The Efficacy of Diet Manipulation for Mitigating Enteric Methane Production in Ruminants

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ABSTRACT

Enteric carbohydrate fermentation within ruminants results in the production of vast quantities of methane, a very potent greenhouse gas. Efforts to alleviate atmospheric methane accumulation, then, concentrate largely on minimizing the excretion of methane gas from livestock. These mitigation techniques include adjusting the composition of the animal feed and supplementing the feed with ionophores or methanogenesis inhibitors. The efficacy of each of these strategies has been studied using *in vitro* or sulfur hexafluoride gas tracer techniques. These studies reveal that cows fed a corn and soybean concentrate diet produce significantly less methane than forage-fed cattle; monensin and/or lasalocid ionophore additives likewise decrease methane production, though only for a short time; and the chemical inhibitors 2-bromoethanesulphonate, lumazine, and ethyl 2-butynoate also briefly depress enteric methane formation. It is apparent, therefore, that effective long-term methane mitigation strategies will involve using a combination of techniques, thereby reducing the efficiency with which the methanogenic microbes can adapt.

INTRODUCTION

Methane is a potent greenhouse gas that is over 20 times more effective at trapping heat than carbon dioxide. However, its chemical lifetime in the atmosphere is approximately 12 years, making it a prime candidate for mitigating global warming over the near-term (Gibbs et al., 1989). Sources of methane include wetlands, paddy fields, energy sectors, ruminants, landfills, and biomass burns, with enteric carbohydrate fermentation constituting the largest source (Wright et al., 2004; Mastepanov et al., 2008). Indeed, ruminants contribute approximately 18-20% of the global methane produced annually. Furthermore, 2-15% of these animals' ingested energy is lost solely as methane, representing a great inefficiency in nutrient conversion and utilization (Moss et al., 2000).

The reduction of methane expulsion from ruminants – particularly livestock – is thus an important endeavor. To achieve these ends, an understanding of the relevant aspects of ruminant digestion and a familiarity with tools available for the manipulation of the ruminal microflora is vital.

Plant tissues – the basis of a cow's diet – contain about 75% carbohydrates, which provide the primary source of energy for the livestock (Umphrey and Staples, 1992). The majority of these carbohydrates undergo pregastric, anaerobic fermentation in the rumen, the largest compartment of a cow's four-chambered stomach. This is accomplished by a complex microbial community that includes nearly 10^{11} bacteria, 10^{9} methanogens, 10^{6} ciliate protozoa, and 10^{6} fungi per mL (Kumar et al., 2009). Warm, moist, neutral conditions within the rumen support the proliferation of these microorganisms.

The main end-products of the fermentation are volatile fatty acids – acetic acid, propionic acid, butyric acid, and valeric acid – which the animal uses as its main energy source. Hydrogen is an intermediate compound formed by cellulolytic bacteria, and, with carbon dioxide or formate as substrate, it is quickly ultilized by methanogens to produce methane and generate ATP (Sutton, 1971). This process, therefore, acts as a hydrogen sink in order to inhibit the accumulation of hydrogen in the

rumen and keep fermentation running efficiently. Methane gas is then expelled from the mouth or anus, and the volatile fatty acids are absorbed into the bloodstream through the rumen wall. Carbohydrates that escape ruminal digestion are broken down in the abomasums, and the end-products are absorbed through the small intestine.

Though most methanogenic species have proven resistant to culture, *Methanobrevibacter ruminantium*, *Methanosarcina barkeri*, *Methanobrevibacter formicicium*, and *Methanomicrobium mobile* are considered of greatest significance, as they have been detected at high concentrations in the rumen using molecular techniques (Kumar et al., 2009). Many of these organisms even form symbiotic relationships with anaerobic fungi and ciliated protozoa.

With an understanding of the relationship of methane production to carbohydrate fermentation and an awareness of the constituents of the enteric microbial community, one can approach the goal of reducing methane production and expulsion. Considering these processes and properties, it is apparent that adjustments can be made either to the livestock diet or to the microbial community itself. The majority of research thus far has focused on diet – namely the performance of forage and concentrate diets and the potential of various diet additives. Studies of enteric microbial communities present in other ruminants have also recently spurred interest in transforming the composition of the ruminal microflora within cows. This paper focuses on three topics related to the effects of diet on methane production – the compositional basis of the feed, the addition of ionophores to the feed, and the supplementation of the feed with methanogenesis inhibitors.

The compositional basis of a cow's diet has been known to have effects on methane expulsion, with corn and soybean meal concentrate diets generally resulting in less gas production than forage diets. Concentrate and forage diets also affect ruminal pH differently, which may contribute to the activity of the enteric methanogens. This connection is explored in a study by Kessel and Russell (1996).

Ionophores are highly lipophilic ion carriers. They pass through the porous peptidoglycan layer of gram-positive bacteria and lodge in the lipid membrane. Therein, they destroy ion gradients at the expense of ATP, ultimately resulting in the depletion of energy reserves, impaired cell division, and the likely death of the microorganism (Tedeschi et al., 2003). Many formate and hydrogen-producing bacteria are gram-negative and sensitive to ionophores, effectively preventing the formation of the necessary substrates for methanogens. Bacteria responsible for cross-feeding hydrogen to methanogens are likewise inhibited, resulting in a dramatic reduction in methane production. Many propionate-producing ruminal bacteria, on the other hand, are not inhibited by ionophores, resulting in an increased proportion of this volatile fatty acid (Callaway et al., 2003). Propionate is very efficiently utilized by ruminants, and thus may enable increased derivation of energy from feed. The efficacy of ionophores in ruminant diets is examined by Guan et al. (2006).

Finally, methanogenesis can also be directly inhibited by the addition of halogenated methane analogues, most commonly 2-bromoethanesulphonate (BES), 3-bromopropanesulfonate (BPS), lumazine, and ethyl 2-butynoate. Chloral hydrate, when converted into chloroform in the rumen, also inhibits methane production. Some inhibitors, however, are more effective against certain species of methanogens than others, and some only offer short-term protection. These issues are addressed by Ungerfeld et al. (2004).

MATERIALS AND METHODS

When determining the effects of various diets and additives on the overall expulsion of methane from cows, the experimental procedure in its most basic sense involves adjusting the desired variable and measuring the resultant methane emission. It is not always easy, however, to adjust the diets of cattle in a way in which all other variables are controlled, nor is it simple to effectively trap and measure the amount of methane expelled in the animals' belches and flatulence. Measurement techniques such

as radio and stable isotopic methods and respiration chambers are complex, costly, and can be impractical for gathering data on a large scale. This has brought a great deal of attention and effort to the generation of accurate and reliable reaction-based models, as well as to the utilization of *in vitro* techniques.

The first of the three aforementioned studies – the role of pH in the activity of methanogens within forage- and concentrate-fed cattle – was tested *in vitro*. Rumen contents were obtained from two ruminally cannulated Holstein cows. The cows were fed either a forage diet to provide a high ruminal pH or a concentrate diet to induce a low pH ruminal environment. The ruminal contents were collected three hours after feeding and the pH was determined immediately. Measurements of bacteria concentrations, methane production (via gas chromatography), and volatile fatty acids in the ruminal fluid (via liquid chromatography) were also obtained (Kessel and Russell, 1996).

Guan et al. (2006) tested the short-term and long-term effects of a single ionophore (monensin) and a rotation of two ionophores (monensin and lasalocid) on enteric methane emissions. Thirty-six Angus steers were randomly assigned to one of six dietary treatments (low-concentrate without supplementation, low-concentrate with monensin supplementation, low-concentrate with a two-week rotation of monensin and lasalocid supplementation, high-concentration without supplementation, high-concentration with monensin supplementation, and high-concentration with a two-week rotation of monensin and lasalocid supplementation) and were evaluated over a 16-week period. Daily enteric methane emissions were measured using a sulfur hexafluoride gas tracer technique.

Lastly, Ungerfeld et al. (2003) use an *in vitro* technique to test the effects of four methanogenesis inhibitors (BES, BPS, lumazine, and ethyl 2-butynoate) on methane production by the ruminal methanogens *Methanobrevibacter ruminatium*, *Methanosarcina mazei*, and *Methanomicrobium mobile*. The methanogens were grown in MS media containing 25% v/v clarified ruminal fluid. BES and BSP were delivered at 0, 10, 50, and 250 µmol/L, and lumazine was used at 0, 25, 50, or 100%

saturation. Ethyl 2-butynoate was directly injected into the tubes to achieve 4 and 8 mmol/L concentrations. Methane production was monitored at 4 and 6 days of incubation using a flame ionization-detection GC, with a 10% v/v methane gas mixture as a standard.

RESULTS

The analysis of three recent studies that represent three different techniques for reducing enteric methane production provides a useful insight into the prospects of lowering global atmospheric methane concentrations.

Kessel and Russell (1996) examined the levels of methane expulsion from forage-fed and concentrate-fed cows in relation to ruminal pH. They found that the pH of a cow fed an all-forage diet remained more or less constant around 6.7-6.9. The ruminal pH of a concentrate-fed cow, on the other hand, decreased dramatically to as low as 5.45 in the period soon after feeding. Mixed ruminal bacteria from the forage-fed cow converted carbon dioxide and hydrogen to methane, while no methane was produced by the concentrate-fed cow. Zero-time intercepts of methane production revealed that the concentrate-fed cow had fewer methanogens than the forage-fed cow. Adjusting the rumen of the concentrate-fed cow to pH 7.0 by the removal of acetic acid resulted in the subsequent detection of methane. Adjusting the rumen of the forage-fed cow to pH 6.0 resulted in the cessation of methane production.

The impact of ionophore administration on enteric methane emission was evaluated by Guan et al. (2006). Daily enteric methane emissions, as measured using a sulfur hexafluoride tracer gas technique, ranged from 55 to 370 L/steer daily. Ionophore supplementation resulted in decreased emissions by 30% for cattle receiving the high-concentrate diet for two days and by 27% for cattle receiving the low-concentrate diet for four days. Cattle fed a rotation of ionophores (monensin and lasalocid) did not exhibit a greater decrease in methane production, nor did they have a longer period of

depressed gas expulsion. Total ciliate protozoal populations dropped by 83% and 76% in the high- and low-concentrate diets, respectively, when treated with the single ionophore or with the ionophore rotation. However, original protozoal populations were restored by the fourth week for those cattle fed the high-concentrate diets. For those fed the low-concentrate diets, ciliated protozoans had returned to their original numbers by the sixth week.

A third method of reducing methane emissions was studied by Ungerfeld et al. (2004). They explored the effects of four particular methanogenesis inhibitors – BES, BPS, lumazine, and ethyl 2-butynoate – on the production of methane by the ruminal methanogens *Mb. ruminantium*, *Ms. mazei*, and *Mm. mobile*. Methane production of *Mb. ruminantium* was decreased by BES at 10 µmol/L, and it was minimal at 50 and 250 µmol/L at four days time. After six days, *Mb. ruminantium* in 50 µmol/L BES had recovered significantly. *Ms. mazei* and *Mm. mobile* were minimally affected by BES at all except for the highest concentration, and *Ms. mazei* showed strong recovery after six days. BPS did not affect methane production by any of the species studied. Methanogen growth was minimal in the media 50 or 100% saturated with lumazine, though inhibition was less at 25% saturation. Ethyl 2-butynoate almost completely inhibited methane production by *Mb. ruminantium* at 4 and 8 mmol/L, inhibited *Mm. mobile* at 8 mmol/L, and did not have any effect on *Ms. mazei*.

DISCUSSION

Cattle farms represent the largest source of methane gas production, as methanogenic microbes within cow rumen generate methane during the process of carbohydrate fermentation. Nevertheless, they constitute one of the prime targets of greenhouse gas reduction efforts, as methane has a relatively short atmospheric life span and research in the area has generated a wealth of useful information and techniques for mitigation.

Three such techniques include the adjustment of the compositional basis of livestock feed, the addition of ionophores to the feed, and the supplementation of the feed with methanogenesis inhibitors. The potential of each of these strategies is explored by Kessel and Russell (1996), Guan et al. (2006), and Ungerfeld et al. (2003), respectively.

With respect to the composition of livestock diets, Kessel and Russell found corn and soybean concentrate diets to severely acidify cow rumen, with the effect of inhibiting methanogen activity. This observation echoes results of previous studies (Beever et al., 1989; Johnson and Johnson, 1995). When acetic acid was removed from the rumen of the concentrate-fed cows and the pH was allowed to shift back to neutral, however, methane production ensued. Likewise, when the pH of the forage-fed cow rumen was decreased, methane production was halted. Kessel and Russell, then, were able to elegantly demonstrate the relationship between feed composition, rumen acidity, and methanogen activity. Indeed, forage-fed cattle – unlike concentrate-fed cows – ingest a high amount of fiber. This leads to the copious secretion of saliva, which buffers the rumen (Parish, 2007). Concentrate-fed cows that do not secrete as much saliva experience dramatic reductions in ruminal pH with apparent toxicity to methanogens.

A diet high in corn and soybean concentrate, then, appears to be useful for reducing enteric methane production. Sure enough, the very high grain diets (greater than 90% concentrate) often fed to cows in U.S. feedlots result in strikingly reduced methane emissions: 2-3% of gross energy intake, relative to the approximately 6% of forage-fed cattle. Nevertheless, researchers have noticed that low pH levels for extended periods of time can shift the rumen microbial population in favor of bacteria that produce high levels of lactic acid, leading to acute acidosis (Parish, 2007). Perhaps a heterogeneous feeding strategy that favors concentrate diets with occasional infusions of forage feed could provide the greatest reduction in methane production with minimal negative effects on cow health.

Ionophores are molecules that embed in the membranes of gram-positive microorganisms and disrupt ion balances, ultimately destroying the cell. A study by Guan et al. (2006) evaluated the efficacy of supplementing cow diets with these molecules in order to decrease hydrogen availability to methanogens and thus decrease enteric methane production. The addition of monensin to the cattle diets did result in a 27-30% decrease in methane emissions for two to four weeks, depending on the energy content of the diet. Because the number of ciliate protozoa in the rumen decreased simultaneously, the reduced methane expulsion following ionophore infusion appears to be linked to the symbiotic relationship of methanogens with these organisms. Indeed, methanogenic bacteria have been observed on the surface of some ciliate protozoa and may account for 9-25% of methanogenesis in the rumen fluid (Kumar et al., 2009). However, the decline in protozoa numbers and methane production was found only to be transitory, and rotation of monensin and lasalocid ionophores every two weeks does not appear to increase the extent or duration of depression in enteric methane emissions. Nevertheless, ionophore supplementation seems to hold a great deal of potential. With further research and development, ionophores may become an extremely important tool for methane mitigation.

Another tool – the use methanogenesis inhibitors – was studied by Ungerfeld et al. (2003). They examined the effects of 2-bromoethanesulphonate (BES), 3-bromopropanesulfonate (BPS), lumazine, and ethyl 2-butynoate on three species of methanogens (*Methanobrevibacter ruminantium*, *Methanosarcina mazei*, and *Methanomicrobium mobile*). *Mb. ruminantium* was the most sensitive to the effects of BES, and all species showed some degree of recovery after six days. BPS had no effect on methane production for any of the methanogens. Lumazine inhibited growth at 50 and 100% saturation, though not significantly at lesser concentrations. *Mb. ruminantium* was most sensitive to ethyl 2-butynoate, *Mm. mobile* was somewhat sensitive, and *Ms. mazei* was unaffected.

BES is a structural analogue of coenzyme M and interferes with the final reductive step of methanogenesis. *Mb. ruminantium* requires coenzyme M in the medium, while *Ms. mazei* has the ability to synthesize coenzyme M. *Mb. ruminantium*, therefore, must transport more external coenzyme M, leaving it more vulnerable to the effects of the BES analogue (Ungerfeld et al., 2003). This variable sensitivity implies that BES supplementation may result in the selection of resistant methanogens to occupy the empty niche left by *Mb. ruminantium*, effectively counteracting any initial decrease in enteric methane production.

Though BPS has been found to be a potent inhibitor of an enzyme involved in methanogenesis in a pure enzyme-substrate system, its inability to inhibit methanogens in cell cultures suggests that BPS is not transported into cells. BPS has a closer structure to methyl-coenzyme M (rather than coenzyme M), and coenzyme M transport systems may not be suitable for transporting this type of molecule (Ungerfeld et al., 2003).

Lumazine is a structural analogue of some important co-factors in methanogenesis. Its ability to inhibit methanogen growth highlights its potential as an effective tool for mitigation. Nonetheless, slight methanogen recovery was observed six days post-feeding, jeopardizing the chance of significant long-term benefits.

Cell envelope differences may be related to the differences observed in toxicity of the methanogens to ethyl 2-butynoate. The presence of an S-layer in *Ms. mazei* and *Mm. mobile* (absent in *Mb. ruminantium*) may have conferred some resistance, which is a problem for the practical use of this inhibitor *in vivo* (Ungerfeld et al., 2003). Like with BES, selective resistance to ethyl 2-butynoate among different species may favor these species over the long-term, rendering obsolete any initial decreases in enteric methane production.

CONCLUSION

In sum, livestock feed composition, ionophore addition, and inhibitor supplementation offer useful ways of mitigating enteric methane production in cows, thereby decreasing the presence of methane in the atmosphere. Cows fed corn and soybean concentrate diets tend to produce nearly half the methane that is expelled from cows fed high forage diets, though unfortunately it comes at the expense of the cow's health. The ionophores monensin and lasalocid are capable of reducing methane production in the short-term, though further research must be conducted to find ways of extending the effects of these feed additives. Chemical methanogenesis inhibitors are likewise useful in the short-term, though differential resistance among methanogen species and strains decreases the ability of any one inhibitor to maintain a marked effect over time. Future strategies to reduce enteric methane production may be most successful if they involve heterogeneous diets and ever-changing combinations of ionophore and inhibitor supplements.

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