MITIGATING ENTERIC METHANE EMISSIONS FROM GRAZING BEEF CATTLE THROUGH FAT SUPPLEMENTATION

By

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Abstract: Ruminant animals produce a significant amount of the greenhouse gases that contribute to climate change, with the majority of emissions arising from grazing ruminant systems. Chief among these gases is methane which arises as a byproduct of ruminal fermentation. Enteric methane also represents a significant energy waste to the system. Due to these negative implications of enteric methane production it is important to identify and examine potential mitigation options for grazing ruminants. A potential option for reducing methane emissions in grazing systems is to supplement feed stuffs that are high in lipid content. These supplements have been shown to reduce methane emissions in cattle fed a total mixed diets, but have not been examined in a grazing system. Therefore, the objective of the experiment described in chapter II is to determine how whole cottonseed (approximately 19% fat) affects animal performance and methane emissions by grazing beef steers. In Chapter II average daily gain increased linearly as the amount of whole cottonseed consumed increased. It was also determined that daily methane production and methane emission intensity (g of methane/kg of gain) had a quadratic relationship to whole cottonseed intake. Minimum daily methane production and emission intensity was found at 1.86 and 2 kg of whole cottonseed intake per day, respectively. Another aspect of fat supplements that could influence the emission mitigation potential is the physical form of the supplements. This possibility was examined (Chapter III) by offering cattle either no fat supplement (control), whole cottonseed, a supplement containing soy bean oil, or a supplement containing bypass fat. In this experiment it was determined that whole cottonseed reduced daily methane production (g of methane/head/d) compared to the control, while no other treatments differed from the control. It was also found that the bypass and soybean oil treatments improved average daily gain compared to the whole cottonseed and control treatments. These effects resulted in an improved emission intensity for all supplemented treatments compared to the control and all supplemented treatments did not differ.

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CHAPTER I

REVIEW OF LITERATURE

Introduction

Sustainability is defined as the use of resources in the present that does not compromise the ability to meet future needs (NRC, 2010). This is often thought of as balancing social, economic, and environmental dimensions (NRC, 2010). Grazing ruminants play a key role in sustainable agriculture due to their ability to utilize food sources that are unusable by humans (i.e. cellulose from plants) and turn them into consumable products for humans (i.e. meat, milk, and wool; Hofmann, 1989; Church, 1979). While utilizing these abundant resources, grazing animals do produce a majority of the greenhouse gas (**GHG**) emissions associated with beef production (Rotz et al., 2015). An estimated 73% of methane (**CH_4**) and 66% of nitrous oxide emissions from beef production in the southern Great Plains result from the cow-calf sector (Rotz, 2015). Therefore, developing means for mitigating emissions from grazing ruminants are important for improving the sustainability of beef production.

Climate Change

Short wave radiation is emitted from the sun as visible light and has more energy than longwave radiation, which is infrared light (Ogolo et al., 2009). The earth is heated by this shortwave radiation. A portion of this energy is absorbed by the atmosphere, while some is reflected back into space, and a portion is absorbed by the Earth's surface (IPCC, 2013). The portion of solar energy that is absorbed by the Earth's surface is emitted back into the atmosphere, in the form of longwave radiation. This energy is then absorbed by the atmosphere, which re-emits longwave radiation in all directions (IPCC, 2013). When longwave radiation is emitted from the atmosphere back towards Earth, it warms the Earth's surface, in a process known as the greenhouse effect (IPCC, 2013).

The greenhouse effect is a key part of Earth's climate. Without it, the surface temperature would be -19 degrees Celsius (Place and Mitloehner, 2010). However, due to increasing concentration of GHG from anthropogenic sources, the greenhouse effect has increased surface temperatures, resulting in climate change (Place and Mitloehner, 2010; Younger et al., 2008). Climate change is defined as the change in average weather over time, generally over a 30-year period (IPCC, 2013). From 1970 to 2004, GHG emissions have increased by 70%, with carbon dioxide (**CO_2**) accounting for 77% of these emissions (Younger et al., 2008). This has caused a significant increase in land surface air temperature (IPCC, 2013).

Methanogenesis and Methane Implications

Methanogenesis

Enteric methane (**CH_4**) is a naturally occurring byproduct of microbial fermentation and is produced by methanogenic archaea. Methane emissions from cattle have been reported to be around 62 (Beauchemin and McGinn, 2005) and 230 (Beck et al., 2017) g of CH_4 per animal per day in feedlot and grazing systems respectively, but this production can change drastically depending on a number of factors. These archaea, commonly referred to as methanogens are located in the rumen and hindgut of ruminant animals (Hook et al., 2010). Methanogens play an important role in rumen health by removing excess hydrogen, which can be toxic to certain microorganisms (Beauchemin et al., 2009). Enteric CH_4 is generally produced through the hydrogenotrophic pathway, which uses hydrogen and CO_2 as substrates. Source of hydrogen and CO_2 in the rumen are primarily through the production of volatile fatty acid (**VFA**), but formate is another source (Hook et al., 2010; Hungate et al., 1970). There is a small amount of CH_4 that is produced from alternative pathways (Hill et al., 2016). Methanogens in the genus *Methanosarcina* grow slowly when hydrogen and CO_2 are the only available substrates and use the methylotrophic pathway primarily, which utilizes methanol or methylamine as the main substrate (Hook et al., 2010). The aceticlastic pathway uses acetate as the main substrate; however, this pathway is minor in the rumen because of how rapidly VFA are transported across the rumen wall (Hill et al., 2016).

Environmental Implications

The global livestock industry accounts for 14.5% of anthropogenic GHG (Gerber et al., 2013). The major GHG that contribute to this estimate include: CO_2, nitrous oxide (**N_2O**), and CH_4. Methane is an especially potent GHG and has 28 times the global warming potential (**GWP**) of CO_2, on a 100-year scale (IPCC, 2013). Enteric CH_4 production accounts for 39.1% of the GHG from the global livestock industry (Gerber et al., 2013). The next two largest contributors are N_2O from manure and CO_2 from feed production, which account for 16.4% and 13.0% respectively (Gerber et al., 2013). The impacts and magnitude that enteric CH_4 production affects the environment has brought negative attention to the beef industry. It is important to note that the areas that are less efficient at ruminant production (i.e. developing nations) there is a larger mitigation potential than developed areas (Gerber et al., 2013). However, due to the large scale of ruminant production in developed nations there is an opportunity for small reductions in emissions that would result large GHG emission mitigation.

Animal Production Implications

Environmental implications are not the only negative side effect of enteric CH_4 by ruminant animals. Methane production also represents an energy loss because of the removal of metabolic hydrogen and carbon that are produced by ruminal fermentation (Martinez-Fernandez et al., 2014). It has been determined that 2 to 12% of gross energy intake (**GEI**) is lost as CH_4 (Johnson and Johnson, 1995). If enteric CH_4 production is mitigated then the substrates (hydrogen and CO_2) could be incorporated into fermentation products, which would allow the animal to be more energetically efficient (Haisan et al., 2014). Therefore, decreasing CH_4 emissions can be economically beneficial and could subsequently motivate producers to implement mitigation strategies.

Methane Measurement Systems

Traditionally technologies such as respiration chambers (**RC**) and the sulfur hexafluoride (**SF_6**) tracer method have been used to determine daily methane production (**DMP**; Hammond et al., 2015; Storm et al., 2012). The GreenFeed emission monitoring (**GEM**; C-Lock Inc., Rapid City, South Dakota) system is a relatively new technology that allows for spot measurements of CH_4 emissions from cattle and uses these measurements to determine DMP (Hammond et al., 2015; Dorich et al., 2015). These technologies have different applications that should be understood before they are used.

Respiration Chambers

The RC system places cattle into enclosed chambers and draws air through the chamber at set intervals, and measures the concentration change of the air coming in and leaving the system (Brown et al., 1984). This method allows for a direct and accurate measurement of the total CO_2 and CH_4 produced (Hammond et al., 2015). The animals are placed in chambers for a relative short amount of time (generally 2-3 days) and are fed a fixed amount so that dry matter intake (**DMI**) is known (Huhtanen et al., 2015). This is important because over 70% of variation in DMP is explained by differences in DMI (Velazco et al., 2016). Further, enclosing the whole animal allows RC to measure the CH_4 produced in the hindgut, which can account for 2-3% of the total CH_4 emitted (Muñoz et al., 2012).

While RC allows for direct and accurate measurements of CH_4 and CO_2, it does have some drawbacks. First, animals are only sampled for a few days at a time. Velazco et al. (2016) state that CH_4 emissions change from season to season, due to change in feed abundance and quality. These seasonal variations will not be observed by an intensive 2 to 3-day measurement time. Additionally, RC remove the animals out of their production setting, which can impact their behavior and potentially reduce normal levels of DMI. This also means that RC cannot be used to estimate CH_4 production in grazing scenarios (Hammond et al., 2015). Finally, RC are expensive and require significant labor, which make studies containing large numbers of animals infeasible in most cases (Huhtanen et al., 2015; Hammond et al., 2015).

There is a recent adaptation to RC, which are commonly referred to as head boxes. These systems are similar in that they place animals into stalls and remain there while being sampled (Andreini et al., 2017; Place et al., 2011). The differences in these system lie in that head boxes only place the animals head into the box, while RC enclose the whole animal and since the box is made to only house the head, there is some cost saved in the production of head boxes (Andreini et al., 2017; Place et al., 2011). Additionally, this style of chamber still allows for high quality data to be collected (Andreini et al., 2017; Place et al., 2011). However, the animal is removed from its production environment and, unlike RC, head boxes do not collect CH_4 emissions from the hindgut.

Sulfur Hexafluoride Tracer

The SF_6 tracer method allows for CH_4 emissions to be estimated in the animal's production environment and therefore can be applied to grazing operations (Johnson et al., 1994). This method works by placing a bolus that is filled with SF_6 into the rumen. These boluses release SF_6 at a known rate. As gases produced by the rumen are eructated, they are captured into PVC canisters that hang around the nose and mouth of the animal. The PVC canisters hold a vacuum of about 90 kPa to draw in the eructated gas. The amount of excreted SF_6 and CH_4 is then analyzed in a laboratory using gas chromatography (Hammond et al., 2015; Muñoz et al., 2012). Once the concentrations of SF_6 and CH_4 are determined, DMP can be calculated by relating these concentrations to the predetermined release rate of the canister that was placed in the rumen (Muñoz et al., 2012).

Using the SF_6 method to estimate CH_4 emissions allows measurements to be taken in a more natural environment, but there are some negative aspects associated with this method. First, placing the SF_6 bolus in the rumen, the PVC canister on the animal, and the frequent handling can negatively affect grazing behavior. Methane yield (**MY**; g of CH_4 per kg DMI) will be biased if grazing intake is lower than expected (Hammond et al., 2015). Next, there is a larger animal to animal variation associated with the SF_6 method compared to using RC (Huhtanen et al., 2015; Hammond et al., 2009). Additionally, the SF_6 method is integrative and is unable to detect diurnal variations of CH_4 emissions. Finally, the SF_6 method is also labor intensive and therefore sampling a large number of animals is difficult (Huhtanen et al., 2015).

GreenFeed System

The GEM system is a relatively new method for measuring enteric methane emissions. There has been a wealth of recent research describing this system. The GEM system is stationary and has a feed hopper, used to bait the animals into using the system. The feed is made accessible when the animal's radio frequency identification tag is read. The GEM system then draws air around the animal to capture the CH_4 and CO_2 that the animal emits while at the feeder. The captured gas is compared to the gas that was present before the animal was in the chamber as well as after it left, so that a change in gas concentration can be determined (Cottle et al., 2015). Measurements from animals lasting longer than 3 minutes are typically used because there are multiple eructation events during that time (Velazco et al., 2016; Hammond et al., 2015). This system minimizes animal handling and allows animals to be sampled in their production environments. Additionally, one system can sample a relatively large number of animals. Fifteen to 20 animals in grazing scenarios or 20 to 25 animals in confinement can be sampled (Hammond et al., 2015; Dorich et al., 2015).

Since the GEM system is a new technology, some studies were unable to find treatment differences that were detected by other systems. Hammond et al. (2015) conducted a 2x2 factorial design experiment with 4 heifers in each treatment. The heifers received either corn silage or grass silage with or without a linseed product. They found that while RC and SF_6 were able to detect treatment differences, the GEM system was not (Hammond et al., 2015). On the other hand, Velazco et al. (2016) found that DMP measured by the GEM system and by RC were not different. Since CH_4 production is typically highest after a feeding, the sampling time could result in a potential bias. It is suggested that this bias can be mitigated if a sampling schedule is built around a diurnal pattern instead of allowing random visits (Dorich et al., 2015). The supplement that the GEM system provides could have an impact on CH_4 measurements. In one study, the GEM supplement provided 17% of the daily metabolizable energy intake. This could affect calculated DMP by increasing fermentation as well as decreasing the amount of forage consumed. It is suggested to use a low-energy supplement in the GEM in an attempt to minimize its effect on CH_4 emissions (Velazco et al., 2016).

Comparison of Systems

With the technologies available to measure enteric CH_4 emission it is important to understand the capabilities of each system in order to determine what method is best suited for a given scenario. Use of RC gives accurate and direct measurements of CH_4, but with high initial investment and labor costs, measuring a large number of animals is infeasible. This system also takes animals out of their production system which makes using this method impractical in grazing scenarios (Hammond et al., 2015; Huhtanen et al., 2015). The SF_6 method allows animals to be sampled in their production environment, but requires a large amount of labor. There is also a potential of affecting grazing behavior while using the SF_6 method (Hammond et al., 2015). The GEM system is a new technology that uses an average of spot measurements to estimate DMP. This system allows a relatively large number of animals to be tested in their production environment, with a minimal amount of animal handling and lower labor input (Hammond et al., 2015). Since this system is new, there are still some uncertainties about its precision and accuracy. However, as more studies are conducted using this system there will be more strategies developed for using the GEM.

Estimating Daily Methane Production from Spot Measurements

As previously mentioned, Hammond et al. (2015) was unable to determine treatment differences with the GEM that both the RC and SF_6 methods were able to detect. It was believed that the GEM was unsuccessful in detecting treatment differences because the system relies on the animals to visit the unit throughout the day and assumes that the CH_4 that is sampled is representative of the CH_4 that is emitted from the animal the rest of the day. This assumption might be wrong because there can be as much as a 5-fold difference in CH_4 emissions throughout the day (Hammond et al., 2015). To avoid this problem, strategies must be implemented in order to acquire accurate data while using the GEM system.

Using the GreenFeed in Grazing Scenarios

Dry matter intake and time of feeding are rarely known for grazing ruminants and so it is not possible to relate feed events with GEM measurements (Cottle et al., 2015). Due to this issue, a power analysis was conducted to determine the relationship between the length of experiment and the number of animals needed to achieve an estimate that was within 5 to 10% of the mean (Cottle et al., 2015). The power analysis found that using 20 animals would require 98 days to achieve an estimate that was within 5% of the mean. When the number of animals was doubled, only 47 days were needed. Likewise, to conduct a 50-day trial it was estimated that 36 animals would be needed to obtain 95% confidence (Cottle et al., 2015). One variable that could impact DMP estimates is the number of allowed visits to the GEM each day. This variable is set by the researcher, but the animal chooses how many visits it will use (Cottle et al., 2015). Using the same power analysis, Cottle et al. (2015) found that 20 animals sampled over 98 days, would require 2 allowed visits per day to achieve 95% confidence. The number of days are only reduced to 91 when animals are allowed to visit 5 times per day. As a result, Cottle et al. (2015) suggests to leave the number of allowed visits at 2 per animal per day. This power analysis suggests that with 20 animals tested over a 70-day period, DMP can be determined with 5 to 10% confidence (Cottle et al., 2015). Averaging short term measurements over a period of 40-70 d can be an effective way to determine treatment differences when feed events are unknown (Velazco et al., 2016).

Management Effects on Methane Production

There is a known correlation between ruminal digestibility of a feedstuff and CH_4 emissions (Hristov et al., 2013). Due to the impact that management practices have on digestibility of feedstuffs there is an opportunity to decrease CH_4 emissions through proper management. In order to determine the efficacy of different management practices on decreasing CH_4 emissions, it is important to consider the magnitude of impact a management practice can have on CH_4 emissions.

Forage Management

It has been estimated that the cow-calf sector accounts for 60 to 84% of the total GHG emissions from the beef industry (Grainger and Beauchemin, 2011). The relatively low percentage of GHG emissions from the growing and finishing sectors is due to the short time that they are fed until harvest and also due to the predominantly concentrate diets that they consume (Grainger and Beauchemin, 2011). This discrepancy of CH_4 production between industry sectors is also due to the fact that the majority of cow herds are managed on pasture and the fermentation of fiber produces more CH_4 than the fermentation of starch (Hristov et al., 2013). This is because the major VFA produced by fiber fermentation is acetate (the relationship between CH_4 production and VFA is discussed below). As a result, the greatest impact on GHG emissions by the beef industry will occur by mitigating the amount of CH_4 produced from forage-based systems (Grainger and Beauchemin, 2011).

DeRamus et al. (2003) conducted a three-year study looking at beef cows and heifers on unimproved pasture that were continuously grazed, as compared to a best management practices (**BMP**) pasture system that utilized management-intensive grazing. Emissions were lower in the spring, when forage quality was high, and higher during the summer and the fall, when forage quality declined (DeRamus et al., 2003). Even with seasonal variations, the cows on BMP always had lower CH_4 emissions than cows in the continuous grazed system throughout the year. Annual CH_4 emissions were decreased by 22% for BMP compared to the continuously grazed system (DeRamus et al., 2003). This was a result of increased pasture quality that resulted from the BMP system over the continuously grazed system (DeRamus et al., 2003).

Beauchemin et al. (2011) conducted a study using a modeling approach to examine the impact that management can have on total farm GHG emissions. One of the strategies examined was improving the forage quality that was fed to the breeding stock during the winter. This was accomplished by harvesting the forage at an earlier stage of maturity, which decreased the amount harvested by 10%, but improved dry matter digestibility (Beauchemin et al., 2011). The improved dry matter digestibility decreased DMP by 5% compared to the baseline (Beauchemin et al., 2011).

A meta-analysis by Archimède et al. (2011) included 22 studies that compare methane production of systems using C3 (i.e. bermudagrass, *Cynodon dactylon*) or C4 (i.e. dallisgrass, *Paspalum dilatatum*) grasses, as well as the implementation of legumes commonly found in tropical (i.e. white clover, *Trifolium repens*) or temperate (i.e. alfalfa, *Medicago sativa*) climates. Tropical grasses use the C4 pathway of photosynthesis, while temperate grasses utilize the C3 pathway (Archimède et al., 2011). It was found that animals consuming C4 grasses had 17% greater MY than those consuming C3 grasses (Archimède et al., 2011). This is due to the C4 pathway depositing more lignin than C3 grasses, reducing digestibility (Wilson, 1994; Archimède et al., 2011). It was also concluded by Archimède et al. (2011) that animals eating tropical legumes emit less CH_4 than those eating temperate legumes and that legumes in general produce less CH_4 than grasses. Methane emissions were 20% less when cattle were fed tropical legumes versus C4 grasses. These findings suggest that there is an opportunity to reduce CH_4 emissions from pastured cattle with the addition of legumes and this is especially true in areas where tropical grasses are utilized (Archimède et al., 2011).

Grain Processing

Processing grains increase the availability of starch in the rumen, which results in an improved digestibility of the feedstuff. The improved digestibility reduces energy losses and increases rate of passage, which can subsequently reduce CH_4 emissions directly (Hristov et al., 2013). Grain processing can also effect CH_4 production by increasing feed efficiency, leading to increased animal performance and decreased number of days until harvest. One study compared precision processing, a process of setting roller width to match kernel size, to a conventional processing, leaving roller width the same for all kernel sizes, of barley and observed an improvement in animal performance (Yang et al., 2012). It was observed that by precision processing the barley, there was a 25-day reduction in days on feed, which saved 163 kg of feed per head throughout the feeding period (Yang et al., 2012). Reducing the amount of feed consumed reduces total CH_4 produced because DMI and CH_4 emissions are highly correlated. Additionally, reducing days on feed could have a significant impact on CH_4 production by reducing the total carbon footprint (**CFP**; kg of CO_2 equivalent per kg of product produced) of the beef industry (Hristov et al., 2013).

Processing grains can have a direct impact on CH_4 emissions. Owens et al. (1997) found that steam flaking corn decreased DMI, without effecting performance, resulting in a 10% improvement in feed efficiency. Hales et al. (2012) also reported improved feed efficiency, with a 4% reduction in DMI. While there was only a 4% reduction in DMI the observed effects on CH_4 production were still significant. When cattle fed steam flaked corn DMP and MY were reduced by 21 and 17% respectively, compared to dry rolled corn (Hales et al., 2012). The losses of gross energy and digestible energy were also reduced by 19 and 21% respectively (Hales et al., 2012). These reductions in energy loss would explain why Owens et al. (1997) found that feeding steam flaked corn had no negative effect on ADG even with a 10% decrease in DMI.

Methane Mitigation Strategies

With the rising concern of the beef industry's environmental impact, it is becoming increasingly important to find sustainable means of lowering enteric CH_4 production. Various supplementation strategies have the potential to significantly reduce the enteric CH_4 emitted by cattle. In order to determine if a method of decreasing CH_4 emissions is appropriate it is important to establish how beneficial these methods are at decreasing CH_4 production. Other factors to determine includes: how long the method will reduce CH_4 production, what the mechanism is for affecting CH_4 production, and what effect the method has on animal performance.

Supplementing Lipids

Abdalla et al. (2012) and Dong et al. (1997) determined that lipids have the potential to reduce CH_4 production *in vitro*. It has also been confirmed *in vivo* that supplementing cattle with oils, or feedstuffs high in lipid content, reduces CH_4 production (Beauchemin and McGinn, 2006; Grainger et al., 2010; McGinn et al., 2004). When adding canola oil at 4.6% of the diet on a dry matter basis, Beauchemin and McGinn (2006), reported a reduction of DMP by 32% compared to the control. Supplemented sunflower oil decreased CH_4 emissions by 22% compared to a control (McGinn et al., 2004). When these studies (Beauchemin and McGinn, 2006; Grainger et al., 2010; McGinn et al., 2004) expressed energy lost through CH_4 production as a percentage of GEI, a 21% reduction was found compared to the control.

The McGinn et al. (2004) and Beauchemin and McGinn (2006) studies were only 21 days, raising the question of the long-term efficacy of lipid supplementation on CH_4 mitigation. Hristov et al. (2013) discussed this in a paper reviewing CH_4 mitigation options and indicated that there had not been enough research on the long-term effects of supplementing oils to elucidate possible ruminal adaptation to oils and its impact on long-term DMP. It was noted that there has been some work done on the long-term effects of oil supplementation on CH_4 production, but with conflicting results (Hristov et al., 2013). In a meta-analysis, Grainger and Beauchemin (2011) concluded that supplemental lipids, in general, do reduce DMP over an extended period of time. One study, conducted on lactating dairy cows supplemented with whole cottonseed over a 12-week period, found that during the first 3 weeks CH_4 emissions was reduced by 13% and by week 12 a 23% reduction was observed (Grainger et al., 2010).

As discussed above, various studies have confirmed the ability of supplementing oils to mitigate CH_4 emissions. There are two proposed modes of action by which supplemental lipids can reduce methane. The first mode is by unsaturated fatty acids providing an alternative hydrogen sink through the process of biohydrogenation (Czerkawski et al., 1966; Johnson et al., 2002; Dong et al., 1997). This has been suggested to have a minor impact *in vivo* (Hristov et al., 2013). The second mode of action for the reduction in CH_4 emission has been attributed to a decrease in DMI (Beauchemin and McGinn, 2006; Hristov et al., 2013). The reduction of DMI is a result of decreased fiber digestibility and McGinn et al. (2004) reported a decrease in NDFD by 20%. This mode of action is corroborated by Abdalla's et al. (2012) findings *in vitro*. Beauchemin and McGinn (2006) observed that cattle supplemented canola oil had a 10%

decrease in DMI when compared to the control. When CH_4 emissions are expressed as MY there is no reported difference between animals supplemented oils and those that are not. This is further evidence of the mechanism that lipids have for reducing CH_4 emissions (Beauchemin and McGinn, 2006).

With the reduction in DMI there has been some concern expressed about the impact that supplemental lipids could have on animal performance (Beauchemin and McGinn, 2006; Hristov et al., 2013; Feiser and Vanzant, 2004). However, adding 4.6% canola oil on DM basis to a diet did not influence ADG, even with a 10% decrease in DMI (Beauchemin and McGinn, 2006). Grainger and Beauchemin (2011) arrived at the same conclusion in their meta-analysis of 27 studies. Having no impact on cattle performance even with a decrease in DMI can only be explained by the increase in energy density of the diet caused by adding the oils. In one experiment, the addition of canola oil increased the energy of the diet by 6% (Beauchemin and McGinn, 2006). The additional energy resulted in no change in GEI between the treatments even with the decrease in DMI (Beauchemin and McGinn, 2006).

Increasing Starch

There is a negative relationship between level of concentrates in the diet and CH_4 emissions (Iqbal et al., 2008). In diets that are predominantly starch, such as those in the U.S. feedlot industry, the proportion of GEI that is converted to CH_4 is typically less than 4% (Beauchemin et al., 2009). This is in contrast to forage based diets, where greater than 6.5% of GEI is lost as CH_4 (Beauchemin et al., 2009). The rate that starch is fermented will also have an effect on CH_4 emissions. Benchaar et al. (2001) examined differences between the level of CH_4 mitigation between slowly degraded starch and a rapidly degraded starch in the diet. When adding the slowly degraded starch, a 14% reduction in DMP was observed and decreased GEI lost as CH_4 production by 16% compared to the control (Benchaar et al., 2001). When a rapidly fermented starch was used there was no change in the reduction of DMP at 14%, but the reduction in GEI lost became 23% (Benchaar et al., 2001).

There is not much debate that increasing starch in diets would cause a sustained decrease in MY (Hristov et al., 2013; Grainger and Beauchemin 2011). The concern with supplementing starch to reduce CH_4 emissions lies in other areas. One concern is that it reduces the benefit of ruminants to convert forages, otherwise unusable by humans, into human consumable products (Grainger and Beauchemin, 2011). An additional concern is the amount of concentrate in the diet required to observe a significant reduction in CH_4 emissions. A review by Hristov et al. (2013) stated that concentrate levels would need to be 35 to 40% of the diet in order to lower CH_4 emissions. This might mean that in pasture-based systems, supplementing starches might not result in reduced CH_4 emissions.

There are two ways that supplemental starch would decrease CH_4. The first is by altering the VFA that are produced during fermentation. The production of propionate through starch fermentation produces less hydrogen as compared to acetate, the predominant VFA in fiber fermentation (Johnson and Johnson, 1995). Theoretically, if the acetate:propionate ratio is 0.5 then 0% of GEI would be lost as CH_4 (Johnson and Johnson, 1995). This ratio would never occur as general acetate:propionate ratios are 3.4 for forage fed (Pesta et al., 2016) and 1.6 for cattle on high concentrate diets (Meyer et al., 2009). The other means by which starches reduce CH_4 emissions is by reducing ruminal pH during fermentation. When ruminal pH is decreased below 6.0, there is a reduction in cellulolytic microorganism, which could cause a decrease in DMI (Fieser and Vanzant, 2004). Methanogens would likewise be affected by reduced ruminal pH, resulting in a decrease in CH_4 production (Grainger and Beauchemin, 2011).

Supplementing Monensin

Monensin has been used for many years to improve efficiency of ruminal fermentation by shifting the VFA profile and decreasing the acetate:proprionate ratio, which reduces energy loss as CH_4 and decreases the loss of dietary protein (Bergen, 1984). As environmental concerns have increased, there has been greater interest in monensin's effect on CH_4 production. Tedeschi et al. (2003), in a summary of literature, reported a 25% reduction in CH_4 production compared to cattle not provided monensin. McGinn et al. (2004) found monensin only reduced CH_4 by 9%, which is still within the range proposed by Johnson and Johnson (1995) who stated that the effects of monensin on CH_4 emissions will range from slight to a 25% reduction.

The above paper (McGinn et al., 2004) looked at monensin's effect on CH_4 emissions on a short-term basis. Some have voiced concerns on the long-term effect that monensin has on DMP and question if it is sustained (Grainger and Beauchemin, 2011; Hristov et al., 2013). After analyzing several papers, Beauchemin et al. (2008) concluded that while CH_4 can be initially reduced by as much as 30% in the short term, baseline levels of CH_4 can be expected to be reestablished after 2 months.

Monensin is a carboxylic polyether ionophore antibiotic. They are considered antibiotics because they target certain bacteria. Ionophores are anions and therefore are able to bind to different metal ions, such as sodium or potassium ions (Duffield et al., 2012). Once bound to a cation, the ionophore can transport across lipid bilayers and cell membranes of bacteria causing an increase in osmotic pressure inside of cells (Bergen, 1984; Duffield et al., 2012). This leads to distension within the cell, which can hinder the bacteria's ability to produce energy (Bergen et al, 1984). Monensin, as well as other ionophores target certain microorganisms in the rumen, causing a shift in the microbial population. Potentially the most important microorganism type targeted is the gram-positive bacteria. A study by Fernando et al. (2005) found that supplementation of monensin reduced gram-positive bacteria from 39% to 30% of the total bacterial population. This is important because gram-positive bacteria account for much of the wasted energy that is associated with fermentation in the rumen (Fernando et al., 2005). These energy losses are in the form of CH_4 and CO_2 (Bergen, 1984).

Monensin has been extensively studied and it has become established that monensin improves feed efficiency and animal health of ruminants in all sectors of the beef industry (Beauchemin et al., 2008). Monensin increased ADG by 13.3% when supplemented to steers on wheat pasture (Fieser, 2007). As reviewed by Duffield et al. (2012), it was found that feeding monensin to growing and finishing cattle increased average daily gain by 2.5% and decreased DMI by 3% resulting in an increased feed efficiency of 6.4%. Monensin also offers health benefits such as decreased incidence of bloat (Fieser, 2007) and coccidiosis (Bergen, 1984).

Supplementing 3-nitrooxypropanol

3-nitrooxypropanol (**NOP**) is a relatively new product that has been successful in its CH_4 mitigation potential (Romero-Perez et al., 2015; Hristov et al., 2013; Romero-Perez et al., 2014; Haisan et al., 2014; Martinez-Fernandez et al., 2014). In a study on

Holsteins in mid lactation, given 2,500 mg of NOP per day, it was found that DMP was reduced by 60% and MY decreased from 17.8 in the control group to 7.18 (Haisan et al., 2014). The results found by Hristov et al. (2015), were not as drastic, with a 30% reduction in DMP observed. A study using 8 cannulated beef heifers offered different levels of NOP (0, 0.75, 2.25, and 4.50 mg per kg of BW) found that CH_4 emission decreased linearly as the level of NOP increased. At the highest level of supplementation, a 33% reduction of CH_4 was observed, compared to the control (Romero-Perez et al., 2014).

In 2014, there was a gap in knowledge on the long-term effects of supplementing NOP, leading to the mention that more research would need to be done (Romero-Perez et al., 2014; Hristov et al., 2013). A more recent study was conducted by Romero-Perez (2015) to determine the long-term effects of NOP. Cattle were offered either 0 or 2 g of NOP per day for 112 days. There was a sustained reduction of CH_4 emissions by 59.2% of the treated compared to the control. Methane yield was also reduced from 22.46 to 9.16 g of CH_4/kg of DMI (Romero-Perez et al., 2015).

Unlike supplemental lipids, which decreased CH_4 emissions by decreasing DMI (Beauchemin and McGinn, 2006; Hristov et al., 2013), NOP had little to no effect on DMI (Romero-Perez et al., 2015; Romero-Perez et al., 2014; Haisan et al., 2014; Martinez-Fernandez et al., 2014). The proposed mode of action for mitigating CH_4 emissions is through an antagonistic effect on methyl-coenzyme M reductase (**MCR**). 3-nitrooxypropanol is a structural analog of methyl-coenzyme M, which is involved in the last step of methanogenesis where a methyl group is transferred to MCR to make CH_4 (Romero-Perez et al., 2014; Haisan et al., 2014).

Without a negative impact on DMI and a reduction in CH_4 production, one would expect there to be a significant increase in animal performance with supplemented NOP. Improved animal performance has been reported in lactating dairy cows (Haisan et al., 2013; Hristov et al., 2015). Hristov et al. (2015) observed a greater ADG (330 g per day compared to 210 g per day) by the cows supplemented NOP compared to the control. The difference in BW change was larger for Haisan et al. (2014) who observed 1.06 kg per day for cows given NOP compared to 0.39 kg per day in the control. Supplementing NOP had no effect on milk production (Haisan et al., 2014; Hristov et al., 2015). In beef cattle, Romero-Perez (2014) found no change in ADG among treatments. This is probably because the BW of the animals were around 620 kg (Romero-Perez et al., 2014) and few performance benefits would be expected at this stage of physiological maturity. There needs to be research conducted on growing or finishing beef cattle in multiple production systems to determine the impact NOP would have on animal performance in the beef industry.

Growth Promoters Effect on Industry Carbon Footprint

Examples of growth promoters (**GP**) include ionophores, implants, and in-feed hormone analogs such as melengesterol acetate and beta-adrenergic agonists. One of the most effective ways to reduce the beef industry's CFP is through utilizing GP technologies, which increase animal production and potentially reduce the number of days required to reach appropriate slaughter weights (Neumeier and Mitloehner, 2013). During 2007, the beef industry needed 69.9% of the number of animals to produce the same amount of product in 1977. This contributed to the 16.3% reduction of the beef industry's CFP per billion kg of beef produced (Capper, 2011). Some of this improvement in efficiency is due to improved genetics, but a significant amount of this is credited to new technologies (Neumeier and Mitloehner, 2013; Capper and Hayes, 2012; Capper, 2011). The removal of GP from the beef industry would have a large effect on the efficiency of animal production. This would result in an increased number of animals needed to meet market demands, which would increase the amount of feed needed and considerably increase the CFP of the beef industry.

Effect on Animals Numbers

Capper and Hayes (2012) examined the impact of removing GP technologies from the beef cattle production system. It was found that in order to produce the same amount of meat without GP, 11.8% more animals would be required (Capper and Hayes, 2012). The need for more animals to produce the same amount of meat is due to the change in average slaughter weight that would occur. Capper and Hayes (2012) determined that if GP were removed there would be a 53 kg difference in average slaughter weights, 521 kg for animals not provided GP and 574 kg for animals produced utilizing all available GP technologies.

Effect on Feed Needed

Improved production efficiency, as a result of technologies and genetics, has decreased the amount of feed needed from 1977 to 2007 by 18.6% per billion kg of beef (Capper, 2011). An increase in the number of animals needed, when GP are removed, will lead to an increase in the amount of required feedstuffs to achieve the same level of production. Capper and Hayes (2012) predicted that without GP there would be a 10.6% increase in the total amount of feedstuffs required. In order to meet the increased demand for animal feed, there would be an increase in farming inputs. A 6.8% increase in fertilizers as well as a 7% increase in fossil fuel energy required has been estimated (Capper and Hayes, 2012).

Carbon Footprint Impacts

The need for more animals and the resulting increase in the required amount of feedstuffs would result in an increased CFP of the beef industry. Neumeier and Mitloehner (2013) estimated that there is a 9.8% reduction in the total CFP of the beef industry when GP are implemented, thus aligning with the findings of Capper and Hayes (2012). If GP technologies were removed from the beef production system, there would be an expected increase in total CH_4 emissions of 9.3% and an 8.9% increase in total GHG emissions (Capper and Hayes, 2012). These increases are a result of the increased emissions from the energy required to grow feedstuffs for the animals, increased electricity, increased required land use, and the CO_2 and CO_2 equivalents produced (Capper and Hayes, 2012).

Summary of Literature Review

Increasing concerns regarding the beef industry’s contribution to GHG emissions has made it necessary to establish methods to measure enteric CH_4 production in ruminant animal’s production environments. The SF_6 method has been used for years and can be used to measure CH_4 produced by animals in their production environment. However, due to high labor costs and increased animal handling, which could affect animal behavior, it might not be appropriate in some cases. Recently, the GEM system has received attention due to its relatively low labor costs and its minimal animal handling requirements. Since the time and duration of sampling is controlled by the animal, there needs to be strategies implemented to insure that the diurnal CH_4

production has been captured. Cottle et al. (2015) suggests that the most impactful considerations when designing a study with the GEM system are number of animals and the duration of the study. If managed appropriately the GEM system has proven to be able to measure treatment differences (Velazco et al., 2016) and therefore can be used to determine CH_4 mitigation strategies.

Suggested CH_4 mitigation strategies include forage management (DeRamus et al., 2003; Archimède et al., 2011) and grain processing (Hales et al., 2012), which have both shown to have an effect on CH_4 production. Other strategies include supplementing energy sources, including lipids and starches, which decrease CH_4 emissions by reducing DMI (Beauchemin and McGinn, 2006; Hristov et al., 2013). Even though DMI is reduced it might not decrease animal performance due to the added energy of the supplement (Beauchemin and McGinn, 2006). It might be difficult to have producers implement lipid or starch supplementation in production operations because no benefit to animal performance is observed. Producers need to have economic incentive to implement a supplement regimen into their operations, which is what makes the new product NOP promising. 3-Nitrooxypropanol has been shown to have a significant reduction of CH_4 emissions, 60% in the case of Haisan et al. (2014). This reduction is seen without affecting DMI (Beauchemin and McGinn, 2006; Hristov et al., 2013) which shows a strong potential to increase animal performance. A product that has a positive impact on animal performance as well as being a significant reducer of CH_4 emissions will make producers more likely to implement it in their operations. Monensin has been used for years to improve animal efficiency and has been shown to reduce CH_4 emissions by as much as 30%. This reduction is not believed to be long lasting and CH_4 emissions can be expected to return to normal levels (Beauchemin et al., 2008).

It is important to consider the effect that GP can have on the beef industry's CFP. Even though GP might not affect DMP directly, any time animal efficiency is increased the amount of CH_4 per unit of product produced is reduced. Additionally, GP can reduce the number of animals needed and the amount of feedstuffs required to produce a given amount of product, which could decrease the total amount of GHG emitted. This is a result of a shortened number of days on feed that is required to reach acceptable slaughter weights and also because of the increase in slaughter weight (Capper and Hayes, 2012; Neumeier and Mitloehner, 2013).

As discussed earlier, there are many opportunities to reduce the CFP of the beef industry. The CFP can be reduced directly, by management and supplements, or indirectly, through GP to improve animal efficiency, allowing the beef industry the opportunity to approach its environmental impact from different fronts. Methane mitigation strategies would lessen the environmental impact and reduce energy losses to potentially improve animal performance, thereby providing economic incentives. This in turn will ensure a sustainable and profitable future for the beef industry.

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CHAPTER II

Whole cottonseed supplementation improves performance and reduces methane emission intensity of grazing beef steers.

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Abstract: The objective of this experiment was to determine the effect lipid supplementation, from whole cottonseed (**WCS**), on average daily gain (**ADG**) and enteric CH_4 production of steers grazing warm-season perennial forages. Steers (n = 18; initial BW = 317 ± 5.5 kg) were adapted to an in-pasture CH_4 measurement device (GreenFeed emission monitoring system (**GEM**); C-Lock Inc., Rapid City, SD) for three weeks. Steers were stratified by adaptation-period use of GEM and randomly assigned to treatments within stratifications. Treatments were either 0, 0.9, 1.8, 2.7, 3.6, or 4.5 kg of WCS (as-fed) per day, offered in individual feeding stanchions. Orts were measured and actual WCS intake was used in the analysis. Body weight was measured weekly before feeding for the duration of the experiment. Mean supplement intake of supplemented animals ranged from 0.89 to 2.86 kg per day. Total fat content of the diet (WCS + forage) at the greatest WCS intake was estimated to be 8.3%. Animal performance increased linearly as WCS intake increased ($P = 0.02$). Two of the three steers assigned to the 0 WCS treatment refused to visit the GEM, and therefore CH_4 emission data were unavailable. Because only one observation was available at 0 WCS, and the Cook's Distance of this point was greater than 1 ($Di \geq 7.48$), the 0 WCS observation was excluded from further analysis. There was a quadratic relationship between daily methane production (g of CH_4 per animal per day) and WCS intake ($P = 0.02$) with a minimum daily methane production at 1.86 kg WCS/d. Emission intensity (**EI**; g of CH_4 per kg of ADG) was less at moderate levels of WCS intake, and after 2.0 kg of WCS intake/d EI increased. This resulted in a significant quadratic relationship between emission intensity and WCS intake ($P = 0.011$). These results suggest that if WCS supplementation is used to mitigate CH_4 EI, 2.0 kg of supplement is the optimal dose.

Key words: Enteric methane, whole cottonseed, beef cattle, grazing, GreenFeed

Introduction

As much as 12% of gross energy intake of grazing cattle is lost to the environment as methane (**CH_4**; Johnson and Johnson, 1995). Enteric CH_4 is also a potent greenhouse gas and has 28 times the global warming potential of carbon dioxide (IPCC, 2013). Due to the negative impact that enteric CH_4 emissions have on both energy utilization of beef cattle and the environment, there is a need to develop strategies to reduce CH_4 emissions without reducing animal performance.

Supplementing fat may reduce CH_4 emissions through two proposed modes of action. Fat may cause a reduction in DMI (Eugène et al., 2008; Rabiee et al., 2012;

Hristov et al. 2013), reducing CH_4 because CH_4 emissions are directly related to DMI. Unsaturated fatty acids may also provide an alternative hydrogen sink as they become saturated in the rumen (Czerkawski et al., 1966; Dong et al., 1997; Johnson et al., 2002). However, this mode may only play a minor role (Hristov et al., 2013).

Supplemental fat has reduced CH_4 production in vitro (Dong et al., 1997) and in sheep fed oleic, linoleic, and linolenic acids (Czerkawski et al., 1966). Beauchemin et al. (2007) observed a reduction in CH_4 emissions when beef heifers were given tallow, sunflower oil, or whole sunflower seeds and all sources were added to provide fat at 5.9% of the total diet. The CH_4-mitigating effect of whole cottonseed (**WCS**) is less consistent (Grainger et al., 2008, Johnson et al., 2002). WCS supplementation has proven to be a popular and effective supplement to grazing beef cattle in the southern Great Plains due to the high energy density and protein content (Rogers et al., 2002). The objective of this experiment was to determine the effect of supplemental WCS on grazing cattle performance and CH_4 emissions.

Materials and Methods

All procedures used in this experiment were approved by the Oklahoma State University Institutional Animal Care and Use Committee (#AG-16-9).

Location and Pasture

The experiment was conducted from May 23, 2016 to July 5, 2016 (45-d) on warm season perennial pastures at the Oklahoma State University Bluestem Research Range, located near Stillwater, OK. The major forage in the pasture was a mixture of tall grass prairie (indian grass, *Sorghastrum nutans*; little bluestem, *Schizachyrium* *scoparium*) and yellow bluestem (*Bothriochloa ischaemum*). Minor herbage included big bluestem (*Andropogon gerardii*) and various forbs.

Acclimation

Twenty-two steers were acclimated to the individual feeding stalls and a CH_4 measurement device (GreenFeed emission monitoring system (**GEM**); C-Lock Inc., Rapid City, SD), which are described in further detail below. The acclimation period began 3 wk prior to the start of the experiment, and proceeded as follows. The GEM has panels that form an alley so that only one animal can visit at a time. The animals were pastured with the GEM with the panels removed. After one week the panels were placed in front of the GEM and then gradually narrowed until they formed a parallel alley of approximately 0.5 m in width. The panels that formed the alley were initially 2.4 m long and 1.8 m high; however, due to inadequate visitation of the GEM, the panels were replaced with panels that were 1.8 m long and 1.2 m high. Beginning the second week of acclimation, the steers were brought to the feeding stalls daily and supplemented WCS. In order to train the steers to eat the WCS, 0.9 kg of WCS was mixed with a protein supplement that the steers were fed prior to the experiment. As the second week progressed less protein supplement was added until the steers consumed only WCS. During the third week of acclimation 1.8 kg of WCS was offered.

Animals and Feeding

After the acclimation period, 18 steers were selected (BW = 317 ± 5.5 kg) that most consistently used the GEM. To ensure that all treatments had adequate CH_4 estimates for the study, the steers were stratified by GEM visits, then 3 steers were randomized into each treatment to receive either 0, 0.9, 1.8, 2.7, 3.6, or 4.5 kg of WCS/d on an as-fed basis. During the first two weeks of the experiment, animals were housed in a 6-ha pasture to keep the steers in close proximity to the GEM. After two weeks, the steers were moved to a 16-ha pasture where they remained for the rest of the study. On d 6, a steer from the 0-kg treatment was replaced with another steer due to an unrelated health issue. Once the experiment began, steers were weighed weekly each Monday before feeding at 0730 h. A regression was fitted for each animal's weight over the duration of the experiment to determine average daily gain (**ADG**; kg/d). The steers were offered supplemental WCS in individual feeding stalls at 0800 h each day. These stalls were 1.8 by 0.9 m. Steers were allowed 30 minutes to consume WCS, orts were weighed, and steers were returned to the pasture.

Samples of the WCS and the GEM supplement were sent to a commercial lab (Dairy One Inc., Ithaca, NY) for analysis of ether extract (**EE**). A subsample was retained for analysis at the USDA-ARS in Woodward, OK. The retained supplements were dried in a freeze drier (FreeZone 6, Labconco, Kansas City, MO) and analyzed for DM and ash (AOAC, 1990), NDF and ADF were determined (Van Soest et al., 1991) in an Ankom 2000 Fiber Analyzer (Ankom Technology, Macedon, NY), and N was measured by combustion (Vario Ma CN; Elementar Americas, Mount Laural, NJ). Nitrogen concentration was multiplied by 6.25 to determine CP. Supplements nutritive values are shown in **Table 2.1.**

Methane Measurement

The GEM system was used to measure CH_4 emissions of the cattle. The GEM drops feed (32 ± 0.30 g/drop) when an animal's radio frequency identification tag (**RFID**) is read in order to encourage the animal to stay at the GEM while it is being sampled (Gunter et al., 2017). The GEM bait for the current experiment was 97% soybean meal and 3% molasses, pelleted into 1-cm diameter pellets. During a visit, air is drawn around the animal's head and shoulders in order to capture the gases that are emitted. The gases are analyzed for oxygen, carbon dioxide, and CH_4 by sensors housed within the GEM. This captured gas concentration is then compared to the background gas that is measured before and after the animal visits. This allows for the emissions from the animal to be separated from the gas concentrations from other sources (Cottle et al., 2015). The GEM was set so that 6 drops were provided per visit with 30-s drop intervals. The animals were limited to 3 visits per day, with 6-h minimum allowed between each visit, to ensure that the GEM visits were spaced throughout the day. Only measurements from animals sampled for longer than 3-min were used (Velazco et al., 2016a).

Analysis

The current experiment was a completely randomized design with 6 levels of WCS offered. Fixed effects regression models were used for statistical analysis of all variables of interest. For ADG, GEM visitation (drops/d), daily methane production (**DMP**; g of CH_4/d), and methane emission intensity (**EI**; g of CH_4 / kg of body weight gain; Velazco, et al., 2016b) as dependent variables, average WCS intake was used as the independent variable. Individual animal was the experimental unit and significance was declared at $\alpha = 0.05$. All statistical analysis was conducted using R (R Core Team, 2015).

Results and Discussion

Supplement Intake

Mean WCS intake ranged from 0.89 to 2.86 kg per d by the steers that were offered WCS. The amount of WCS consumed increased as the amount offered increased, up to approximately 2.5 kg of WCS, after which intake appeared to plateau. This resulted in a significant quadratic relationship between actual WCS intake and the amount of WCS offered ($P < 0.01$; $R^2 = 0.94$; **Table 2.2**). While intake increased to a point, the percentage of WCS offered that was consumed decreased quadratically ($P < 0.01$; $R^2 = 0.92$; **Table 2.2**). The cattle that received the higher levels of WCS were variable in the amounts consumed per day (**Figure 2.1**) and this finding is corroborated by others (Schauer et al., 2005; Bowman and Sowell, 1997).

Animal Performance

Average daily gain ranged from 1.16 to 1.66 kg/d by steers that consumed, respectively, 0 and 2.3 kg of WCS/d. We found that WCS intake had a positive linear relationship with ADG ($P = 0.02$; $R^2 = 0.29$; **Table 2.2**). For every additional kg of WCS intake there was an observed 0.09 ± 0.03 additional ADG (**Figure 2.2**).

All animals achieved a relatively high level of ADG, with the intercept of the linear regression line being 1.24 ± 0.07 kg/d (**Figure 2.2**). This high performance could be a result of compensatory gain (Hornick et al., 2000) as the steers were grazing dormant tall grass prairie during the winter and had low ADG prior to this experiment. Another explanation for the high ADG could be the relative high nutritive value of the forage during the period of the experiment. The experiment took place from May to July and therefore forage was at its highest quality (15-19% CP; Basurto et al., 2000).

GreenFeed visits

GreenFeed visitation tended to have a linear relationship with WCS intake ($P = 0.08$; **Table 2.2**). However, the most GEM visits were by animals who consumed moderate to high amounts of WCS per day (**Figure 2.3**) and we believe that visitation was less related to WCS intake and more to animal behavior. Only one steer from the 0 WCS treatment used the GEM. Because this point is one of the extreme values the 0 kg/d of WCS intake is at the end of the regression, it could be an influential point. An analysis for a leverage point was conducted for DMP and EI using Cook's Distance (Cook, 1977). The values of the Cook's Distance for the 0 WCS observation were above the acceptable threshold (D_i = 7.87 and 9.07, for DMP and EI respectively) indicating that this observation was biasing the regression. The 0 WCS observation was therefore removed from further analysis.

Methane Emissions

Average DMP for all animals was 228.45 ± 4.53 g CH_4 per d. With the 0 WCS intake observation removed, there was a quadratic relationship of DMP and WCS intake (P = 0.02; **Table 2.2**; R^2 = 0.44; **Figure 2.4**). The minimum of the quadratic DMP regression line was at 1.86 kg of WCS intake/d. There was a quadratic relationship observed between EI and WCS intake (P = 0.011; **Table 2.2**; R^2 = 0.60; **Figure 2.5**). Intake of moderate amounts of WCS decreased EI with the minimum of the regression line at 2.0 kg of WCS intake/d. Mean EI, for all levels of WCS intake was 161.26 ± 5.21 g of CH_4/kg of BW gain.

Using the reported forage quality for the time of year this experiment was conducted (May 23 to July 5; around 15% CP; Basurto et al., 2000) and the known amount of WCS intake, the 2016 Beef Cattle Nutrient Requirements Model (The NASEM, 2016) predicts that a 317 kg steer will consume about 8.7 kg of DM/d. The fat content of the diet for the greatest and lowest level of WCS intake would be 8.3% and 4.6% respectively. Patra et al. (2013) determined that a 1% of diet DM addition of fat would reduce methane yield (g of CH_4 / kg DMI) by 5.6%, regardless of fat source. It is possible that methane yield was reduced during the current experiment, but an estimate of forage intake would be required.

Using the GEM

Hristov et al. (2015) discussed using the GEM with categorical treatments in a crossover design and a randomized block design. It was suggested that a crossover design would require 8 to 12 animals per treatment with 7-day treatment periods and 12 to 15 animals per treatment over 42 to 70 day would be needed for a randomized block design (Hristov et al., 2015). The current experiment utilized a continuous treatment structure over 45 d and had CH_4 estimates from only 13 animals. Length of the experiment can play a large role in experiments with the GEM by decreasing the animal-to-animal variation (Cottle et al., 2015). To investigate this effect, the daily CH_4 measurements were separated into increasing 5 day increments (0-5, 0-10, etc.) and the CV of DMP was calculated. The CV of DMP decreased linearly ($P = 0.01$; $R^2 = 0.60$; **Table 2.2**) as the length of experiment increased from 5 days to 45 days, with a final CV of 7.42%.

Implications

Despite acclimation, only 13 animals visited the GEM during the experiment. This was problematic due to the lack of observations at the 0 intake of WCS level, which narrowed the scope of inference of the current experiment. There is a need for more data to confirm the efficacy of WCS supplementation to beef cattle as a means of GHG mitigation. The EI regression line indicates a minimum at 2.0 kg of WCS intake/d. Therefore, if producers choose to supplement WCS and wish to minimize EI then they should target a consumption of 2.0 kg/hd/d.

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Table 2.1. Ingredient and nutrient content of the supplements.

	Supplements [a]	
Item	Pelleted GreenFeed bait	Whole Cottonseed
Formulation, % DM		
Soybean meal	97	---
Molasses	3	---
Whole Cottonseed	---	100
Nutritive Value, %		
CP	49.5	22.1
NDF	8.9	40.5
ADF	5.4	30.3
EE	3.4	19.6

[a] WCS = Whole Cottonseed, supplement offered to animals at either 0, 0.9, 1.8, 2.7, 3.6, or 4.5 kg/d.

Table 2.2 Dependent and independent variables, and regression equations and *P* –values.

			P- Value	
Dependent Variable	Independent Variable	Model [a]	Lin.	Quad.
Supplement Intake	WCS offered	$y = -0.04 x^2 + 0.74 x - 0.46$	<0.01	<0.01
% Supplement Consumed	WCS offered	$y = -0.31 x^2 + 1.28 x + 43.35$	<0.01	<0.01
GreenFeed Visits	actual WCS intake	$y = 13.8 x + 23.8$	0.08	0.11
Average Daily Gain, kg/d	actual WCS intake	$y = 0.09 x + 1.24$	0.02	0.36
Daily Methane Production, g of CH_4/hd/d [b]	actual WCS intake	$y = 28.6 x^2 - 106.1 x + 314.1$	0.76	0.02
Emission Intensity, g of CH_4/kg of gain [b]	actual WCS intake	$y = 32.2 x^2 – 129.8 x + 276.4$	0.13	<0.01
Coefficient of Variation [b]	Length of Experiment	$y = -0.18 x + 14.9$	0.01	0.11

[a] Highest significant or tendency order model

[b] The 0 kg of WCS treatment per day was excluded.

Figure 2.1. Daily intakes of supplemented steers are more variable for the higher levels of offered WCS. Each panel is one animal, and the red line indicates how much they were offered each day.

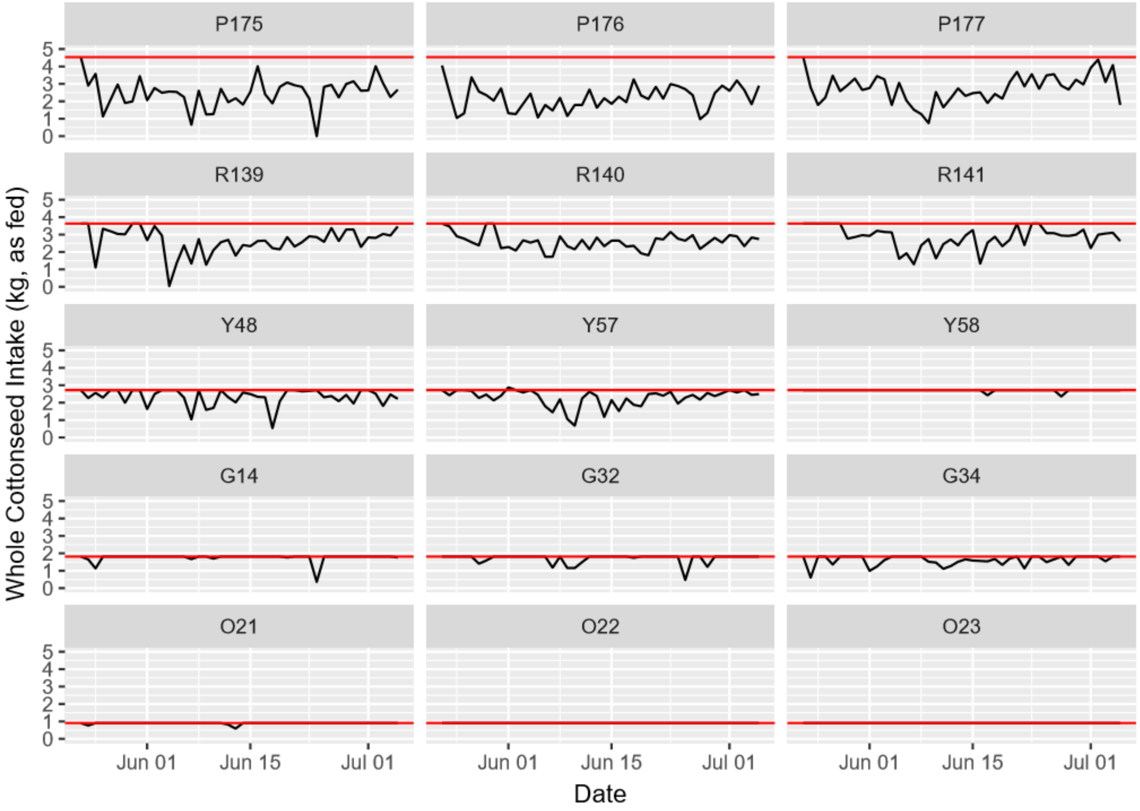


Figure 2.2. Average daily gain of grazing beef steers increased linearly as WCS intake increased (*P* = 0.02).

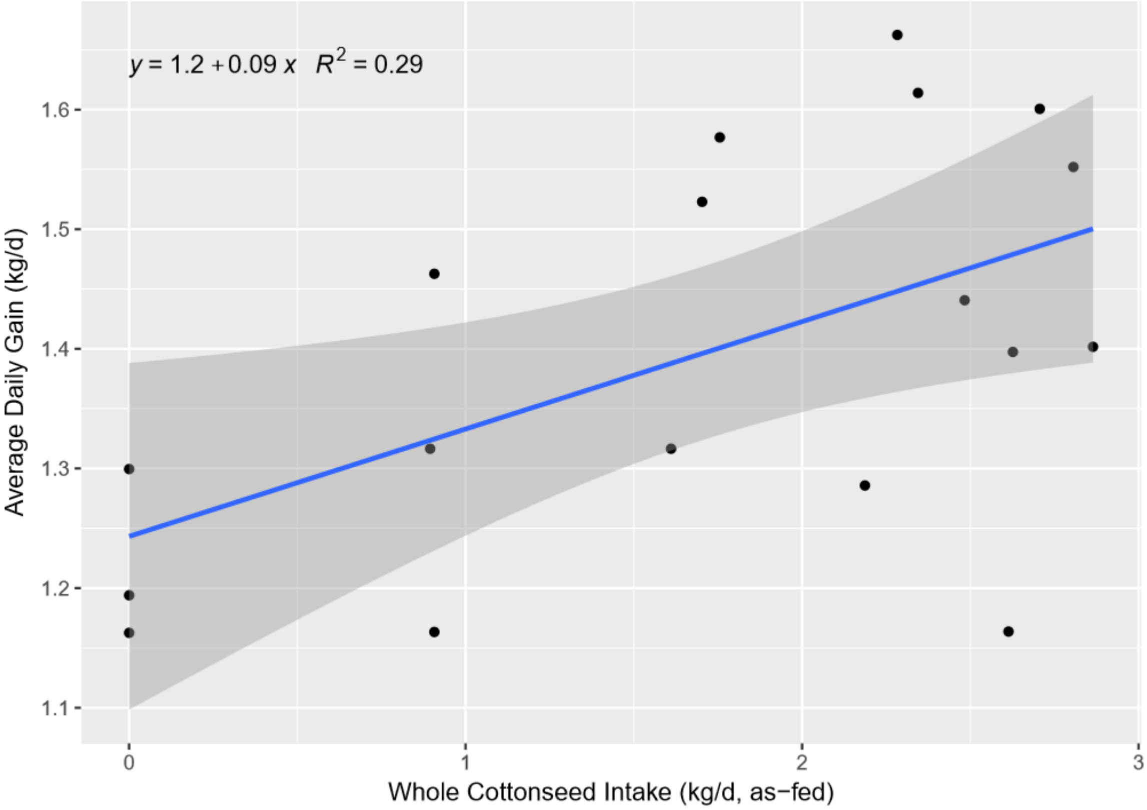


Figure 2.3. GreenFeed visits tended (*P* = 0.08) to have a linear relationship with WCS intake.

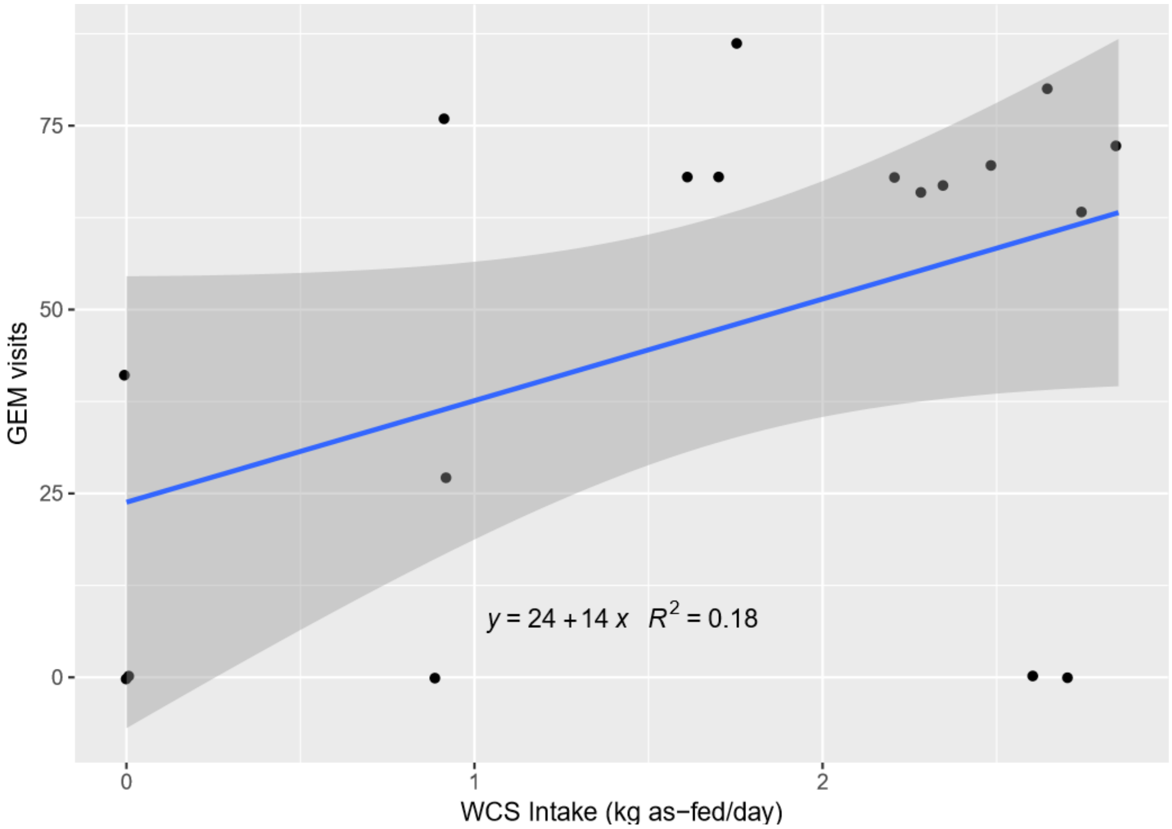


Figure 2.4. Daily methane production had a quadratic relationship to WCS intake (*P* = 0.02).

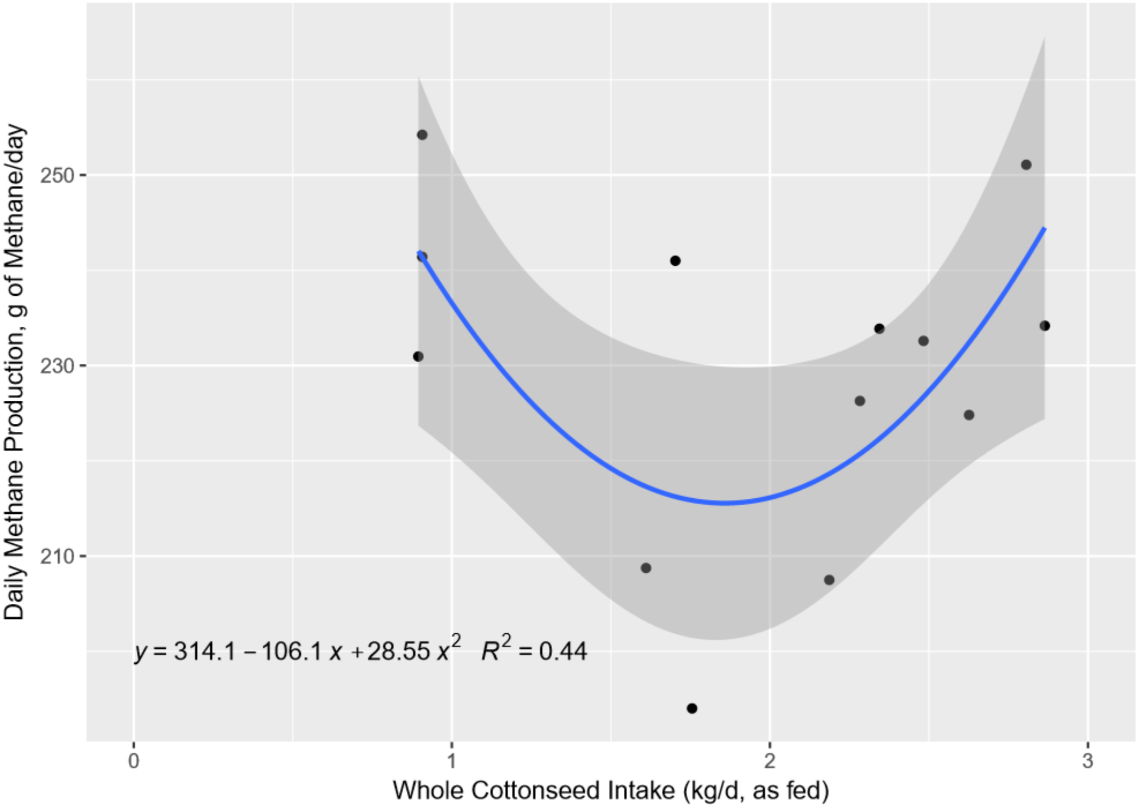
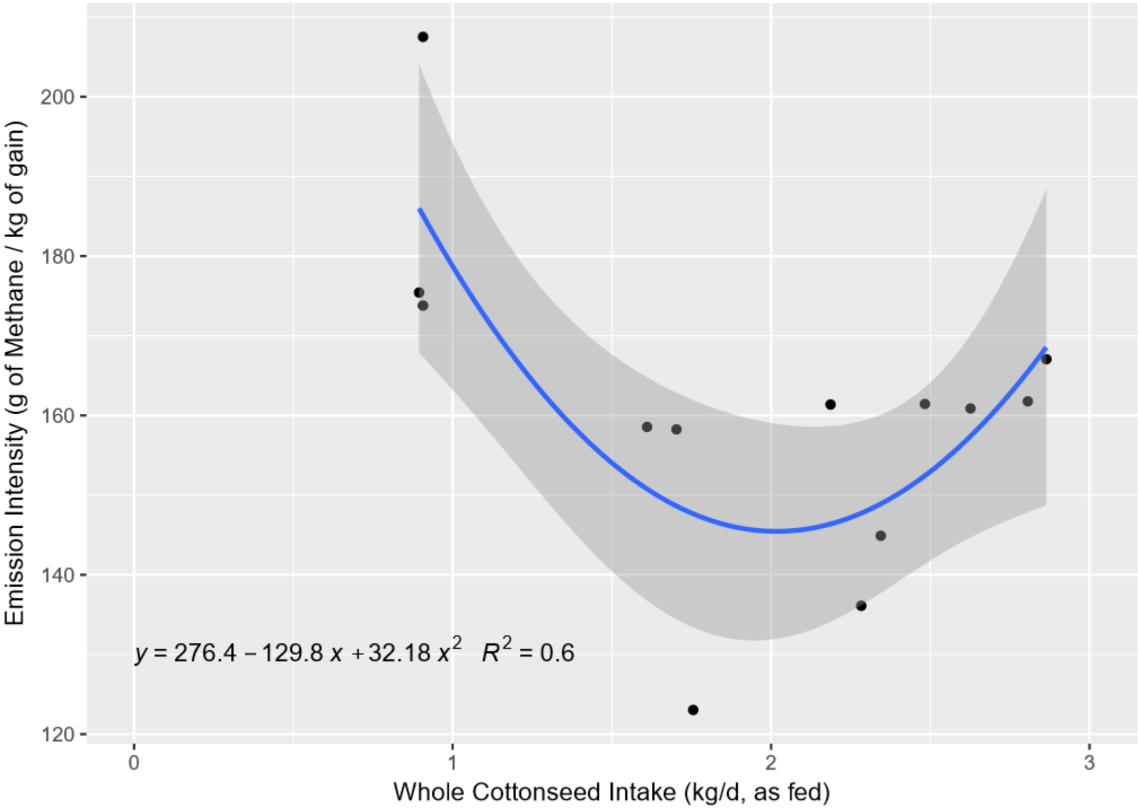


Figure 2.5. Emission intensity responded quadratically (*P* = 0.01) to WCS intake.



CHAPTER III

Physical form of fat supplements affects methane emissions of grazing beef cattle.

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Abstract: The objective of this experiment was to determine how physical form of lipid supplements affect forage intake, average daily gain (**ADG**), and enteric methane (**CH_4**) emissions from steers grazing tall grass prairie in late summer. Steers (n = 20; BW = 279 ± 8 kg) were acclimated to a GreenFeed emission monitoring system (**GEM**; C-Lock Inc., Rapid City, SD) which uses a bait feed and were randomly assigned to one of four treatments, either no supplement (**CON**), whole cottonseed (**WCS**), a supplement with rumen bypass fat (**BYP**; Megalac, Arm and Hammer Animal Nutrition, Princeton, NJ), or a supplement containing soybean oil (**SBO**). The BYP treatment was added in order to determine if the results were due to the energy provided by the supplement or as a result

of the fats acting in the rumen. The BYP and SBO supplements were formulated on an as-fed basis to provide the same amount of fat as WCS, and all supplements were offered at 1.59 kg per day. Indigestible acid detergent fiber (**IADF**) and TiO_2 were used as internal and external markers, respectively, to determine forage intake. Whole cottonseed supplemented cattle and CON had similar levels of ADG ($P = 0.15$). The SBO and BYP were not different ($P = 0.69$) but produced greater ADG than CON and WCS ($P \leq 0.04$). The WCS treatment had a significantly lower DMP (18% lower than CON) than the other treatments ($P \leq 0.03$), while SBO and BYP did not differ from CON ($P \geq 0.61$). We believed that BYP would be similar to the CON in DMP; however, we thought SBO would have the same effect on DMP as WCS. The SBO and BYP CH_4 emission intensity (**EI**; g of CH_4/kg of gain) were not different from WCS ($P \geq 0.20$), while all supplemented treatments had lower EI than the CON ($P \leq 0.02$). Forage intake tended ($P = 0.10$) to be lower under WCS compared to the other treatments. There was a tendency ($P = 0.08$) for WCS to have a decreased NDF digestibility (P = 0.08) and WCS significantly reduced ADF digestibility compared to the other treatments ($P \leq 0.006$). There was a tendency for WCS and SBO to have reduced methane yield ($P = 0.081$). CON had a significantly higher CH_4 emissions as a percentage of GEI than WCS and SBO ($P \leq 0.02$), and BYP was not different from the other treatments ($P \geq 0.11$). As Forage intake ($P = 0.05$) and ADF digestibility ($P = 0.004$) increased, DMP increased, whereas NDF digestibility had no effect on DMP ($P = 0.52$). Supplementing WCS directly reduced CH_4 by decreasing DMP, while the other treatments were not different from CON; however, the additional ADG from BYP and SBO reduced EI so that all supplemented treatments were not different from each other and were lower than CON.

These result suggest that all supplements decreased the environmental impact of the system, albeit in different ways.

Key words: Enteric methane, whole cottonseed, bypass fat, soybean oil

Introduction

Enteric methane (**CH_4**) emissions from beef cattle are a concern to consumers. Enteric CH_4 represents 5.7% of global and 2.5% of U.S. total anthropogenic greenhouse gas emissions (Gerber et al., 2013; USEPA, 2017). Enteric CH_4 contribution to global greenhouse gas emissions is partly due to CH_4 having 28 times the global warming potential of carbon dioxide (**CO_2**; IPCC, 2013). Enteric CH_4 also represents a significant energy loss to the animal as 2 to 12% of gross energy intake is lost as CH_4 (Johnson and Johnson, 1995).

Supplementing lipids is one strategy to reduce enteric CH_4 production. Lipid supplementation was found to decrease CH_4 *in vivo* across ruminant species (Czerkawski et al., 1966; Grainger et al., 2008; Beauchemin et al., 2007). This strategy has two proposed modes of action. The first is that lipids provide an alternative hydrogen sink as unsaturated fatty acids become saturated in the rumen (Czerkawski et al., 1966; Johnson et al., 2002). This mode is believed to account for only a small amount of reduction (Hristov et al., 2013; Johnson and Johnson, 1995). The second mode of action of reducing CH_4 is by decreasing dry matter intake (**DMI**; Hristov et al., 2013; Eugène et al., 2008; Raibee et al., 2012).

Physical form of lipid (free or bound in an oilseed) could influence its ability to mitigate CH_4 emissions (Brask et al., 2013). Brask et al. (2013) and Fiorentini et al. (2014) did not find an impact of lipid form on CH_4, while Beauchemin et al. (2007)

determined that whole sunflower seeds reduced CH_4 more than sunflower oil. These experiments were conducted with dairy or beef cattle fed total mixed rations. The effect of the physical form of lipid supplements on CH_4 emissions from grazing cattle is unknown. Therefore, the objective of this experiment is to determine the effect that physical form of fat supplements has on forage DMI, ADG, and CH_4 emissions from cattle grazing tall grass prairie in late summer.

Materials and Methods

All procedures used in the current experiment were approved by the Oklahoma State University Institutional Animal Care and Use Committee.

Location and Pasture

The 60-day (August 16, 2016 - October 14, 2016) experiment was conducted at the Oklahoma State University Bluestem Research Range, near Stillwater. Pastures consisted of tall grass prairie (big bluestem, *Andropogon gerardii*; little bluestem, *Schizachyrium scoparium*; indian grass, *Sorghastrum nutans*) and yellow bluestem (*Bothriochloa ischaemum*). Animals were pastured in a 22-ha pasture for 5 weeks and were then moved to a 16 ha pasture where they remained for the rest of the experiment. Forage mass was measured when animals were first placed into the pastures and when they were removed from the pasture (Moffet et al., 2014). Ninety plate meter readings were taken on the first day of the experiment when animals were placed in the 22 ha pasture. Once the steers were moved to the 16-ha pasture, 50 plate meter readings were taken on the original and new pastures. The last week of the study, 50 plate meter readings were taken before the animals were removed. After the plate meter heights were recorded on the sampling days, ten additional plate meter heights were taken at selected locations, in order to encompass the whole range of forage mass in the pasture, and then were clipped to ground level. The clippings were weighed, dried in a 40ºC oven, and weighed again, to determine DM content. Clipped weight was regressed on plate height and the equation was applied to the plate meter readings in order to estimate forage mass of the pastures. The forage mass of the pastures throughout the experiment averaged 5,929 kg/ha.

Animals, Treatments, and Feeding

Cross bred Bos taurus steers (n = 20; BW = 279 ± 8 kg), all originating from the same ranch in central Oklahoma, were randomly assigned to one of four treatments: no fat supplement (**CON**), or 1.59 kg/d of either whole cottonseed (**WCS**), a supplement containing rumen bypass fat (**BYP**; Megalac, Arm and Hammer Animal Nutrition, Princeton, NJ), or a supplement containing soybean oil (**SBO**). The BYP and SBO supplements were formulated to be identical except for the source of lipid (**Table 3.1**). These supplements were formulated to be similar to the fat percentage of WCS in order to provide the same amount of supplemental lipids to the steers. The BYP was used to determine if the obtained results were from effects of the fat on ruminal fermentation or just as a result from the additional energy. Since the fat in BYP is rumen unavailable we expected to see observations similar to CON, and for SBO and WCS to have similar observations.

The steers were in the pasture except for when they were fed at 0800 each morning. Supplements were offered by placing the animals in individual feeding stalls for 30 min. After 30 min the steers were placed back into the pasture and any orts were weighed to determine actual supplement intake. Steers were weighed (unshrunk BW) before feeding once weekly and these weights were used to determine ADG by regression.

Forage Intake, Laboratory Analysis and Calculations

Forage intake was estimated by the double marker method (Kartchner, 1981). For the last 19 d of the experiment, CON steers received 0.45 kg of additional GEM supplement and all animals were dosed with 10 g titanium dioxide (**TiO_2**) daily in their supplement so that each steer was offered 6 g of titanium (**Ti**) each day. During the last 5 d of the experiment, fecal samples were taken twice daily (once in the morning before feeding (0630) and once in the afternoon (1600)), in the squeeze chute via rectal grab. In order to obtain an accurate representation of grazed forage, a cannulated steer was rumen evacuated and turned out to the pasture to graze during the last week of the experiment (Lesperance et al., 1960). After one hour, samples of the grazed forage were taken from the rumen and then the evacuated rumen contents were returned to the cannulated steer. A subsample of the masticate forage sample and a composited sample of the supplements were sent to Dairy One (Dairy One Inc., Ithaca, NY) to determine CP, NDF, ADF, fatty acid (**FA**) profile, and ether extract (**EE**). Additional samples of supplement and forage were freeze dried (FreeZone 6, Labconco, Kansas City, MO) and fecal samples were dried in a 60°C oven. Once dry, samples were ground to pass a 1-mm screen (Thomas A. Wiley Laboratory Mill, model 4). Fecal samples were then composited across days with animal. Fecal samples were analyzed for DM and ash (AOAC, 1990), NDF and ADF using the method of Van Soest et al. (1991) in an Ankom 2000 Fiber Analyzer (Ankom Technology, Macedon, NY), and for N by combustion (Vario Ma CN; Elementar

Americas, Mount Laural, NJ). Nitrogen concentration was multiplied by 6.25 to determine CP.

A subsample of the supplements, forage, and fecal were analyzed for indigestible ADF (**IADF**) using the procedure described by Bohnert et al. (2002), with one minor change. The cannulated steer used for *in situ* digestion was grazing wheat pasture instead of consuming low-quality forage. In brief, triplicate samples (0.5 g) of forage and supplements were weighed into Ankom filter bags (F57; Ankom Co., Fairport, NY) and, excluding fecal samples, incubated for 16 h at 39°C in a solution containing 0.1% pepsin (Catalog # 41707-5000, Acros Organics, Fair Lawn, NJ) and 10% 1NHCl using a DaisyII incubator (24 sample bags and 2 L per incubation vessel; Ankom Co., Fairport, NY). Samples were then rinsed with warm (39°C) tap water, placed into a mesh bag along with the fecal samples, and incubated for 96 h in the rumen of a cannulated steer. The sample bags were then removed from the rumen, rinsed with warm (39°C) tap water until the water was clear, and analyzed for ADF as described above. Orts and fecal samples were analyzed for Ti by portable X-ray fluorescence (Barnett et al., 2016). The amount of Ti remaining in orts was subtracted from the daily dosage for that animal for further calculations. The IADF from the supplements were removed so that only IADF from forage was considered in further calculations. Indigestible ADF was used to determine DM digestibility (**DMD**), Ti was used to calculate fecal output, and DMD and fecal output were used to calculate forage DMI using equations described by Kartchner (1981), with one exception. Total digestible nutrients were used to calculate fecal output from the supplements rather than using IVOMD. Neutral and acid detergent fiber digestibility

(**NDFD** and **ADFD** respectively) were calculated from the amount of NDF and ADF in the feces and the total amount consumed.

Gross energy (**GE**) of the supplements, forage, and feces was measured in an adiabatic bomb calorimeter (AC600; Leco, St. Joseph, MI; ISO, 1988). Gross energy intake (**GEI**) was determined by multiplying the GE content of the forage and supplements by each animals' respective intakes. Fecal energy was subtracted from GEI to determine digestible energy (**DE**) intake (**DEI**). The energy content of enteric CH_4 was assumed to be 13.3 Mcal per kg and was used to calculate the percentage of GEI and DEI that was lost as enteric CH_4 (Beauchemin and McGinn, 2005).

Methane Measurement

Methane was measured by averaging the spot measurements obtained from the GreenFeed emission monitoring (**GEM**; C-Lock Inc., Rapid City, SD; Gunter et al., 2017) system from each animal throughout a day. Once the animals head is in the hood, its radio frequency identification tag is read and a pelleted supplement is dropped (32 ± 0.3 g/drop). While visiting the GEM, a fan draws air around the animal's head in order to capture the emitted gases. The captured gas concentration is then compared to background gases when the animal is not present so that the background gas concentration can be separated from what the animal actually emits (Cottle et al., 2015). The GEM was set so 6 drops were provided per visit with 30 second interval between each drop. This is to keep the animal there for at least 3 minutes to ensure that several eructation events occur (Velazco et al., 2016). Data from visits < 3 min were removed from the data set (Velazco et al., 2016; Arthur et al., 2017).

Statistical Analysis

The experimental design was a completely random design, with 4 levels of lipid supplements. The statistical model to determine treatment effects was: $y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$, where y_{ij} is the observation of animal j within supplement i, μ is the overall mean, α_i is the effect of supplement i, and ϵ_{ij} is the random error associated with animal j in supplement i. Individual animal was the experimental unit, statistical significance was declared at $\alpha = 0.05$ and tendencies were declared at $0.05 < P \leq 0.1$. ANOVA was used for initial analysis and, upon significance, Fisher's LSD was used for separation of means. Initial BW was included in the model as a covariate to determine if there was any effect. Linear models were fit to determine the effects of forage intake, NDFD, and ADFD on DMP. All statistical analyses were conducted using R (R Core Team, 2015).

Results and Discussion

Supplements and Forage Intake

The nutrient compositions of supplements and forage are presented in **Table 3.1**. The EE of BYP and SBO was lower than the original formulation and the content was less than the WCS supplement (**Table 3.1**). The supplements also differed in their FA profile (**Table 3.2**). The BYP supplement had the largest percentage of saturated and monounsaturated FA. The SBO supplement had intermediate and WCS was the lowest percentage of saturated FA. The SBO and WCS supplements had similar polyunsaturated FA concentrations and BYP had the lowest, with linoleic acid being the primary polyunsaturated FA for all supplements. The WCS treatment had an average supplement intake of 1.54 kg per day, while SBO and BYP consumed their target supplement intake of 1.59 kg per day (**Table 3.3**). However, as WCS had a larger percentage of fat, the supplement provided 0.3 kg/d of fat provided through the treatment supplements, while SBO provided 0.29 kg/d, and BYP provided 0.26 kg/d. This resulted in SBO and WCS being similar ($P = 0.79$) in amount of additional fat provided by the treatment supplements but greater than BYP ($P \leq 0.008$).

One animal in WCS was removed from the forage intake and digestibility portion of the experiment because it had significant amounts of orts each day of the fecal collection period causing incomplete recoveries of Ti resulting in unrealistic fecal output estimates. There was no significant treatment effect on forage intake ($P = 0.15$) but WCS did have numerically lower forage intake (**Table 3.3**). There was observed differences in the percent fat of the diet which were 2.4%, 6.2%, 5.4%, and 5.2% for CON, WCS, SBO, and BYP, respectively (**Table 3.3**). WCS had a significantly higher fat percentage of the diet than the other treatments ($P < 0.01$), SBO and BYP did not differ ($P = 0.19$) but were higher than the CON ($P < 0.01$). The SBO had a higher GEI and DEI than CON ($P \leq 0.009$), but there were no other significant differences detected ($P \geq 0.07$; **Table 3.3**).

BW and Animal Performance

There was a tendency for initial BW to be different between treatments ($P = 0.08$); however, when included in further analysis as a covariate it did not explain a significant amount of error. The CON and WCS ADG were not different ($P = 0.15$), at 0.47 and 0.64 kg/d respectively. The SBO and BYP had similar ($P = 0.69$) levels of performance at 0.88 and 0.92 kg/d respectively. Average daily gain from SBO and BYP was greater than CON and WCS ($P \leq 0.04$; **Table 3.3**).

Methane Emissions

There was a tendency ($P = 0.10$) for BYP to visit the GEM less than the other treatments and BYP did consume less supplement from the GEM than the other treatments ($P \leq 0.02$). The CON consumed the most GEM supplement and SBO and WCS were not different from each other ($P = 0.79$; **Table 3.3**). The CON, SBO, and BYP had similar levels of DMP ($P \geq 0.61$), at approximately 194 g of CH_4 per head per day (**Table 3.4**). The WCS treatment had significantly lower DMP than CON, SBO, and BYP ($P \leq 0.03$; **Table 3.4**). Daily methane production observed in this experiment is similar to other experiments with grazing beef cattle (DeRamus et al., 2003; Pavao-Zuckerman et al., 1999) and cattle offered fresh cut forage (Hart et al., 2009). The WCS treatment had an average DMP of 161.4 g of CH_4 per head per day and reduced DMP by 18% compared to the CON. Grainger et al. (2008) found a 12% reduction in DMP in dairy cows fed WCS and Beauchemin et al. (2007) found a 17% reduction from sunflower or tallow derived lipid sources, which is similar to the 18% reduction in the current experiment.

SBO and WCS had divergent impacts on CH_4 emissions, possibly because the supplements contained fat from different sources, however their FA profiles were similar (**Table 3.2**). Biohydrogenation of unsaturated FA is believed to have a minor effect on CH_4 mitigation (Beauchemin et al., 2007; Grainger and Beauchemin, 2011; Hristov et al., 2013) and only an estimated 1% of metabolic hydrogen is used in this process (Johnson and Johnson, 1995). Due to these considerations we believe that the differences observed between the WCS and SBO are due to their physical form.

Fiorentini et al. (2014) and Brask et al. (2013) both found no effect on DMP by the physical form of the fat compared to the control. While the differences observed between the experiments may be due to the different oil type and oil seeds that were used, we postulate that our observations differ due to the basal diet that the animals were consuming. In our scenario, the cattle grazed pasture and then the oil was provided through a supplement once a day. If there were differences in ruminal passage rate between WCS and SBO, this may account for the difference we observed between the two treatments. This likely would not be as big a consideration in a scenario feeding a TMR, such as in the case of Fiorentini et al. (2014) and Brask et al. (2013). In fact, the results of SBO and BYP treatments were not significantly different (**Table 3.3**; **Table 3.4**) furthering the evidence that SBO was not having ruminal effects.

When CH_4 emissions were expressed as emission intensity (**EI**; g of CH_4 per kg of gain) the additional ADG for SBO and BYP diluted the larger DMP, resulting in EI that were not different from WCS ($P \geq 0.20$; **Table 3.4**). The EI for CON was greater ($P \leq 0.02$; **Table 3.4**) than the other treatments at 442.8 g of CH_4 per kg of gain and WCS, SBO, and BYP resulted in a reduction of EI by 34.2%, 50.6%, and 52.1%, respectively.

There was a tendency ($P = 0.08$) for CON to produce a greater CH_4 yield (CH_4/kg of intake; **Table 3.4**). When CH_4 was expressed as a percent of GEI, CON produced the greatest (9.0%) and was greater than WCS and SBO ($P \leq 0.02$), and BYP was not different than the other treatments ($P \geq 0.11$; **Table 3.4**). Methane emissions as a percent of GEI are within the range reported by Johnson and Johnson (1995).

Forage intake, NDFD, and ADFD effects on DMP

While we were unable to determine a treatment effect on forage intake, there was a significant linear relationship between forage intake and DMP ($P = 0.05$; **Figure 3.1**). This linear relationship suggests that 1 kg of additional forage intake increased DMP by 13.9 ± 6.6 g CH_4/d (**Figure 3.1**). There was a tendency for a treatment effect of NDFD ($P = 0.08$) but it did not appear to influence DMP as there was no linear relationship ($P = 0.52$; **Figure 3.2**). Brask et al. (2013) likewise found no relationship between NDFD and DMP. The WCS decreased ADFD ($P \leq 0.006$) by 10.6%, while all other treatments were not different from CON ($P \geq 0.39$). There was a significant linear relationship between ADFD and DMP ($P = 0.004$) so that every 1% increase in ADFD resulted in an increase of DMP by 5.12 ± 1.52 g of CH_4/d (**Figure 3.3**). This relationship would indicate that the reduction of DMP by WCS was associated with decreased ADFD.

Implications

From this study, we conclude that WCS had a direct effect on CH_4 emissions by reducing DMP that was not observed with the SBO and BYP treatments. The added performance of SBO and BYP reduced EI so that WCS, SBO, and BYP did not differ. Reducing EI improves sustainability by decreasing the environmental impact of producing beef. These results would imply that, while the different fat supplements did not reduce CH_4 emissions in the same manner, all supplements improved the sustainability of the current system by reducing EI compared to CON.

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Table 3.1. Forage composition, ingredient and nutrient composition of supplements.

	Forage and Supplements [a]				
Item	Forage	WCS	SBO	BYP	GEM
Formulation, % as-fed					
Whole Cottonseed	---	100	---	---	---
Cottonseed meal	---	---	73.3	73.3	---
Cottonseed hulls	---	---	6.0	6.0	---
Soybean oil	---	---	20.7	---	---
Megalac			---	20.7	
Soybean meal	---	---	---	---	97.0
molasses	---	---	---	---	3.0
Nutritional Composition					
GE, Mcal/kg	4.4	5.3	5.1	5.2	4.7
TDN	56.0	73.0	92.0	89.0	81.0
CP	9.6	20.9	30.4	27.5	48.1
NDF	72.1	50.6	40.0	39.4	12.8
ADF	46.1	35.0	28.6	24.5	6.0
EE	2.4	19.6	18.4	16.1	3.4

[a] Forage = the masticate sample obtained from the cannulated steer; WCS =whole cottonseed; SBO = supplement containing soybean oil; BYP = the same supplement as SBO but with rumen by-pass fat as fat source. GEM = pelleted supplement provided through the GreenFeed.

Table 3.2. Fatty acid content of Forage and Supplements.

	Forage and Supplements [a]				
FA, % of total FA	Forage	WCS	SBO	BYP	GEM
Saturated	32.77	26.83	19.97	45.34	24.05
Palmitic	20.25	22.83	15.96	39.63	19.49
Stearic	4.57	2.59	2.94	4.11	3.72
Monounsaturated	6.46	18.32	24.85	34.12	17.02
Palmitoleic	0.41	0.55	0.16	0.20	0.12
Oleic	5.96	17.71	24.51	33.81	16.79
Polyunsaturated	46.05	54.06	53.57	19.20	58.00
Linoleic	17.54	53.81	48.93	18.59	51.39
Linolenic	28.50	0.20	4.64	0.61	6.61
Other [b]	14.72	0.79	1.33	1.61	0.94

[a] Forage = the masticate sample obtained from the cannulated steer; WCS =whole cottonseed, offered to WCS treatment at 1.59 kg / d; SBO = supplement containing soybean oil, offered to SBO treatment at 1.59 kg / d; BYP = the same supplement as SBO but with rumen by-pass fat as fat source, offered to BYP treatment at 1.59 kg / d; GEM = supplement that was used in the GreenFeed.

[b] Includes fatty acids that were not analyzed.

Table 3.3. Animal performance, forage digestibility, and forage and supplement intake.

	Treatment [w]					
Item	CON	WCS	SBO	BYP	SEM	*P*-value [d]
n	5	5	5	5	---	---
Initial BW, kg	565	543	657	611	17.4	0.08
ADG, kg	0.47^{b}	0.64^{b}	0.88^{a}	0.92^{a}	0.05	<0.01
GreenFeed Visits [x]	1.3	1.1	1.3	0.8	0.1	0.10
GreenFeed Intake, kg [y]	0.7^{c}	0.3^{b}	0.3^{b}	0.2^{a}	0.05	<0.01
Fat Supp. Intake, kg [z]	0^{c}	1.54^{b}	1.59^{a}	1.59^{a}	0.2	0.03
Forage Intake, kg [e]	5.9	4.9	5.9	5.5	0.2	0.16
Total Intake, kg [e]	6.6	6.7	7.6	7.2	0.2	0.10
GE Intake, Mcal [e]	29.2^{a}	31.1^{ab}	34.7^{b}	32.6^{ab}	0.8	0.05
DE Intake, Mcal [e]	15.0^{a}	16.0^{ab}	18.0^{b}	16.2^{ab}	0.4	0.05
EE, % of diet [e]	2.4^{c}	6.2^{a}	5.4^{b}	5.2^{b}	0.3	0.01
NDF Digestibility, % [e]	56.4	53.7	54.9	56.1	0.4	0.08
ADF Digestibility, % [e]	54.8^{a}	50.3^{b}	54.8^{a}	55.1^{a}	0.6	<0.01

[a-c] rows with different superscripts differ ($P < 0.05$).

[e] n = 4 for the WCS treatment.

[w] CON = control, no fat supplement; WCS = offered 1.59 kg of whole cottonseed per day; SBO = offered 1.59 kg of a supplement containing soybean oil; BYP = offered 1.59 kg of the same supplement as SBO but with rumen by-pass fat as fat source.

[x] Average visits to the GreenFeed, a visit was when an animal remained at GreenFeed >3min.

[y] Amount of supplement received from the GreenFeed.

[z] Intake of a fat supplement, does not include supplement intake provided through the GreenFeed or offered to the control during titanium dioxide dosing.

Table 3.4. Methane emissions expressed as daily methane emissions, emission intensity, methane yield, and as a percent of gross and digestible energy intake.

	Treatment [u]					
Item	CON	WCS	SBO	BYP	SEM	*P*-value
n	5	5	5	5	---	---
DMP [v]	197.0^a	161.4^b	190.8^a	193.3^a	5.1	0.03
Emission Intensity [w]	442.8^a	291.4^b	218.9^b	212.1^b	28.8	<0.01
Methane Yield [e,x]	30.2	24.5	25.0	27.0	0.9	0.08
% GEI [e,y]	9.0^a	7.1^b	7.3^b	7.9^{ab}	0.3	0.04
% DEI [e,z]	17.5	13.9	14.1	16.0	0.6	0.08

[a-c] rows with different superscripts differ ($P \leq 0.05$).

[e] n = 4 for the WCS treatment.

[u] CON = control, no fat supplement; WCS = offered 1.59 kg of whole cottonseed per day; SBO = offered 1.59 kg of a supplement containing soybean oil; BYP = offered 1.59 kg of the same supplement as SBO but with rumen by-pass fat as fat source.

[v] Daily methane production averaged across the experiment, in g per d.

[w] Emission intensity in g of CH_4 per kg of ADG.

[x] g of CH_4 per kg of intake.

[y] Methane as a percent of gross energy intake.

[z] Methane as a percent of digestible energy intake.

Figure 3.1. Increasing forage intake increased daily methane production (g of CH_4/d; $P = 0.05$).

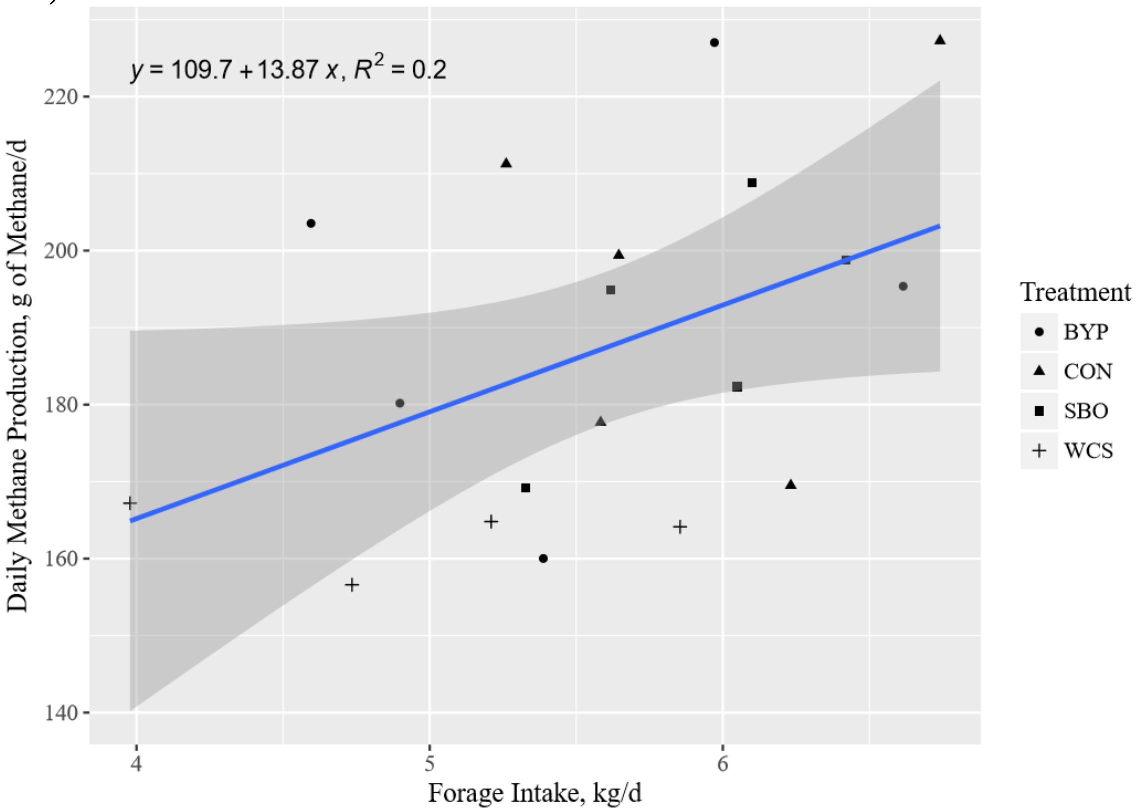


Figure 3.2. NDF digestibility had no effect on daily methane emissions from the grazing steers (*P* = 0.52).

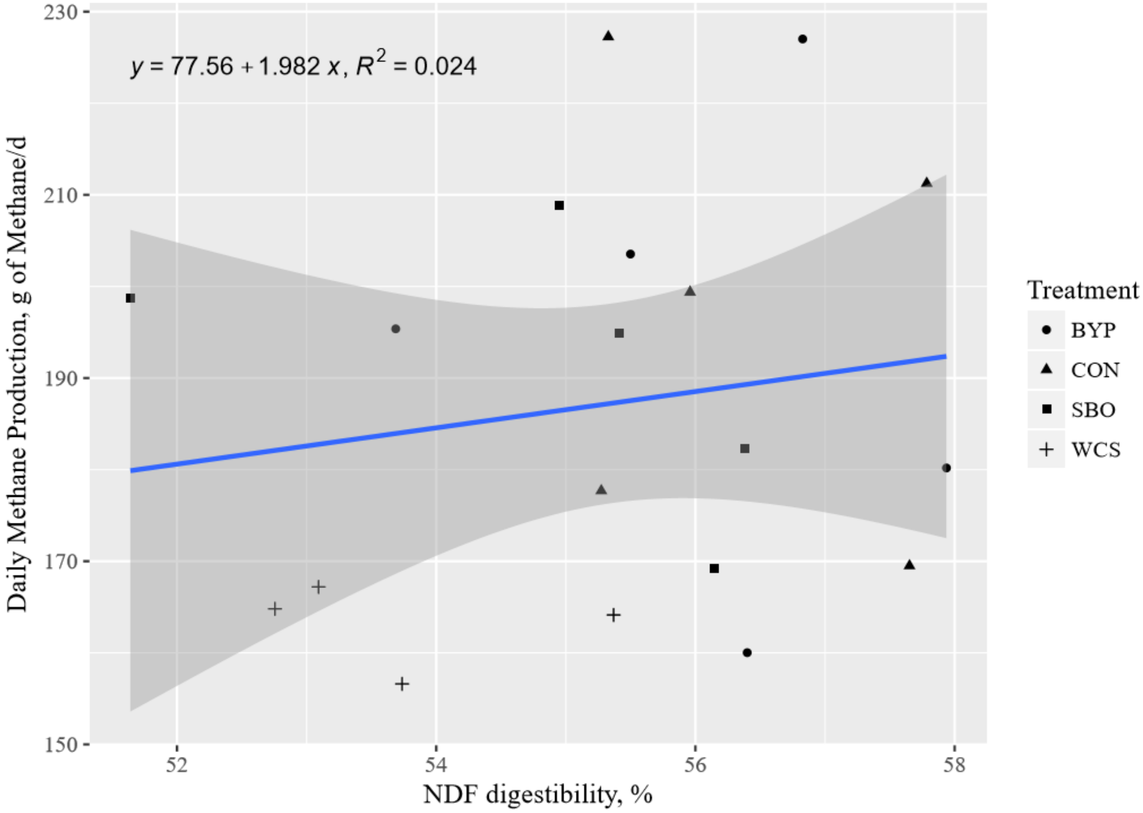
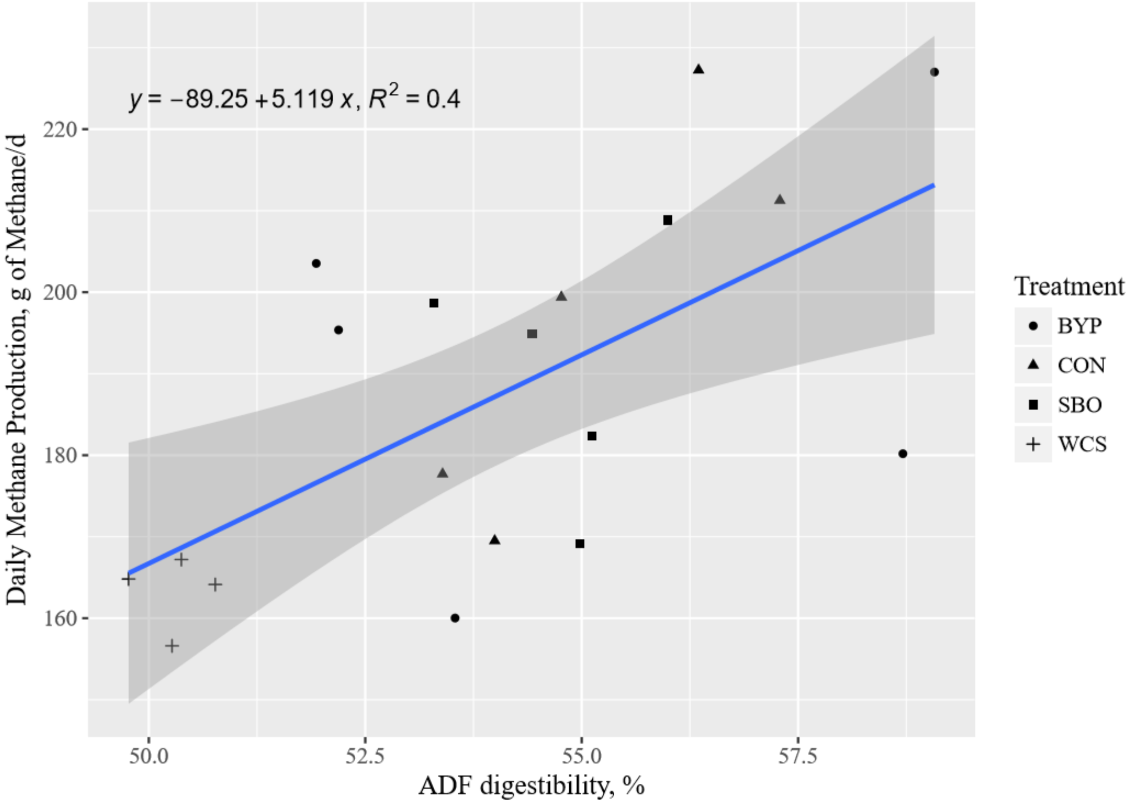


Figure 3.3. As ADF digestibility increases there was a linear increase in daily methane emissions (*P* = 0.004).



VITA

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