A REVIEW OF THE ROLE OF CATTLE IN THE CARBON CYCLE AND MONENSIN: A META-ANALYSIS

A Thesis

by

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ABSTRACT

Ruminal fermentative processes allow cattle to consume a variety of feeds that are converted into human edible product, resulting in methane (CH_4) as a byproduct. Feed additives, like monensin, can be added to cattle rations to alter ruminal fermentation and reduce CH_4 emissions. The purpose of this thesis is to illustrate the role of cattle in cycling carbon between the atmosphere, plants, cattle products and emitted CH_4, and to conduct meta-analyses that provide robust summary statistics of effects of monensin on CH_4 emissions. Data used to conduct the meta-analyses were collected from published literature and analyzed using Comprehensive Meta-Analysis v.3 (Biostat Inc., Englewood, NJ). Monensin reduced CH_4 production by 14.39 ± 2.81 g/d, 6.05 ± 0.99 g/Mcal DE/kg, and 1.99 ± 0.43 g/kg DMI. Monensin has proven to be a viable feed additive technology that enhances the efficiency of cattle by reducing CH_4 emissions.

DEDICATION

Both of my parents have poured so much effort, time, and financial support into my sister and I's education. They consistently made sacrifices in order to send my sister and I to Catholic school during our elementary and intermediate years. After transferring to public school, my parents always gave their best effort to attend and be a part of all extracurricular activities that my sister and I participated in. Mom and dad, you are nothing short of the best parents Morgan and I could have ever asked for. You both always make us feel so loved, always encourage us, support us, and have done an amazing job providing for us. Without each of your support and dedication to our family, Morgan and I would have never achieved such great success. It is because of each of you that Morgan and I are who we are today. I am forever grateful. For this, I dedicate my thesis to both of you.

May God continue to guide, protect, and provide for both of you and our family.

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This work was supervised by a thesis committee consisting of Dr. Tryon Wickersham (advisor) of the Department of Animal Science, and Dr. David Anderson of the Department of Agricultural Economics, and Dr. Jason Sawyer of the King Ranch® Institute for Ranch Management.

All work for Chapter III was completed by the student, in collaboration with Dr. Tryon Wickersham of the Department of Animal Science and Dr. Jason Sawyer of the King Ranch® Institute for Ranch Management and Dr. Ben Holland of Cactus Feeders.

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CHAPTER I

INTRODUCTION AND REVIEW OF THE LITERATURE

Introduction

Carbon is fundamental to life and serves as the energetic backbone of animal systems (Capper, 2011; Garnett et al., 2017). Cattle aid in the transformation of carbon from one form to another where the amount of carbon intake is equivalent to carbon retained as product (i.e. protein and fat), carbon excreted in feces and urine, and carbon released as carbon dioxide (CO_2) and CH_4 gas, a byproduct of ruminal fermentation (Capper, 2011; Garnett et al., 2017). Yet despite these gaseous losses, the consumption of photosynthetically derived feed sources of cereal grains, byproducts, and forages by cattle actually transfer carbon from one form to another, allowing cattle to participate in the biogenic carbon cycle as temporary carbon sinks. Additional facets to cattle production, such as plant growth on croplands and grasslands, provide an atmospheric carbon sink and can reduce realized emissions from cattle when included in assessments.

Though CH_4 is a byproduct of the beef industry, cattle production systems have made tremendous efforts towards addressing economical, societal and environmental sustainability (Baber et al., 2019b) by increasing production efficiency. A shift towards greater production efficiency has allowed the U.S. beef cattle industry fewer inputs per kg of beef output. There has been a gradual decrease in the number of cattle used for beef production in the U.S. from 1975 to 2017 (FAOSTAT, 2020). Because greenhouse gas (GHG) emissions are tightly linked to population numbers (Crutzen et al., 1986), it is reasonable to conclude that U.S. cattle CH_4 emissions have decreased over time (Johnson et al., 1993; Johnson and Johnson; 1995). Capper

et al. (2011) reported that in 2007, production of one billion kg of U.S. beef required 70% of cattle, 81% of feedstuffs, and 67% of the land as did production in 1977, which led to a reduction of 16.3% and 17.7% in the carbon footprint and CH_4 production of cattle, respectively. This gradual process of increasing efficiency has in part been made with the application of technology such as ionophores, namely monensin (Johnson and Johnson, 1995; FAO, 2010; Capper, 2011). Monensin is a widely adopted feed additive that has been included in cattle diets for over 50 years. Monensin has earned its place by improving the performance of cattle and has been a key player in reducing CH_4 emissions. Yet, reports of monensin's CH_4 reductions have been variable; therefore, a review, in the form of a meta-analysis, will be helpful in synthesizing past reports to produce a robust statistic.

Ionophore introduction:

Ionophores, or ion carriers, are chemical compounds that can be added to cattle diets. Multiple ionophores are marketed in the U.S. including laidomycin propionate, lasalocid, and monensin, yet monensin is the most commonly used ionophore (Tedeschi et al., 2003). Monensin was discovered in 1967 and was approved by FDA for feedlot cattle and commercially marketed as Rumensin™ (Elanco, Greenfield, IN) in 1975. Since then, monensin has been approved for other classes of cattle including calves, stocker cattle, replacement heifers and mature beef and dairy cows. Monensin spares amino acids by inhibiting proteolytic bacteria, prevents coccidiosis, and enhances production efficiency increasing the propionate to acetate ratio resulting in a decrease in CH_4 production. Both grazing cattle and feedlot cattle benefit from monensin, but variation in the response exists. Grazing cattle exhibit an increase in average daily gain (ADG), whereas feedlot cattle express a decrease in feed to gain (F:G) due to decreased intake with minute changes in ADG (Tedeschi et al., 2003).

Monensin mode of action:

Monensin is a lipophilic ion carrier that is able to cross microbial cell membranes, facilitating the energy exhausting exchange of ions and H^+ across bacterial cell lipid bilayers (Russell and Strobel, 1989). This exchange of molecules consumes microbial energy in the form of ATP. Monensin possesses an affinity for Na^+ ions, whereby Na^+ ions are transported out of the cell, while H^+ are concurrently transported into the cell. Transport of H^+ into the cell negates normal ATPase processes that actively work to maintain cellular homeostasis, optimal pH, and proton gradient by continually pumping H^+ out of the cell. Eventually, monensin activity depletes cellular energy reserves, and the cell can no longer counter monensin's effects.

Bacterial susceptibility to ionophores:

Susceptibility to ionophore action is a function of cell structure. Gram-negative bacteria have cell walls that contain 3 layers comprised of a cytoplasmic membrane, thin peptidoglycan, and outer membrane, whereas gram-positive bacteria only have two layers consisting of a cytoplasmic membrane and thick peptidoglycan. Hence, gram-positive bacteria are more susceptible to ionophore effects since they lack an outer membrane, which serves as a protective barrier. A few gram-negative bacteria, like *Butyvibrio fibrosolvens,* contain a mutation altering their outer membrane making them susceptible to ionophore action (Bergen and Bates, 1983). Yet, they can negate the negative effects of an ionophore, since most gram-negative bacteria have the capacity for electron-transport-dependent phosphorylation (Kroger, 1977), where ATP is generated.

Monensin alteration of ruminal fermentation:

Knowing bacterial susceptibility to monensin allows us to anticipate changes in ruminal fermentation products when feeding monensin. A monensin-induced decrease in gram-positive

bacteria stimulates an increase in gram-negative bacteria, like cellulolytic *Fibrobacter succinogens* and *Butyvibrio fibrosolvens* and amylolytic *Selenomas ruminatium*, and their resulting fermentation products. This mechanism of selection for *S. ruminatium* is hypothesized to be one of the major leverage points for an increase in total propionate production (Chen and Wolin, 1979). Likewise, gram-negative bacteria increase hydrogen sink availability by increasing succinate and propionate production (Bergen and Bates, 1983). Cellulolytic gram-positive bacteria, namely *Ruminococcus albus* and *Ruminococcus flavefaciens*, are the primary producers of acetate, formate, H_2, and CO_2. Therefore, when the growth of these gram-positive bacteria is inhibited by monensin (Chen and Wolin, 1979), there is a decrease in acetate:propionate (A:P) ratio. Monensin also prompts a decrease in gram-negative intracellular pH from an influx of H^+ (Russell and Strobel, 1989), which increases proton motive force and electron transport, stimulating succinate production by the fumarate-reductase system (Bergen and Bates, 1984).

Monensin effects on methanogenesis:

It is generally accepted that monensin does not directly act on methanogens; instead, monensin decreases the supply of substrates used by methanogens for CH_4 production (Russell and Strobel, 1989). Monensin-induced CH_4 reductions are variable, ranging from 4-26% (Benz and Johnson, 1982; Wedegaertner and Johnson, 1983; Vyas et al., 2018). Some reports suggest this range in CH_4 reduction is related to the duration of feeding monensin, but a past meta-analysis did not draw this conclusion (Appuhamy et al., 2013). Guan et al. (2006) reported that monensin supplemented cattle on high concentrate and high forage diets returned to original CH_4 levels by week 3 and 6 of feeding. However, regardless of this, Goodrich et al. (1984) reported

that monensin continued to depress intake of high concentrate diets and enhance ADG on high forage diets by 6.4% and 13.5%, respectively, which increases efficiency of the animal overall.

Monensin summary

Monensin has the capacity to alter many production parameters like ADG and DMI, but also positively influence's the environmental contribution from beef cattle by altering ruminal fermentation, reducing CH_4 emissions. Monensin possesses an affinity for Na^+/H^+ exchange within gram-positive (and some gram-negative) bacteria that aids in reducing substrate available for methanogenesis. As a result, CH_4 emissions are reduced, leaving more energy available to the animal for growth.

Overall summary

Consumer-based industries world-wide strive for a common goal, and that is to meet the needs of consumers by producing and delivering wholesome products in the most efficient manner while generating a profit. This remains true for the beef cattle industry, yet the goal is expanded to not only provide the growing population with wholesome, high-quality beef that cattle provide, but also to be stewards of these animals, including the land and environmental consequences it takes to continue their production. The beef cattle industry's stake in food production can be supported and promoted by reinforcing the source of food consumed by cattle, how it is utilized as becoming a consumer product or when expelled back to the environment as a gas, and how producers aid in making this process more efficient by technological application, like monensin.

CHAPTER II

THE ROLE OF CATTLE IN THE CARBON CYCLE

Ruminant CH_4 production introduction

Enteric CH_4 production from cattle is mainly subject to the type of carbohydrate consumed and intake. Carbohydrate source and intake affect ruminal bacteria populations and their products, VFA concentrations, pH, amount of CH_4 production, and overall animal performance. The role of cattle in the carbon cycle is to aid carbon movement by recycling energy from feedstuffs into consumable product, or CH_4 that is transformed within the atmosphere and reabsorbed as CO_2 by plants for growth. Both forage and concentrate feedstuffs are products of recent carbon storage by plants growing on grasslands and/or croplands. Enteric CH_4 emissions from cattle do not positively contribute to the earth's atmospheric burden as being part of the short-term carbon cycle. This concept is crucial in addressing emission source whose emissions either contribute to the atmospheric GHG concentration or not.

Ruminal fermentative processes

Ruminal fermentative processes occur in a highly reductive environment, giving rise to several product pathways that accept and continually transfer H^+ and electrons to final electron acceptors (Russell and Strobel, 1989). Glucose in the rumen can undergo glycolysis, which yields pyruvate that is further reduced to form ATP used for work. To drive the continuation of fermentative processes after pyruvate is produced from glycolysis, anaerobic bacteria must oxidize NADH back to NAD^+, whereby significant amounts of H^+ are produced. Electrons and H^+ are shuttled along complex ruminal reductive pathways, producing intermediate products like

lactate, succinate, and formate, reactants required for VFA and CH_4 production (Hungate et al., 1970; Wolin, 1981).

Ruminal CH_4 production

Rumen microbial communities consist of cellulolytic and amylolytic gram-positive and gram-negative bacteria, protozoa, fungi, and archaea, which include the methanogens, strict anaerobic archaea responsible for CH_4 formation (Cicerone and Oremland, 1988). Fermentation is affected by chemical and physical properties of cattle diets that dictate the final substrates available for methanogenesis and the methanogen population (Cicerone and Oremland, 1988); hence, ruminal bacteria support methanogen activity by supplying primary products needed for methanogenesis. Whether amylolytic or cellulolytic, ruminal bacteria are highly substrate specific (Morgavi et al., 2010). Concentrate-rich diets favor starch fermenting bacteria like gram-positive *Streptococcus bovis* and gram-negative *Selenomas ruminatium*, whereas fiber-based diets favor gram-negative *Fibrobacter succinogens*, and gram-positive *Ruminococcus albus* and *Ruminococcus flavefaciens* (Morgavi et al., 2010). Of the three major cellulolytic bacteria, *Ruminococcus albus* and *Ruminococcus flavefaciens* produce H_2, while *Fibrobacter succinogens* does not (Morgavi et al., 2010).

Methanogens are end users of fermentative products whose central role is removal of excess H_2 from oxidative processes, producing ATP that promotes cell growth via utilization of H_2 (the main electron donor) and reduction of CO_2 (the main electron acceptor) to CH_4 (Wolin, 1980; Morgavi et al., 2010) Other substrates for CH_4 production like formate, and less significant compounds comprising a methyl group such as methanol and *N*-methylated amines and acetate exist, but CO_2 is more available (Hungate et al., 1970; Patterson and Hespell, 1979; Cicerone and Oremland, 1988; Morgavi et al., 2010). Formate itself generates about 18% of the electrons used

for methanogenesis, as reported by Hungate et al. (1970). Growth of each of these electron acceptors is specific to the methanogen. *Methanobacterium formicum, Methanobrevibacter ruminatium,* and *Methanomicrobium mobile* use H_2, CO_2, and formate, whereas *Methanosarcina barkeri* uses H_2, CO_2, methanol, methyl amines, and acetate as its substrate for CH_4 production. Overall, methanogenesis is an imperative process, as it allows fermentation to occur that would otherwise be impaired by a buildup of H^+ from the oxidation of NADH to NAD^+ (Wolin, 1981; Morgavi et al., 2010). Other hydrogen sinks exist such as acetogenesis, biohydrogenation of unsaturated fatty acids (UFA), and propionate formation, yet these alternate processes consume smaller amounts of H_2 (Wolin, 1981; Morgavi et al., 2010).

Diet effects on ruminal VFAs, pH, and CH_4 production:

Ruminal microbes form acetate, propionate, and butyrate, the three primary VFAs, at different levels in response to the diet and the fermentation environment. Forage diets, composed of structural carbohydrate (cell-wall), tend to have a greater A:P ratio compared to grain diets, composed primarily of non-structural carbohydrates (less cell wall) and commonly accompanied by lipid additions. Penner et al. (2009) reported A:P ratios of 3.98 vs. 2.36 when comparing an 8% to 64% concentrate diet on a dry matter basis. These VFA ratios are closely tied to CH_4 emissions, as the acetate production pathway produces H_2, a precursor of CH_4, while propionate formation serves as a hydrogen sink reducing potential CH_4 production (Johnson and Johnson, 1995; Morgavi et al., 2010). Forage versus concentrate diets also affect ruminal pH, which affects fermentation and CH_4 production (Beauchemin and McGinn, 2006). High-forage diets stimulate increased rumination, saliva, and buffer production creating a higher ruminal pH and more favorable environment for cellulolytic microbial activity and H_2 production for potential CH_4 formation, than high-concentrate diets (Penner et al., 2009). Conversely, high-concentrate

diets fed at high levels can stimulate lower ruminal pH, creating a less favorable environment for microbial activity and production of precursors for methanogenesis (Doreau et al., 2011). Penner et al. (2009) reported average ruminal pH of 6.48 vs. 6.03 when fed an 8% vs. 64% concentrate diet, respectively.

Diet effects on animal performance and CH_4 production

Animal performance is closely linked to diet and substrate effects; when CH_4 production on a given diet is reduced, more energy is available for growth. The plane of nutrition for cattle can vary considering stage of production, type of carbohydrate, level of dietary fat, feedstuff processing, digestibility, and interactions between level of intake and digestibility (Blaxter and Clapperton, 1965; Johnson and Johnson, 1995; Hales and Cole, 2017). Blaxter and Clapperton (1965) reported that feeding high levels of high-quality diets resulted in a greater decrease of CH_4 production when compared to feeding high levels of low-quality diets on an intake basis. Conventional cow-calf and stocker production rely heavily on forage-based inputs. Conversely, feedlot systems are often characterized by feeding high levels of high-quality diets, rich in energy and protein, with the addition of fat, grain processing, and technology application (Johnson and Johnson, 1995) which all play a role in increasing animal efficiency by reducing days to finish and CH_4 losses as compared to other sectors of beef production (Pelletier et al., 2010). Beauchemin et al. (2010) and de Vries and de Boer (2010) reported that the cow-calf sector is responsible for 84% of GHG, attributing nearly 16% to stocker and feedlot sectors (Table A1).

Byproducts from crop production, such as straw, are generally lower quality and contain more fiber than the grain they originated from; therefore, it would be anticipated that CH_4 production from crop production byproducts would increase. Approximately two-thirds of a

starch source is starch, with one-third remaining as a feed source. Therefore, after the ethanol production process converts the original grain's starch to ethanol, the remaining byproduct is 3x more concentrated in fat, fiber, and protein (FAO, 2014). Hales et al. (2013) demonstrated this increase in CH_4 production from additional fiber as wet distillers grains plus solubles (WDGS) inclusion increased from 0 to 45% in a steam-flaked corn-based diet. Byproducts from biodiesel production, such as glycerol, a glucose precursor, stimulate production of propionate thereby decreasing available substrates for CH_4 production (FAO, 2014).

It is typical for feedlot rations to have dietary fat added to increase energy intake. Addition of fat to the diet provides slight hydrogen sinks through biohydrogenation of UFA to saturated fatty acids (SFA), enhanced propionate production, and protection of feed particles from enzymatic attack (Johnson and Johnson, 1995). Beauchemin et al. (2007, 2008) and Martin et al. (2010) reported CH_4 reductions between 3.8 to 5.6% for every 1% increase in added fat.

Feedstuff processing to increase microbial access to feedstuffs allows for greater nutrient absorption by the animal while affecting CH_4 production. Hales et al. (2012) reported that steam-flaking corn increased starch availability and apparent digestibility, whereas dry-rolling corn increased apparent neutral detergent fiber (NDF) digestibility. Dry-rolled corn-based diets had greater CH_4 production (L/steer, L/kg DMI, % GE and DE) than steam-flaked corn-based diets. Digestibility is a good predictor of CH_4 emissions but is also a function of the type of carbohydrate. Generally, CH_4 production is stimulated when microbes have more access to easily digestible feeds. Yet, for structural carbohydrates that are less digestible, their chemical components produce a greater A:P ratio. Doreau et al. (2011) reported that, although not statistically significant, enteric CH_4 emissions were lowest for corn grain diets when compared to hay and corn silage.

Manure CH_4 emissions, though less than enteric CH_4, are also a function of digestibility. As digestibility of a feed increases, the absorption of nutrients by the animal increases, leaving less carbon available for excretion. Garnett et al. (2017) found that manure CH_4 emissions were highest for hay diets and lowest for corn diets, with corn silage ranking between the two.

Conversely, interactions exist between level of intake and rate of digestion. As level of intake increases, substrate rate of passage increases and extent of digestion decreases, allowing a subsequent reduction in CH_4 production per unit of intake (Blaxter and Clapperton, 1965; Benchaar et al., 2001). Blaxter and Clapperton (1965) reported that when fed at maintenance, as diet digestibility increased from 65 to 90%, CH_4 production (kcal/100 kcal of feed) increased from 7.5 to 9%. Yet, when fed at 2x and 3x maintenance, CH_4 yields (kcal/100 kcal of feed) were either independent of intake level or decreased from 6 to 5%, respectively. Others like Beauchemin and McGinn (2006) and Johnson et al. (1993) reported that CH_4 production (% GEI) decreased by 0.773% and 1.8%, respectively, for every level of intake above maintenance. Overall, feedstuff source and level of intake are the two biggest factors dictating CH_4 production; as total intake increases, the absolute amount of CH_4 increases, yet CH_4 production per intake (kcal/100 kcal of feed) decreases (Blaxter and Clapperton, 1965).

Impact of CH_4 emission source:

Though most CH_4 undergoes oxidation to CO_2 by OH^- radicals in the atmosphere, natural and anthropogenic CH_4 emissions differ depending on the age of the source, originating as either "dead carbon methane", "modern biogenic methane" sometimes referred to as contemporary CH_4, or "old biogenic methane". Source magnitude is a function of whether the respective emission positively contributes to radiative forcing or not. Cicerone and Oremland (1988) define dead carbon CH_4 (age millions of years) as CH_4 having no radiocarbon content, while sources

containing radiocarbon content are classified as “modern biogenic” (high radiocarbon content, age 0-200 years, considered part of the short-term carbon cycle) or “old biogenic” (low radiocarbon, age 201-50,000 years; Prentice et al., 2001). Dead carbon is responsible for roughly 10-25% emissions generated from fossil CH_4 geologically and from fossil fuel extraction and use, while the remainder of CH_4 emissions are from biogenic sources derived from wetlands, enteric fermentation from domestic and wild ruminants, human and agriculture waste, landfills, termites, plant biomass burning, and oceans (Cicerone and Oremland, 1988; Wuebbles and Hayhoe, 2002; Ciais et al., 2013; Garnett et al., 2017). Modern biogenic CH_4 is part of the short-term carbon cycle, so emissions from beef cattle production are neutral, or negative, and do not positively contribute the total CO_2 burden (Prentice et al., 2001; Garnett et al., 2017). This is because carbon is continually cycled between the atmosphere, plants, and cattle. Cattle cycle the recently absorbed carbon in the plant as CO_2 through respiration, and as CH_4 from enteric fermentation and manure. Opposite of biogenic CH_4, dead carbon CH_4 from fossil fuels does positively contribute to the total CO_2 burden, because it has been stored for millions of years and is not a recent component of the carbon cycle (Cicerone and Oremland, 1988; Wuebbles and Hayhoe, 2002; Boucher et al., 2009). Boucher et al. (2009) demonstrated this emission source concept by positive indirect CO_2 effects from fossil fuel sources compared to anthropogenic biogenic sources whose indirect CO_2 effects were neutral or negative; therefore, it is proven that due to consumption of recently sequestered carbon, emissions from cattle may actually remove or precisely transfer atmospheric CO_2 (Boucher et al., 2009; Soussana et al., 2010). A carbon cycle without fossil fuels would likely reduce CH_4’s effect on trapping heat in the earth’s atmosphere, because no new CH_4 would be responsible for accentuating CO_2’s atmospheric burden.

Impacts of wild versus domesticated ruminants

Methane production from ruminants is not a new phenomenon, as wild ruminants existed before cattle were domesticated (Crutzen et al., 1986; Garnett et al., 2017). Khalil and Rasmussen (1987) reported that CH_4 concentrations have significantly increased in only the last 100 to 200 years, after being constant for nearly 20,000 years, suggesting that ruminant fermentation, whether from ancient wild animals or today's domestic animals, are not the main causes of GHG increases. If domesticated beef cattle production were eradicated, CH_4 production from not only fossil fuels, but also from wild ruminants and termites, would still exist, and emissions could potentially be greater due to the incidence of wildfire from litter accumulation (Manzano and White, 2019). At equal intakes, wild ruminants, such as buffalo, could potentially contribute greater CH_4 production per day due to their reduction in efficiency (Manzano and White, 2019). Crutzen et al. (1986) reported CH_4 production yields at 9% for buffalo, compared to domesticated feedlot cattle and pasture cattle who have CH_4 yields of 1.9 to 2.2% up to 7.7 to 8.4%, respectively. Yet buffalo are predicted to have fewer total emissions than domestic cattle because of a smaller population and being fed at maintenance (Crutzen et al., 1986).

In the U.S. prior to the 15th century, the majority of CH_4 emissions were from bison who comprised nearly 50% of wild ruminant population (Hristov, 2012). Based on DMI, total wild ruminant (i.e. bison, elk, white-tailed and mule deer) emissions were estimated at 3.51 to 7.91 Tg CH_4/yr (or 98 to 221 Tg CO_2e; Hristov, 2012). Current domestic non-dairy cattle enteric CH_4 emissions of nearly 125 Tg CO_2e fall within the lower end of this range (FAOSTAT, 2020). Despite old biogenic CH_4 from ancient wetlands and hydrates, if the population of beef cattle

stays constant or continues to decrease (FAOSTAT, 2020), their CH_4 emissions will continue to cycle at consistent or decreasing levels.

Carbon movement introduction

Carbon movement throughout beef cattle production is continuous because of carbon cycling between emissions, sequestration in soils, storage among crops and grasses, and animals (Garnett et al., 2017). Carbon consumed by cattle comes from plants in the forms of forage, high-energy crop products, and byproducts, and is emitted in two forms as CO_2 via respiration and CH_4 via fermentation in the rumen and large intestine. Nearly 65-75% of consumed carbon is respired into the atmosphere as CO_2 shortly after ingestion, with the remaining carbon retained as product, excreted in the feces (25-40%), or eructated as CH_4 (5-10%) (Wolin, 1981; Soussana et al., 2004). Source of carbon emission is important due to lifetime, especially when comparing CH_4 that has a substantially shorter lifetime of nearly 9-12.4 years to CO_2 with a lifespan of thousands of years, as discussed by Myhre et al. (2013a,b). Yet, because cattle consume carbon previously sequestered by plant matter, emission counterparts do not contribute to atmospheric CO_2 burden, as opposed to fossil fuel emissions. Beef cattle production can, however, contribute to the atmospheric burden if transportation of products and equipment used to harvest and process feed sources into more readily available forms like steam-flaking are considered, as demonstrated in a life cycle assessment by Doreau et al. (2011). Therefore, for beef cattle systems to be carbon neutral, sequestration may need to be explored.

Carbon sequestration and storage

Cropland and grazing lands enhance the environment through soil carbon sequestration and preservation of soil organic matter (SOM). Carbon sequestration is the transfer of atmospheric CO_2 into stable, undisturbed reservoirs that increase soil organic carbon pools (Lal,

2004). Nearly 58% of soil organic dry matter is made up of carbon (Prentice et al., 2001). Carbon storage occurs by the process of photosynthesis as plants use stomata to consume CO_2 from the atmosphere where it is then converted to glucose for plant growth (Waring et al., 1998; Prentice et al., 2001). Tracer studies were used to analyze carbon transfer from plant shoot to root and revealed that about 50% of aboveground carbon is transferred belowground with half of the carbon remaining in the roots, nearly one-third lost to soil respiration, and over 15% deposited as soil organic matter (Smith et al., 2008). Carbon belowground is sequestered for long periods of time in root systems and eventually becomes part of the soil as stable carbon via root turnover and rhizodeposition (Nguyen, 2009). Rhizodeposition is the direct transfer of organic carbon from roots to soil, which provides the largest carbon input to the soil due to root's composition that are resistant to soil degradation (Soussana et al., 2004; Smith et al., 2008; Garnett et al., 2017). Rhizodeposition, therefore, may explain why less disturbed grasslands are richer in soil organic carbon than croplands (Jones and Donnelly, 2004; Soussana et al., 2010). Improved management practices such as decreased tillage, irrigation, sowing of perennial vs. annual crops for extended rotation periods, planting crops that generate increased yields with less reliance on nitrogen fertilization, and change of land use from cropland to grassland help to increase soil carbon stocks (Soussana et al., 2004). Carbon sequestration rates rise until equilibrium is reached over a length of decades, and this is dependent on plant species and productivity, soil type and nutrient availability, land and grazing management practices, previous land use, climate and season changes, length of growing season, and precipitation, where eventually new equilibrium levels are established (Lemaire and Chapman, 1996; Soussana et al., 2004). Though land management practices tend to be consistent in the U.S. leading to soil carbon

levels at or near equilibrium (Rotz et al., 2019), rapid plant productivity aboveground, in crops and grazing lands, can aid in short-term carbon storage by drawing down large amounts of CO_2.

Carbon sequestration and storage in croplands

Cropland production is a key component of livestock systems, serving an integral role in feed production. Cropland carbon is subject to multiple fates: 1) released back into the atmosphere, 2) consumed by humans and animals, or 3) integrated into stable SOM. Tillage practices contribute to oxidation of soil organic matter and CO_2 emissions, yet implementation of soil management using conservation tillage, improved crop varieties, and improved crop nutrition and biomass increase terrestrial carbon sinks (Bruce et al., 1999; Johnson et al., 2005).

Plant biomass yield can be even further enhanced under increased levels of atmospheric CO_2, triggering what is known as "CO_2 fertilization" (Prentice et al., 2001; Keenan et al., 2016). Rubisco, the carbon-fixing enzyme, is altered by CO_2 fertilization stimulating photosynthesis by increasing the reaction rate with CO_2 and decreasing the rate of oxygenation. CO_2 fertilization causes direct climate feedback on plant growth and crop yields, by extending growing season, increasing leaf area and decomposition of plant litter, and indirectly increasing water and transpiration efficiency through stomata pore reduction (Prentice et al., 2001; Keenan et al., 2016). Carbon dioxide fertilization is mildly expressed in C_4 plants and is especially expressed in C_3 plants used for agricultural crop production (Koch and Mooney, 1996; Mooney et al., 1999). Koch and Mooney (1996) found that when CO_2 levels were doubled, net primary productivity (NPP) of C_3 plants increased by 33%. Plant response to increased atmospheric CO_2 levels are highest initially and remain, although at a decreasing rate, as long as plant productivity outweighs stress of water limitation and high temperature.

USDA-ERS (2020) reported that corn, sorghum, barley, and oats are the major crops of feed grain production in the United States, with corn comprising more than 95% of total feed grain production and use. USDA-NASS (2020) stated that in 2019, corn crops produced 168.0 bushels per acre on a total of 81.5 million grain-harvested acres. Assuming that one bushel of corn is equivalent to 25.4 kg, this is equivalent to 347,776,800,000 kg of harvested corn grain in 2019. Latshaw and Miller (1924) further estimated that carbon comprises 44.72% of corn grain. Applying 44.72% carbon to a total yield of 347,776,800,000 kg of harvested corn grain, this is equivalent to 155,525,784,960 kg of total carbon predicted to be harvested in corn grain in 2019. Therefore, estimates of potential carbon storage in U.S. corn grain production alone in 2019 can be found by multiplying 155,525,784,960 kg of total carbon in the grain by 3.67 (ratio of molecular weight of CO_2 to C) for a total of 570,779,630,803 kg of CO_2 drawn down from the atmosphere. This is equivalent to 453% of the reported 126 Tg CO_2e enteric CH_4 emissions by non-dairy cattle in the United States in 2017 (FAOSTAT, 2020).

In addition to carbon storage in crop grains, carbon can be stored for longer periods of time in crop residue, roots and sequestered in soil, if undisturbed. Bruce et al. (1999) estimated that well-managed croplands have the potential to sequester 0.2 to 0.4 tonnes of C/ha/yr in the soil. This estimate falls within the range of 10 to 33 percent (proportion of net fixed carbon exudated by roots) of the net carbon fixed by corn grain calculated above (Beauchamp and Voroney, 1994). Long-term sequestration is dependent on crop yield, harvest index (HI), and deposition of manure (Beauchamp and Voroney, 1994). Crop residue can be predicted using a crop's HI that is the ratio of grain production to total plant yield, excluding the roots (Beauchamp and Voroney, 1994). Beauchamp and Voroney (1994) reported that roots, and carbon excreted belowground contribute to 20% of corn, soybean, and other cereal's total carbon

content. Using estimates of 80% for carbon storage in above-ground biomass, and an HI of 0.50, nearly 40% of above-ground corn crop carbon is carbon residue, with potential of enhancing SOM (Beauchamp and Voroney, 1994). Bolinder et al. (1999) suggested that 12.2 and 21.1 % of corn crop residue carbon from shoots and roots can be integrated into SOM, with some estimates even higher in humid areas due to enhanced root turnover (Beauchamp and Voroney, 1994). Additionally, direct manure deposition can enhance SOM. Manure deposition varies by animal and type of intake. Intake that is high in digestibility will return less carbon to the soil as manure. Assuming a corn grain total digestible nutrient (TDN) value of 90%, an 80% biomass, and an HI of 0.50, nearly 4% of ingested C would be returned to the soil (Beauchamp and Voroney, 1994).

Carbon sequestration and storage in grasslands

Grasslands (characterized as being dominated by grasses and forbes, NRCS, 1997) have the potential to store carbon short-term with proper grazing management, and have greater capacity than croplands at storing carbon for longer bouts of time due to increased root turnover, protection, and stabilization by reduced incidence of tillage and erosion (Soussana et al., 2004). Soil carbon concentrations are generally greatest in clayey and moist soils (Lal, 2004), and in the uppermost 30 cm of soils (80-90%; Soussana et al., 2004) where grazing management is likely to be most influential. Likewise, absolute carbon concentrations are greater at deeper depths (Garnett et al., 2017) due to lower rates of disturbance. Beauchamp and Voroney (1994) reported alfalfa contribution to soil carbon over 3 years. Year 1 generated 10,000 kg of alfalfa per ha that decreased to 7,000 kg of alfala per ha by year 3, but soil carbon yielded from the alfalfa crop increased from 71 to 80%, explaining the effects of rhizodeposition and root turnover with time. Because long-term carbon sequestration in established grasslands, soil organic levels in

established grasslands may be near equilibrium (Johnson et al., 2005); therefore, lower sequestration rates may be seen in these areas (Garnett et al., 2017).

Grazing benefits to carbon sequestration

Intensive grazing management using proper stocking density on pastureland and rangeland also has the potential to enhance short-term storage and potential soil carbon sequestration. Cattle aid in the balance between plant growth and harvesting, stimulating continual plant growth by drawdown of atmospheric CO_2 (NRCS, 1995; FAO, 2010). Grassland soil carbon sequestration has been proposed at 0.12 tonnes of C/ha/yr for improved pastures, and 0.40 tonnes of C/ha/yr for unmanaged pastures used for intensive grazing (Phetteplace et al., 2001; Pelletier et al., 2010). Others have proposed that intensive management improves soil organic carbon levels by 0.20-0.35 tonnes of C/ha/yr to a high range of 1-3 tonnes of C/ha/yr (Bruce et al., 1999; Smith et al., 2008; FAO, 2010; Garnett et al, 2017). Henderson et al. (2015) similarly estimated that North American rangelands and pasturelands can sequester 0.21 tonnes of CO_2/ha and 0.14 tonnes of CO_2/ha, respectively, when improved grazing is established in areas that have positive carbon sequestration capacity. Grazing management allows cattle to directly deposit manure to the ground, where it acts as fertilizer (Capper, 2011). Additionally, it is reported that increasing production inputs through introducing the addition of manure to lands can sequester nearly 0.42 to 0.76 tonnes of C/ha/yr with respect to location (Smith et al., 2008; FAO, 2010).

In summation, carbon sequestration rates are highly debated and lots of variation exists mainly due to differences in measuring method, temperature, precipitation, and past land use, but also due to plant species, soil capacity and available nutrients, and tillage practices (Jones and Donnelly, 2004; Sousanna et al., 2010; Picasso et al., 2014). Feed production and harvest

processes have the potential to contribute to GHG emissions, yet grasslands and croplands that have positive sequestration capacity can serve as carbon sinks achieving carbon neutralization (Picasso et al., 2014). Cattle, crops, and grasslands have a symbiotic relationship in that consumption and growth of these feedstuffs contributes positively to the growth of cattle and carbon sequestration. Doreau et al. (2011) showed that although hay diets stimulated CH_4 production from cattle, adjustments for carbon sequestration potential of grasslands decreased the overall contribution of hay diets to GHG emissions. Likewise, Pelletier et al. (2010) estimated that pasture-finished and feedlot finished beef could reduce their total emissions by 8.2 and 1.8 kg per kg live weight if grassland soil carbon sequestration is accounted for.

CHAPTER III

MONENSIN: A META-ANALYSIS

Synopsis

Methane production is an energetic cost to beef cattle; therefore, reducing CH_4 losses reduces the loss of energy to cattle. Producers may choose to incorporate feed additives, such as monensin, into their managerial regimen to reduce losses of CH_4, improve performance, and increase profitability. Addition of monensin to beef cattle rations produces variable reductions in CH_4 production, which may be due to differences in DMI, diet composition, dose, duration of feeding and delivery of monensin, and method of measurement. To test the absolute effectiveness of monensin at reducing CH_4 production, a meta-analysis was performed comparing cattle fed monensin and control cattle. A random effects analysis was used to establish the effect size of monensin's CH_4 reduction. The random effects meta-analysis demonstrated that monensin significantly ($P < 0.05$) reduced CH_4 emissions by 14.39 g CH_4/d, 6.05 g CH_4/Mcal DE/kg, and 1.99 g CH_4/kg DMI. To account for differences in projects, a mixed effects model was used to assess heterogeneity. The full model included predictors monensin dose (mg/d), level of dietary fat (% diet), dietary NDF (% diet), and control DMI (kg/d). Predictors were eliminated from the full model if they were insignificant ($P > 0.05$). Between study heterogeneity was not present for g CH_4/kg DMI analysis ($P = 0.07$) but was present for g CH_4/d ($P = 0.001$) and g CH_4/Mcal DE/kg analyses ($P = 0.008$). Dietary fat was the only significant predictor explaining 9.24% of the between study variance in the g CH_4/d analysis, compared to both fat and NDF that significantly affected monensin-induced CH_4 reduction per Mcal DE/kg, responsible for 100% of between study variance. Further research is

warranted to address more potential covariates that may have effects on monensin's efficacy in reducing CH_4 per day.

Introduction

Increased production efficiency benefits producers and consumers by generating greater outputs with fewer inputs and may offer potential long-term benefits to the environment (McGinn et al., 2004). Adoption of production enhancing technology has been an important driver of enhancing production efficiency (Johnson and Johnson, 1995). Due to the high degree of technical competitiveness in commodity industries, efficiency enhancing technologies are rapidly adopted; today, feed additives like ionophores, are nearly ubiquitous among U.S. feedyards.

Monensin is an ionophore that has been included in feedlot diets since its introduction in the 1970s (Russell and Strobel, 1989). Monensin improves production parameters like ADG and DMI to improve F:G (Potter et al., 1976; Benz and Johnson, 1982; O'Kelly and Spiers, 1992;), and has been reported to affect ruminal methanogenesis by altering fermentation patterns, resulting in decreased A:P ratios (Potter et al., 1976; Thornton and Owens, 1981; McGinn et al., 2004; Tomkins et al, 2015). Since acetate contributes to CH_4 production and formation of propionate requires more hydrogen serving as a hydrogen sink, monensin reduces energetic losses of CH_4 to the atmosphere, sparing more energy for gain.

To summarize monensin's overall effect on CH_4 reduction from previously conducted studies, a meta-analysis was performed. Meta-analyses are used to condense multiple independent studies to draw a conclusion regarding a treatment's effect (Normand, 1999). Meta-analytic research can also be used to assess individual study treatments degree of impact, and variability in the studies included (Normand, 1999). This meta-analysis aimed to quantify the

summary effect of monensin-induced CH_4 reduction, assess the variability between studies, and to consolidate and identify some of the strongest effects between monensin and study-specific predictors of ruminal CH_4 production and their contribution to between study variance.

Materials and Methods

Data sources:

Texas A&M University Library databases and Google Scholar were used to collect relevant peer-reviewed journal articles by using a combination of keywords "beef cattle", "methane", "monensin", "rumensin", "*vivo*" and –"*vitro*". Our search was further narrowed by considering only studies where CH_4 production was measured *in-vivo* directly from animal specimens and studies that included a control and treatment group(s), specifically monensin. Multiple combinations of relevant web-based keywords and reviewing citations within accumulated articles resulted in 15 studies collected for analysis. After inspecting each of the 15 studies in-depth, five were discarded due to inaccessible data or data that did not meet our criterion specifically comparing treatment to control groups. Our final meta-analysis included a total of 10 primary studies ranging from 1981-2018 (Table B1). Different treatments within studies were further separated (to increase statistical power of our meta-analysis- Normand, 1999), resulting in a total of 19 experiments.

Data gathering and calculation:

All primary studies reported dietary ingredients fed, yet only some studies included dietary composition, such as gross energy (GE) or digestible energy (DE). Digestible energy was used as it accounts for digestibility of the diet. Since inclusion of dietary information varied across studies, many back calculations were made to determine DE. For primary studies, Thornton and Owens (1981), Benz and Johnson (1982), Wedegaertner and Johnson (1983),

McGinn et al. (2004), and Beauchemin and McGinn (2006), where GE of the diet and dry matter digestibility (DMD) were reported, DE was simply calculated at GE × DMD. Mwenya et al. (2005) ration DE was calculated first by finding the digestibility of the diet by dividing reported digestible energy intake (DEI) by gross energy intake (GEI), which was then applied to the GE of the diet. Ration DE for Hemphill et al. (2018) was calculated by dividing reported DEI by DMI. Three studies, O'Kelly and Spiers (1992, Tomkins et al. (2015), and Vyas et al. (2018), reported the dietary ingredient fed but did not report DE, NDF, or fat; therefore, book values from NASEM (2016), FeedTables (FeedTables, 2021), or Feedipedia (Feedipedia, 2021) were used. If the exact ingredient (i.e. same ingredient and processing method applied) was not available, the most similar ingredient was used. Forage:concentrate (F:C) was determined as a percent of the diet, where grains, byproduct meals, and premixes were considered concentrates, whereas hays, grasses, silages and byproduct roughages (i.e. cottonseed hulls) were considered as forages.

Due to the compilation of multiple independent studies included for this meta-analysis, all 10 primary study's reported continuous data did not reflect common units. Methane data was standardized into three responses g CH_4/d, g CH_4/Mcal DE/kg, and g CH_4/kg DMI. Denominators, Mcal DE/kg and kg DMI, were chosen to account for differences in intake.

For studies reporting CH_4 values as CH_4 kcal/hr the following equations were used:

$$\frac{CH_4\ Mcal}{d} = \frac{CH_4\ kcal}{h} \times \frac{24\ h}{d} \times \frac{Mcal}{1000\ kcal}$$

$$\frac{CH_4\ kg}{d} = \frac{CH_4\ Mcal}{d} \times \frac{4.184\ MJ}{Mcal} \times \frac{CH_4\ kg}{55.55\ MJ}$$

$$\boldsymbol{\frac{CH_4\ g}{Mcal\ DE}} = \frac{CH_4\ kg}{d} \times \frac{d}{Mcal\ DE} \times \frac{1000\ g}{kg}$$

$$\frac{\boldsymbol{CH_4\ g}}{\boldsymbol{DMI\ kg}} = \frac{CH_4\ kg}{d} \times \frac{d}{DMI\ kg} \times \frac{1000\ g}{kg}$$

For studies reporting CH_4 values as % of GE the following equations were used:

$$\frac{CH_4\ Mcal}{d} = \frac{GE\ kcal}{MBS} \times \frac{CH_4\ kcal}{GE\ kcal} \times MBS \times \frac{Mcal}{1000\ kcal}$$

$$\frac{CH_4\ kg}{d} = \frac{CH_4\ Mcal}{d} \times \frac{4.184\ MJ}{Mcal} \times \frac{CH_4\ kg}{55.55\ MJ}$$

$$\frac{\boldsymbol{CH_4\ g}}{\boldsymbol{Mcal\ DE}} = \frac{CH_4\ kg}{d} \times \frac{d}{Mcal\ DE} \times \frac{1000\ g}{kg}$$

$$\frac{\boldsymbol{CH_4\ g}}{\boldsymbol{DMI\ kg}} = \frac{CH_4\ kg}{d} \times \frac{d}{DMI\ kg} \times \frac{1000\ g}{kg}$$

For studies reporting CH_4 values as kcal of CH_4/MBS^{-1}/d^{-1} the following equations were used:

$$\frac{CH_4\ Mcal}{d} = \frac{CH_4\ kcal}{MBS} \times MBS \times \frac{Mcal}{1000\ kcal}$$

$$\frac{CH_4\ kg}{d} = \frac{CH_4\ Mcal}{d} \times \frac{4.184\ MJ}{Mcal} \times \frac{CH_4\ kg}{55.55\ MJ}$$

$$\frac{\boldsymbol{CH_4\ g}}{\boldsymbol{Mcal\ DE}} = \frac{CH_4\ kg}{d} \times \frac{d}{Mcal\ DE} \times \frac{1000\ g}{kg}$$

$$\frac{\boldsymbol{CH_4\ g}}{\boldsymbol{DMI\ kg}} = \frac{CH_4\ kg}{d} \times \frac{d}{DMI\ kg} \times \frac{1000\ g}{kg}$$

For studies reporting CH_4 ml/min, CH_4 l/d, or CH_4 l/kg DMI the following equations were used:

$$\frac{CH_4\ g}{d} = \frac{CH_4\ ml}{min} \times \frac{60\ min}{h} \times \frac{24\ h}{d} \times \frac{16\ g}{1\ mol\ CH_4} \times \frac{1\ mol}{22.4\ l} \times \frac{1\ l}{1000\ ml}$$

$$\frac{CH_4\ g}{d} = \frac{CH_4\ l}{d} \times \frac{16\ g}{1\ mol\ CH_4} \times \frac{1\ mol}{22.4\ l}$$

$$\frac{\boldsymbol{CH_4\ g}}{\boldsymbol{Mcal\ DE}} = \frac{CH_4\ g}{d} \times \frac{d}{Mcal\ DE}$$

$$\frac{\boldsymbol{CH_4\ g}}{\boldsymbol{DMI\ kg}} = \frac{CH_4\ g}{d} \times \frac{d}{DMI\ kg}$$

$$\frac{\boldsymbol{CH_4\ g}}{\boldsymbol{DMI\ kg}} = \frac{CH_4\ l}{kg\ DMI} \times \frac{16\ g}{1\ mol} \times \frac{1\ mol}{22.4\ l}$$

For studies reporting CH_4 values as CH_4 g/hd^{-1}/d^{-1} the following equations were used:

$$\frac{\boldsymbol{CH_4\ g}}{\boldsymbol{Mcal\ DE}} = \frac{CH_4\ g}{d} \times \frac{d}{Mcal\ DE}$$

$$\frac{\boldsymbol{CH_4\ g}}{\boldsymbol{DMI\ kg}} = \frac{CH_4\ g}{d} \times \frac{d}{DMI\ kg}$$

For studies reporting CH_4 values as CH_4 MJ/100 MJ GEI the following equations were used:

$$\frac{CH_4\ Mcal}{d} = \frac{GEI\ MJ}{d} \times \frac{CH_4\ MJ}{100\ MJ} \times \frac{1\ Mcal}{4.184\ MJ}$$

$$\frac{CH_4\ kg}{d} = \frac{CH_4\ Mcal}{d} \times \frac{4.184\ MJ}{Mcal} \times \frac{CH_4\ kg}{55.55\ MJ}$$

$$\frac{\boldsymbol{CH_4\ g}}{\boldsymbol{Mcal\ DE}} = \frac{CH_4\ kg}{d} \times \frac{d}{Mcal\ DE} \times \frac{1000\ g}{kg}$$

$$\frac{\boldsymbol{CH_4\ g}}{\boldsymbol{DMI\ kg}} = \frac{CH_4\ kg}{d} \times \frac{d}{DMI\ kg} \times \frac{1000\ g}{kg}$$

For studies reporting CH_4 values as % of GEI the following equations were used:

$$\frac{CH_4\ Mcal}{d} = \frac{GEI\ Mcal}{d} \times \frac{CH_4\ Mcal}{GEI\ Mcal}$$

$$\frac{CH_4\ kg}{d} = \frac{CH_4\ Mcal}{d} \times \frac{4.184\ MJ}{Mcal} \times \frac{CH_4\ kg}{55.55\ MJ}$$

$$\frac{\boldsymbol{CH_4\ g}}{\boldsymbol{Mcal\ DE}} = \frac{CH_4\ kg}{d} \times \frac{d}{Mcal\ DE} \times \frac{1000\ g}{kg}$$

$$\frac{\boldsymbol{CH_4\ g}}{\boldsymbol{DMI\ kg}} = \frac{CH_4\ kg}{d} \times \frac{d}{DMI\ kg} \times \frac{1000\ g}{kg}$$

Statistical model and analysis:

Comprehensive Meta-Analysis version 3 software (Biostat Inc., Englewood, NJ) was used to perform all meta-analyses. To quantity the effectiveness of monensin for reducing CH_4 emissions, raw mean difference (RMD) was used as the effect size, calculated by the model as the difference between control (CON) and monensin (MON) means. Conversion of all primary studies mean CH_4 values to one meaningful, common unit (as either g CH_4/d, g CH_4/Mcal DE/kg, or g CH_4/kg DMI) made using the RMD applicable. Forest plots and funnel plots were built using the RMD as the effect size to serve as a visual in assessing variation for each individual experiment and summary effect, and publication bias.

Model assumptions and selection:

A random effects model was used as the basic analyses, since the studies included were performed independently and were therefore not assumed to share an identical effect size (Borenstein et al., 2009). The meta-analytic program used method of moments as the computational method to derive mean effect size shown in the forest plots (Fig. B1, B3, and B5). Mean summary effect and corresponding heterogeneity (Table B3) were derived from meta-regression, using restricted maximum likelihood (REML) as the computational method. By performing a random effects analysis, the results can be more generally applied to the common population, beef cattle. It was assumed that monensin's observed effect would be the result of the true effect plus variance from sampling error and between study error, or heterogeneity. Funnel plots can be constructed and assessed for publication bias and heterogeneity using regression tests, or visually where the summary effect is drawn as the middle vertical line, confidence intervals bounded by ±1.96 × SE are drawn by the outer diagonal lines, range of CH_4 reduction

outcomes is reported along the x-axis, and respective SE is reported along the y-axis. Visually assessing the funnel plot, the absence of publication bias would reflect symmetry about the mean effect size. Conversely, the presence of publication bias would be reflected by asymmetry about the mean effect, where either more studies fall to the right or left of the mean effect size. For our purpose funnel plots (Fig. B2, B4, and B6) were constructed for random effects models using RMD and, to eliminate subjectivity, were assessed for publication bias using Duval and Tweedie's *Trim and Fill*.

When heterogeneity was significant, the random effects models weree extended to a mixed effects models using meta-regression. Original predictors hypothesized to affect the response to monensin included monensin dose and type of delivery, duration of monensin feeding, dietary fat level, NDF concentration, F:C, control DMI, and method of CH_4 measurement. After final compilation of all relevant information from each primary study, it was concluded that there were an insufficient number of levels of the two covariates delivery of monensin and method of CH_4 measurement. Additionally, the covariate duration of feeding was also excluded due to incomplete washout periods between treatment groups. Correlation between NDF concentration and F:C eliminated F:C as a possible covariate in the model. Therefore, the full models included monensin dose, dietary level of fat, NDF concentration, and control DMI. Reduced models were developed by removing predictors that were nonsignificant *(*$P > 0.05$). Both our full and final models' parameter estimates (Table B4) were fit using the REML function, due to its unbiased and efficient manner (Viechtbauer et al., 2010). Multicollinearity was assessed using the variance inflation factor (VIF), considered large at values greater than 10 (Craney and Surles, 2002). Substantial VIF values can have adverse effects on predictor coefficients and SE values.

Results

Ten primary studies provided 19 individual experiments (Table B1, B5) whose characteristics were assessed in three meta-analyses. A range of days of adaptation and duration from 2 to 161 days was reported. Three types of monensin delivery were used, with premix as the most common. Two methods of CH_4 measurement were used, with whole animal chamber respiration calorimetry the most utilized. Covariate values are summarized in Table B2. Monensin dose ranged from 60 to 309.2 mg per day, with an average value of 200.44 mg per day. Average ration level of dietary fat and NDF concentration was calculated at 3.14% and 39.59%, respectively. Reported daily consumption of control cattle varied from 4.1 to 10.47 kg per day, with an average of 6.53 kg of feed per day.

Effects of monensin using a random-effect and mixed-effect models:

Visual representation using a forest plot (Fig. B1) reported that 17 experiments favored monensin's effect on CH_4 reduction per day. Publication bias was not present in the g CH_4/d analysis, assessed both visually by symmetric dispersion in the funnel plot (Fig. B2) and statistically by Duval and Tweedie's *Trim and Fill* test, where the summary estimate remained unchanged. Average CH_4 production from control cattle (Table B3) was 128.58 g per day. Monensin significantly ($P < 0.001$) reduced CH_4 production by 14.39 g per day. Heterogeneity, characterized by tau (T^2), for the g CH_4/d analysis was significant ($P = 0.0013$) at 74.91. Ratio of true heterogeneity to observed variation (I^2) was considered close to moderate (defined as 50%) (Borenstein et al., 2009) at 56.60%. Level of dietary fat was the only significant ($P = 0.0499$) predictor included in the final model, explaining 9.24% of the between study variance (T^2 67.98 vs 74.91) in monensin's effect on CH_4 (Table B4).

Forest plot for the g CH_4/Mcal DE/kg analysis (Fig. B3) visually reported that 18 experiments favored application of monensin for reducing CH_4 production. Publication bias was not present in the g CH_4/Mcal DE/kg analysis, assessed both visually by symmetric dispersion in the funnel plot (Fig. B4) and statistically by Duval and Tweedie's *Trim and Fill* test, where the summary estimate remained unchanged. Average CH_4 production (Table B3) of control cattle was 48.43 g/Mcal DE/kg. Monensin significantly ($P < 0.001$) reduced CH_4 production by 6.05 g/Mcal DE/kg. Heterogeneity was significant ($P = 0.0077$) at 7.51 for the g CH_4/Mcal DE/kg analysis, and I^2 was near moderate at 49.61%. Level of dietary fat and NDF concentration significantly ($P = 0.001$ and $P = 0.0041$, respectively) explained 100% of the between study variance (T^2 0 vs 7.51) in monensin's effect on CH_4 (Table B4).

Visual assessment of the forest plot (Fig. B5) for the g CH_4/kg DMI analysis reported that 18 experiments favored monensin's effect on reducing CH_4 production. Publication bias was not present in the g CH_4/kg DMI analysis, assessed both visually by symmetric dispersion in the funnel plot (Fig. B6) and statistically by Duval and Tweedie's *Trim and Fill* test, where the summary estimate remained unchanged. Control cattle produced an average of 20.72 g CH_4/kg DMI (Table B3). Monensin significantly ($P < 0.001$) reduced CH_4 production by 1.99 g/kg DMI. Heterogeneity was not significant ($P = 0.0668$) in the g CH_4/kg DMI analysis, quantified by a T^2 value of 1.09. Ratio of true heterogeneity to observed variance was reported by an I^2 value below moderate at 35.01%.

Discussion

Meta-analyses are powerful tools that combine relevant studies into one summary statistic (Viechtbauer, 2010). This meta-analysis was helpful in assessing the effect of ionophores to provide insight on how monensin can help reduce CH_4 emissions from beef cattle

production. With respect to publication bias, this study aimed to quantify the summary effect of monensin-induced CH_4 reduction and assess the variability between previously conducted studies included here. Meta-regression aimed to consolidate and identify some of the strongest effects between monensin and study-specific predictors of ruminal CH_4 production and their relative contribution to between study variance.

Forest plots (Fig. B1, B3, and B5) below provide a cohesive report of each experiment's RMD, SE, confidence interval, and *P*-value. Following the hypothesis of monensin to reduce CH_4 production per day, per Mcal DE/kg, and per kg DMI, it was expected that although every individual experiment may not favor monensin's effect, most of the experiment's individual RMD and the summary effect size would fall to the left of the middle line in the forest plots. Funnel plots were constructed to assess publication bias, both visually and statistically. Studies that are biased can potentially transpose this bias into the study effect, so it is critical to address the presence of publication bias from studies where the summary effect is drawn (Borenstein et al., 2009). Assessing funnel plots below (Fig. B2, B4, and B6), each analysis looks fair in that most studies tend to be dispersed throughout the funnel plot, yet one greater study fell to the right of the mean effect. To statistically prove whether or not asymmetry was present, Duval and Tweedie's *Trim and Fill* method was used to detect if studies were missing to the left of the mean. Because mean effect sizes for each analysis remained unchanged after looking for missing studies to the left of the mean, it was determined that publication bias was not present in either of the analyses. Extensive review of the web to include all previous studies thought to be relevant to this study's aims may have aided in the insignificance of publication bias.

The hypothesis of the application of monensin to reduce CH_4 production per day was proven as effective through the summary effect. A monensin-induced CH_4 reduction of 11.19%

was reported, from the average CH_4 production per day of control cattle. Monensin-induced CH_4 emission reductions per day is well supported by previous studies where reductions of 1-37% were calculated (Thorton and Owens, 1981; Benz and Johnson, 1982; Wedegaertner and Johnson, 1983; O'Kelly and Spiers, 1992; McGinn et al., 2004; Mwenya et al., 2005; Beauchemin and McGinn, 2006; Tomkins et al., 2015; Vyas et al., 2018). Heterogeneity is the variance in true (real) effects. Simply put, heterogeneity is only due to between study variation (real reasons that these studies may have differed), without regard to within study variation, or sampling error (Borenstein et al., 2009). Heterogeneity was significant in the g CH_4/d analysis; therefore, meta-regression was used to attempt to extrapolate which predictors were likely responsible for causing monensin's effect size to differ between studies, and how much those predictors were able to improve the model by accounting for between study variation. A mixed effects model was used to further investigate significant heterogeneity. Dietary level of fat was the only explanatory variable that influenced monensin's effect. Adding monensin to a diet with an average fat value of 3.14% significantly reduced CH_4 emissions. Every 1% increase in the level of dietary fat, above 3.14%, contributed to monensin's effect by decreasing CH_4 an additional 6.61 g. Fats are added to cattle diets, either as UFA or SFA, to increase energy density. Unsaturated fatty acids undergo biohydrogenation in the rumen, where they are converted to SFA, serving as a hydrogen sink. However, the amount of UFA supplementation determines the rate of ruminal biohydrogenation. The percent of biohydrogenation decreases as the level of UFA intake is increased (Hall and Eastridge, 2014), likely from exceeding the microbe's ability to hydrogenate UFA (McGinn et al., 2004). Beam et al. (2000) reported that for every percentage unit increase in linoleic acid added to the diet, biohydrogenation rate decreased by 1.20% per hour. By increasing the level of UFA fed in the diet, the rate of biohydrogenation

can be reduced, allowing hydrogen to be incorporated into propionate production by gram-negative bacteria. Digestibility of a feed source can be used as a predictor of energy content, and this is reflected in TDN values. As the level of dietary fat content and consequential energy values increase in a diet, TDN is expected to increase. Low-quality forages are characterized by TDN <55, and high-quality forages and concentrates characterized by TDN >55 (NASEM, 2016). Cattle fed diets with high TDN concentrations and diminishing NDF concentrations decrease DMI because of reduced microbial efficiency and satiety effects (Hall and Eastridge, 2014). A decrease in DMI reduces substrate available for fermentation and overall CH_4 production per day. Therefore, diets supplemented with monensin and fat containing a high TDN may decrease daily intake, which would reduce CH_4 production per day. Surprisingly, NDF concentration did not have an effect on monensin's effect on a daily basis. This may have occurred from a reduction in ruminal fiber digestion from increased fat supplementation. McGinn et al. (2004) demonstrated this interaction between dietary fat, NDF, and CH_4 where when fed 400 g/d (~5% added fat) of sunflower oil, ruminal fiber digestion, acetate, and A:P were decreased, while propionate concentration was higher. In the g CH_4/d analysis, 81.76% (R^2 = 0.0924) of between study variance was left unexplained.

Summary effect measure for the g CH_4/Mcal DE/kg analysis demonstrated a monensin-induced CH_4 reduction of 12.49% from the average CH_4 production per Mcal DE/kg of control cattle. Monensin-induced CH_4 emission reductions per Mcal DE/kg is well supported by previous studies where reductions of 1-37% have been calculated (Thorton and Owens, 1981; Benz and Johnson, 1982; Wedegaertner and Johnson, 1983; O'Kelly and Spiers, 1992; McGinn et al., 2004; Mwenya et al., 2005; Beauchemin and McGinn, 2006; Tomkins et al., 2015; Vyas et al., 2018). Heterogeneity was significant in the g CH_4/Mcal DE/kg analysis; therefore, meta-

regression using a mixed effects model was used. Full model predictors differed between the g CH_4/d and g CH_4/Mcal DE/kg analyses by exclusion of control DMI for g CH_4/Mcal DE/kg analysis since energy per kg of intake is a function of total intake. Study diets were comprised of an array of diet ingredients, contributing to large differences in dietary energy. Primary study diet compositions ranged from 1.84 to 4.15 Mcal DE/kg, yet in this analysis, ranges in energy were controlled for. When controlling for differences in DE, both NDF concentration and dietary fat level contributed to 100% (R^2 = 1.00) of monensin's effect. Adding monensin to a diet with an average NDF concentration and dietary fat value of 39.59% and 3.14%, respectively, significantly reduced CH_4 emissions. Every 1% increase in NDF concentration and level of dietary fat, contributed to a monensin-induced CH_4 reduction of 0.11 and 3.21 g CH_4/Mcal DE/kg, respectively. Digestible energy is defined by gross energy minus fecal energy and reflects diet digestibility. Interactions exist between CH_4 production and digestibility. Blaxer and Clapperton (1965) highlighted this interaction where, when fed at maintenance, as diet digestibility increased from 65 to 90%, CH_4 emissions increased from 7.5 to 9% of DEI. Therefore, when digestibility is controlled, the affects it has on CH_4 are also controlled for. Forages, and crop residues and byproducts, are characterized by high NDF fractions 35 to 70% (NASEM, 2016). Previous studies by Blaxter and Clapperton (1965), Doreau et al. (2011), Hales et al. (2013), and Garnett et al. (2017) reported that diets containing higher amounts of fiber tend to stimulate CH_4 production. Likewise, when monensin is added to high fiber diets, there are more gram-positive bacteria to act on, leading to a greater reduction in hydrogen for methanogens to use for CH_4 production, over grain diets and corresponding gram-negative bacterial population.

Summary effect measure for the g CH_4/kg DMI analysis demonstrated a monensin-induced CH_4 reduction of 9.61% from the average CH_4 production per kg DMI of control cattle. Monensin-induced CH_4 emission reductions per kg DMI is well supported by previous studies where reductions of 1-30% were calculated (Thorton and Owens, 1981; Benz and Johnson, 1982; Wedegaertner and Johnson, 1983; O'Kelly and Spiers, 1992; McGinn et al., 2004; Mwenya et al., 2005; Beauchemin and McGinn, 2006; Tomkins et al., 2015). Heterogeneity did not exist in the g CH_4/kg DMI analysis; therefore, no further investigation of the source of heterogeneity was warranted. Methane emissions are closely related to the amount of DMI consumed; as total intake increases, the absolute amount of CH_4 increases. O'Kelly and Spiers (1992) reported that when feeding monensin, nearly 55% of the reduction in CH_4 emissions results from an anorectic effect, leaving the remaining effect of monensin's CH_4 reduction to be directly related to changes in fermentation. Therefore, as monensin significantly reduced CH_4 emissions when fixed for DMI, differences between individual study's intakes were eliminated. By controlling the effect of DMI on CH_4 emissions, the margin for CH_4 differences reported by studies was reduced, and homogenous variance was achieved.

CHAPTER IV

SUMMARY

Beef cattle serve an integral role in the biogenic carbon cycle as they aid in carbon cycling while simultaneously providing benefits to humanity through protein production (Capper, 2011). Utilization of beef cattle for protein production allows the consumption of low-quality and high-quality diets that correspond to the most efficient use of marginal land and productive areas of cropland. As the beef cattle industry strives towards improved efficiency and productivity, expected emissions of CO_2 and CH_4 related to crop harvest and feed processing, enteric fermentation, and fossil fuel inputs will decrease. Beef cattle producers are stewards of the land and animal's they produce, and actively work to reduce CH_4 emissions through feeding high-quality diets and applying technology, like monensin. Intensive production systems, like feedlots, reduce the environmental impact of beef cattle while still providing global demands for beef protein, due to lower CH_4 emissions and a reduction in days to finish. Our meta-analysis proved that monensin successfully reduced CH_4 emissions per day, per Mcal DE, and per kg DMI. A reduction in these losses allows more energy to be used by cattle for growth.

REFERENCES

Appuhamy, J. A. D. Ranga Niroshan, A. B. Strathe, S. Jayasundara, C. Wagner-Riddle, J. Dijkstra, J. France, and E. Kebreab. 2013. Anti-methanogenic effects of monensin in dairy and beef cattle: A meta-analysis. J. Dairy Sci. 96:5161-5173. doi:10.3168/jds.2012-5923

Baber, J. R., J. E. Sawyer, and T. A. Wickersham. 2019a. Evaluation of net protein contribution, methane production, and net returns from beef production as duration of confinement increases in the cow-calf sector. J. Anim. Sci. 97:2675–2686. doi:10.1093/jas/skz145

Baber, Jessica R, J. E. Sawyer, B. P. Holland, K. J. Karr, A. B. Word, and T. A. Wickersham. 2019b. Net protein contribution of beef feedlots from 2006 to 2017. Transl. Anim. Sci. 3:1575–1584. doi:10.1093/tas/txz142

Beam. T. A., T. C. Jenkins, P. J. Moate, R. A. Kohn, and D. L. Palmquist. 2000. Effects of amount and source of fat on the rates of lipolysis and biohydrogenation of fatty acids in ruminal contents. J. Dairy Sci. 83:2564-2573.

Beauchamp, E. G. and R. P. Voroney. 1994. Crop carbon contribution to the soil with different cropping livestock systems. J. Soil Water Conserv. 49(2):205-209.

Beauchemin, K. A., and S. M. McGinn. 2006. Enteric methane emissions from growing beef cattle as affected by diet and level of intake. Canadian J. Anim. Sci. 86:401–408.

Beauchemin, K. A., S. M. McGinn, and H. Petit. 2007. Methane abatement strategies for cattle: Lipid supplementation of diets. Canadian J. Anim. Sci. 87:431–440.

Beauchemin, K. A., M. Kreuzer, F. O'Mara, and T. A. McAllister. 2008. Nutritional management for enteric methane abatement: A review. Aust. J. Exp. Agric. 48:21-27.

Beauchemin, K. A., H. Henry Janzen, S. M. Little, T. A. McAllister, and S. M. McGinn. 2010. Life cycle assessment of greenhouse gas emissions from beef production in western Canada: A case study. Agric. Syst. 103:371–379. doi:10.1016/j.agsy.2010.03.008

Benchaar, C., C. Pomar, and J. Chiquette. 2001. Evaluation of dietary strategies to reduce methane production in ruminants: A modelling approach. Canadian J. Anim. Sci. 81:563–574.

Benz, D. A. and D. E. Johnson. 1982. The effect of monensin on energy partitioning by forage fed steers. Proc. West. Sec. Ameri. Soc. Anim. Sci. 33:60-63.

Bergen, W. G. and D. B. Bates. 1984. Ionophores: their effect on production efficiency and mode of action. J. Anim. Sci. 58(6):1465-1483.

Blaxter, K. L., and J. L. Clapperton. 1965. Prediction of the amount of methane produced by ruminants. British J. Nutr. 19:511–522. doi:10.1079/bjn19650046

Bolinder, M. A., D. A. Angers, M. Giroux, and M. R. Laverdiere. 1999. Estimating C inputs retained as soil organic matter from corn (Zea Mays L.). Plant and Soil. 215:85-91.

Borenstein, M., L. V. Hedges, J. P. T. Higgins, and H. R. Rothstein. 2009. Introduction to meta-analysis. John Wiley and Sons, Ltd. Chichester, UK.

Boucher, O., P. Friedlingstein, B. Collins, and K. P. Shine. 2009. The indirect global warming potential and global temperature change potential due to methane oxidation. Environ. Res. Lett. 4:1–5. doi:10.1088/1748-9326/4/4/044007

Bruce, J. P., M. Frome, E. Haites, H. Janzen, R. Lal, and K. Paustian. 1999. Carbon sequestration in soils. J. Soil Water Conserv. 54:382-389.

Capper, J. L. 2011. The environmental impact of beef production in the United States: 1977 compared with 2007. J. Anim. Sci. 89:4249–4261. doi:10.2527/jas.2010-3784

Chen, M. and M. J. Wolin. 1979. Effect of monensin and lasalocid-sodium on the growth of methanogenic and rumen saccharolytic bacteria. Appl. and Environ. Micro. 38(1):72-77.

Ciais, P., C. Sabine, G. Bala, L. Bopp, B. Brovkin, J. Canadell, A. Chhabra, R. DeFries, J. Galloway, M. Heimann, C. Jones, C. le Quere, R.-B. Myneni, S. Piao, and P. Thornton. 2013. Carbon and other biogeochemical cycles. In: T. F. Stocker, D. Qin. G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P. M. Midgley, eds. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, United Kingdom and New York, NY, USA. p. 465-570.

Cicerone, R. J., and R. S. Oremland. 1988. Biogeochemical aspects of atmospheric methane. Global Biogeochem. Cycles. 2:299–327. doi:10.1029/GB002i004p00299

Cooprider, K. L., F. M. Mitloehner, T. R. Famula, E. Kebreab, Y. Zhao, and A. L. van Eenennaam. 2011. Feedlot efficiency implications on greenhouse gas sustainability. J. Anim. Sci. 89:2643–2656. doi:10.2527/jas.2010-3539

Craney, T. A. and J. G. Surles. 2002. Model-dependent variance inflation factor cutoff values. Quality Eng. 14(3):391-403

Crutzen, P. J., I. Aselmann, and W. Seiler. 1986. Methane production by domestic animals, wild ruminants, other herbivorous fauna, and humans. Tellus B Chem. Phys. Meteorol. 38:271–284. doi:10.3402/tellusb.v38i3-4.15135

Doreau, M., H. M. G. van der Werf, D. Micol, H. Dubroeucq, J. Agabriel, Y. Rochette, and C. Martin. 2011. Enteric methane production and greenhouse gases balance of diets differing in concentrate in the fattening phase of a beef production system. J. Anim. Sci. 89:2518–2528. doi:10.2527/jas.2010-3140

FAO. 2010. Challenges and opportunities for carbon sequestration in grassland systems: A technical report on grassland management and climate change mitigation. Integrated Crop Management. 9:1-57. Rome.

FAO. 2014. Biofuel co-products as livestock feed- Opportunities and challenges, Technical Summary. Rome.

FAOSTAT. 2020. Compare Data. http://www.fao.org/faostat/en (Accessed 17 March 2020.)

Feedipedia. 2021. feedipedia.org. (Accessed 8 January 2021.)

FeedTables. 2021. https://feedtables.com/content/table-feed-profile. (Accessed 8 January 2021.)

Garnett, T., C. Godde, A. Müller, E. Röös, P. Smith, I. J. M. de Boer, E. zu Ermagessen, M. Herrero, C. van Middelaar, C. Shader, and H. van Zanten. 2017. Grazed and confused? Ruminating on cattle, grazing systems, methane, nitrous oxide, the soil carbon sequestration question- and what it all means for greenhouse gas emissions. FCRN, University of Oxford

Goodrich, R. D., J. E. Garrett, D. R. Gast, M. A. Kirick, D. A. Larson, and J. C. Meiske. 1984. Influence of monensin on the performance of cattle. J. Anim. Sci. 58:1484–1498.

Guan, H., K. M. Wittenberg, K. H. Ominski, and D. O. Krause. 2006. Efficacy of ionophores in cattle diets for mitigation of enteric methane. J. Anim. Sci. 84:1896-1906. doi:10.2527/jas.2005-652

Hales, K. E., N. A. Cole, and J. C. MacDonald. 2012. Effects of corn processing method and dietary inclusion of wet distillers grains with solubles on energy metabolism, carbon−nitrogen balance, and methane emissions of cattle. J. Anim. Sci. 90:3174–3185. doi:10.2527/jas2011-4441

Hales, K. E., N. A. Cole, and J. C. MacDonald. 2013. Effects of increasing concentrations of wet distillers grains with solubles in steam-falked, corn-based diets on energy metabolism, carbon-nitrogen balance, and methane emissions of cattle. J. Anim. Sci. 91:819-828. doi:10.2527/jas2012-5418

Hales, K. E., and N. A. Cole. 2017. Hourly methane production in finishing steers fed at different levels of dry matter intake. J. Anim. Sci. 95:2089–2096. doi:10.2527/jas2016.1023

Hall, M. B. and M. L. Eastridge. 2014. Invited review: Carbohydrate and fat: Considerations for energy and more. PAS 30:140-149.

Harper, L. A., O. T. Denmead, J. R. Freney, and F. M. Byers. 1999. Direct measurements of methane emissions from grazing and feedlot cattle. J. Anim. Sci. 77:1392–1401.

Hemphill, C. N., T. A. Wickersham, J. E. Sawyer, T. M. Brown-Brandl, H. C. Freetly, and K. E. Hales. 2018. Effects of feeding monensin to bred heifers fed in a drylot on nutrient and energy balance. J. Anim. Sci. 96:1171-1180. doi:10.1093/jas/skx030

Henderson, B. B., P. J. Gerber, T. E. Hilinski, A. Falcucci, D. S. Ojima, M. Salvatore, and R. T. Conant. 2015. Greenhouse gas mitigation potential of the world's grazing lands: Modeling soil carbon and nitrogen fluxes of mitigation practices. Agriculture, Ecosystems and Environment. 207:91–100. doi:10.1016/j.agee.2015.03.029

Hristov, A. N. 2012. Historic, pre-European settlement, and present-day contribution of wild ruminants to enteric methane emissions in the United States. J. Anim. Sci. 90:1371–1375. doi:10.2527/jas.2011-4539

Hungate, R. E., W. Smith, T. Bauchop, I. Yu, and J. C. Rabinowitz. 1970. Formate as an Intermediate in the Bovine Rumen Fermentation. J. Bacteriol. 102:389–397.

Johnson, D. E., T. M. Hill, G. M. Ward, K. A. Johnson, M. E. Branine, B. R. Carmean, and D. W. Lodman. 1993. Ruminants and other animals. In: M. A. K. Khalil, ed. Atmospheric methane, sources, sinks and role in global change. p. 199–229.

Johnson, K. A., and D. E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. 73:2483–2492.

Johnson, J. M. F., D. C. Reicosky, R. R. Allmaras, T. J. Sauer, R. T. Venterea, and C. J. Dell. 2005. Greenhouse gas contributions and mitigation potential of agriculture in the central USA. Soil and Tillage Res. 83(1):73-94. doi.org/10.1016/j.still.2005.02.010

Jones, M. B., and A. Donnelly. 2004. Carbon sequestration in temperate grassland ecosystems and the influence of management, climate and elevated CO2. New Phytologist. 164:423–439. doi:10.1111/j.1469-8137.2004.01201.x

Keenan, T. F., I. C. Prentice, J. G. Canadell, C. A. Williams, H. Wang, M. Raupach, and G. J. Collatz. 2016. Recent pause in the growth rate of atmospheric CO2 due to enhanced terrestrial carbon uptake. Nature Comm. doi:10.1038/ncomms13428

Khalil, M. A. K., and R. A. Rasmussen. 1987. Atmospheric methane: Trends over the last 10,000 years. Atmos. Environ. 21:2445–2452.

Koch, G. W., and H. A. Mooney. 1996. Response of terrestrial ecosystems to elevated CO2: A synthesis and summary. In: G. W. Koch and H. A. Mooney, eds. Carbon Dioxide and Terrestrial Ecosystems. Academic Press, San Diego. p. 415–429.

Kroger, A. 1977. Phosphorylative electron transport with fumarate and nitrate as terminal hydrogen acceptors. Microbial Energetics: 27th Symp. Soc. Gen. Microbiol. London. p. 61-93.

Lal, R. 2004. Carbon sequestration impacts on global climate change and food security. Science. 304:1623-1627.

Latshaw, W. L. and E. C. Miller. 1924. Elemental composition of the corn plant. J. Agric. Res. 27(11):845-861.

Lemaire, G., and D. Chapman. 1996. Tissue flows in grazed plant communities. In: J. Hodgson, ed. The Ecology and Management of Grazing Systems. CAB International, Palmerston North, New Zealand. p. 1–36.

Manzano, P., and S. R. White. 2019. Intensifying pastoralism may not reduce greenhouse gas emissions: Wildlife-dominated landscape scenarios as a baseline in life-cycle analysis. Clim. Res. 77:91–97. doi:10.3354/cr01555

Martin, C., D. P. Morgavi, and M. Doreau. 2010. Methane mitigation in ruminants: From microbe to the farm scale. Animal. 4:351–365. doi:10.1017/S1751731109990620

McAllister, T. A., E. K. Okine, G. W. Mathison, and K.-J. Cheng. 1996. Dietary, environmental, and microbiological aspects of methane production in ruminants. Can. J. Anim. Sci. 76:231-243

McGinn, S. M., K. A. Beauchemin, T. Coates, and D. Colombatto. 2004. Methane emissions from beef cattle: effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. J. Anim. Sci. 82:3346-3356.

Mooney, H. A., J. Canadell, F. S. Chapin III, J. R. Ehleringer, Ch. Korner, R. E. McMurtrie, W. J. Parton, L. F. and Pitelka, and E.-D. Schulze. 1999. Ecosystem physiology responses to global change. In: B. H. Walker, W. L. Steffen, J. Canadell, and J. S. I. Ingram, eds. Implications of Global Change for Natural and Managed Ecosystems. A Synthesis of

GCTW and Related Research. IGBP Book Series No. 4. Cambridge University Press, Cambridge. p. 141–189.

Morgavi, D. P., E. Forano, C. Martin, and C. J. Newbold. 2010. Microbial ecosystem and methanogenesis in ruminants. Animal. 4:1024–1036. doi:10.1017/S1751731110000546

Mwenya, B. C. Sar, B. Santoso, T. Kobayashi, R. Morikawa, K. Takaura, K. Umetsu, S. Kogawa, K. Kimura, H. Mizukoshi, and J. Takahashi. 2005. Anim. Feed Sci. and Tech.118:19-30. doi:10.1016/j.anifeedsci.2004.10.014

Myhre, G., D. Shindell, F.-M. Breon, W. Collins, J. Fuglestvedt, J. Huang, D. Koch, J.-F. Lamarque, D. Lee, B. Mendoza, T. Nakajima, A. Robock, G. Stephens, T. Takemura, and H. Zhang. 2013a. Anthropogenic and natural radiative forcing. In: T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, P. M. Midgley, eds. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. p. 659-740.

Myhre, G., D. Shindell, F.-M. Breon, W. Collins, J. Fuglestvedt, J. Huang, D. Koch, J.-F. Lamarque, D. Lee, B. Mendoza, T. Nakajima, A. Robock, G. Stephens, T. Takemura, and H. Zhang. 2013b. Anthropogenic and natural radiative forcing supplementary material. In: T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, P. M. Midgley, eds. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. p. 8SM1-8SM44.

National Academies of Sciences, Engineering, and Medicine (NASEM). 2016. Nutrient requirements of beef cattle: Eighth revised edition. Washington, DC: The National Academies Press. doi:10.17226/19014

Nguyen, C. 2009. Rhizodeposition of organic C by plant: Mechanisms and controls. In: E. Lichtfouse, M. Navarette, P. Debaeke, S. Veronique, and C. Alberola, eds. Sustainable Agriculture. Springer, Doredrecht. doi:10.1007/978-90-481-2666-8_9 p. 97-123.

Normand, S-L. T. 1999. Tutorial in biostatistics. Meta-analysis: formulating, evaluating, combining, and reporting. Statist. Med. 18: 321-359.

NRCS. 1995. Grazing lands: RCA issue brief #6. nrcs.usda.gov/wps/portal/nrcs/detail/national/technical/nra/rca/?cid=nrcs143_014209 (Accessed 10 March 2020.)

NRCS. 1997. National Range and Pasture Handbook. https://www.nrcs.usda.gov/wps/portal/nrcs/detail/national/landuse/rangepasture/?cid=stelprdb1043084 (Accessed 29 April 2020.)

O'Kelly, J. C. and W. G. Spiers. 1992. Effect of monensin on methane and heat productions of steers fed lucerne hay either *ad libitum* or at the rate of 250 g/hour. Aust. J. Agric. Res. 43:1789-1793.

Patterson, J. A., and R. B. Hespell. 1979. Trimethylamine and methylamine as growth substrates for rumen bacteria and methanosarcina barkeri. Curr. Microbiol. 3:79–83.

Pelletier, N., R. Pirog, and R. Rasmussen. 2010. Comparative life cycle environmental impacts of three beef production strategies in the Upper Midwestern United States. Agric. Syst. 103:380–389. doi:10.1016/j.agsy.2010.03.009

Penner, G. B., M. Taniguchi, L. L. Guan, K. A. Beauchemin, and M. Oba. 2009. Effect of dietary forage to concentrate ratio on volatile fatty acid absorption and the expression of genes related to volatile fatty acid absorption and metabolism in ruminal tissue. J. Dairy Sci. 92(6):2767-2781. doi:10.3168/jds.2008-1716

Phetteplace, H. W., D. E. Johnson, and A. F. Seidl. 2001. Greenhouse gas emissions from simulated beef and dairy livestock systems in the United States. Nutrient Cycling in Agroecosystems. 60:99-102.

Picasso, V. D., P. D. Modernel, G. Becoña, L. Salvo, L. Gutiérrez, and L. Astigarraga. 2014. Sustainability of meat production beyond carbon footprint: A synthesis of case studies from grazing systems in Uruguay. Meat Sci. 98:346–354. doi:10.1016/j.meatsci.2014.07.005

Potter, E. L., C. O. Cooley, L. F. Richardson, A. P. Raun, and R. P. Rathmacher. 1976. Effect of monensin on performance of cattle fed forage. J. Anim. Sci. 43(3):665-669.

Prentice, I. C., G. D. Farquhar, M. J. R. Fasham, M. L. Goulden, M. Heimann, V. J. Jaramillo, H. S. Kheshgi, C. le Quéré, R. J. Scholes, D. W. R. Wallace, Contributing:, D. Archer, M. R. Ashmore, O. Aumont, D. Baker, M. Battle, M. Bender, L. P. Bopp, P. Bousquet, K. Caldeira, P. Ciais, P. M. Cox, W. Cramer, F. Dentener, I. G. Enting, C. B. Field, P. Friedlingstein, E. A. Holland, R. A. Houghton, J. I. House, A. Ishida, A. K. Jain, I. A. Janssens, F. Joos, T. Kaminski, C. D. Keeling, R. F. Keeling, D. W. Kicklighter, K. E. Kohfeld, W. Knorr, R. Law, T. Lenton, K. Lindsay, E. Maier-Reimer, A. C. Manning, R. J. Matear, A. D. Mcguire, J. M. Melillo, R. Meyer, M. Mund, J. C. Orr, S. Piper, K. Plattner, P. J. Rayner, S. Sitch, R. Slater, S. Taguchi, P. P. Tans, H. Q. Tian, M. F. Weirig, T. Whorf, and A. Yool. 2001. Chapter 3: The Carbon Cycle and Atmospheric

Carbon Dioxide. In: L. Pitelka, and A. Ramirez Rojas., eds. Climate Change 2001: The Scientific Basis. Cambridge University Press. p. 183–237.

Rotz, C. A., S. Asem-Hiablie, S. Place, and G. Thoma. 2019. Environmental footprints of beef cattle production in the United States. Agric. Syst. 169:1–13. doi:10.1016/j.agsy.2018.11.005

Russell, J. B., and H. J. Strobel. 1989. Effects of ionophores on ruminal fermentation. Appl. and Envir. Micro. 55(1):1-6.

Smith, P., D. Martino, Z. Cai, D. Gwary, H. Janzen, P. Kumar, B. McCarl, S. Ogle, F. O'Mara, C. Rice, B. Scholes, O. Sirotenko, M. Howden, T. McAllister, G. Pan, V. Romanenkov, U. Schneider, S. Towprayoon, M. Wattenbach, and J. Smith. 2008. Greenhouse gas mitigation in agriculture. Phil. Trans. R. Soc. B. 363:789–813. doi:10.1098/rstb.2007.2184

Soussana, J.-F., P. Loiseau, N. Vuichard, E. Ceschia, J. Balesdent, T. Chevallier, and D. Arrouays. 2004. Carbon cycling and sequestration opportunities in temperate grasslands. Soil Use Manag. 20:219–230. doi:10.1079/sum2003234

Soussana, J. F., T. Tallec, and V. Blanfort. 2010. Mitigating the greenhouse gas balance of ruminant production systems through carbon sequestration in grasslands. Animal. 4:334–350. doi:10.1017/S1751731109990784

Tedeschi, L. O., D. G. Fox, and T. P. Tylutki. 2003. Potential environmental benefits of ionophores in ruminant diets. J. Environ. Qual. 32:1591-1602.

Thornton, J. H. and F. N. Owens. 1981. Monensin supplementation and *in vivo* methane production by steers. J. Anim. Sci. 52(3):628-634.

Tomkins, N. W., S. E. Denman, R. Pilajun, M. Wanapat, C. S. McSweeney, and R. Elliott. 2015. Manipulating rumen fermentation on methanogenesis using an essential oil and monensin in beef cattle fed a tropical grass hay. Anim. Feed Sci. and Tech. 200:25-34. doi:10.1016/j.animfeedsci.2014.11.013

USDA-NASS. 2020. Crop Production 2019 Summary. p. 1-122.

USDA-ERS. 2020. Feedgrains sector at a glance. (https://www.ers.usda.gov/topics/crops/corn-and-other-feedgrains/feedgrains-sector-at-a-glance/ (Accessed 29 April 2020).

Viechtbauer, W. 2010. Conducting meta-analysis in R with the metafor package. J. Stat. Soft. 36(3):1-48. Doi: DOI: 10.18637/jss.v036.i03

de Vries, M., and I. J. M. de Boer. 2010. Comparing environmental impacts for livestock products: A review of life cycle assessments. Lives. Sci. 128:1–11. doi:10.1016/j.livsci.2009.11.007

Vyas, D., A. W. Alemu, S. M. McGinn, S. M. Duval, M. Kindermann, and K. A. Beauchemin. 2018. The combined effects of supplementing monensin and 3-nitrooxypropanol on methane emissions, growth rate, and feed conversion efficiency in beef cattle fed high-forage and high-grain diets. J. Anim. Sci. 96:2923-2938. doi:10.1093/jas.sky174

Waring, R. H., J. J. Landsberg, and M. Williams. 1998. Net primary production of forests: A constant fraction of gross primary production? Tree Phys. 18:129–134. doi:10.1093/treephys/18.2.129

Wedegaertner, T. C. and D. E. Johnson. 1983. Monensin effects on digestibility, methanogenesis and heat increment of a cracked corn-silage diet fed to steers. J. Anim. Sci. 57(1):168-177.

Wolin, M. J. 1981. Fermentation in the rumen and human large intestine. Science. 213:1463–1468. doi:10.1126/science.7280665

Wuebbles, D. and K. Hayhoe. 2002. Atmospheric methane and global change. Earth-Science Reviews. 57:177-210.

APPENDIX A

CHAPTER II TABLES

Table A1. Summary of methane emissions from production systems and finishing scenarios.

Production System	CH_4, g/steer/d	CO_2 eq. kg/kg CW gain	CO_2-eq. kg/kg body protein	Reference
Cow-calf	0.153	19.95[a]	62.71	Phetteplace et al. (2001)[f]
Cow-calf	0.400	20.42[a]	67.92	Beauchemin et al. (2010)
Cow-calf	0.372	-	95.76[g]	Baber et al. (2019a)[b]
Cow-calf	-	10.14[c]	-	Rotz et al. (2019)[b]
Range	0.138	9.12[a]	43.57	Crutzen et al. (1986)[bd]
Pasture-finish	0.231	15.24	72.81	Harper et al. (1999)[bi]
Stocker	0.231	14.97[a]	56.79	Phetteplace et al. (2001)[f]
Pasture-finish	0.160	12.03[a]	52.67	Pelletier et al. (2010)[b]
Sileage-finish	0.214	6.03	34.65	Doreau et al. (2011)[h]
Hay-finish	0.203	6.57	38.02	Doreau et al. (2011)[h]
Stocker	-	-	39.95[g]	Baber et al. (2019a)[b]
Stocker	-	2.10[c]	-	Rotz et al. (2019)[b]
Feedlot	0.108	3.10[e]	18.04	Crutzen et al. (1986)[bd]
Feedlot	0.066	1.91[e]	11.11	Harper et al. (1999)[bi]
Feedlot	0.098	2.78[e]	15.57	Phetteplace et al. (2001)[f]
Feedlot	0.170	5.61	29.53	Beauchemin et al. (2010)
Feedlot	0.185	5.78[e]	31.10	Pelletier et al. (2010)[b]
Feedlot	0.295	7.92	40.93	Cooprider et al. (2011)
Corn grain-finish	0.118	2.76[e]	17.48	Doreau et al. (2011)[h]
Feedlot	-	-	19.87[g]	Baber et al. (2019a)[b]
Feedlot	-	0.98[c]	-	Rotz et al. (2019)[b]

All reported emissions are a total of enteric fermentation and manure (unless otherwise stated) with an applied GWP_{100} of 28.

[a] Assuming a DP of 58%.

[b] Assuming just enteric methane.

[c] Using only Southern plains data.

[d] Range assumptions: diet gross energy consumption of 110 MJ/hd/d, methane conversion factor= 7%, 1 kg methane = 55.65 MJ, incoming weight 158.8 kg, exiting weight 340.3 kg, ADG= .732 kg/d, DOF= 217. Feedlot assumptions: diet gross energy consumption of 150 MJ/hd, methane conversion factor= 4%, 1 kg methane= 55.65 MJ, incoming weight 340.3 kg, exiting weight 612.5 kg, ADG= 1.51 kg/d, DOF= 180.

[e] Assuming a DP of 64.5%.

[f] Cow-calf assumptions: incoming weight 39 kg, exiting weight 158.8 kg, DOF= 320 (using study ADG). Stocker assumptions: incoming weight 158.8 kg, exiting weight 340.3 kg, DOF= 244. Feedlot assumptions: incoming weight 340.3 kg, exiting weight 612.5 kg, DOF= 171.

[g] HeP= body protein* (1- % inedible product), assuming an inedible product average of 24.5%.

[h] Assuming incoming weight of 400 kg.

[i] Pasture-finish assumptions: incoming weight 340.3 kg, exiting weight 503.6 kg, ADG= .732 kg/d, DOF= 217. Feedlot assumptions: incoming weight 340.3 kg, exiting weight 612.5 kg, ADG= 1.51 kg/d, DOF= 180.

APPENDIX B

CHAPTER III TABLES AND FIGURES

Table B1. Primary studies and characteristics used

Study	Experiment	n[1]	Adaptation (duration), d	Monensin delivery[2]	Monensin dose, mg/d	Method of CH_4 measurement[2]
Thornton and Owens (1981)	1	3	17 (20)	premix	200.0	1
	2	6	17 (20)	premix	200.0	1
	3	3	17 (20)	premix	200.0	1
Benz and Johnson (1982)	1	4	14 (21)	premix	200.0	1
	2	4	28 (35)	premix	200.0	1
Wedegaertner and Johnson (1983)	1	6	21 (28)	premix	174.3	1
	2	6	21 (28)	premix	174.3	1
O'Kelly and Spiers (1992)	1	6	54 (55)	premix	309.2	1
	2	3	50 (51)	premix	181.5	1
McGinn et al. (2004)	1	4	16 (21)	premix	254.4	1
Mwenya et al. (2005)	1	4	14 (22)	premix	184.3	2
Beauchemin and McGinn (2006)	1	4	17 (21)	premix	254.4	1
Tomkins et al. (2015)	1	5	25 (26)	ruminal	60.0	1
	2	5	25 (26)	ruminal	250.0	1
Hemphill et al. (2018)	1	8	14 (16)	top dress	150.0	2
	2	8	42 (44)	top dress	150.0	2
	3	7,8	161 (163)	top dress	150.0	2
Vyas et al. (2018)	1	5	48 (105)	premix	171.3	1
	2	5	63 (133)	premix	268.0	1

[1] n = number per treatment (CON vs MON), except Hemphill et al. (2018) Experiment 3 where CON=7 and MON=8

[2] 1= whole animal chamber, 2= headbox

Table B2. Summary values for predictors assessed

Variables	Minimum	Maximum	Mean
Dose, mg/d	60.00	309.21	200.44
Fat, %	1.50	4.93	3.14
NDF, %	13.32	68.80	39.59
Control DMI, kg/d	4.10	10.47	6.53

Neutral detergent fiber (NDF)
Dry matter intake (DMI)

Table B3. Mean summary effect and heterogeneity of each analyses

		RMD		Heterogeneity		
Response variable	Average[a]	RMD ±SE	*P*-value	T^2	I^2	*P*-value
g CH_4/d	128.58	-14.39 ± 2.81	<0.0001	74.91	56.60%	0.0013
g CH_4/Mcal DE	48.43	-6.05 ± 0.99	<0.0001	7.51	49.61%	0.0077
g CH_4/kg DMI	20.72	-1.99 ± 0.43	<0.0001	1.09	35.01%	0.0668

[a] average of control group
Raw mean difference (RMD)
Standard error (SE)
Methane (CH_4)
Digestible energy (DE)
Dry matter intake (DMI)

Table B4. Final model: summary effect, explanatory variable(s), and heterogeneity

	Final model			
Variable	RMD ± SE	*P*-value	T^2	R^{2a}
g CH_4/d				
Intercept	-14.39 ± 2.81	<0.0001	67.98	
Fat	-6.61 ± 3.37	0.0499		9.24%
g CH_4/Mcal DE				
Intercept	-6.05 ± 0.99	<0.0001	0.00	
Fat	-3.21 ± 0.84	0.0001		100.00%
NDF	-0.11 ± 0.04	0.0041		
g CH_4/kg DMI				
Intercept	-1.99 ± 0.43	<0.0001	1.09	

[a] predictors' total contribution to between-study variability (R^2)
Raw mean difference (RMD)
Standard error (SE)
Heterogeneity (T^2)
Methane (CH_4)
Digestible energy (DE)
Neutral detergent fiber (NDF)
Dry matter intake (DMI)

Fig B1. Forest plot (g CH_4/d) showing individual study effect size (RMD), standard error (SE), and 95% confidence interval (CI). Middle line showing no effect.

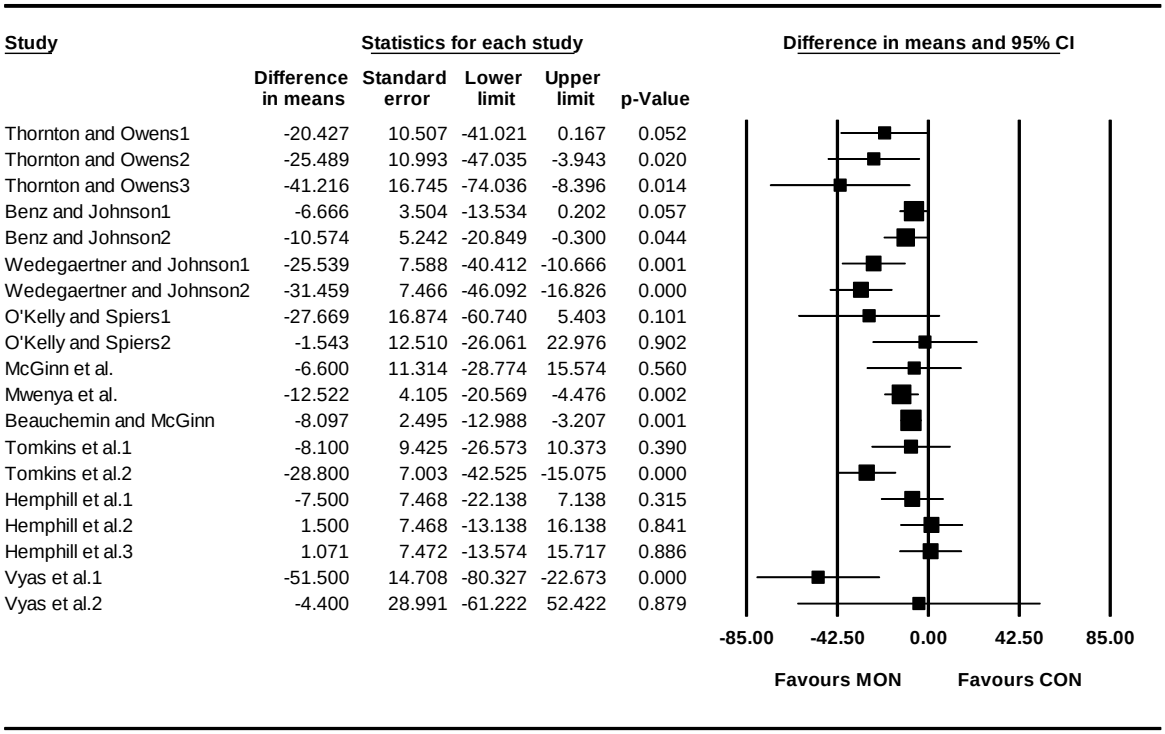

Fig B2. Funnel plot (g CH_4/d) showing summary effect and primary study dispersion.

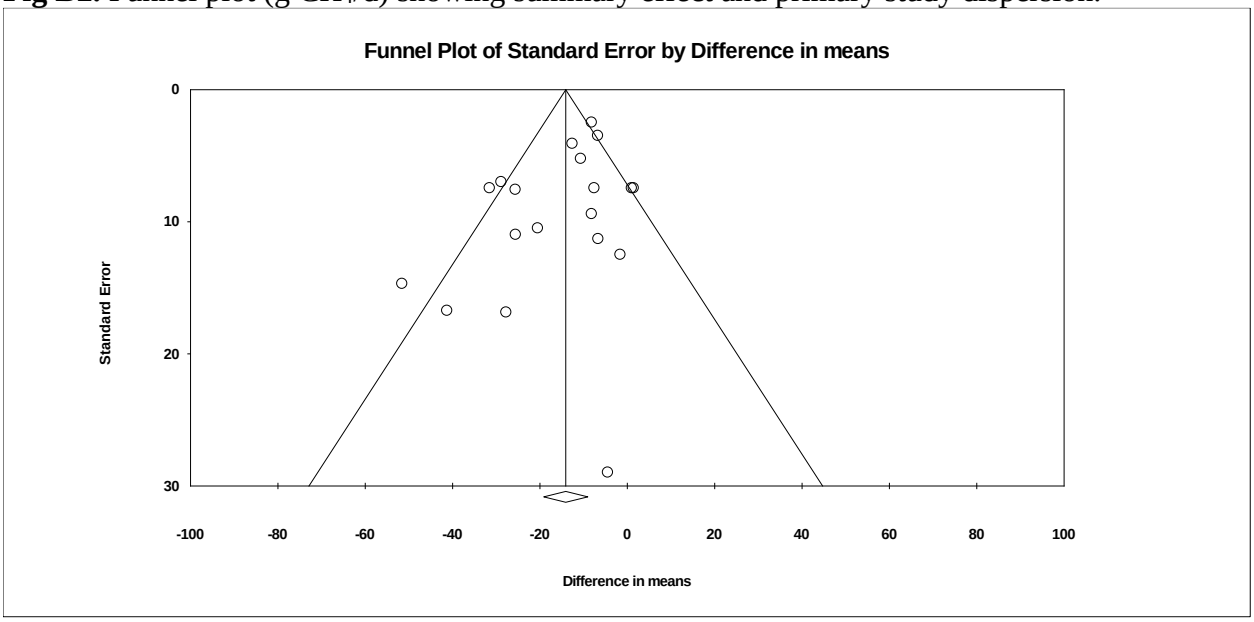

Fig B3. Forest plot (g CH_4/Mcal DE/kg) showing individual study effect size (RMD), standard error (SE), and 95% confidence interval (CI). Middle line showing no effect.

Study	Difference in means	Standard error	Lower limit	Upper limit	p-Value
Thornton and Owens1	-7.611	3.326	-14.131	-1.092	0.022
Thornton and Owens2	-9.866	4.119	-17.939	-1.794	0.017
Thornton and Owens3	-18.578	6.666	-31.644	-5.513	0.005
Benz and Johnson1	-4.173	1.339	-6.797	-1.548	0.002
Benz and Johnson2	-3.672	2.026	-7.644	0.299	0.070
Wedegaertner and Johnson1	-8.484	2.230	-12.855	-4.114	0.000
Wedegaertner and Johnson2	-11.214	2.367	-15.853	-6.576	0.000
O'Kelly and Spiers1	-11.375	6.945	-24.987	2.237	0.101
O'Kelly and Spiers2	-0.624	5.148	-10.715	9.466	0.903
McGinn et al.	-3.851	3.869	-11.435	3.733	0.320
Mwenya et al.	-3.411	1.001	-5.373	-1.450	0.001
Beauchemin and McGinn	-4.370	3.966	-12.144	3.403	0.270
Tomkins et al.1	-3.389	3.944	-11.118	4.340	0.390
Tomkins et al.2	-12.050	2.930	-17.793	-6.308	0.000
Hemphill et al.1	-3.917	4.063	-11.880	4.045	0.335
Hemphill et al.2	-2.675	3.911	-10.342	4.991	0.494
Hemphill et al.3	1.428	3.726	-5.876	8.732	0.702
Vyas et al.1	-14.914	4.259	-23.262	-6.566	0.000
Vyas et al.2	-1.130	5.263	-11.445	9.186	0.830
	-6.035	0.974	-7.944	-4.126	0.000

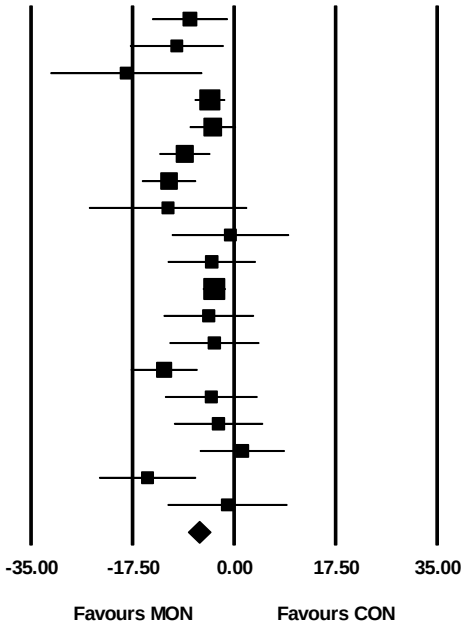

Fig B4. Funnel plot (g CH_4/Mcal DE/kg) showing summary effect and primary study dispersion.

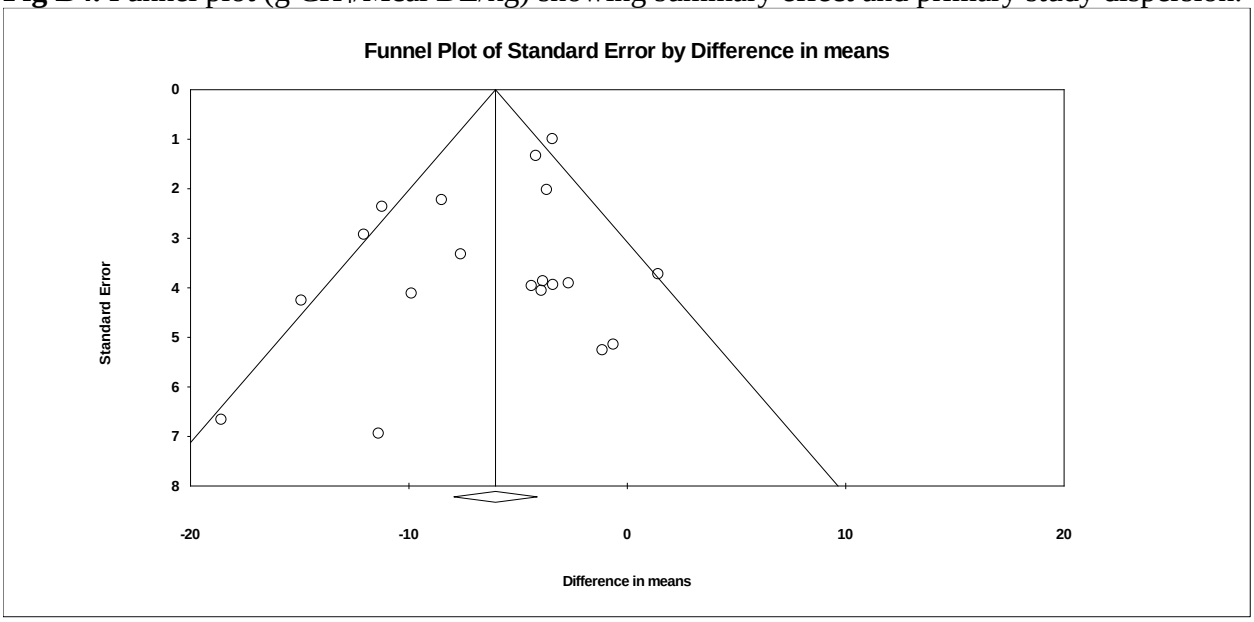

Fig B5. Forest plot (g CH_4/kg DMI) showing individual study effect size (RMD), standard error (SE), and 95% confidence interval (CI). Middle line showing no effect.

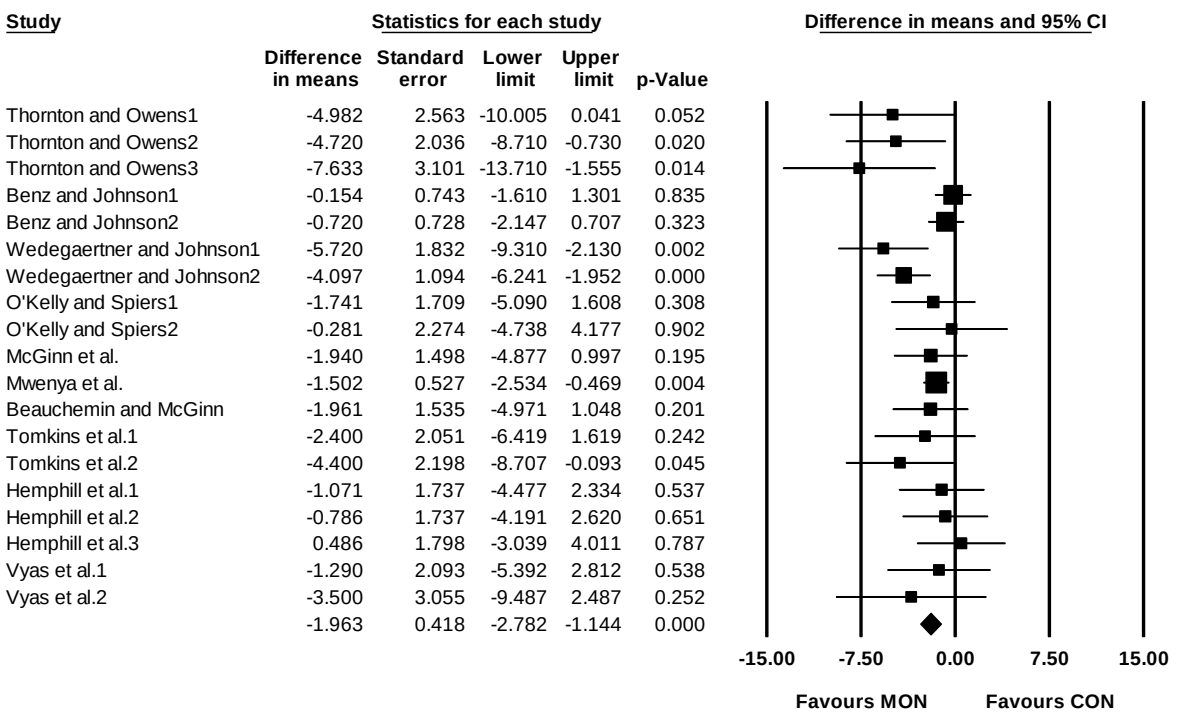

Fig B6. Funnel plot (g CH_4/kg DMI) showing summary effect and primary study dispersion.

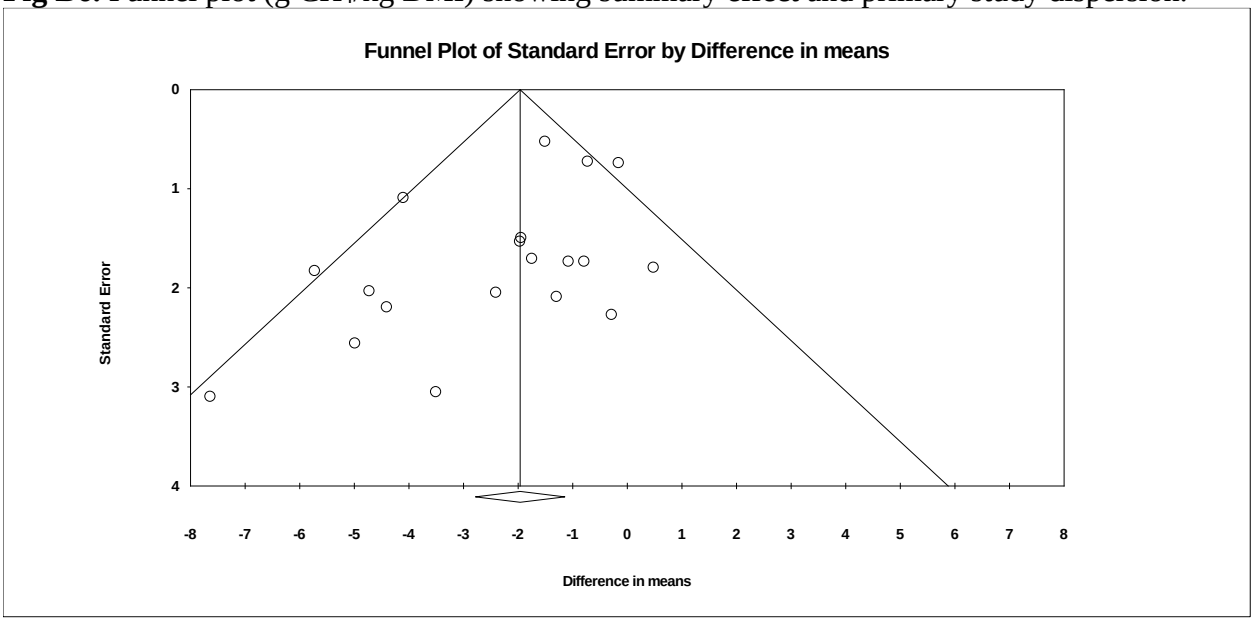

Table B5. Summary values of each analyses forest plot

	g CH_4/d		g CH_4/Mcal DE/kg		g CH_4/kg DMI	
Study	Effect size	SE	Effect size	SE	Effect size	SE
Thornton and Owens1	-20.43	10.51	-7.61	3.33	-4.98	2.56
Thornton and Owens2	-25.49	10.99	-9.87	4.12	-4.72	2.04
Thornton and Owens3	-41.22	16.75	-18.58	6.67	-7.63	3.10
Benz and Johnson1	-6.67	3.50	-4.17	1.34	-0.15	0.74
Benz and Johnson2	-10.57	5.24	-3.67	2.03	-0.72	0.73
Wedegaertner and Johnson1	-25.54	7.59	-8.48	2.23	-5.72	1.83
Wedegaertner and Johnson2	-31.46	7.47	-11.21	2.37	-4.10	1.09
O'Kelly and Spiers1	-27.67	16.87	-11.38	6.95	-1.74	1.71
O'Kelly and Spiers2	-1.54	12.51	-0.62	5.15	-0.28	2.27
McGinn et al.	-6.60	11.31	-3.85	3.87	-1.94	1.50
Mwenya et al.	-12.52	4.11	-3.41	1.00	-1.50	0.53
Beauchemin and McGinn	-8.10	2.50	-4.37	3.97	-1.96	1.53
Tomkins et al.1	-8.10	9.43	-3.39	3.94	-2.40	2.05
Tomkins et al.2	-28.80	7.00	-12.05	2.93	-4.40	2.20
Hemphill et al.1	-7.50	7.47	-3.92	4.06	-1.07	1.74
Hemphill et al.2	1.50	7.47	-2.68	3.91	-0.79	1.74
Hemphill et al.2	1.07	7.47	1.43	3.73	0.49	1.80
Vyas et al.1	-51.50	14.71	-14.91	4.26	-1.29	2.09
Vyas et al.2	-4.40	28.99	-1.13	5.26	-3.50	3.06

Methane (CH_4)
Standard error (SE)
Digestible energy (DE)
Dry matter intake (DMI)