RUMINANT METHANOGENIC ACTIVITY IN THE UNITED STATES

BEEF CATTLE INDUSTRY

A Thesis

by

SARAH B. KLOPATEK

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Chair of Committee,	Tryon A. Wickersham
Co-Chair,	Luis O. Tedeschi
Committee Members,	Jason E. Sawyer
	Todd R. Callaway
Head of Department,	H. Russell Cross

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ABSTRACT

In the U.S., gastrointestinal fermentation from cattle is estimated to account for approximately 25% of total anthropogenic related methane (CH_4) emissions. In addition, 2-8% of gross energy consumed by cattle is lost in the form of CH_4, representing an energetic cost to the animal. Thereby, in order to decrease greenhouse gas emissions (GHG) and improve the efficiency of cattle production additional research on gastrointestinal CH_4 emissions from cattle is needed. In ruminants carbohydrate (CHO) catabolism and nitrogen (N) utilization have a tremendous impact on ruminal methanogenesis. However, the impact of purified carbohydrates in the presence of a variety of N sources on rates of CH_4 and VFA production remains unknown. In order to determine these rates for use in predictive models of the ruminal fermentation, we formulated a fractional rate equation to fit the rate of CH_4 production and measured the concentration of CH_4 and VFA and using purified CHO with a variety of N sources in two *in vitro* mixed ruminal microorganism fermentation studies. In both studies, a CHO treatment × incubation time (IT) effect was observed for both VFA and CH_4 ($P < 0.01$). There was also a N × IT interaction for CH_4 production at 24 h in Study 2, where nitrogen free and NH_3 treatments produced greater concentrations of CH_4 than treatments with amino acids ($P < 0.01$). A nonlinear equation for the conversion of carbohydrates to CH_4 was able to fit starch treatments in Study 1 and glucose treatments in Study 2. Overall, this study demonstrated different fermentation patterns among all CHO and N sources and was the first step in determining rates for *in vitro* CH_4 production. Although cattle contribute with high amounts of anthropologic GHG, they

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are not the only methanogenic producing food source in the U.S. Rice and wild ruminants (e.g. bison, elk, deer) are also methanogenic producing food sources. The objective of this final study was to compare the efficiency of beef and milk production to pre-settlement wild ruminants and rice production on a kilogram of CH_4 emitted to kilogram human-edible protein production basis. Bison had the highest ratio of 13.93 kg CH_4: Protein, followed by elk (12.50) deer (6.66) and beef (2.47). Overall, wild ruminants emitted 296 to 564 percent more CH_4 per kilogram of human-edible protein produced than current beef cattle production systems. Rice yielded the second lowest CH_4 to human-edible protein ratio (0.83), followed by dairy cattle milk production (0.50). We believe, this analysis provides insight on the efficiency of methanogenic food sources that may aid in the development of regulatory guidelines of CH_4 production.

DEDICATION

I dedicate this thesis to my mother and father. Without their continual love and support

both as parents and as professors, this thesis would not have been possible. Thank you

Ma'ma and Daddy, I love you both.

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CHAPTER I

INTRODUCTION

Ruminants are able to degrade cellulose due to the symbiotic relationship they have with the native anaerobic microorganisms that inhabit their rumen. However, cellulose utilization under anaerobic conditions requires the regeneration of reducing equivalents, often by interspecies hydrogen transfer resulting in CH_4 production. Methane represents an energetic loss from 2 to 8% of gross energy to the animal, as well as a loss of profit to the producer (Dong et al., 2006). In 2014, the EPA listed beef cattle as the leading cause of agriculturally-related CH_4 emissions in the United States, accounting for approximately 38% of total agricultural CH_4 emissions (U.S. EPA, 2014). Furthermore, CH_4 has a 12-year atmospheric lifetime and global warming potential 25 times greater than that of CO_2 (U. S. EPA, 2016), thus strategies to mitigate enteric CH_4 production are of critical interest worldwide to environmentalists, as well as to cattle producers.

Many strategies have been evaluated to reduce CH_4 including modifying dietary composition, inclusion of ionophores, organic acids, and plant compounds, defaunation and vaccinations. These abatement strategies directly or indirectly target ruminal methanogen populations, resulting in varying degrees of efficacy. Although strides have been made in the reduction of methanogenesis, there is a continual need for ruminal CH_4 mitigating research.

Ruminal CH_4 production models can also be utilized as an instrument to help reduce emissions at the animal, farm, regional and global scale (McAllister et al., 2011). These models are not only vital from an agricultural systems perspective, but from both policy-making and regulatory perspectives. Although, predictive modeling has become an essential tool in animal nutrition, its accuracy is difficult to assess and at times is less precise than desired (McAllister et al., 2011). It is we known that carbohydrate catabolism and nitrogen (N) utilization have a tremendous impact on ruminal methanogenesis as well as ruminant nutrition. However, the impact of purified carbohydrates in the presence of a variety of N sources on rates of CH_4 and VFA production remains unknown. In order to clarify these rates for use in predictive models of ruminal fermentation, it is important to formulate fractional rate equations to fit the rate of CH_4 production. Ultimately, by constructing nonlinear rate equations for the conversion of carbohydrates to CH_4, the predictive accuracy of current CH_4 models can be improved upon.

Although cattle production provides the population with an abundant and complete source of protein (National Academy of Sciences, 2005; U.S. Department of Health and Human Services, 2016), some believe because cattle contribute to anthropogenic GHG that they are a non-sustainable food source (Pollan, 2002; Bittman, 2008). However, one factor that has been remiss from the dialog is that beef cattle have not always been the dominant source of agricultural CH_4 emissions nor are they the only current methanogenic food source.

Bison, elk and deer, like cattle, are ruminants and it has been theorized that prior to European settlement, wild ruminants produced sizable amounts of CH_4 (Subak, 1994; Hristov, 2012). In addition to wild ruminants the grain rice, one of the most heavily consumed food sources in the world, is methanogenic, constituting one of the largest sources of agricultural CH_4 emissions (van Groenigen et al., 2013; IPCC, 2007). Therefore, in order to understand and address the impending issues of food sustainability, the efficiencies of pre-settlement and current methanogenic agricultural food sources should be compared on an CH_4 output and human-edible protein basis. Ultimately, by performing innovative comparison studies, we can provide insight on the efficiency of methanogenic food sources and one day may aid in the development of environmental and governmental food sustainability guidelines.

CHAPTER II

LITERATURE REVIEW

Introduction

Ruminants are an evolutionary marvel because their symbiotic relationship with anaerobic microbes in their reticulorumen allows for the conversion of cellulose and other structural carbohydrates into meat, milk, and fiber. However, this ability to utilize forages comes at a cost. Methane (CH_4) is an inevitable end product of all ruminal fermentation and is a sink for reducing equivalents. Therefore, a certain amount of methanogensis is inevitable from ruminal fermentation.

Methane is a potent greenhouse gas (GHG), with a global warming potential 23 times greater than that of carbon dioxide (U.S. EPA, 2016). In 2012, the Environmental Protection Agency (EPA) listed gastrointestinal fermentation from beef and dairy cattle as one of the leading cause of agriculturally related CH_4 emissions in the United States, accounting for approximately 25% of total agricultural CH_4 emissions (U.S. EPA, 2014). Additionally, CH_4 production is a cost to animal production, representing a loss of 2 to 8% of gross energy (Gerber et al., 2013). Therefore, it is of critical interest to cattle producers as well as environmentalists that CH_4 emissions from gastrointestinal fermentation be reduced.

Many strategies including diet modification, ionophores, and feed additives, have been used to reduce methanogenesis, with varying degrees of success (Van Nevel and Demeyer, 1977; Beauchemin and McGinn., 2006; Ellis et al. 2007). Over the last

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quarter century, gastrointestinal CH_4 production from the entire beef cattle industry has increased by 0.6% while beef production has increased by 14%, meaning the U.S. produces more beef with fewer emissions. However, the continuing improvement of CH_4 mitigation will be dictated by the availability and quantity of feed, physiological state of the animals, governmental regulatory agencies, public perception, and the development of new technologies.

The ruminant

Food animals can be categorized as either non-ruminants or ruminants. Non-ruminants, such as pigs and horses, are postgastric fermenters and have simple, single chamber stomachs. Ruminants, such as cattle and sheep, are pregastric fermenters and have a complex, four chambered "stomach"s. Ruminant digestion differs from non-ruminant digestion as the majority of microbial fermentation occurs in the rumen, prior to passage through the gastric stomach (abomasum) opposed to non-ruminants where the majority of fermentation occurs in the large intestine or cecum. In cattle, the rumen is a blind pouch that is an anaerobic fermentation chamber with a volume of 100 to 180 L that comprises approximately 50% of the gastrointestinal tract volume. The Rumen is populated by a wide variety of microorganisms including protozoa, bacteria, and fungi that synergistically degrade and ferment feedstuffs. Remarkably, the rumen is one of the world's richest microbial habitats in the world. In a droplet of rumen fluid there are more bacteria present than there have been people that have ever lived on earth (Russell

and Hespell, 1981; Prescott et al., 2005). Thus far, over 200 species of bacteria and 20 species of protozoa have been cultured in rumen fluid (Russell and Hespell, 1981).

The Ruminal microbial fermentation end-products include volatile fatty acids (VFA), microbial crude protein, NH_4, CO_2, and CH_4. Volatile fatty acids are absorbed across the epithelium and serve as the primary energy source for ruminants. The most common VFA are acetate, propionate, and butyrate and their relative ratios vary according to dietary composition. Branched-chain VFA, include isovalerate, valerate, and isobutyrate, are also produced. Although BC VFA comprise only a small percentage of the total VFA production, they are imperative to ruminal syntrophic processes (Pitt et al., 1996).

Production of VFA decreases ruminal pH; to counteract this, ruminants attempt to maintain ruminal pH homeostasis by producing large quantities of saliva that contains bicarbonate, a buffer. Domestic cattle secrete between 100 to 150 liters of saliva per day, depending on diet and size of the animal (Bowen, 2009). If ruminal pH falls below 5.5, severe health problems can occur (Garrett et al., 1999).

Cattle typically meet the majority of their protein requirements by degrading and digesting ruminal microbes that pass out of the reticulorumen. This ability to utilize microbial crude protein derived from fermentation of forage enables cattle to effectively fill a unique environmental niche. Although, microbial protein is also synthesized in the large intestine, most herbivores cannot use this as a protein source because amino acids, dipeptides and tripepdides, the end products of protein digestion, cannot be absorbed from the large intestine.

Carbohydrate degradation

Cellulose is the most abundant polymer on land comprising 20-40% of plant matter on a DM basis (Leschine, 1995). Cellulose is a β-1,4 linked glucose homopoloyer with glucose molecules oriented linearly, allowing polymerized molecules to stack (Leschine, 1995). Thus far, no vertebrate animals have been identified with the ability to produce cellulose-degrading enzymes, and only specific varieties of microbes, possess the enzymatic ability to hydrolyze β 1,4 glycosidic bonds (Watanabe and Tokuda, 2000).

Microorganisms have evolved several strategies to degrade cellulose. One strategy, is the release of cellulose degrading enzymes into the ruminal fluid. The other dominant strategy is direct adhesion. Carbohydrate-binding modules (CBM) adhere their enzymes directly to cellulose to create a maximum interface between the catalytic domain and the substrate before the enzyme diffuses from the cellulose particle (Wilson, 2011). In addition to CMB, Cellulosomes are multi-enzyme complexes that use scaffoldin subunits or cellulose-binding domains, to adhere carbohydrates to the microbial cell wall (Ding et al., 2008). Overall, adhesion strategies are advantageous to the microorganisms because they allow for the protection of their enzymatic resources by directly attaching to plant cell walls and closely capturing degradation products.

Pure cellulose is a biological rarity. In nature, cellulose is predominantly associated with hemicellulose, lignin, pectin, and proteins in a plant (Van Soest, 1994; Leschine, 1995). The degree to which cellulose is bound to other structural components affects nutrient availability and digestibility. Lignin, an organic polymer important to

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plant structural integrity, is an anti-nutritional factor that slows down fiber degradation by ruminal microbes. As plants mature, lignin is deposited and cross-linked with cellulose within the cell-wall. Thus, as the plant matures, the ability of ruminal microorganisms to degrade cellulose decreases due to the increased lignification (Jung and Allen, 1995; Moore and Jung, 2001).

Both physical and chemical factors that alter the rumen environment can influence rate of cellulose degradation. One of the principal chemical factors impacting cellulose degradation is pH. Bacteria responsible for cellulose degradation, such as *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens* are pH sensitive. If the pH declines below the optimum fibrolytic enzyme pH, 6.2 (Greve et al., 1984; Matte and Forsberg, 1992), fiber digestion is inhibited (Russell and Dombrowski, 1980; Hoover, 1986; Grant and Mertens, 1992).

Cellulose molecules can be oriented in tightly packed crystalline constructions, or more loosely arranged in amorphous structures. The percent of crystallinity within native cellulose ranges from 60-90% (Leschine, 1995).   Although crystallinity may affect cellulose digestibility, it has been observed that surface area or cellulose may have a greater impact on digestibility (Weimer et al., 1990). Particles with larger surface areas have a higher rate of microbial colonization than smaller surface areas and thus have a higher rate of cellulose degradation (Weimer et al., 1990).

Plants primarily store energy in the form of starch (Van Soest, 1994). Starch is predominantly composed of amylopectin and amylose, also both glucose polysaccharides. Amylose is a linear α-1,4 glucopyranosid consisting of several hundred

chains (Van Soest, 1994). Amylopectin, is a much larger polymer than amylose and consists of both α-1,4-linkages and α-1,6 linkages (Van Soest, 1973). Incorporation of α-1,6 linkages causes branching in starch and increases accessibility to microbes, opposed to amylose which is structurally condensed. Unlike β-1,4 bonds, α-1,4 and α-1,6 bonds can be hydrolyzed by microbial or vertebrate enzymes. Factors that affect the rate of starch digestion include the proportion of amylose to amylopectin, and degree of crystallinity (Theurer, 1986). Starch, like cellulose, can be divided into amorphous or crystalline regions. Crystalline starch's compact structure makes it nearly impermeable to microbial attack, therefore as the percentage of crystalline structure increases, starch digestibility decreases (Sveinbjörnsson, 2006).

Processing methods such as steam flaking and grinding increase the rate and extent of starch degradation by ruminal microbes (Theurer, 1986). Amylolytic starch degradation from of starch from cereal grains by both ruminal microbial and pancreatic enzyme sources are increased by processing methods. It is thought that the improvement in starch digestion by processing techniques is the primary reason for enhanced feed conversion of cattle fed high grain diets (Theurer, 1986).

As starch enters the rumen, starch-fermenting bacteria, such as *Prevotella ruminicola*, *Selenomonas ruminatium*, and *Streptococcus bovis,* begin to ferment starch into lactate and acetate (Ørskov, 1986; Russell and Rychlik, 2001). Because lactate has a low pKa (3.9), it dissociates into lactate and Hydronium ions which causes a decrease in pH. When the ruminal pH decreases below 6.0 many species of bacteria grow more slowly, including fibrolytic bacteria, but many amylolytic bacteria are not pH sensitive

until pH falls below 5.5 (Reece et al., 2015). In addition, starch fermenting bacteria have a generation time significantly faster than fiber fermenting bacteria (Hungate, 1966). Accordingly, inclusion of starch in the diet alters the rumen environment in favor of starch fermenting microorganisms, inhibiting fiber digestion (Piwonka and Firkins, 1996).

Starch is added to the diet to increase energetic efficiency by increasing the propionate to acetate ratio. Propionate is gluconeogenic and provides more moles of ATP per mole of propionate than acetate (Russell and Rychlik, 2001). The rapid fermentation of starch can also cause ruminal health issues. If the VFA production rate is greater than the rate of absorption, ruminal pH begins to fall. If pH falls below 5.5 then ruminal acidosis, either acute or subacute ruminal acidosis, can occur (Blood and Radostits, 1989; Plaizier et al., 2008). Clinical signs of acidosis depend on the severity of the pH depression as well the time the pH remains depressed. Signs include poor body condition, decreased rumen motility, laminitis, abscesses, and even result in the mortality of the animal (Blood and Radostits, 1989; Underwood, 1992).

Methanogenesis

Ruminal methanogens are autotrophic archaea that live in symbiosis with the ruminal consortium of protozoa, fungi and bacteria (Van Soest, 1982). Two classes of methanogenic archaea predominate the rumen, *Methanobacteriales* and *Methanomicrobiales* (St-Pierre et al., 2015); thus far, microbiologists, have isolated seven species from rumen fluid (Janssen and Kirs, 2008).

Ruminal methanogens produce CH_4 as an end product of ruminal fermentation. Although, the production of CH_4 represents an energetic loss to the animal, the presence of methanogens in the rumen are important for microbial energetic efficiency. Aerobic organisms produce ATP via oxidation phosphorylation, but because the rumen is anaerobic, microbes cannot utilize oxygen as the terminal electron acceptor (Murray, 2012). Coupled redox reactions are utilized for energy generation within the rumen, utilizing carbon, instead of oxygen, as the final electron acceptor (Russell, 2002). Through the process of interspecies hydrogen transfer, CO_2 and hydrogen protons are converted to CH_4 by methanogens (Scheifinger et al., 1975; Latham and Wolin, 1977). Methanogens are a major means of reducing equivalent disposal. By keeping the partial pressure of H_2 low via hydrogenase, methanogens allow for ruminal bacteria to more efficiently metabolize energy.
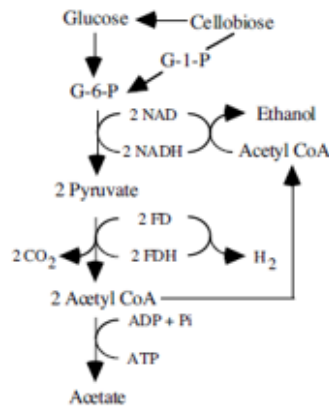
Figure 1.1: The Fermentation of cellobiose by R. *albus* grown as a pure culture. (Taken from Russell, 2002).
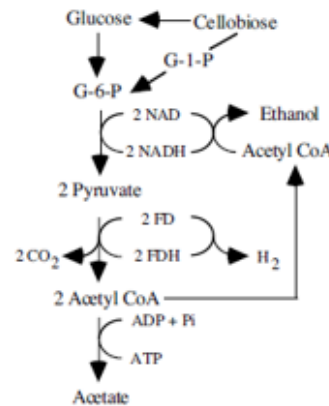
Figure 1.2: The Fermentation of cellobiose by R. *albus* grown in a co-culture with a methanogen. (Taken from Russell, 2002).

In a pure culture of R. *albus* (Fig. 1.1) hydrogenases are unable to oxidize NADH, thereby alcohol production becomes the alternative method for reducing equivalent disposal reducing the total ATP yield. In contrast when grown in a co-culture of R. *albus* and a methanogen (Fig. 1.2), the methanogen is able to use hydrogens produced by the reductions of G-6-P to Pyruvate and Acetyl CoA to acetate. Ultimately, this allows for the regeneration of reducing equivalents, enabling the production of two acetate and two ATP. Accordingly, methanogens are crucial for microbial energetic efficiency and the complete elimination of methanogens from the rumen would be detrimental to both microbes as well as the host animal.

Methane mitigation strategies and impactors

Despite the intrinsic necessity for a certain level of CH_4 production in the rumen, minimizing CH_4 production is a goal of all producers. Gastrointestinal CH_4 production can be inhibited by directly inhibiting methanogens or indirectly by decreasing substrate availability to the methanogens. Numerous strategies have been developed and implemented to reduce gastrointestinal CH_4 production with varying degrees of efficacy.

Dietary impacts

One of the most utilized strategies to reduce methanogenic activity is diet modification. Dietary factors that influence rate of passage, rate of fermentation, rumination or pH, alter H^+ concentrations affecting microbiota present, including methanogens. Specific dietary factors include DMI, metabolizable energy intake, digestibility, and the percent of forage in the diet (Ellis et al., 2007).

Dry matter intake may account for as much as 52 to 64% of the variation in daily CH_4 production (Boadi et al., 2002; Hammond et al., 2009). In general, as DMI increases total CH_4 production increases (Blaxter, 1967; McAllister, et al. 1996). However, when intake is increased from maintenance, the amount of energy lost as CH_4 per unit of feed intake decreases (Blaxter, 1967). Studies have indicated that when forage DM intake increases by 30% kg $^{-0.75}$, Y_m (percent of gross energy lost in the form of CH_4) values decreased from 7% to 6.5% (McAllister et al., 1996).

In addition to feed intake, diet digestibility also has a substantial impact on CH_4 production (Hegarty et al., 2010). Factors that affect the digestibility of the feedstuff include, but are not limited to, plant type, plant maturity, physical processing methods, lignification, neutral detergent fiber content, and acid detergent fiber content. As plants mature, the fraction of insoluble fiber increases, which causes a decrease in plant digestibility. Thus, ruminal CH_4 production generally increases concurrently with the maturity of the forage fed (Tyrrell and Moe, 1992; Jung and Allen, 1995; Boadi et al., 2002). Digestibility of feedstuffs can be improved by physical processing methods (e.g. chopping, pelleting, and grinding) which can lower GHG emissions. Studies have indicated that CH_4 production is lower for finely ground and pelleted forages than chopped and/or long stem forages (Hironaka et al. 1996).

Forage type also has an effect on ruminal methanogenesis (e.g., legume forages generally yield lower CH_4 production from ruminal fermentation than do grass forages) (Varga et al. 1985). Varga et al. (1985) suggested that because the legume diet had a higher proportion of soluble carbohydrates compared to the grass diet that the grass diet

was favorable to ruminal methanogensis. Strategies that have been recommended to decrease emissions without negatively affecting intake include replacing grass silage with either corn or legume silages, and incorporate highly digestible fibers to the diet, such as beet pulp (Kujawa, 1994; Hristov et al., 2013).

Increasing the proportion of concentrate to the diet significantly effects methanogenic activity (Moe and Tyrrell, 1979; Kebreab et al., 2008). Johnson and Johnson (1995) reported that animals fed at maintenance lost on average 6-7% Y_m on 100 percent forage diets compared to 2-3% Y_m for animals fed high concentrate diets (up to 90 percent). This decline in CH_4 production caused by the inclusion of concentrate feedstuffs happens because of several factors. Adding concentrate to cattle diet increases the propionate to acetate ratio by providing substrate to amylolytic bacteria (van Kessel and Russell, 1996). When propionate is produced the hydrogen availability for methanogens is decreased, ultimately decreasing CH_4 production (van Kessel and Russell, 1996). Additionally, the decreased pH and increased rate of fermentation caused by the inclusion of dietary starch, may decrease methanogen activity by inhibiting ciliate protozoa (Demeyer, 1975; McAllister et al., 1996). Methanogenic archaea are metabolically and synergistically associated with ciliate protozoa (Finlay et al., 1994). Due to protozoa's intricate relationship with ruminal methanogens, it has been estimated that ciliate protozoa are responsible for 9 to 37% of the CH_4 production within the rumen (Newbold et al., 1995). Thereby, if protozoa are reduced methanogenesis is impacted.

Forage generally has a slower rate of digestion compared to starch and prolonging the ruminal residence time of feed particles increases the amount of CH_4 produced per unit of feed digested (McAllister, 1996). Overall, incorporating concentrates into cattle diets decreases methanogenic activity and is a crucial strategy for providing sustainable beef that limits GHG emissions.

Ionophores

Monensin is a naturally occurring polyether ionophore produced from *Streptomyces cinnamonensis*. The benefits of ionophores to ruminants occur because they alter the end products of the ruminal fermentation through secondary effects of a disruption of the ion gradients maintained by Gram-positive bacteria, killing the gram-positive bacteria (Russell and Strobel, 1989). Ionophores were first utilized to help combat coccidiosis in the poultry industry. When poultry litter was fed to cattle, it led to the discovery that ionophores inhibited methanogenesis. Early research determined that monensin resulted in improvements in feed utilization, increasing feed to gain ratios up to 17% (Raun et al., 1976). *In vitro* research determined that monensin did not have a direct effect on methanogens, but rather inhibited of formate degradation decreasing hydrogen availability for methanogens (Van Nevel and Demeyer, 1977). Today, monensin is the predominant ionophore used in the feedlot cattle industry increasing feed efficiency by 6% and 15% for feedlot and grass-fed animals, respectively (Russell, 2002).

Chlorinated Hydrocarbons

Chlorinated hydrocarbons inhibit CH_4 production both *in vitro* and *in vivo* (Leng, 2014). One of the most successful chlorinated hydrocarbons tested *in vivo* is bromochloromethane (BCM). When BCM was added to ruminal fluid *in vitro*, it reduced CH_4 production by 94% (Martinez-Fernandez et al., 2015). In an *in vivo* study, when BCM was included in Brahman cattle diets, the Y_m values were significantly decreased as BCM inclusion levels increased (Tomkins and Hunter, 2004).

The chlorinated hydrocarbon, 9, 10-anthraquinone (AQ) has also been shown to decrease CH_4 production (Garcia-Lopez et al., 1996; St-Pierre et al., 2015) by directly affecting methanogenic bacteria (Kung et al., 2003). Inclusion of AQ in ovine diets decreased CH_4 emissions without compromising digestibility or animal health (Kung et al., 2003). However, long-term studies are needed to determine the longevity and persistence of efficacy of AQ as a CH_4 abatement strategy for cattle.

3-Nitrooxypropanol

The compound 3-Nitrooxypropanol (NOP) is a structural analog to methyl-coenzyme M (MCR), a cofactor involved in the final reduction stages of methanogenesis (Haisan et al., 2014). NOP inhibits MCR, subsequently causing a decrease in methanogenesis (Van Nevel and Demeyer, 1995). During *in vivo* trials, NOP was shown to reduce CH_4 emissions in lactating dairy cows without decreasing DMI or milk production (Haisan et al., 2014). However in a contrasting study, although a reduction of CH_4 was observed the addition of NOP also reduced diet digestibility (Reynolds et al.,

2014). Overall, additional research is needed to determine NOP dose, delivery method,

as well as the longevity of efficacy (Reynolds et al., 2014).

Vaccinations

One of the most recent CH_4 mitigating strategies being investigated is an anti-

methanogenic vaccination. In one vaccine trial, sheep where assigned one of two

different treatment, either a 3-methanogen cocktail or a 7-methanogen cocktail (Wright

et al., 2004). It was thought that the vaccine would use the ruminant's immune system

to produce antibodies against methanogens and directly inhibit CH_4 production. The 3-

methanogen cocktail reduced CH_4 production per kg dry matter intake by 7.7%, but the

seven-methanogen cocktail had no effect on methanogenic activity.   In a later trial, a

vaccine based on five-methanogen strains was developed and administered to sheep at 0,

28, and 103 days (Williams et al., 2009). Although the vaccine targeted 52% of the

known methanogen population, total CH_4 output actually increased by 18%. Currently, it

does not appear that vaccination is a viable CH_4 abatement strategy.

Fats

Unsaturated fats, which are often included in the ruminant diet, have inhibitory

effects on ruminal CH_4 emission. Unsaturated fatty acids may be used as hydrogen

acceptors as an alternative to the reduction of carbon dioxide. In addition, fatty acids

have the ability to directly inhibit methanogens by binding to the methanogen cell

membrane interrupting CH_4 transport (Dohme et al., 2001). The addition of fat is also

thought to decrease CH_4 by decreasing protozoa (Newbold et al., 2015).

Refined soy oil, linseed oil, coconut oil and sunflower oil have all been shown to reduce gastrointestinal CH_4 (Jordan et al., 2006; Beauchemin and McGinn., 2006; McGinn et al., 2004). *In vitro* studies demonstrate that the addition of fat, at 5.3%, reduced total CH_4 production by as much as 20% (Dohme et al., 2001). Overall, the addition of fats to the diet has been considered a more favorable means of CH_4 mitigation for it is more natural then chemical additives (Toprak, 2015). However, if dietary fat is fed in excess, feed intake and fiber digestibility decreases, which significantly impacts animal performance and lowers feed efficiency (McGinn et al., 2004).

Dicarboxylic organic acids

Dicarboxylic Organic acids such as malate, aspartate, and fumarate have been evaluated as dietary additives to improve animal performance and decrease methanogenesis (Newbold and Rode, 2006). It was hypothesized that organic acids would increase the propionate to acetate ratio, thereby decreasing H_2 availability for methanogens. However, the effectiveness of organic acids as a cattle CH_4 mitigating strategy are highly variable between experimentations. When the organic acids DL malate and fumarate were added to ruminal *in vitro* fermentations there was little effect on CH_4 concentrations (Callaway et al, 1997). Interestingly, when DL malate was fermented in the presence of monensin, CH_4 production decreased as inclusion levels of DL malate increased (Callaway et al., 1997). In an *in vivo* study when 100 g/kg fumarate was supplemented to growing lambs, depending on type of encapsulation of the acid, CH_4 emissions were reduced by 62% to 76% (Williams et al., 2009).

Contrastingly, in other *in vivo* trials when fumarate was fed to growing beef cattle it was not found to significantly reduce CH_4 emissions (McGinn, 2004; Beauchemin et al., 2010). Ultimately, additional studies need to be performed to determine the effectiveness of dicarboxylic organic acids usage in large scale cattle operations.

Defaunation

The anti-methanogenic effects of many feed additives and dietary treatments have been directly or indirectly associated with decreasing protozoa activity (Ffoulkes and Leng, 1988; Morgavi et al., 2012; Newbold et al., 2015). Elimination of protozoa (defaunation) has been suggested as a way to mitigate CH_4 emissions strategy (Newbold et al., 1995; Boadi et al., 2004; Hristov and Jouany, 2005), and the decrease in CH_4 production in the absence of protozoa has been observed both *in vitro* and *in vivo*. Studies have reported a range in CH_4 reduction from 5 to 20% (Hegarty, 1999; Martin et al., 2010). Although short term trials have demonstrated efficacy in reducing methanogen populations no long term defaunation trials have demonstrated efficacy.

U.S. cattle gastrointestinal CH_4 production

The beef cattle production chain can be divided between sectors including cow-calf, stocker, and feedlot. Life cycle analysis have determined that It was determined that the cow–calf system accounted for about 80% of total GHG emissions and the feedlot system only 20% (Beauchemin et al., 2010). Of the cow-calf sector approximately 84% of gastrointestinal CH_4 was from mature cows. A review by Broucek (2014) determined that CH_4 emissions from beef cattle ranged from 161 to 323

g of CH_4 per day. For mature beef cows, emissions range from 240 g to 396 g of CH_4 per day (Broucek, 2014). It has been suggested that to reduce emissions, additional CH_4 mitigation research should be devoted to the cow-calf sector.

U.S. EPA ruminant CH_4 estimates

There are two dominant factors when estimating livestock production of CH_4, average daily feed intake as gross energy (GE; MJ/d) and CH_4 conversion rates (Y_m; Crosson et al., 2011). Animal intake and digestibility are the factors impacting of Y_m, while DMI is the largest contributing factor for total CH_4 production (Hristov et al. 2013). Dry matter intake for cattle varies depending on diet, age, sex, region and stage of production. International Panel of Climate Change (IPCC) assessments have estimated the Y_m of feedlot cattle 3.0 ± 1.0 while cattle consuming temperate-climate grasslands were estimated at 6.5 ± 1.0 (IPCC 2006).

Using IPCC's calculation and Tier 2 methodology (characterization of diets and animal growth curves for each category) the U.S. EPA estimated that for the year 2012 gastrointestinal CH_4 emissions for the U.S. cattle industry (both dairy and beef) were 6.71 CH_4 Tg yr^{-1}. For beef cattle 4.79 Tg yr^{-1}, approximately 170 g of CH_4 per head per day. In total, gastrointestinal CH_4 emissions from livestock in the U.S. constituted 25% of total U.S. anthropogenic GHG emissions.

Although this contribution may seem high, it is consistent with other estimates. The Emissions Database for Global Atmospheric Research (EDGAR) estimated that total U.S. cattle gastrointestinal CH_4 emissions for 2005 were 6.45 Tg y^{-1}, which was within 5% of EPAs assessment. Hristov et al. (2014) using a "bottom-up" approach,

similar to the U.S. EPA, estimated that total gastrointestinal CH_4 emissions from the total beef and dairy cattle herd to be were 6.24 Tg yr^{-1} which was comparable to current, U.S. EPA estimates.

However, other reports have suggested that the U.S. EPA underestimated ruminant GHG emissions. Using a "top-down" approach, Miller et al. (2013) concluded that the current U.S. EPA assessment substantially underestimated ruminant CH_4 emissions. In this study, U.S. anthropogenic CH_4 sources were estimated from atmospheric CH_4 observations, extensive spatial datasets, and a high-resolution atmospheric transport model. According to Miller's estimates, U.S. cattle CH_4 emissions from gastrointestinal fermentation were approximately 12.7 ± 5.0 Tg yr^{-1}, which was double the U.S. EPA assessments. However, this assessment was unable to be substantiated by animal scientists (Hristov et al., 2014).

Comparing emissions: Grass-fed vs. grain-fed beef

In the past 10 years, the demand for grass-fed beef has grown annually at a rate of 25-30% (Windrock International, 2012), in part due to the belief that grass-fed is more environmental friendly than conventionally raised beef (Walsh, 2008). Despite the recent increase in demand for grass-fed beef a life cycle analysis has demonstrated that grain-fed beef has a lower environmental footprint than grass-fed beef systems (Crosson et al., 2011; Peters et al, 2011; Capper, 2012).

Capper (2012) determined that if the U.S. resorted to consuming only grass-fed animals to meet the current beef demands, the number of cattle would need to increase by 64.6 million animals and this would require more than 200,000 square miles be

devoted to beef rearing, equivalent to 75% of the state of Texas (Capper, 2012). To

produce a billion kg of conventional beef, production systems require 56.3% fewer

animals, 24.8% less water, 55.3% less land, and require 71.4% of the fossil fuels

compared to grass-fed systems (Capper, 2012). To switch to an all grass-fed beef from a

GHG standpoint CH_4 emissions would increase by 0.35 Tg yr^{-1} (Capper, 2012). Overall,

the combination of technologies and management strategies used in conventional cattle

production greatly reduce resource use and GHG emissions per unit of beef produced as

compared to grass-fed operations (Capper, 2012).

Trends in the industry

Over the last several decades the beef industry has made tremendous

improvements in animal efficiency.   In 1977 it took 25% more animals to produce the

same amount of beef than in 2007 (Capper, 2011). In addition, the time to produce beef,

from calving to harvest, has been dramatically reduced. Today cattle on average are

harvested at 485 days compared to 30 years ago when cattle where harvested at

approximately 609 days (Capper, 2011). Ultimately, with improved genetics, nutrition,

and management, cattle are grown more efficiently and each animal produces greater

quantities of edible beef (Capper, 2011). Today's beef production requires 81.4% of

feedstuffs, 67% of the land resources and 87.9% of water as compared to systems from

the late 1970's (Capper, 2011).

According to the U.S. EPA from 1990 to 2012, emissions from beef cattle

gastrointestinal fermentation increased by 0.6 percent (US EPA, 2014). Yet, beef cattle

populations actually declined by 5 percent while beef production increased 14 percent

(U.S. EPA, 2014). This indicates that although emission factors per head are increasing, emission factors per unit of product are going down and the U.S. is able to produce more beef with fewer emissions.

GHG emissions for protein sources-rice and wild ruminants

The world population is expected to grow to 9.5 billion people by 2050 (FAO, 2009). In order to meet the nutritional needs of a growing population, world-wide food production must increase by 100% and protein resources need to increase by 70% (FAO, 2011). However, in order to counter the effects of climate change, GHGs need to be immediately reduced, rendering food production a conundrum. Although there is no food production "silver bullet", life cycle analyses can be performed to help determine which food sources produce the greatest amounts of specific nutrients at the lowest GHG impact.

While beef production produces a significant amount of CH_4, it is not the only methanogenic food source. Rice, one of the most widely consumed human foodstuffs in the world, produces large quantities of CH_4 (Yan et al., 2009; Neue, 2014). Specifically, global rice production accounts for 31-112 Tg CH_4 yr^{-1} compared to the estimate of worldwide total ruminant production of 76 to 92 Tg CH_4 yr^{-1} (IPCC, 2007). In the United States rice is the third largest agricultural CH_4 source, producing more than 0.35 Tg CH_4 yr^{-1} (U.S. EPA, 2014).

Historically and currently, cattle are not the only large ruminants in the United States. Wild ruminants, such as bison, elk, and deer produce large quantities of CH_4 on a per head basis (Crutzen et al., 1986; Galbraith et al., 1998; Kelliher and Clark, 2010).

It has been estimated that wild ruminant produce 9% of GE as CH_4 (Crutzen et al.,

1986), which is significantly higher than either grass-fed or grain fed cattle, 6.5% and

3% respectively (Dong et al., 2006). Currently, wild ruminants contribute a low

proportion of U.S. greenhouse gases, approximately 0.28 Tg yr^{-1} (Hristov, 2012).

However, prior to European settlement (pre-settlement) approximately 30-75 million

bison inhabited the United States (McHugh, 1972; Dary, 1989; Isenberg, 2001). Pre-

settlement CH_4 emissions for wild ruminants for the contiguous U.S. has been estimated

from 2.9 to 7.3 Tg yr^{-1}, depending on assumed bison population size (30-75 million).

Assuming a population of 50 million, bison would have historically produced 86 percent

of the present-day CH_4 emissions from domestic ruminants in the U.S. (Hristov, 2012).

Overall, this points out that there has always been CH_4 production from a large ruminant

population in North America and brings into question whether or not todays cattle

production should be condemned for their associated GHG emissions.

Conclusion

Cattle production, ruminant nutrition, GHG production, and food security and

sustainability are complex and inextricably linked. With continual improvements and

improvements in technology and beef cattle management, beef production in the U.S.

has become more efficient. Currently, the U.S. is able to produce more beef, with fewer

animals and resources, without increasing herd gastrointestinal CH_4 emissions (Capper,

2011; U.S. EPA, 2014). Although cattle production requires a significant investment of

resources, it still plays an important role in the United States food supply, and

production of beef and dairy when viewed as part of the larger world-wide food supply

is a sustainable source of high quality protein for a growing world population.

CHAPTER III

METHANE AND VFA PRODUCTION RATES BY *IN VITRO* MIXED RUMINAL

MICROORGANISMS FERMENTATIONS OF PURIFIED CARBOHYDRATES AND

A VARIETY OF N SOURCCES

Introduction

Ruminants are able to degrade cellulose due to the symbiotic relationship they

have with the native anaerobic microorganisms that inhabit their rumen (pre-gastric

fermentation chamber). However, cellulose utilization under anaerobic conditions

requires the regeneration of reducing equivalents, often by interspecies hydrogen transfer

resulting in CH_4 production. Methane represents an energetic loss from 2 to 8% of gross

energy to the animal, as well as a loss of profit to the producer (Dong et al., 2006). In

2012, the U.S. EPA described gastrointestinal fermentation from livestock, particularly

beef cattle, as one of the leading causes of agriculturally-related CH_4 emissions in the

United States, accounting for approximately 25% of total anthropogenic CH_4 emissions

(U.S. EPA, 2014). Furthermore, CH_4 has a 12-year atmospheric lifetime and global

warming potential 25 times greater than that of CO_2 (U.S. EPA, 2016), thus strategies to

mitigate gastrointestinal CH_4 production are of critical interest worldwide, to

environmentalists, as well as to cattle producers.

Ruminal CH_4 production models can be utilized as an instrument to help reduce

emissions at the animal, farm, regional and global scale (McAllister et al., 2011). These

models are not only vital from an agricultural systems perspective, but from both policy-

making and regulatory perspectives. Although, predictive modeling has become an essential tool in animal nutrition, its accuracy is difficult to assess and at times is less precise than desired (McAllister et al., 2011). Carbohydrate degradation kinetics have been extensively researched (Groot et al., 1996) and are incorporated into the Cornell Net Carbohydrate and Protein System (CNCPS) model (Russell et al., 1992; Sniffen et al., 1992); however, research has not established a definitive link between the kinetics of carbohydrate degradation and the rate and extent of CH_4 production. Therefore the objective of this study was to 1) determine the concentrations of VFA and CH_4 from mixed ruminal microorganism degradation of purified carbohydrate substrates fermented in a the presence of a variety of nutrient conditions and 2) formulate a fractional rate for the conversion of carbohydrates into CH_4.

Materials and methods

Study 1: Methane and VFA production rates by in vitro mixed ruminal microorganism fermentations of purified carbohydrates

All animals were maintained in accordance with a protocol approved by the Southern Plains Agricultural Research Center Animal Care and Use Committee. One Holstein steer (BW 550 kg) and one Jersey cow (BW 360 kg) were provided *ad libitum* access to water, minerals, and Bermudagrass pasture at all times. Ruminal contents were collected by hand from at least five locations (at random) from the ventral sac of the rumen at 0700. Immediately after removal from the rumen, contents were squeezed through a fine mesh nylon strainer (Reaves and Co., Durham, NC) and pooled together,

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filling a 1000 ml flask (500 ml per animal). Ruminal fluid was then transported to the laboratory and incubated at 39 °C for 45 min, to allow gas production to buoy feed particles to the top of the flask and protozoa to sediment to the bottom. The middle layer of mixed ruminal fluid was combined (33% vol/vol) with an anoxic basal medium containing (per liter): 292 mg K_2HPO_4, 202 mg KH_2PO_4, 436 mg NH_4SO_4, 480 mg NaCl, 100 mg $MgSO_4 \cdot 7H_2O$, 64 mg $CaCl_2 \cdot H_2O$, 4,000 mg Na_2CO_3, 600 mg cysteine hydrochloride (Cotta and Russell, 1982). The resultant suspensions were anaerobically transferred to 18×150 mm Balch tubes (Bellco Glass, Vineland, NJ; 10 ml per tube) that contained 0 or 0.5 grams of carbohydrate substrate (amorphous cellulose, corn starch, or glucose), that were flushed under a CO_2 gas phase. Amorphous cellulose was cordially provided by Dr. P. Wiemer (USDA-ARS Dairy Forage Research Center). The amorphous structure of the cellulose was determined by x-ray crystallography (Isogai and Atalla, 1991). Tubes in triplicate for each of 8 time points (n = 24 tubes/substrate) were then sealed using butyl rubber stoppers with aluminum crimps and incubated for 24 h at 39 °C under a CO_2 headspace. Samples were collected from tubes (n=3 at each time point) after 0, 1, 2, 4, 8, 12, 18, and 24 h of incubation. Prior to liquid sample removal after 4, 12, and 24 h of incubation, a headspace gas sample (1.0 ml) was removed from each tube via gastight syringe and analyzed for CH_4 using a Gow Mac thermal conductivity series 550 gas chromatograph (Gow Mac Instrument, Bridgewater, NJ) equipped with a Carbosieve S 8100 column (Supelco, Inc., Bellefonte, PA). The gas flow (N_2) was 20 ml/min, and the column temperature was 125 °C and the detector temperature was 150 °C. Immediately upon opening, pH for each fermentation tube was

measured using an pH meter (Orion 2 Star) and ruminal fluid samples (5 ml) were collected and centrifuged ($10,000 \times g$, 10 min, 24 °C) to remove particulate matter, and the resultant supernatant was frozen at -20 °C until further analysis. Volatile fatty acids were determined using an Agilent 7890A gas chromatograph equipped with an FID detector, and an Agilent 7693 autosampler. Agilent DB-FAPP capillary columns were used and the inlet temperature was 230 °C, the oven temperature ranged from 40 °C to 200 °C, and the detector was heated to 300 °C.

Study 1 was conducted as a completely randomized design with a 6×3 factorial treatment structure. Factors included incubation time (IT) and carbohydrate (CHO) as fixed effects, and triplicate was treated as a random effect. Carbohydrates included corn starch, purified glucose and amorphous cellulose. Data were analyzed using the mixed models procedure of SAS® 9.4. Model effects included IT and CHO and all 2-way interactions. Means within time were separated using Tukey's HSD (honest significant difference) using the PDMIX800 macro of SAS. The exponential equation, $[CH_4]=A-B*exp^{-kf*time}$, was formulated to fit CH_4 concentration using PROC NLIN of SAS. Kf was representative of the fractional rate, A was the asymptote, and B was the slope.

Study 2: Methane and VFA production rates by in vitro mixed ruminal microorganism fermentations of purified carbohydrates and a variety of N sources.

All animals were maintained in accordance with a protocol approved by the Texas A&M University Animal Care and Use Committee. Two ruminally cannulated Angus cross steers (BW 350kg) were provided *ad libitum* access to water and minerals at all times. Rumen fluid was collected from cattle fed 0.9 kg/d of dried distiller's grains

with *ad libitum* access to pasture and Bermudagrass hay at the time of ruminal fluid collection. Ruminal contents were collected by hand from at least five locations (at random) in the ventral sac of the rumen at 0700. Ruminal contents were squeezed through 8 layers of cheesecloth and transported to the laboratory and prepared as described in Study 1.

Rumen fluid containing mixed ruminal bacteria was anaerobically transferred (33% v/v) to nitrogen-free anoxic media as described above (Chen and Russell, 1991). Mixed ruminal microorganism media was subdivided into four aliquots, based upon added nitrogen source. Each aliquot contained 0 mg/L added nitrogen (NF; negative control), or 900mg/ L of either: ammonia (NH_3), casamino acids and trypticase (AA), or an equimolar mixture of NH_3 and the casamino acids and trypticase mixture (AA+NH_3). Each ruminal fluid aliquot containing these nitrogen sources were anaerobically transferred into Balch fermentation tubes that contained one of the three carbohydrate sources used previously (amorphous cellulose, glucose, starch) to achieve final concentrations of 1600 mg/L. Fermentations of each N source with no added carbohydrate were used to estimate the contribution of the residual carbohydrate in the ruminal fluid and these negative controls were subtracted from production values.

Triplicate tubes (n = 3) for each time point (n = 36 tubes/substrate) were then sealed using butyl rubber stoppers with aluminum crimps and incubated for 24 h at 39 °C under a CO_2 atmosphere. Headspace gas samples and pH were measured at times 4, 12, and 24 h, samples were frozen for later analysis. Immediately upon opening, pH for each fermentation tube was measured using an pH meter (Orion 2 Star) and ruminal

fluid samples (5 ml) were collected and centrifuged (10,000 $\times$ g, 10 min, 24 °C) to remove particulate matter, and the resultant supernatant was frozen at -20 °C until further analysis. Procedures for gas and VFA measurements were as described above.

Study 2 was implemented as a randomized complete block design with a 3 $\times$ 4 factorial treatment structure, and day was treated as a blocking factor. Data were analyzed using PROC MIXED of SAS. Model effects included the fixed effects of IT, N and CHO source, and all 2- and 3-way interactions. Means within time were separated using Tukey's HSD in the PDMIX800 macro of SAS. Estimation of exponential production of CH_4 production was determined using a nonlinear regression. The exponential equation, $[CH_4]=A-B*exp^{-kf*time}$, was formulated to fit CH_4 concentration using PROC NLIN of SAS. Kf was representative of the fractional rate, A was the asymptote, and B was the slope. Equations for both studies were divided by CHO treatment.

Results

Study 1

There was a CHO treatment effect and an IT $\times$ CHO treatment interaction on pH ($P < 0.01$; Figure 2.1). During fermentation there were no differences in pH between substrates until 2 h, when glucose presented with lower pH, followed by starch and cellulose ($P < 0.05$). The pH declined for all CHOs over the 24 hour period ($P < 0.05$). By 24 h glucose and starch fermentations reached their nadir at pH's of 4.6 and 4.9, respectively; whereas cellulose fermentations was 5.6 ($P < 0.05$). During the

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fermentation the pH did fall below 5.5 suggesting that fibrolytic bacteria may have been

inhibited (Russell and Dombrowski, 1980; Hoover, 1986).

Figure 2.1: pH of fermentations for each carbohydrate source (2500 mg/L) in mixed ruminal microorganism fermentation in Study 1

There was both a CHO treatment effect, and an IT $\times$ CHO treatment interaction

for CH_4 production ($P < 0.01$; Figure 2.2). Methane production differed between

substrates at all time points except at 1 h. By 2 h, glucose was fermented the most

rapidly and produced significantly higher concentrations of CH_4 than both starch and

cellulose ($P < 0.05$). Fermentations containing glucose continued to produce greater

concentrations of CH_4 through 8 h ($P < 0.05$). Starch fermentation CH_4 concentrations

at 12 h were 29% higher than glucose and 52% greater than cellulose ($P < 0.05$). By 18

h, starch containing fermentations contained the greatest concentration of CH_4, followed

by cellulose, then glucose ($P < 0.05$). At 24 h, cellulose concentrations were similar to starch ($P > 0.05$) and 49% greater than glucose ($P < 0.05$).
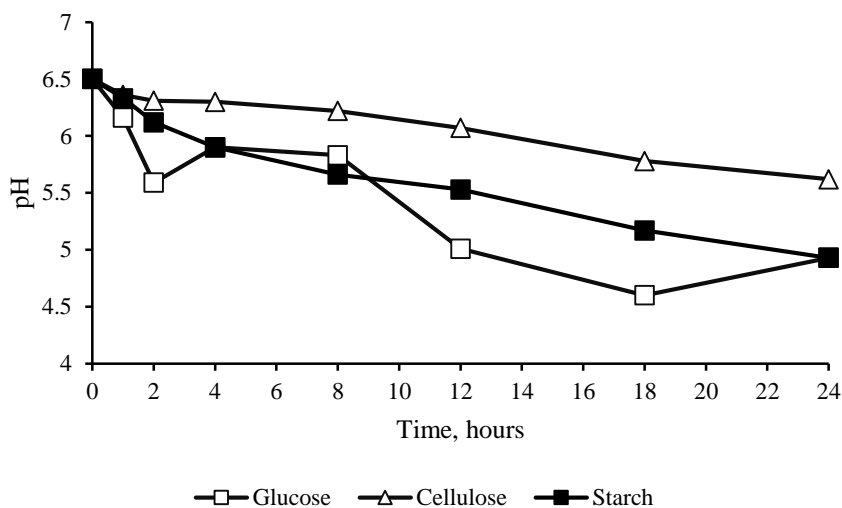

Figure 2.2: Methane production of fermentations of each carbohydrate source (2500 mg/L) in mixed ruminal microorganism fermentation in Study 1

Volatile fatty acid (VFA) production demonstrated a CHO treatment effect and an IT $\times$ CHO treatment interaction ($P < 0.01$; Figure 2.3). From 2 h through 4 h fermentations of glucose produced more VFA than those of starch or cellulose ($P < 0.05$). However, at 8 h starch fermentations contained the highest concentrations of VFA; concentrations were 32% greater than glucose ($P < 0.05$) and 96% higher than cellulose ($P < 0.05$). At 12 h VFA concentrations produced from fermentations of cellulose increased from 1.27 mM to 12.36 mM. Although VFA production from cellulose fermentation increased, starch and glucose fermentations contained higher concentrations than cellulose fermentations, (24% and 66% greater, respectively [$P <$

0.05]). By 18 h glucose and cellulose fermentations VFA concentrations were similar ($P > 0.05$), and starch concentrations were 31% greater than the other CHO fermentations ($P < 0.05$). By 24 h, VFA concentrations in starch fermentations were 25% greater than those in cellulose fermentations ($P < 0.05$) with VFA concentrations from glucose fermentation being intermediate between starch and cellulose ($P > 0.05$ to each). There was no effect of CHO source on the acetate to propionate (A:P) ratio ($P > 0.05$) at any time point.

The nonlinear regression equation, $A - B*\exp^{-kf*time}$, criteria was met for glucose CH_4 production (Table 2.1). A kf value for CH_4 concentrations was valued at 0.31 (Table 2.1). Cellulose CH_4 production was unable to converge. Starch CH_4 production was able to converge, but the kf value of zero was in the confidence interval, suggesting a zero rate.

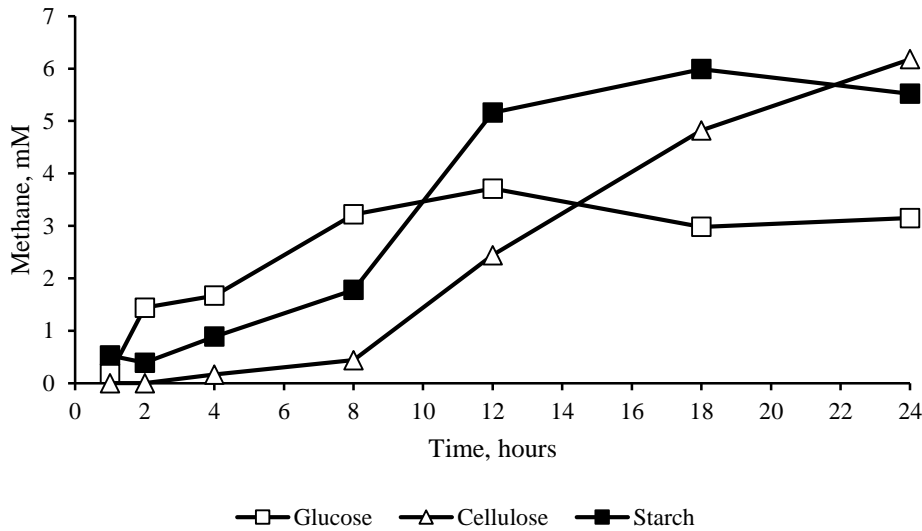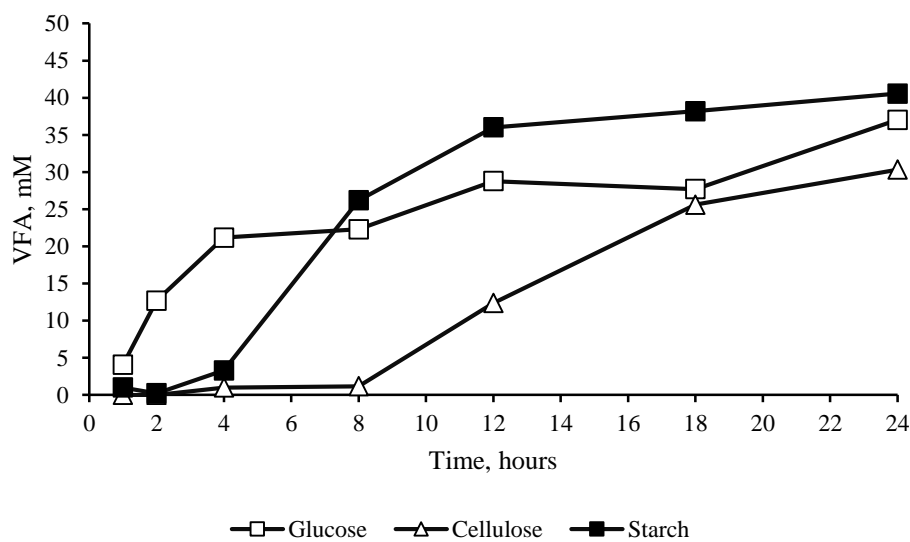Figure 2.3: Volatile fatty acid (VFA) production of fermentations of each carbohydrate source (2500 mg/L) in mixed ruminal microorganism fermentation in Study 1

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Table 2.1: Fractional rates of CH$_4$ production among CHO sources

CHO	Variable	Estimate	Approx. STD Error	Approximate 95% Confidence Limits	
Study 1					
Glucose	a	3.31	0.17	2.95	3.68
	b	4.18	0.54	3.03	5.32
	kf	0.319	0.084	0.14	0.5
Study 2					
Starch	a	5.52	0.296	4.92	6.12
	b	10.59	1.33	7.89	13.3
	kf	0.18	0.038	0.11	0.26

Study 2

Glucose pH decreased the most rapidly of the CHO sources, from 6.3 to 5.6 within the first four hours ($P < 0.05$; Figure 2.4). The pH of both cellulose- and starch-containing fermentations did not decrease until 12 h; but by 24 h, glucose fermentation pH increased to 5.8, while cellulose- and starch-containing fermentation pH decreased to 5.94 and 5.76, respectively ($P < 0.05$). At no time did the pH fall below 5.5, suggesting that fibrolytic bacteria were not completely inhibited in these fermentations (Russell and Dombrowski, 1980; Hoover, 1986). In this study there was no effect of N source on pH ($P > 0.05$).

There was a CHO treatment effect and an IT × CHO treatment interaction in CH$_4$ production ($P < 0.01$ Figure 2.5). During initial fermentation at 4 h, glucose was fermented the most rapidly and resulted in CH$_4$ concentration approximately 61% greater than both starch- and cellulose-containing fermentations ($P < 0.05$). At 12h of

fermentation, starch fermentations contained the concentrations of CH_4 that were 43%

higher ($P < 0.05$) than glucose- and cellulose-containing fermentations which had

similar CH_4 concentrations ($P > 0.05$).

This pattern continued at 24 h and starch fermentation produced approximately

30% more CH_4 than did cellulose or glucose fermentations ($P < 0.05$). There was an

effect of N source on CH_4, but this only became significant at 24h ($P < 0.05$; Figure 2.6),

at which time NH_3 and NF containing fermentations produced 24% more CH_4 than did

fermentations containing amino acids ($P < 0.05$).

Figure 2.4: pH of fermentations for each carbohydrate source (1600 mg/L) in mixed ruminal microorganism fermentation in Study 2

Figure 2.5: Methane production of fermentations of each carbohydrate source (1600 mg/L) in mixed ruminal microorganism fermentation in Study 2

Figure 2.6: Methane production of fermentations of each nitrogen source (900 mg N/L) in mixed ruminal microorganism fermentation in Study 2

Volatile fatty acid concentrations were affected by N source, CHO source, and their subsequent two and three-way interactions with IT (to be consistent) ($P < 0.01$; Figure 2.7 and 2.8). At 4 h, glucose fermentations contained greater proportions of VFA than either cellulose or starch fermentations, (56% and 73% greater, respectively [$P < 0.05$]). By 12 h, both glucose and starch fermentations contained higher VFA concentrations than did cellulose fermentations ($P < 0.05$). At 24 h, glucose fermentations contained marginally higher VFA concentrations than starch fermentations ($P = 0.05$), and significantly greater concentrations than from cellulose fermentations ($P < 0.05$). There were no distinguishable differences between N sources on VFA production at 4 h or 12 h ($P > 0.05$; Figure 2.8). However at 24 h, NH_3 treatments produced on average 40% greater VFA concentrations than all other N treatments ($P < 0.05$).
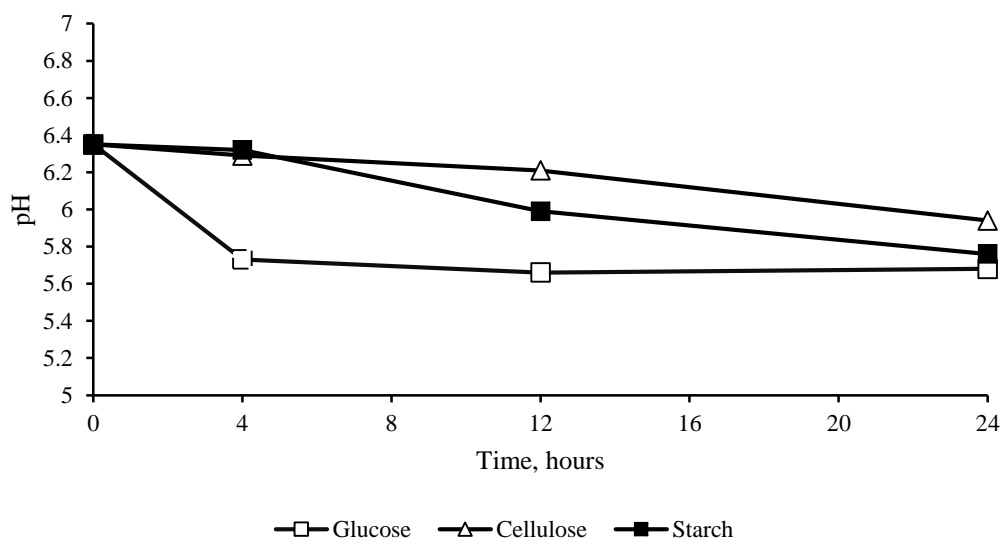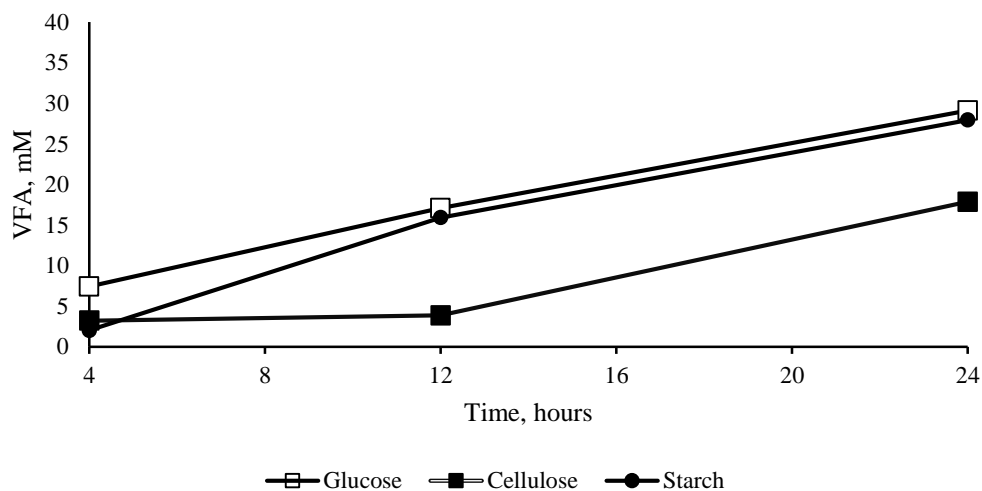

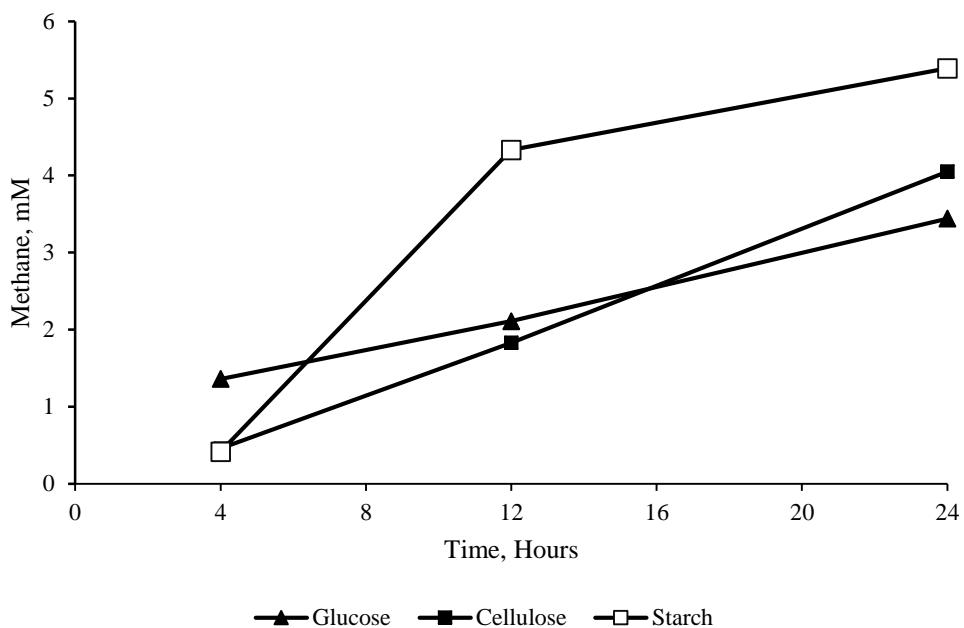Figure 2.7: Volatile fatty acid (VFA) production of fermentations of each carbohydrate source (1600 mg/ L) in mixed ruminal microorganism fermentation in Study 2

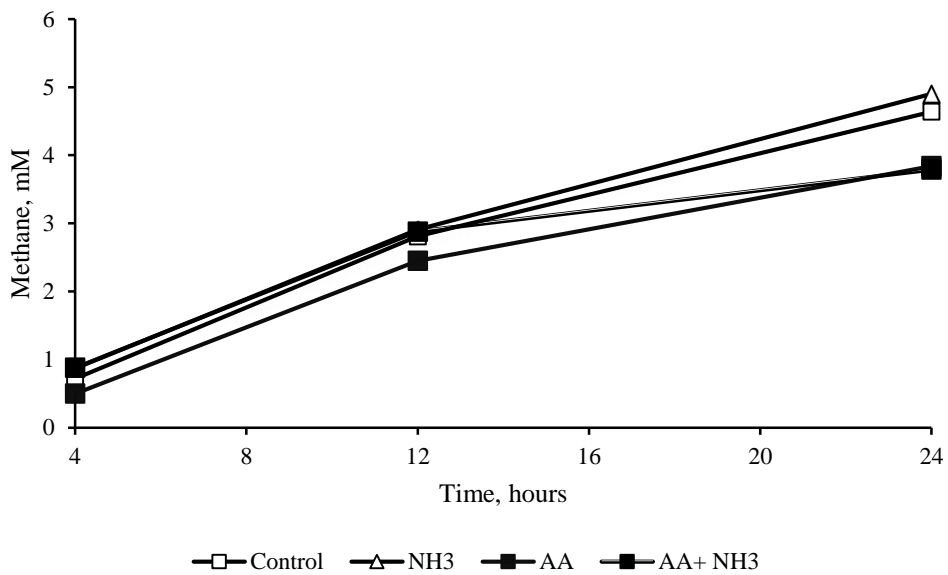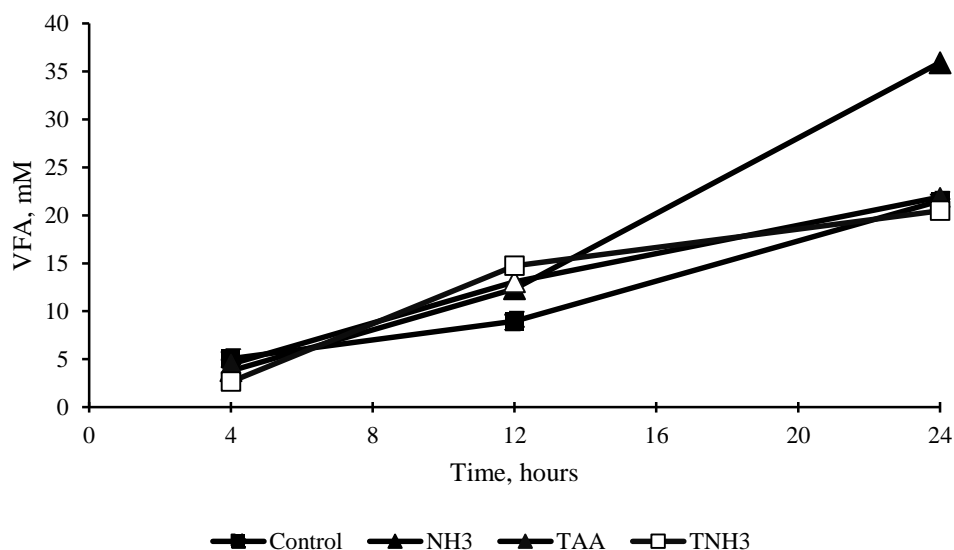Figure 2.8: Volatile fatty acid (VFA) Production of fermentations of each nitrogen source (900 mg N/L) in Study 2

There was an IT $\times$ CHO treatment interaction on A:P ratio ($P < 0.01$). At 24 h, cellulose had the highest A:P ratio, followed by starch and glucose ($P < 0.05$). There was no effect of N treatment on A:P ratio. In Study 2, CH_4 production rates from starch fermentation converged to fit the nonlinear rate equation, $A-B*exp^{-kf*time}$, with a kf value of 0.18. Neither cellulose nor glucose CH_4 productions converged with the equation (Table 2.1).

Discussion

In both studies, there were effects of CHO source on CH_4 concentrations at different times. As expected, glucose was the most readily available carbohydrate.

In Study 1 and Study 2, glucose fermentations produced the highest concentrations of CH_4 within the first four hours, followed by starch and cellulose fermentations. This result was expected because complex carbohydrates, such as starch and cellulose, take longer to be fermented than do more simple carbohydrates, such as glucose (Beuvink and Spoelstra, 1992; Cone and van Gelder, 1999). Although, glucose initially (< 4 h) had 50% greater CH_4 concentrations than cellulose or starch, in both studies CH_4 concentrations during this early period were minute compared to later time points.

In Study 1, at 24 h cellulose fermentations had greater CH_4 concentrations than starch ($P > 0.05$) or glucose ($P < 0.05$; Figure 2.2). Contrastingly, during Study 2, starch-containing fermentations produced significantly greater concentrations of CH_4 than from cellulose at 24 h (Figure 2.5). One hypothesis for the different outcomes, may have been due to the varying diets in each trial. In the first study, animals were fed a diet lacking concentrate, compared to Study 2 which incorporated 0.9 kg of DDG. Rumen fluid collected from animals in Study 2 initially had lower pH than did rumen fluid collected during Study 1 (6.25 compared to 6.5). The incorporation of DDG may have caused the depression in ruminal pH, selecting for more starch fermenting bacteria in the ruminal microbiome rather than fibrolytic bacteria.

Fermentations lacking amino acids resulted in a higher level of CH_4 production compared with fermentations containing amino acids (Figure 2.6). Previous studies have indicated that the inclusion of protein on *in vitro* fermentations decreased total CH_4 production (Cone and van Gelder, 1999). The increase in methanogenic activity in fermentations without amino acids may be similar to the phenomenon of energy spilling.

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Bacteria spill energy in non-growth functions if their growth medium is nitrogen-limited and the energy source is in 'excess' (Russell, 2007). It has been concluded that many bacteria have this reaction in order to dissipate excess ATP when the catabolic rate is faster than the anabolic rate (Russell and Cook, 1995). As a result when energy spilling occurs large amounts of H_2 are dissipated that subsequently can be used by methanogens, which can lead to an increase in CH_4 production.

Ammonia inclusion resulted in the greatest VFA production amongst all N sources (Figure 2.8). This result could also be linked to an energy spilling effect. Without a source of amino acids bacteria were unable to use their N source rapidly enough along with the carbon skeletons derived from CHO fermentation for microbial crude protein synthesis, and as a result diverted their carbon to VFA. Treatments lacking any N source produced VFA concentrations similar to those of treatments that contained amino acids. This may be due to the fact that although there was no additional nitrogen added to the treatments, residual nitrogen was included in the rumen fluid and bacterial protein from cell lysis and death would be available over time. This would be plausible because the ration was not nitrogen limiting and DDG are included as a high quality protein source (Belyea et al., 2004).

As expected both experiments had a CHO $\times$ IT interaction on VFA production. However, results differed between experiments. In Study 1 and 2, glucose fermentation produced the greatest concentrations of VFA compared to both starch and cellulose treatments at 4 h ($P <0.05$). This result was expected for unlike polymers (starch and glucose), glucose can immediately be converted to VFA and fermentation of glucose

does not generally demonstrate a lag time before fermentation (Beuvink and Spoelstra, 1992). In Study 1, at 24 h starch produced marginally higher concentrations of VFA than glucose. Yet in Study 2, glucose fermentation produced greater VFA concentrations as compared to starch.

In Study 2, at 24 h glucose fermentations contained the lowest A:P ratio (P <0.05) as well as the lowest CH_4 concentrations compared with starch or cellulose fermentations (P <0.05). An increase in propionate concentration decreases in hydrogen availability for methanogens, which can lead to a reduction in CH_4 in the rumen (Russell and Rychlik, 2001). Surprisingly, starch and cellulose fermentations produced similar A:P ratios, which is atypical of these very different substrates. Starch fermentations are known to have a lower A:P ratio than cellulose fermentations (Armentano and Young, 1983), so it is unknown why in this experiment starch and cellulose had similar A:P ratios, but it may be related to the amorphous nature of the cellulose used in this study.

Rates were unable to be determined for all of the CHO treatments in both Study 1 and 2. Although a formula converged for starch in Study 1 and cellulose in Study 2; the kf value of zero was in the confidence interval, indicating a linear relationship for fermentations. However, carbohydrate degradation is known to be an exponential relationship (Russell et al., 1992; Sniffen et al., 1992; Russell, 2009), making it very unlikely that the conversion of amorphous cellulose to CH_4 is a truly linear relationship. Lengthening the fermentation time of cellulose beyond 24 h could exhibit a nonlinear production rate for CH_4. In addition to more accurately fit fractional rate equations to

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CH$_4$ production, additional measurements would need to be taken over the fermentation period amongst all CHO treatments.

Conclusion

This is one of the first studies to determine the effects of a purified CHO and variety of N sources on *in vitro* ruminal fermentation. Results indicated that both CHO and N sources had a profound impact on CH$_4$ and VFA production over time. Collectively, the results gathered in these studies are the first steps necessary to build nonlinear rate equations for the conversion of carbohydrates to CH$_4$ and one day may be used to improve the predictive accuracy of CH$_4$ models.

CHAPTER IV

COMPARING METHANE GENERATING PROTEIN SYSTEMS

Introduction

The recent discussion over food sustainability and livestock production comes at a time when the awareness of the effects of global climate change has never been greater (Tedeschi et al., 2015). According to the U.S. EPA (2014); "Methane emissions from enteric (gastrointestinal) fermentation represent 25.0 percent of anthropogenic activities." Methane, a potent greenhouse gas (GHG) with a 12-year atmospheric lifetime and global warming potential 25 times higher than that of carbon dioxide (EPA, 2016), has significant environmental ramifications (Stephenson, 2009). Mitigating climate change must include greenhouse gasses (GHG) reduction. Yet, over the next 25 years, the predicted exponential human population growth necessitating protein production to increase by 70% (IPCC, 2006). Although cattle production provides the population with an abundant and complete source of protein (National Academy of Sciences, 2005; U.S. Department of Health and Human Services, 2016), some believe because cattle contribute to anthropogenic GHG that they are a non-sustainable food source (Pollan, 2002; Bittman, 2008). This conflicting issue involving environmental vs. human protein needs has been referred to as "wicked problem" (Peterson, 2011). However, one factor that has been remiss from the dialog is that beef cattle have not always been the dominant source of agricultural CH_4 emissions nor is it the only source that should be examined. Therefore, in an effort to determine the efficiency of current

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animal protein producing systems both on nutritional and environmental bases life cycle

assessments (LCA) have been performed.

In a recent LCA, it was determined that beef production was the least efficient

protein source and produced five times more GHG than the average of the other

livestock (egg, swine, dairy, and poultry) protein producing categories (Eshel et al.,

2014). In another LCA it was determined that the increase in dairy consumption

suggested in 2010 USDA dietary guideline recommendations, was not a sustainable

decision because of dairy cows associated GHG emissions (Heller and Keoleian,

2015). Subsequently, cattle production have been thought to be a non-sustainable food

source (Pollan, 2002; Bittman, 2008).

Although these assessments addressed cattle substantial production of

agricultural CH_4, over the course of United States' history, there has been a multitude of

agricultural food sources, other than cattle, that have substantially contributed to CH_4

emissions that were not taken into account. For example, Bison, elk and deer, like cattle,

are ruminants and it has been theorized that prior to European settlement (pre-

settlement) produced sizable amounts of CH_4 (Subak, 1994; Hristov, 2012). In addition

to wild ruminants the grain rice, one of the most heavily consumed food sources in the

world, is methanogenic, constituting one of the largest sources of agricultural CH_4

emissions (van Groenigen et al., 2013; IPCC, 2007). In order to understand and address

the impending issues of food sustainability, the efficiencies of pre-settlement and current

methanogenic agricultural food sources should be compared on an CH_4 output and

human-edible protein basis. To our knowledge, such comparisons have not been

performed. Thus, the objective of this study was to compare current cattle producing food sources (beef and milk) to pre-settlement populations of bison, elk and deer, and current rice production on a CH_4 emitted to human-edible protein output basis.

Materials and methods

Literature sources, both historic and present day, were used to determine historic ruminant CH_4 emission and human-edible protein production. The following equation were used to calculate CH_4 emissions and human-edible protein for wild ruminants:

Human-edible Protein Produced = Animal Live Weight (kg) × percent of hot   [1]
carcass × percent of human-edible meat per hot carcass × protein content
(g/kg of product) × population size × sustainable harvest rate

Annual Herd CH_4 Production = CH_4 yield per unit of DM ((g of CH_4)/(kg of   [2]
DMI)) × DMI ((kg per head)/day) × 365 days × population size

Methane Emitted to Human-edible Protein Produced = Herd CH_4 Emissions   [3]
/ Annual Herd Human-edible Protein Produced

Assumptions that had to be made for this species included intake, weight, and population size. It was postulated that pre-settlement, prior to the settlement of Europeans, bison populations could have exceeded 75 million, although many sources

have suggested that the maximum carrying capacity for bison could not exceed 30 million head, this estimate was used for this analysis (Dary, 1989; Isenberg, 2001). Bison intake was based on Kelliher and Clark (2010), calculated dry matter intake (DMI) based on IPCC Tier 2 methodology, estimated that bison consumed 2.2% of body weight as DM. Previous assessments of bison mass for females and males ranged from 318-554 and 544 to 977 kg, respectively (Meagher, 1986). Assuming a 1:1 male to female sex ratio the bison weight on average 600 kg.

The annual sustainable harvest rate for bison populations was determined to be 15% of the herd, or 4.5 million animals (Frost, 2015). This value represented the largest population that could be harvested without decreasing population growth the subsequent year. Dressing percentage used for bison was 57% (NBA, 2016). Approximately, 70% of the hot carcass was boneless lean meat, which equated to 227 kg of human-edible meat (Hawley, 1986; Koch et al., 1995b). Average protein yield per carcass, assuming grass fed, averaged 202 g per kg of meat (USDA, 2015b). Total herd protein was calculated using Equation [1].

The amount of CH_4 produced per kg of DMI per day for this model was based on Hristov (2012) calculations, who determined that bison produced 21 CH_4 per kg of DMI consumed. Hristrov (2012) calculated this value off of the average of two previous estimates: 1) Galbraith et al. (1998) empirically based CH_4 losses of 20 g of CH_4 per kg of DMI per day, and 2) Kelliher and Clark (2010) who calculated that animals produced 21.4 g of CH_4 per kg of DMI per day. Dry matter intake was based off of Kelliher and

Clark (2010), who concluded using IPCC tier 2 methodology, that bison consumed 2.2% body weight as DM. Total CH_4 production was calculated using Equation [2].

Elk

Historic elk populations was valued at 10 million in the contiguous U.S. pre-settlement (Rockey Mountain Elk Foundation [RMEF], 2016). Body weight for elk averaged 227 kg for cows and 318 kg bulls (RMEF, 2016). Elk body weight, assuming a 1:1 sex ratio averaged 272 kg (RMEF, 2016). Field dressed weight (without hide, head, or feet) was determined to be 58% (Field, 2003b). The percent of consumable hot carcass was 58%, yielding 92 kg of human-edible meat per animal (Field, 2003b). Elk's sustainable harvest was based on 15% of population per year, or 1,500,000 animals (Frost, 2015). Protein yield per kg of human-edible meat averaged 213 g (USDA, 2015b). Total herd protein was calculated using Equation [1].

Based on Galbraith et al. (1998) and Hristov (2012) calculations, elk produced 16 g of CH_4 per kg of DMI per day. For the purpose of this analysis the amount of DM consumed relative to body weight (kg) was based on the Small Ruminant NRC (2007), valued at 2.3%. Total CH_4 production was calculated using Equation [2]. Methane to human-edible protein ratio was calculated using Equation [3].

Deer

Although a variety of deer species and sub-species currently exist in the United States, historically, there were two dominant species, White Tailed and Mule deer. Pre-settlement populations for White Tailed and Mule deer where approximately 30 million and 13 million, respectively (Peek, 2003). The size of Mule deer ranged from 60 kg to

128 kg (Bauer and Bauer, 1995). White Tailed deer averaged 50 to 136 kg (Fulbright and Ortega-S, 2006). Average weight between species, assuming a 1:1 female to male sex ratio, averaged 58 kg (Smith, 1991; Ferguson, 2005). The field dressed body weight (without skin, feet, or head) and dressing percentage among deer species averaged 34 kg and 59% (Field, 2003a). Approximately, 57% of the hot carcass was consumable, yielding 18 kg of human-edible meat (Field, 2003a). Protein yield per kg of human-edible meat averaged 223 g (USDA ARS, 2015). Sustainable harvest rate's for deer were higher than that of elk or bison, averaged 22.5% or 9.6 million head (Guynn, 1985; Frost, 2015). Total herd protein was calculated using Equation [1].

Methane emissions for both species of deer were based on Galbraith's (1998) and Hristov (2012) studies that calculated 10 g CH_4 were emitter per kg of DMI per day. Average DMI per animal was assumed to be 3.2% of total body weight, based on the NRC (2007). Total CH_4 production was calculated using Equation [2]. Methane to human-edible protein ratio was calculated using Equation [3].

Beef cattle

In 2012, total beef cattle populations in the U.S. totaled 77 million head (USDA National Agricultural Statistical Service [USDA NASS], 2012). This figure included, all beef cows, beef replacement heifers, bulls, beef heifers and steers. From this population a total of 43%, approximately 38 million animals, where harvested (USDA NASS, 2012). Federally inspected live weights and hot carcass weights across all beef cattle harvested for the year 2012 averaged 593 kg and 359 kg, respectively (Bertramsen, 2015). Percent of hot carcass consumable was 74.4%, based on yield 3 grade carcass

with 0.64 cm fat trim, yielding 251 kg of human-edible (Griffin et al., 1992). Total

edible protein per kg of human-edible meat was determined to be 214 g (United States

Department of Agriculture [USDA], 2015b). Using EPA's estimations for

gastrointestinal CH_4 emissions for 2012, total herd emissions were valued at 4,789 CH_4

Gg yr^{-1}, approximately 170 CH_4 g per head per day (U.S. EPA, 2014). Total herd

protein was calculated using Equation [1]. Annual herd CH_4 production was then

divided by annual herd human-edible protein produced to formulate a CH_4 emitted to

human-edible protein ratio.

Dairy cattle

In 2012, there were 13.7 million head of dairy cattle in the United States

including cows and replacement heifers (USDA NASS, 2012). In the same year the total

herd produced 9.10×10^{10} kg of milk (USDA NASS, 2012). For a kg of milk, there were

36.6 g of protein (USDA Nutrition, 2015). Methane production for dairy cattle was

based on the EPA Inventory of U.S. Greenhouse Gas Emission and Sinks: 1990-2012,

which utilized Tier 2 IPCC methodology and CH_4 values generated by the COWPOLL

model (Kebreab et al., 2008) and other models. Total herd CH_4 for the year 2012 was

estimated to be 1,668 Gg yr^{-1} or averaging 388 CH_4 g d^{-1} per head (U.S. EPA, 2014). It

was assumed that 100% of milk produced was consumable. Annual herd CH_4

production was then divided by total human-edible protein produced to formulate the

CH_4 to human-edible protein ratio.

Rice production

We chose to compare rice production to ruminant production for rice is one of the world's most common crops and it is known to produce CH_4. Predominate rice growing regions in the United States include Arkansas, California, and Louisiana (USDA, 2014). Long grain is grown almost exclusively in the south and accounts for more than 70 percent of U.S. production (USDA, 2012). Medium grain, grown both in California and the South, account for more than one-fourth of total U.S. production and forms most of California's rice crop. Arkansas accounts for most of the southern medium-grain production. Short grain accounts for 1-2 percent of total U.S. rice production and is grown almost exclusively in California. Among U.S. rice species harvested protein content averaged 68 g per kg of product (USDA, 2015b). Methane emissions from rice production was based on EPA Inventory of U.S. Greenhouse Gas Emission and Sinks: 1990-2012, valued at 351 CH_4 Gg yr^{-1} (U.S. EPA, 2014). In 2012, 2.6 million acres were harvested yielding 10.1×10^3 kg (USDA NASS, 2012). Approximately, 68% of the crop was consumable and consumable and retail ready (Wilson, 2015). Protein yield was determined by amount of kg of rice produced $\times$ percent consumable $\times$ g protein produced per kg or rice. Methane to human-edible protein ratio for rice was calculated by CH_4 emissions for the year 2012 divided by annual human-edible protein produced.

Results and discussion

Before European settlement the United States, plains and forests were dominated by wild ruminants including bison (*Bison Bison*), elk (*Cervus canadensis*), and deer (*Odocoileus virginianus* and *Odocoileus hemionus*). Like cattle, wild ruminants produce CH_4 as an end product of microbial fermentation. It has been speculated that during pre-settlement times, 30 to 75 million bison, along with millions of deer and elk, inhabited the U.S. (McHugh, 1972; Dary, 1989; Isenberg, 2001) and at this time were responsible for producing substantial amounts of CH_4 (Subak, 1994).

Determining CH_4 emissions from pre-settlement animals is a difficult task because neither daily animal emissions nor populations could be measured at this time. As such, many assumptions had to be made in this comparison. Factors determining emissions include forage type, DMI, and the size of the animal, which can be difficult to measure. Ruminants are classified as grazers and browsers, which makes it problematic to decipher DMI and type of forage being consumed. Crutzen et al. (1986) theorized that because wild ruminants lived entirely on roughage and herbs at maintenance levels, ruminants would produce on average 9 percent of gross energy lost in the form of CH_4 (Y_m). However, this value is difficult to confirm. Therefore, in order to empirically record wild ruminant emissions, Galbraith et al. (1998) built respiratory chambers for yearling female bison (195.7 kg $\pm$ 7.52), elk (151.3 kg $\pm$ 4.1), and deer (34.4 kg $\pm$ 1.45) (Galbraith et al., 1998a). Using the chambers, Y_m values for bison, elk and deer measured 6.6, 5.2, and 3 percent, respectively. Interestingly, these values were significantly lower than Crutzen's (1986) previous assessments. Animals utilized in

Galbaith's (1998) trials were fed a diet of alfalfa pellets and no long stem

forages. Feeding pellets rather than long stem hay, reduces rumen retention time, which

can decrease total CH_4 production (Galbraith et al., 1998b). This may be why

Galbraith's Y_m values were lower than Cruzen's (1986) estimates. To date, these are the

only empirically collected CH_4 data for wild ruminants and were used as the source for

elk and deer emissions and one of two sources for bison emissions in this model.

Bison

According to the National Bison Association (2015), average dressing

percentages of bison was valued at 57 percent. Dressing percentages in the literature

evaluated the dressed carcasses higher than the National Bison Association, closer to 60

percent (Hawley, 1986; Koch et al., 1995a; López-Campos et al., 2013). However, these

estimates were biased on farmed raised bison consuming a diet that consisted of both

forage and grain. For beef cattle fed 100 percent forages diet, animals yielded lower

dressing percentages than those that were finished on grain (Leheska et al.,

2008). Therefore, it can be reasonably assumed that free range bison dressing

percentages would be lower than grain fed animals. As such, the dressing percentage of

57, based on the estimate provided by National Bison Association, was used in this

analysis. Using the USDA Nutrition Database (2015), each animal produced 4.8×10^4 g

of protein (Table 3.2). Overall, this study determined that bison weighing 600 kg,

consuming 2.2 percent DMI of BW, producing 21 CH_4 g per kg DMI produced 277 CH_4

g per head per day (Table 3.1). For 30 million bison, producing 277 CH_4 g per head per

day a total of 3,035 Gg CH_4 yr^{-1} was emitted (Table 3.2). On a CH_4 to protein biases,

bison emitted 13.93 kg of CH_4 for every kg of human-edible protein produced (Table 3.2).

Table 3.1: Historic Ruminant Gastrointestinal CH_4 Emissions

Items	Bison	Elk	Deer
Body Weight, kg	600[1]	272[2]	57.6[3]
DMI of BW, %	2.2[4]	2.3[5]	3.1[5]
DMI, kg/head/d[6]	13.2	6.26	1.79
CH_4 Emissions, g/kg of DM/d	21	16	10
CH_4 Emissions, g/head/d	277.2	100.1	17.9

1 Meagher (1986)
2 Peek (2003)
3 Fulbright and Ortega (2006)
4 Kelliher and Clark (2010)
5 NRC (2007)
6 Galbraith et al. (1998)

Elk

For historic elk populations (10 million), assuming a skinned carcass percentage of 58 percent (Fields et al., 2003b), elk produced 19.5 kg of protein per animal or annually 2.9×10^7 kg of protein for the herd (Table 3.2). Elk weighing 272 kg, consuming 2.3 percent of body weight as DM, emitting 16 g CH_4 per kg of DMI, produced 100.1 g CH_4 d^{-1} (Table 3.1). For a population of 10 million elk, 365 Gg of CH_4 yr^{-1} was emitted (Table 3.2). For every kilogram of human-edible protein produced, 12.50 kg of CH_4 would be emitted, which was only slightly lower than bison's ratio (Table 3.2).

Deer

Although there were two species of deer used for this analysis, CH_4 emissions for both populations were based on Galbraith's et al. (1998) study using White Tailed deer

at measured 10 g of CH_4 per kg of DMI. Historic deer populations, assuming a field

dressing percentage of 59 percent, produced 4.04 kg of protein per animal. The herd

annually produced 4.2×10^7 kg of protein (Table 3.2). For deer weighing 58 kg,

consuming 3.1 percent of their BW as DM, producing 10 g of CH_4 per kg of DMI, deer

emitted 17.86 CH_4 d^{-1} (Table 3.1). For a population of 43 million deer, emission rate

was valued at 280 Gg of CH_4 yr^{-1} (Table 3.2). Thereby, 6.66 kg of CH_4 was emitted for

every kg of human-edible protein produced (Table 3.2).

Beef cattle

Beef cattle CH_4 emissions were based on the EPA Inventory of U.S Greenhouse

Gas Emissions and Sinks for 2012 (U.S. EPA, 2014). The U.S. EPA assessment used

IPCC's Tier 2 methodology incorporating a total of 177 input variables for cattle

emissions. Based on this assessment, gastrointestinal fermentation from beef cattle for

the year 2012 valued 4,789 Gg CH_4 yr^{-1}, averaging 170 g d^{-1} per head (U.S. EPA, 2014).

The EPA assessment included all beef cows, beef replacement heifers, bulls, and all

feedlot animals. The average hot carcass weight of all federal inspected cattle for 2012

averaged 359 kg (Bertramsen, 2015). Federally inspected cattle encompassed 98.5

percent of cattle slaughtered in the United States (Bertramsen, 2015). It was assumed

that the proportion of consumable meat from the hot carcass was 74.4 percent, based on

previous studies by Griffin et al. (1992) that broke down sub-primal yields of beef

carcasses (Griffin et al., 1992). Using USDA's Nutritional Database the raw beef

Table 3.2: Pre-settlement wild ruminant and current beef cattle gastrointestinal emissions of CH_4 and production of human-edible protein

Protein Source	Bison	Elk	Deer	Beef Cattle
Herd Size[1], in millions	30	10	43	77
Daily Animal CH_4[1,2], g/d/hd	277.2	100.1	17.86	170.4
Daily Herd CH_4, kg/d	8,316,000	1,000,960	767,808	13,113,100
Annual Herd CH_4, Gg/yr	3,035	365	280	4,790
Sustainable Harvest[3,4]	0.15	0.15	0.23	0.44
Head Harvested[5]	4,500,000	1,500,000	9,675,000	33,880,000
Live Weights[1,5], kg	600	272	58	593
Hot Carcass Weight[5], kg	342	190	34.2	359
Percent of Hot Carcass Consumable	0.70	0.58	0.57	0.74
Human Edible Product, kg	239	91.5	18.1	267
Human Edible Protein[7], g/kg	202	213	223	214
Protein Yield per Head, kg	48.4	19.5	4.35	57.1
Herd Protein Yield, Gg	218	29.2	42.1	2000
CH_4 per Human-edible Meat, kg/kg	2.82	0.40	0.33	0.23
CH_4 per Human-edible Protein, kg/kg	13.9	12.5	6.66	2.47

[1] See Table 3.1
[2] U.S. EPA (2014)
[3] Frost (2015)
[4] Guynn (1985)
[5] Bertramsen (2015)
[6] USDA (2015a)

composite of trimmed retail cuts, separable lean and fat, trimmed to 1/8 in. (0.32 cm) fat, all grades, averaging 214 g of protein per kg of beef, approximately 57.1 kg protein per harvested beef animal (Table 3.2). This equated to 2.47 kg CH_4 emitted for every kg of human-edible protein produced (Table 3.2, Table 3.5).

Historic wild ruminant herd emissions

We showed in this model that wild ruminates produced 77 percent of CH_4 emissions as compared to beef cattle emissions. However, this calculation was derived from Galbraith's empirically based data that could have underestimated bison emissions.

Using Crutzen's (1986) Y_m value of 9 percent, and Galbraith's et al. (1998) calculations of bison daily energy intake (kj), bison would produce 370 g of kg CH_4 d^{-1}. Using this value, wild ruminants produced approximately 4,047 Gg of CH_4 yr^{-1}, equating to 98% of beef cattle gastrointestinal CH_4 emissions for the year 2012.

Dairy

Heller and Keoleian (2015) determined that the increase in dairy consumption recommended by 2010 USDA dietary guidelines would contribute significantly to increased GHG emissions and would not be an ideal protein alternative to meat. Furthermore, this statement was repeated in the USDA Scientific Report of the 2015 Nutritional Guidelines Recommendations (USDA, 2015a). However, of all the food sources evaluated in our model, milk produced the fewest emissions per kg of human edible protein produced. For 13.7 million dairy cattle emitting 1,668 Gg CH_4 yr^{-1}, producing 91,000 Gg of milk yr^{-1}, with a protein content of 36.6 g per kg of milk, the CH_4 to human-edible protein ratio was 0.50 (Table 3.3). Overall, dairy produced 20.2% of beefs CH_4 emissions per kilogram of protein produced (Table 3.5). Our model shows the U.S. dairy production is extremely efficient at producing a food source.

Rice

We compared ruminants to rice production, not to condemn one food producing system over another, but merely to contrast it with CH_4 production for beef and milk protein productions. Rice, one of the most widely consumed human feedstuffs in the world, is methanogenic, constituting a predominant source of anthropogenic CH_4 (Agnihotri et al., 1999; Yan et al., 2009; Neue, 2014). It is expected that total rice

consumption will continue to increase concurrently with the rate of population growth

(Mohanty, 2012).

Table 3.3: Dairy cattle gastrointestinal CH_4 emissions and human consumable protein ratio

Herd Size[1], millions	13.7
Daily Head CH_4, g/d/hd	334
Daily Herd CH_4, kg/d	4,600,000
Annual Herd CH_4[2], Gg/yr	1,668
Annual Herd Milk Production[1], Gg/yr	91,000
Annual Milk Production per Hd, kg/yr	9,873.6
Percent Consumable, %	1.0
Consumable Product, kg	91,000
Consumable Protein[3], g/kg	36.6
Protein Yield per Head, kg	243
Herd Protein Yield, Gg	3,330
CH_4 per Milk, kg/kg	0.02
CH_4 per Human-edible Protein, kg/kg	0.50

[1] USDA NASS (2012)
[2] U.S. EPA (2014)
[3] USDA (2015a)

Therefore, it can be expected that rice production and its associated CH_4 emissions will

increase over the course of this century. Currently, worldwide rice production accounts

for 31-112 Tg of CH_4 yr^{-1}, which is similar to total ruminant production that accounts for

76 to 92 Tg CH_4 yr^1 (IPCC, 2007). Most of the world's rice, and all rice in the United

States, is grown on flooded fields (Borris, 2006). The flooding of field's induces the

aerobic decomposition of organic material that depletes soil oxygen creating anaerobic

growing conditions. Methanogens present in the soil produce CH_4 through the anaerobic

decomposition of soil organic matter (Holzapfel-Pschorn et al., 1985).

In 2012, 351 Gg of CH_4 was emitted from 2.68×10^5 acres of rice patties across the U.S. (Table 3.4; U.S. EPA, 2012; USDA NASS, 2016). When rice was compared to beef production (2.47 kg CH_4 per kg of human- edible protein) rice produced 66% fewer CH_4 emissions on a human-edible protein basis (Table 3.4, Table 3.5). When rice was compared to dairy, the dairy CH_4 ratio (0.50 kg CH_4 per kg of human-edible protein) lower than rice's ratio (Table 3.5).

Table 3.4: Rice CH_4 emissions and human-edible protein

Acre harvested[1], acre	268,000
Yield per Acre[1], kg/yr	3,400
Total Produced, Gg/yr	9,110
CH_4 per Hectare, g/d/ha	359
Daily CH_4, kg/d	962,000
Annual Total CH_4[2], Gg/yr	351
Percent of Crop Consumable[3], %	0.68
Human Edible Product, kg	6,195
Human Edible Protein[4], g/kg	68.0
Protein Yield per Acre, kg	157
Harvest Protein Yield, kg	421
CH_4 Emissions per Human-edible Rice, kg/kg	0.06
CH_4 Emissions per Human-edible Protein, kg/kg	0.83

[1]USDA NASS (2012)
[2]U.S. EPA (2014)
[3]Wilson (2015)
[4]USDA (2015a)

Table 3.5: CH_4 emissions to human-edible protein yield

Protein Source	CH_4 to Protein (kg/kg)	Relative to Beef, %
Bison	13.93	564
Elk	12.50	506
Deer	6.66	296
Beef	2.47	100
Rice	0.83	33.6
Milk	0.50	20.2

Conclusion

Currently, the U.S. has a population of 320 million people that is steadily increasing. In order to meet the protein demands of the country's growing population, both in terms of quantity and quality, food production must be produced as efficiently as possible. Additionally, in order to minimize the impact on the environment we need to generate food that minimizes negative environmental feedback. Thus, it is imperative that we evaluate and compare all current methanogenic food producing systems along with historic methanogenic food sources in order to make informed agricultural decisions that are advantageous for both human and environmental health. This model was the first of its kind to compare pre-settlement wild ruminants, and current rice, beef, and milk production on a CH_4 to human-edible protein basis. The implications of this model demonstrate that, when compared to other methanogenic producing food sources, beef may not be as an inefficient food source speculated previously. Ultimately, this model provides insight on the efficiency of methanogenic food sources and one day may aid in the development of environmental and governmental food sustainability guidelines.

CHAPTER V

SUMMARY

Approximately 2-8% of energy consumed by cattle is converted into CH_4 (Yan et al., 2009) a significant energy loss from the animal. Many strategies have been evaluated to reduce CH_4 including modifying dietary composition, inclusion of ionophores, organic acids, and plant compounds, defaunation and vaccinations. Abatement strategies directly or indirectly target ruminal methanogen populations, resulting in varying degrees of efficacy. Methane production not only comes at a cost to the animal, it also contributes to anthropogenic GHG. Specifically, according to the U.S. EPA (2014) gastrointestinal fermentation accounts for 38% of total agriculturally related CH_4 emissions in the U.S. Furthermore, CH_4 has a 12-year atmospheric lifetime and global warming potential 25 times greater than that of CO_2 (U.S. EPA, 2016), thus strategies to mitigate gastrointestinal CH_4 production are of critical interest to environmentalists, as well as to cattle producers.

In ruminants carbohydrate (CHO) catabolism and nitrogen (N) utilization have a tremendous impact on ruminal methanogenesis. However, the impact of purified carbohydrates in the presence of a variety of N sources on rates of CH_4 and VFA production remains unknown. In order to determine these rates for use in predictive models of the ruminal fermentation, we formulated a fractional rate equation to fit the rate of CH_4 production and measured the concentration of CH_4 and VFA and using purified CHO with a variety of N sources in two *in vitro* mixed ruminal microorganism

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fermentation studies. Results indicated that both CHO and N sources had a profound

impact on CH_4 and VFA production over time. Collectively, the results gathered in

these studies are the first steps necessary to build nonlinear rate equations for the

conversion of carbohydrates to CH_4 and one day may be used to improve the predictive

accuracy of CH_4 models.

The objective of the final study was to compare the efficiency of beef and milk

production to pre-settlement wild ruminants and rice production on a kilogram of CH_4

emitted to kilogram human-edible protein production basis. Currently, the U.S. has a

population of 320 million people that is steadily increasing. In order to meet the protein

demands of the country's growing population, both in terms of quantity and quality, food

production must be produced as efficiently as possible. Additionally, in order to

minimize the impact on the environment we need to generate food that minimizes

negative environmental feedback. Thus, it is imperative that we evaluate and compare all

current methanogenic food producing systems along with historic methanogenic food

sources in order to make informed agricultural decisions that are advantageous for both

human and environmental health. Overall, this model was the first of its kind to

compare pre-settlement wild ruminants, and current rice, beef, and milk production on a

CH_4 to human-edible protein basis. Bison had the highest ratio of 13.93 kg CH_4:

Protein, followed by elk (12.50) deer (6.66) and beef (2.47). Wild ruminants emitted

296 to 564 percent more CH_4 per kilogram of human-edible protein produced than

current beef cattle production systems. Rice yielded the second lowest CH_4 to human-

edible protein ratio (0.83), followed by dairy cattle milk production (0.50). Ultimately,

this model provides insight on the efficiency of methanogenic food sources and one day may aid in the development of environmental and governmental food sustainability guidelines.

In conclusion, cattle production, ruminant nutrition, GHG production, and food security and sustainability are complex and inextricably linked. With continual improvements in technology and beef cattle management, the U.S. able to produce more beef without increasing herd gastrointestinal CH_4 emissions (Capper, 2011; U.S. EPA, 2014). Although beef require a great amount of recourses, they still play an intricate role in the United States food system. For it is a balance between the myriad of protein production systems that will ensure U.S livestock's economic, social and environmentally sustainability.

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