleted wheat middlings and each drop from the GEM weighed  $28 \pm 2$  g. While the animal's head was in the hood, air is drawn around the animal's head to capture the emitted gases which are then analyzed for CH<sub>4</sub>, CO<sub>2</sub>, and O<sub>2</sub> (Hristov et al., 2015). Emitted gases are then compared to background gases obtained when animals were not present (Cottle et al., 2015). Each animal was allowed 4 visits per d with a minimum of 4 hr between each visit. Each visit consisted of 6 drops per visit with 30 seconds between each drop. This was done to keep the animal in the hood long enough to obtain accurate estimates, and to encourage animals to visit throughout the day. Only visits that had a minimum of 3 minutes were used to estimate gas emissions (Velazco et al., 2016). The GF was calibrated once weekly and CO<sub>2</sub> recoveries were completed every 30 d (Hristov

et al., 2015). A 2-wk period prior to experiment initiation was used to determine background emission rates for each animal for covariate analysis (Hristov et al., 2015).

### Urea Nitrogen

Blood was drawn from the jugular vein on d 1, 26, and 49 for plasma urea nitrogen analysis. EDTA blood tubes (BD Vacutainer EDTA blood tube; Fisher Scientific) were used and samples were placed on ice immediately after sampling, then centrifuged for 10 minutes at 1,000 rpm. The plasma was removed and stored at -80°C until further analysis. Plasma samples were analyzed using a Urea Assay Kit (MAK006; Sigma Aldrich) and a Spectrophotometric multiwall plate reader (Molecular Devices; Sunnyvale, CA). It was subsequently used to estimate urinary N excretion as described by Kohn et al. (2005; **Equation 1**).

$$\text{Equation 1: Urinary N, g/d} = \text{CR} \times \text{BUN} \times \text{BW}$$

Where CR is clearance rate from the kidneys (1.3 used as a standard clearance rate; Kohn et al., 2005), BUN is blood urea nitrogen and BW is body weight. Plasma urea nitrogen was substituted for BUN (Kohn et al., 2005). Urine N was estimated to examine if provided supplement increased urinary N excretion. Increasing urinary N would increase the amount of N that is available for rapid volatilization in the form of ammonia or nitrous oxide, and therefore may limit the efficacy of the mitigation strategy (Hristov et al., 2014; Place, 2016).

### Forage Quality and Intake

Cattle were bolused with 10g of titanium dioxide, TiO<sub>2</sub>, daily at 0700 (Titgemeyer et al., 2001). During the last 5 days of the experiment fecal samples were collected twice daily at 0700 and 1600 in a squeeze chute via rectal grab (Titgemeyer et al., 2012). A diet sample was obtained by compositing 10 random hand-clipped forage samples on the first day of fecal collection. All samples were frozen after collection at 20°C until further analysis, except for a subsample of forage and supplement samples that were oven dried at 40°C for 48 hours to determine DM. Fecal, forage, and supplement samples for laboratory analysis were lyophilized, and ground to pass through a 1-mm screen (Thomas A. Wiley Laboratory Mill, model 4). After grinding, fecal samples were composited by weight within animal.

Fecal, forage and supplement samples were analyzed for DM and ash (AOAC, 1990), NDF and ADF in triplicate using an ANKOM 2000 Fiber Analyser (Ankom Technology, Macedon, NY), and for N by combustion (Vario Mac CN; Elementar Americas, Mount Laurel, NJ). Nitrogen content was multiplied by 6.25 to determine CP. All samples were analyzed for indigestible ADF (**iADF**) using the procedure described by Bohnert et al. (2002). Samples were analyzed in triplicate with diet and supplement samples incubated for 16 h at 39°C in a solution containing 0.1% pepsin (Catalog #9001-75-6 Fisher Scientific; Hampton, NH) and 10% 1 N HCl using a Daisy<sup>II</sup> incubator (9 sample bags and 2 L per incubation vessel; Ankom Co., Fairport, NY). Samples were rinsed with warm (39°C) tap water and placed in a lingerie bag along with the fecal samples. All samples were incubated for 96 h in the rumen of a cannulated steer consuming low-quality forage. Once removed, the sample bags were rinsed with warm (39°C) tap water until the water was clear and were then dried at 50°C. Samples were

then analyzed for ADF. Forage and supplement samples were analyzed for TDN, NEm, NEg using wet lab procedures at a commercial lab (DairyOne, Ithaca, New York). Titanium dioxide concentration was analyzed using a Delta XRF Analyzer (DP-6000, Olympus Scientific Solutions Americas, Waltham, MA) equipped with a Rh anode tube. Indigestible ADF and TiO<sub>2</sub> was used to calculate forage intake using the two marker method (Kartchner, 1981).

**Equation 2.1:** Fecal output (g/d) = TiO<sub>2</sub> bolused (g/d)/TiO<sub>2</sub> in feces (g)

**Equation 2.2:** Digestibility (%) = 100 - (100(% iADF Intake/% iADF feces))

**Equation 2.3:** Fecal output from forage (g/day) = fecal output (g/day) - (Supplement fed x (1 - Supp. Digestibility))

**Equation 2.4:** Forage intake (g/d) = Fecal output from forage (g/d) / (1 - forage digestibility)

### Statistical Analysis

Actual supplement intake of each animal over the course of the trial was averaged within animal, and used for analysis. All data were analyzed using SAS (SAS Institute Inc., Cary, NC, v 9.4). Average daily gain was determined with PROC REG by regressing BW over time. Models were determined in PROC GLMSELECT, with prediction variables subjected to backwards stepwise regression with baseline CH<sub>4</sub>, total supplement intake, forage intake, initial body weight, sex, monensin dose, and day for PUN and urinary N in the model. Models were selected using Mallows Cp (Thompson, 1978), which examines mean square error of prediction in selecting the best fit model.



Total supplement intake (TSI) included GEM bait feed so no true zero level of intake was available. Animal was the experimental unit (Bello et al., 2016) and significance was declared at  $P \leq 0.05$  and tendencies were declared at  $0.05 < P \leq 0.10$ .

## **Results and Discussion**

### Pasture

Forage mass on d 1 was significantly lower than on d 15, 1189 vs. 1355 kg/ha respectively ( $P < 0.05$ ), but was not different than day 29 (1235 kg/ha). Day 49 had a significantly lower forage mass than any of the previous three measurement days (736 kg/ha;  $P < 0.05$ ). Initial stocking rate of a pasture was 4.94 kg forage DM/kg of BW. This is higher than the recommended level to achieve maximum performance (3.5 kg forage DM/kg average BW; Beck et al., 2013). By the end of the trial forage allowance of the pasture fell to 1.84 kg forage DM/kg of pasture. Flooding in the pasture is a possible explanation for the decrease in forage mass. Precipitation was 4.47 cm higher than average at the trial site and potentially caused waterlogging (<http://www.mesonet.org>). Waterlogging causes soil to become depleted of oxygen within a few hours and can be detrimental to forage growth (Malik et al., 2002).

### Supplement Intake and Animal Performance

Actual supplement intake ranged from 0.5 to 1.84 kg as fed per feeding (0.21 to 0.78 kg/d as fed) for supplemented cattle. Once supplement was offered over 0.5 kg/feeding, no animal consistently consumed all offered ENE. Previous studies have shown that feeding monensin at low levels does not cause palatability issues (Potter et al., 1976; Horn et al., 1981). Therefore, we believe that the cause of the inconsistent levels of

supplement intake was animal variability. Previous research has noted that cattle supplemented with a corn-based supplement do not consume it as readily as high-fiber energy supplements (Horn et al., 2005).

Average daily gain ranged from 0.64 to 1.67 kg/d with a mean ADG of 1.07 kg/hd/d. Average daily gain did had a significant positive quadratic relationship (**Table 2; Figure 1**;  $P = 0.02$ ;  $R^2 = 0.47$ ) with DMI and total supplement intake. These results are consistent with past literature that found increasing supplement intake increased animal performance. Fieser (2007) reviewed studies of cattle grazing wheat pasture supplemented with energy and monensin dating back to 1990. Supplement intake ranged from 0.40 to 2.28 kg/d with an average of 1.14 kg/d. Of the 11 trials with similar energy intake levels as the current trial, ADG ranged from 0.89 to 1.45 kg/d (Fieser, 2007). Supplement conversion, kg of energy supplement per kg of additional gain, ranged from 0.65 to 5.61. As energy supplement intake increased, the conversion of supplement to additional gain increased ( $P = 0.002$ ). That indicates that as supplement intake increases, more is necessary for each kg of additional gain. This results are similar to similar literature (Fieser, 2007; Rouquette et al., 1982; Fieser et al., 2003; Fieser et al., 2005). Fieser et al. (2003) fed a similar energy supplement to the current trial at 0.91 kg every other day and reported a supplement conversion of 3.6. Overall, these results agree with Fieser (2007), who stated that supplement conversion is improved when the amount of supplement fed is decreased.

### Forage Intake

Estimates of forage dry matter intake ranged from 1.57 to 2.95% BW (5.00 to 8.93 kg DM/d). Forage intake had a significant quadratic relationship with total supplement intake (**Table 2; Figure 2**;  $P < 0.01$ ;  $R^2 = 0.53$ ). Dry matter intake of forage was greatest at 0.9 kg of supplement intake and decreased with increasing supplement intake. Dry matter intake as a percentage of BW had a significant quadratic relationship with supplement intake, with heifers having a higher intercept ( $P < 0.01$ ;  $R^2 = 0.80$ ). While DMI levels of the current trial were lower than those reported in similar experiments (Ebert, 2016; Horn et al., 1981), a possible explanation was that at the time of fecal collection the forage mass of the pasture was at its lowest point of the trial (737 kg/ha) and could have reduced the amount of forage consumed (Allison, 1985). McCollum et al. (1992) reported that forage mass levels within the range of the current trial would result in forage intake of 1.8% to 2.3% of BW, which is similar to the current trial. Substitution rate of forage by energy supplement increase linearly with increasing levels of supplement intake ( $P = 0.01$ ). Substitution rate was defined as change in forage intake ( $\text{g/kg BW}^{0.75}$ ) per unit increase in energy supplement ( $\text{g/kg BW}^{0.75}$ ). The model found that as supplement intake increased there was a decrease in forage intake. It estimated that at 0.3 kg of supplement intake, there was a substitution rate of 0.262 and at 0.8 kg of supplement intake the rate was 3.332. The substitution ratios were higher than previously observed, but the increased substitution rate with increasing supplement intake agrees with similar published literature (Boadi et al., 2002; Young et al., 1980, Faverdin et al., 1991).

#### GEM Visits

All animals combined for 1218 total useful GEM visits and averaged 220 seconds in duration. Animals displayed a circadian pattern when visiting the GEM (**Figure 3**). In the current study, animals visited the GEM the most from 0800 to 1100 and 1300 to 1500. The fewest visits occurred from 0400 to 0600 and 1900 to 2100. Alemu et al. (2017) found that animals visited the GF most often at midnight (0000 h), 0600-0700 h, and around 1100 h, with the fewest visits at 0400 and 2200. A possible explanation for the difference in GEM visitation pattern could be the weather at the time of the current trial. Animals typically graze latter in the day and are not as active at night during the winter in an attempt to decrease cold stress (Castle and Halley, 1953; Arnold, 1984). Arnold (1984) found that although cattle do not graze much at night during the winter, there is a small grazing bout from 2000 to 0100 h. The temperature range of the current trial was similar to Arnold (1984; -6°C to 30°C max temperature; <http://www.mesonet.org>) and could explain why the GEM received a small spike in activity from 2300 to 0100 in the current trial.

### Emissions

Mean DMP and CO<sub>2</sub> production were 173 ± 12 (g/d) and 6125 ± 412 (g/d), respectively. Daily methane production was lower than that of a similar study completed by Ebert (2016) who reported DMP levels ranging from 334 to 351 g/d for cattle grazing winter wheat. Jonker et al. (2015) found similar values with cattle being fed a high quality fresh forage. Methane production did have a significant positive linear relationship with initial body weight, sex, and DMI ( $P < 0.01$ ;  $R^2 = 0.74$ ; **Table 2**), with heifers producing more CH<sub>4</sub> (**Figure 4**). Daily methane production was moderately correlated with DMI ( $r = 0.46$ ). Emission intensity had a significant negative quadratic

relationship with total supplement intake and animals that had a high baseline CH<sub>4</sub> had a higher EI ( $P= 0.03$ ;  $R^2= 0.41$ ; **Table 2; Figure 5**). These results do not agree with Ebert (2016) who found that EI was increased when supplemental energy was offered to cattle grazing winter wheat at 0.5% BW with no monensin (approximately 2 kg). The supplement in the current trial contained monensin, which published literature has established its anti-methanogenic properties, with some variability (Appuhamy et al., 2012). The initial BW of cattle in the current trial was 160 and 180 kg less for steers and heifers compared to Ebert (2016), respectively, potentially resulting in lower DMP. Methane yield (g CH<sub>4</sub>/kg of total intake) had a negative linear relationship with DMI and initial body weight, with heifers having a lower MY than steers ( $P < 0.01$ ;  $R^2= 0.84$ ; **Table 2; Figure 6**), where heifers had a lower MY than steers. The MY was similar to previous trials for cattle grazing high quality forages (Ebert, 2016; Grainger et al., 2007; Grainger et al., 2010) with MY values ranging from 18.1 to 27.2. There were no significant relationships between emission variables and nutrient intake or digestibility.

The effect of sex on DMP was unexpected, and subsequently impacted MY. Heifers produced significantly less DMP than steers ( $P < 0.05$ ; 167 vs. 180 g/d, respectively). This is in conflict with published literature such as Jiao et al. (2013) which observed no significant differences for dairy steers and heifers from 6 to 24 months of age, although they did notice a tendency at 18 months of age for steers to produce more methane than heifers ( $P= 0.06$ ). This was particularly interesting as average DMI was numerically greater for heifers than steers, which has been established as a main driver of DMP (Johnson and Johnson, 1995). A possible explanation for this is the inherent variability that can occur with the GEM system (Hill et al., 2016), although there was no

difference between time of visits. The body weight differences between heifers and steers could also have influenced methane emissions. Initial body weight of steers was 21.88 kg greater than heifers, although this was not significant ( $P > 0.05$ ).

### Nitrogen

Plasma urea nitrogen was not affected by any variables in model ( $P > 0.05$ ). Mean PUN levels for the treatment ranged from 0.14 to 0.2 mg/dL. These PUN levels were within a reasonable range with similar studies reporting PUN in this range (Koenig et al., 2015; Lagrange et al., 2017). Mean Urinary N excretion rate ranged from 39.64 to 56.17 g/d. Urinary N excretion had a significant linear relationship with sex, initial body weight, and day (**Table 2; Figure 7**;  $P < 0.01$ ,  $R^2 = 0.52$ ). Nitrogen excretion increased by day of sampling and was greater for heifers. The N excretion level estimated by the equation was considerably lower than similar literature. Shreck et al. (2017) fed fresh cut wheat forage and supplemented a steam-flaked corn based energy supplement and N excretion from urine ranged from 91.4 to 110.1 g/d from cattle of similar BW as those of the current trial. Therefore, this equation may not be appropriate to estimate urinary N excretion from cattle grazing high quality forage.

### **Conclusion**

We conclude that energy supplementation improves the EI and animal performance of stocker cattle grazing winter wheat. It was surprising to detect a difference in DMP and MY between heifers and steers, but we postulate that this may have been due to animal variability and the limited number of animals tested. Additional

research needs to be conducted with wheat grazing cattle to validate the DMP estimates for future modeling research and examination of alternative mitigation option.

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**Table 1.** Forage and Supplement Composition

Item	Forage and Supplements		
	Wheat <sup>a</sup>	Energy Supplement	GEM Pellet
Formulation, % as-fed			
Ground corn	---	62.29	---
Wheat middlings	---	21	100
Molasses	---	5	---
Limestone	---	4.3	---
Dicalcium phosphate	---	2.55	---
Magnesium mica	---	4	---
Salt	---	0.5	---
Magnesium oxide	---	0.22	---
Rumensin 90	---	.000825	---
Vitamin A 30,000	---	0.0005	---
Nutritive Value			
% DM	46.22	91.35	89.88
TDN	58.0	83	73
CP	21.7	10.5	19.1
NDF	44.9	32.88	45.85
ADF	35.6	27.61	32.35
NE <sub>m</sub> (Mcal/kg DM)	1.57	2.03	1.82
NE <sub>g</sub> (Mcal/kg DM)	0.97	1.37	1.19

<sup>a</sup>Wheat= obtained from forage clippings



**Table 2.**  
Regression Models Selected From Backward Stepwise using Mallows Cp.

Model	<i>P-Value</i>		<i>R</i> <sup>2</sup>		Mallows Cp
	Linear	Quadratic	Linear	Quadratic	
ADG= -0.432+ 0.331(TSI) <sup>2</sup> + 0.147(DMI) <sup>a</sup>	--	<b>0.016</b>	--	0.472	0.945
DMI= -6.45- 9.86(TSI) <sup>2</sup> + 18.5(TSI)+ 0.023(IBW)+ 0.624(Sex) <sup>e</sup>	--	<b>0.002</b>	--	0.771	3.029
DMP= 98.33+ 0.17(IBW)+ 5.22(DMI) -11.31(Sex) <sup>b</sup>	<b>&lt;0.001</b>	--	0.741	--	4.253
EI= 87.122- 25.32(TSI) <sup>2</sup> + 0.69(BAS) <sup>c</sup>	--	<b>0.031</b>	--	0.413	2.098
MY= 27.84+ 0.02(IBW)- 1.52(Sex)- 1.59(DMI) <sup>d</sup>	<b>&lt;0.001</b>	--	0.837	--	2.765
UN= -11.017+ 6.23(Sex)+ 0.36(Day)+ 0.20(IBW) <sup>f</sup>	<b>&lt;0.001</b>	--	0.519	--	1.473

<sup>a</sup> TSI= Total Supplement Intake (including GEM bait feed; kg/d); DMI= Dry Matter Intake of Forage (kg/d)

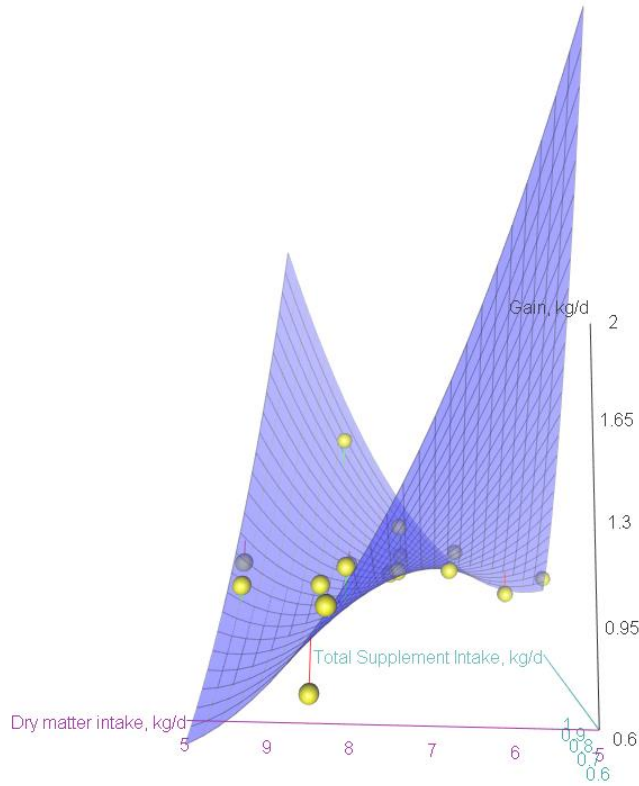
<sup>b</sup> IBW= Initial Body Weight (kg); DMI= Dry Matter Intake of Forage (kg/d); Sex= 1 for heifers, 0 for steers

<sup>c</sup> BAS= baseline CH<sub>4</sub>. Background CH<sub>4</sub> was obtained during a two-week pretrial period where animals were not supplemented but allowed access to the GreenFeed; TSI= Total Supplement Intake (including GEM bait feed; kg/d)

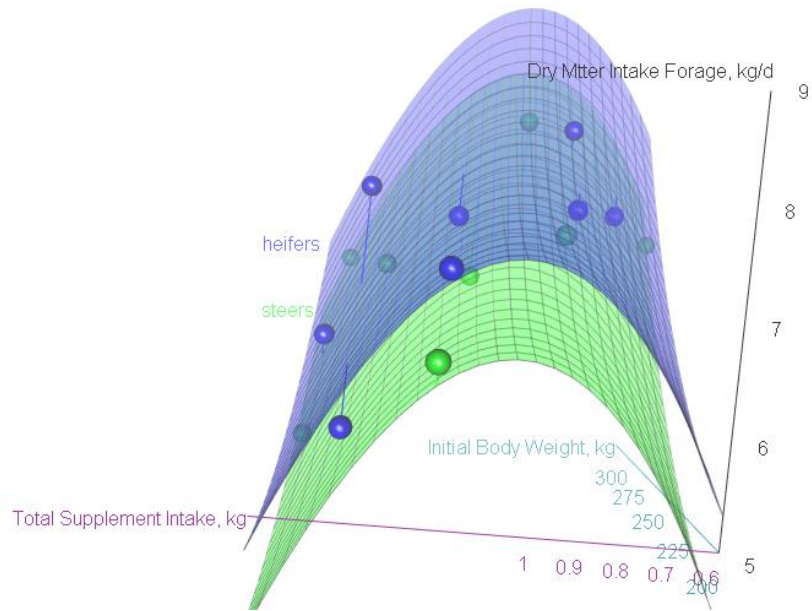
<sup>d</sup> IBW= Initial Body Weight (kg); Sex= 1 for heifers, 0 for steers; DMI= Dry Matter Intake of Forage (kg/d)

<sup>e</sup> IBW= Initial Body Weight (kg); Sex= 1 for heifers, 0 for steers; DMI= Dry Matter Intake of Forage (kg/d)

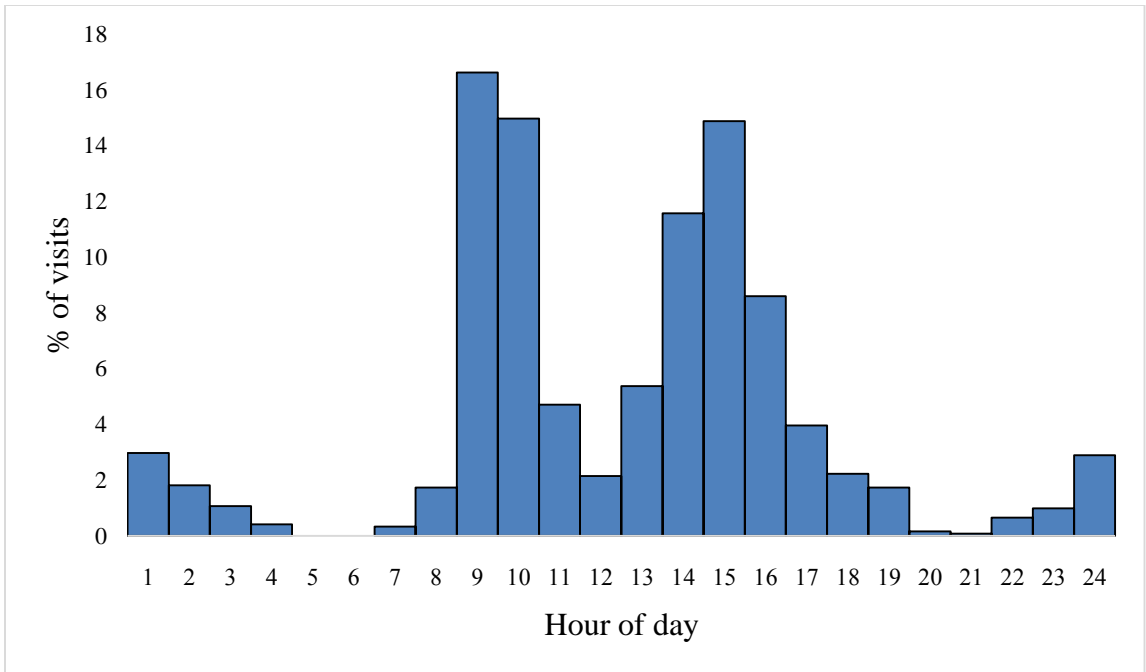
<sup>f</sup> S= 1 for heifers, 0 for steers; Day= 1, 26, or 49; IBW= Initial Body Weight (kg)



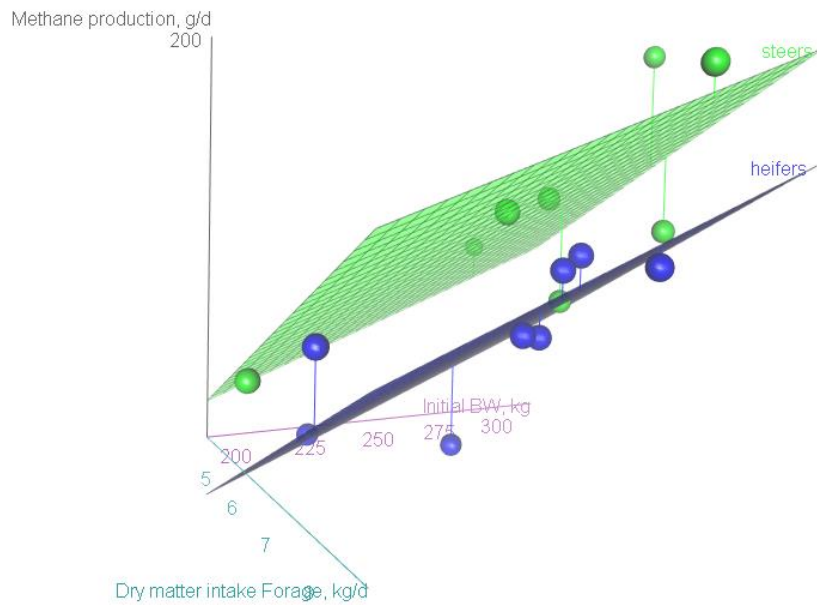
**Figure 1.** Total supplement intake (energy supplement plus GEM bait feed, kg/d) and dry matter intake of forage (kg/d) impacted ADG (kg/d) quadratically. ADG tended to improve when both forage and supplement intake increased (kg/d;  $P < 0.016$ ;  $R^2 = 0.472$ ).



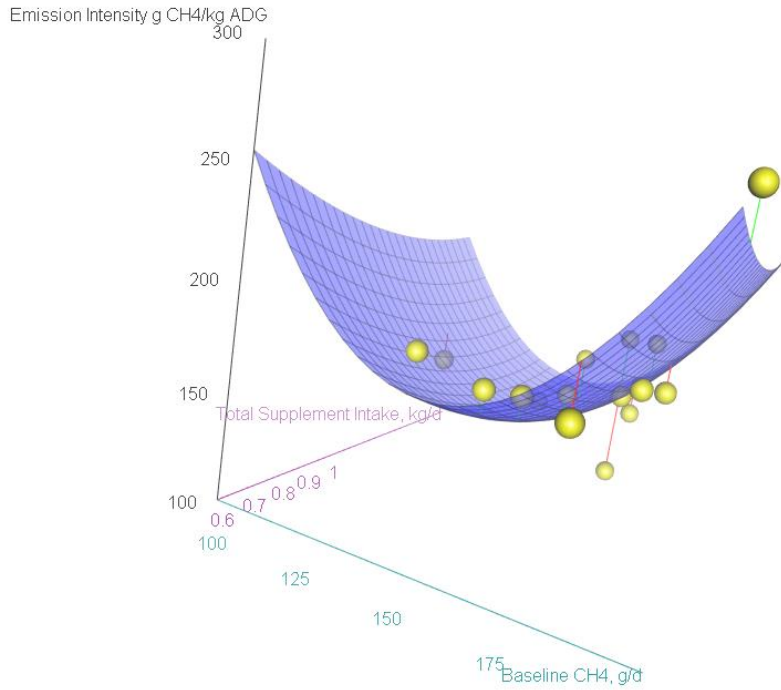
**Figure 2.** Forage intake (kg/d) increased with increasing body weight, and decreased rapidly when supplement intake surpassed 1 kg/d. Heifers consumed more forage than steers (kg/d;  $P < 0.002$ ;  $R^2 = 0.771$ ).



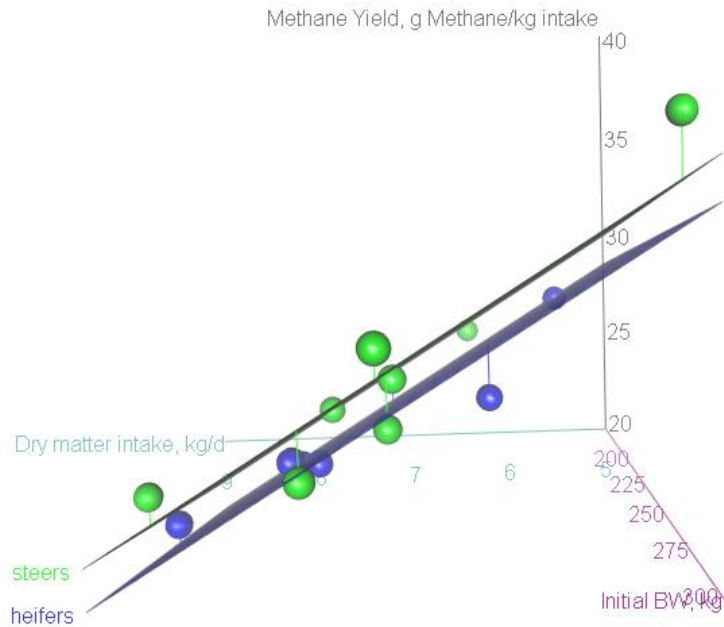
**Figure 3.** GreenFeed visits displayed a diurnal pattern of visitation throughout the day. Animals visited the GreenFeed predominately during mid-morning and mid-afternoon



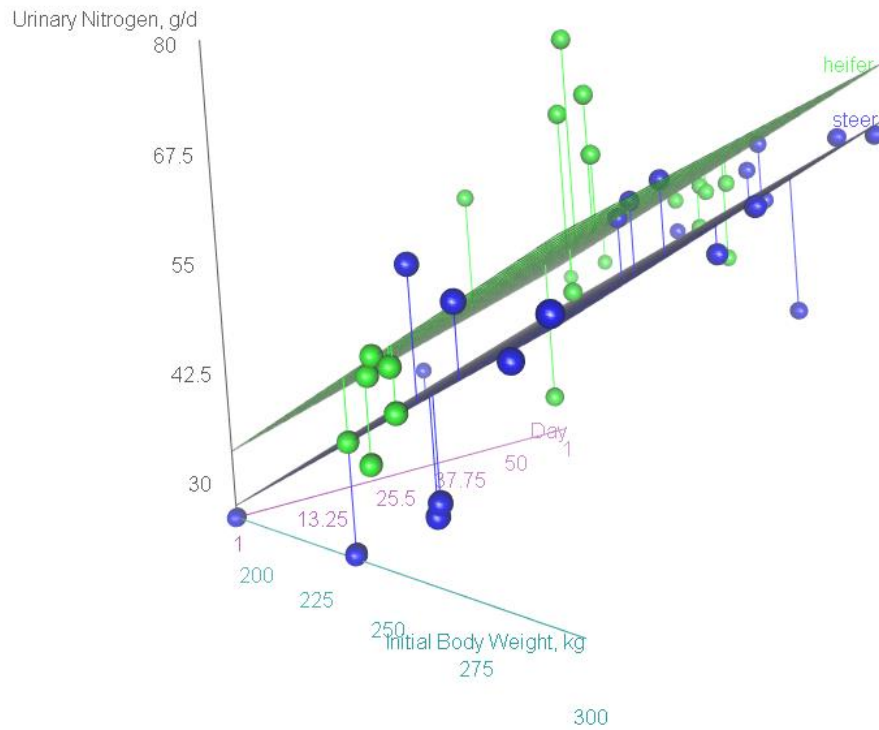
**Figure 4.** Initial body weight (kg) and dry matter intake of forage had a positive linear relationship with DMP (g/d). As forage intake and body weight increased animals produced more methane, with heifers producing less CH<sub>4</sub> than steers ( $P < 0.001$ ;  $R^2 = 0.741$ ).



**Figure 5.** Total supplement intake (energy supplement plus GEM bait feed; kg/d) and baseline CH<sub>4</sub> (g/d) had a negative quadratic relationship with EI (g CH<sub>4</sub>/kg of gain;  $P = 0.031$ ;  $R^2 = 0.413$ ). Emission intensity improved with moderate levels of supplement intake, and animals that produced more methane pretrial had a higher emission intensity.



**Figure 6.** Dry matter intake of forage (kg/d) and initial body weight had a negative linear relationship with methane yield (g CH<sub>4</sub>/kg total intake), with heifers having a lower methane yield than steers ( $P < 0.001$ ;  $R^2 = 0.837$ ). As forage consumption increased methane yield decreased.



**Figure 7.** Day of sampling and initial body weight had a positive linear relationship with urinary N excretion (g/d) with heifers excreting greater amounts of N ( $P < 0.001$ ;  $R^2 = 0.519$ ). As day on trial and body weight increased the amount of N excreted in urine increased.



APPENDICES

**Appendix 1: SAS Code for Emission, Performance, and Intake**

```
data methane;

input ID Sex $ CH4 CO2 O2 SI ADG InitialBW BackgrCH4 Drops DMD DMI ndfd
adfd cpd sub indf iadf dmbw;

EI= CH4/ADG;

ibw=InitialBW/2.205;

di=(drops*28.875)/1000;

tsi=((si*3)/7)+di;

grg=(si*3)/7;

my=ch4/dmi;

ti=dmi+tsi;

m2=ch4/ti;

supp=(si*3)/7;

backgrch4bw = BackgrCH4 / ibw;

monensin = grg * 34;

datalines;

16551 S      199.5 6816.09      6190.53      1.82  1.09  656  173.87 21.69
73.74 7.11 0.695 0.68 0.82 -0.373 3.74 2.95 2.08

16552 H      153.87 5515.9 5034.66      1.37  0.92  473  154.89 25.82 75.35
5.61 0.72 0.69 0.81 -0.168 3.06 2.40 2.21

16553 H      176.1 6176.77      5622.76      0      0.64  566  205.41 25.55
71.52 7.92 0.68 0.66 0.81 0 3.90 3.06 2.81

16554 S      193.98 6787.98      6298.53      0.5    1.09  592 184.02 26.08
75.51 8.93 0.73 0.70 0.83 1.24 4.43 3.48 2.83

16555 S      180.22 6286.24      5658.98      0.55  1.09  532  158.01 22.12
74.09 7.63 0.71 0.69 0.797 -0.223 3.80 2.98 2.63
```

16556	H	175.47	5766.66	5402.42	0.72	1.01	568	128.23	19.16
73.60 8.83 0.69 0.67 0.84 1.79 4.32 3.40 2.91									
16557	H	168.37	6042.07	5516.98	0.56	1.02	532	148.86	21.24
74.15 7.91 0.71 0.68 0.82 -0.327 3.91 3.08 2.74									
16558	S	175.97	6466.51	5930.84	1.84	0.98	608	163.27	23.53
66.88 5.00 0.62 0.59 0.78 -0.185 2.82 2.26 1.57									
16559	S	178.75	6585.36	6134.54	0	0.99	636	182.54	20.10
71.50 7.67 0.68 0.65 0.81 0 3.71 2.92 2.28									
16560	H	152.96	5824.4	5360.76	1.28	1.11	548	169.34	29.98 74.28
6.31 0.72 0.69 0.81 -0.189 3.42 2.68 2.15									
16561	H	167.02	5581.79	5011.17	1	1.03	442	173.85	24.82
71.67 7.19 0.69 0.67 0.78 -0.245 3.70 2.91 2.94									
16562	H	174.62	6066.74	5532.86	1.31	1.67	554	167.32	27.02
73.07 7.94 0.70 0.67 0.81 -0.469 4.11 3.23 2.67									
16563	S	182.06	6451.25	6050.45	0.97	1.05	582	187.19	23.00
73.58 7.07 0.71 0.68 0.78 -0.232 3.62 2.84 2.28									
16564	H	167.73	6086.97	5675.13	0.5	1.07	550	119.16	31.63
72.56 7.72 0.69 0.66 0.81 -0.176 3.96 3.10 2.60									
16565	S	162.51	5671.93	5323.62	0.99	1.05	423	158.21	25.24
73.97 6.41 0.70 0.67 0.82 -0.166 3.35 2.63 2.71									
16566	S	170.59	5878.98	5355.95	1.6	1.24	590	143.72	21.00
72.73 7.11 0.68 0.66 0.82 -0.347 3.70 2.91 2.22;									

**proc glmselect** data= methane plots=all;

class sex;

model dmi = tsi|tsi backgrch4 ibw sex monensin/ selection=backward(select=SL  
choose=cp)showpvalues;

**run;**

**proc glmselect** data= methane plots=all;

class sex;

```
model adg = tsi|tsi backgrch4 ibw sex dmi monensin/ selection=backward(select=SL  
choose=cp)showpvalues;
```

```
run;
```

```
proc glmselect data= methane plots=all;
```

```
class sex;
```

```
model ch4 = tsi tsi*tsi backgrch4 ibw sex dmi monensin/ selection=backward(select=SL  
choose=cp)showpvalues;
```

```
run;
```

```
proc glmselect data= methane plots=all;
```

```
class sex;
```

```
model m2 = tsi tsi*tsi backgrch4 ibw sex dmi monensin/ selection=backward(select=SL  
choose=cp)showpvalues;
```

```
run;
```

```
proc glmselect data= methane plots=all;
```

```
class sex;
```

```
model EI = tsi tsi*tsi backgrch4 ibw sex dmi monensin/ selection=backward(select=SL  
choose=cp)showpvalues;
```

```
run;
```

## Appendix 2: SAS Code for Nitrogen

```
data pun;
input id sex $ si day pun adg tsi initialbw backch4 dmi un;
dsi=(si*3)/7;
IF ti <0.90 then trt=1;
if 0.90< ti <1.20 then trt=2;
if ti >1.20 then trt=3;
monensin=dsi * 150;
ibw= initialbw/2.205;
datalines;
16551 s 1.82 1 .126 1.09 1.41 656 173.87 7.11 48.67
16551 s 1.82 26 .129 1.09 1.41 656 173.87 7.11 55.95
16551 s 1.82 49 .143 1.09 1.41 656 173.87 7.11 63.61
16552 h 1.37 1 . 0.92 1.34 473 154.89 5.61 .
16552 h 1.37 26 .192 0.92 1.34 473 154.89 5.61 60.7
16552 h 1.37 49 .155 0.92 1.34 473 154.89 5.61 51.11
16553 h 0 1 .132 0.64 0.74 566 205.41 7.92 44.06
16553 h 0 26 .188 0.64 0.74 566 205.41 7.92 69.97
16553 h 0 49 .138 0.64 0.74 566 205.41 7.92 50.76
16554 s 0.5 1 .094 1.09 0.96 592 184.02 8.93 32.85
16554 s 0.5 26 . 1.09 0.96 592 184.02 8.93 .
16554 s 0.5 49 .140 1.09 0.96 592 184.02 8.93 57.63
16555 s 0.55 1 0.83 1.09 0.87 532 158.01 7.63 25.96
16555 s 0.55 26 . 1.09 0.87 532 158.01 7.63 .
16555 s 0.55 49 .145 1.09 0.87 532 158.01 7.63 54.72
```

16556 h 0.72 1 .119 1.01 0.86 568 128.23 8.83 39.97  
16556 h 0.72 26 .165 1.01 0.86 568 128.23 8.83 63.38  
16556 h 0.72 49 .153 1.01 0.86 568 128.23 8.83 60.32  
16557 h 0.56 1 .117 1.02 0.85 532 148.86 7.91 36.63  
16557 h 0.56 26 .102 1.02 0.85 532 148.86 7.91 36.39  
16557 h 0.56 49 .157 1.02 0.85 532 148.86 7.91 58.87  
16558 s 1.84 1 .138 0.98 1.47 608 163.2 5 49.71  
16558 s 1.84 26 .150 0.98 1.47 608 163.2 5 59.64  
16558 s 1.84 49 .138 0.98 1.47 608 163.2 5 43.84  
16559 s 0 1 .120 0.99 0.58 636 182.54 7.67 45.11  
16559 s 0 26 .117 0.99 0.58 636 182.54 7.67 51.73  
16559 s 0 49 .146 0.99 0.58 636 182.54 7.67 63.99  
16560 h 1.28 1 .108 1.11 1.42 548 169.34 6.31 34.89  
16560 h 1.28 26 .184 1.11 1.42 548 169.34 6.31 68.5  
16560 h 1.28 49 .144 1.11 1.42 548 169.34 6.31 54.99  
16561 h 1 1 . 1.03 1.15 442 173.84 7.19 .  
16561 h 1 26 . 1.03 1.15 442 173.84 7.19 .  
16561 h 1 49 .155 1.03 1.15 442 173.84 7.19 49.17  
16562 h 1.31 1 .137 1.67 1.34 554 167.32 7.94 44.95  
16562 h 1.31 26 .207 1.67 1.34 554 167.32 7.94 76.6  
16562 h 1.31 49 .154 1.67 1.34 554 167.32 7.94 59.55  
16563 s 0.97 1 .154 1.05 1.08 582 187.19 7.07 52.96  
16563 s 0.97 26 .144 1.05 1.08 582 187.19 7.07 56.23  
16563 s 0.97 49 .153 1.05 1.08 582 187.19 7.07 61.62  
16564 h 0.5 1 .133 1.07 1.12 550 119.16 7.72 43.09

```
16564 h 0.5 26 .131 1.07 1.12 550 119.16 7.72 48.47
16564 h 0.5 49 .157 1.07 1.12 550 119.16 7.72 60.43
16565 s 0.99 1 .102 1.05 1.15 423 158.21 6.41 25.52
16565 s 0.99 26 .133 1.05 1.15 423 158.21 6.41 38.41
16565 s 0.99 49 .142 1.05 1.15 423 158.21 6.41 43.55
16566 s 1.6 1 0.91 1.24 1.30 590 143.72 7.11 31.66
16566 s 1.6 26 .146 1.24 1.30 590 143.72 7.11 57.87
16566 s 1.6 49 .155 1.24 1.30 590 143.72 7.11 64.66
;
```

```
proc glmselect data=pun plots=all;
```

```
class sex;
```

```
model pun = ti ti*ti sex day monensin dmi ibw backch4 / selection=backward(select=sl
choose=cp)showpvalues;
```

```
run;
```

```
proc glmselect data=pun plots=all;
```

```
class sex;
```

```
model un = ti ti*ti sex day monensin dmi ibw backch4 / selection=backward(select=sl
choose=cp)showpvalues;
```

```
run;
```

VITA

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