REVIEW PAPER ON THE MITIGATION STRATEGIES TO REDUCE METHANE EMISSIONS FROM LARGE RUMINANTS: SPECIFIC INTENTION TO THE DAIRY AND BEEF CATTLE'S

Ibrahim Mehdi,

MSc in Range Ecology and Management, College of Dryland Agriculture and Natural Resources, Jigjiga University, Jigjiga, Ethiopia;

ABSTRACT

Enteric methane (CH_4) emission is a major contributor to greenhouse gas emissions, and a loss of feed energy during production. The objective of this paper is to provide an update on current management practices and new dietary strategies recently proposed to reduce CH_4 emissions from ruminants. The existing mitigation strategies for dairy, i.e. the addition of ionophores, fats, use of high-quality forages, and increased use of grains, have been well researched and applied. These nutritional changes reduce CH_4 emissions by manipulating ruminal fermentation, directly inhibiting methanogens and protozoa, or by diverting hydrogen ions away from methanogens. Current literature has identified new CH_4 mitigation options. These include the addition of probiotics, acetogens, bacteriocins, archaeal viruses, organic acids, plant extracts (e.g., essential oils) to the diet, as well as immunization, and genetic selection of cows. These new strategies are promising, but more research is needed to validate these approaches and to assess in vivo their effectiveness in reducing CH_4 production by dairy cows. It is also important to evaluate CH_4mitigation strategies in terms of the total greenhouse gas budget and to consider the cost associated with the various strategies. The more basic understanding of the natural differences in digestion efficiencies among animals as well as a better knowledge of methanogens and their interaction with other organisms in the rumen would enable us to exploit the potential of some of the new CH_4mitigation strategies for dairy cattle production.

Key words: *Dairy, Methane, Mitigation, Rumen*

Introduction

Methane is a potent greenhouse gas that contributes to a global warming. Over the past three centuries, the amount of atmospheric CH_4 has grown by 2.5 fold (Lassey, 2008). The world's estimated 1.3 billion cattle, 75% of which are found in developing countries, account for onefourth of the total CH_4that arises from human activity (Lassey, 2008). Most methane (CH_4) that is emitted from livestock originates in the fore stomach, also called the rumen, of ruminants (cattle and sheep). This source of methane is called enteric CH_4. Only about 10% of the total CH_4 from ruminants in Canada is from manure. However, the digestion process enables ruminants to convert forages into usable energy; a portion of the feed energy (3 to 12%) is used to produce enteric CH_4, and is released into the atmosphere as the animal breathes. Minimizing the production of CH_4 can improve efficiency of livestock production and is an environmentally sound practice. About 25% of the enteric CH_4 produced by the 16.25 million cattle in Canada generated by the dairy industry. The remaining 75% is produced by beef cattle, which comprise about 84% of the country's total cattle population.

Global warming and air quality concerns have focused attention on animal agriculture as one source contributing to these problems. Methane is the greenhouse gas that has received the most attention relative to emissions from animals. In 2005, the total greenhouse gas emissions in the USA were 7,260 Tg CO_2 equivalents (EPA, 2007). This value has increased by 16.3% from 1990 to 2005. Methane emissions were 539 Tg on a CO_2 equivalents basis. This value has decreased 11.4% since 1990. Methane emissions from enteric fermentation were 112.1 Tg on a CO_2 equivalent basis in 2005 versus 115.7 in 1990. This is a decrease of 3.1%. Thus, there has already been some decrease in both total and enteric fermentation methane emissions in the U.S., since 1990. Enteric methane emissions are produced in ruminant animals because of microbial degradation of carbohydrates in the rumen. Enteric methane accounted for about 21% of the total U.S. CH_4 emissions in 2005. Methane emissions from dairy cattle represented about 25% of total enteric CH_4 emissions while beef cattle accounted for 71%. Methane emissions from all cattle in the U.S. account for about 11% of the world methane emissions from cattle (Westberg*et.al.*, 2001).While carbon dioxide receives the most attention as a factor in global warming, there are other gases to consider including methane.

In an effort to combat global warming, reducing methane emissions is an attractive target. Firstly, methane has a global warming potential 21 times that of carbon dioxide (IPCC, 2001). Secondly, methane is broken down quite rapidly in the atmosphere within 9-15 years (FAO, 2006). Therefore, a fall in methane emission would quickly result in a reduction in atmospheric greenhouse gas concentration.Methane production in the digestive tract of ruminants called enteric fermentation is one of the major sources of

global methane emissions. According to the FAO report 'Livestock's Long Shadow', enteric methane emissions amount to almost 86 million tons of methane each year (FAO, 2006). With an extra 17.5 million tons of methane produced from manure, livestock are responsible for 37% of anthropogenic methane (FAO, 2006). The total share of livestock in CO_2emissions is 9%.

Methane, which is produced in the rumen called enteric methane, CH_4 as part of the normal process of feed digestion. Typically, about 6 to 10% of the total gross energy consumed by the dairy cow is converted to CH_4, and it is released via the breath. In addition, CH_4 is a potent greenhouse gas that contributes to global warming. Reducing CH_4 losses is an environmentally sound practice that can improve production efficiency (Karen *et al*., 2008). The different approaches have been proposed to reduce CH_4 production by ruminants. Therefore, the aim of this paper is to review the current management practices for mitigation and new strategies proposed to mitigate enteric CH_4 emissions from ruminants, as they relate in particular to dairy cattle.

2. Methane Production in the Rumen

2.1. Methanogenesis

Enteric CH_4 emission is produced as a result of microbial fermentation of feed components. Methane, a colorless, odorless gas, which is produced predominantly in the rumen (87%) and to a small extent (13%) in the large intestines (Torrent and Johnson, 1994). Rumen $CH_4$is primarily emitted from the animal by eructation. The conversion of feed material to CH_4 in the rumen involves the integrated activities of different microbial species, with the final step carried out by methanogenic bacteria (Moss *et al*. 2000). Primary digestive microorganisms (bacteria, protozoa and fungi) hydrolyze proteins, starch and plant cell wall polymers into amino acids and sugars. These simple products are then fermentedto volatile fatty acids (VFA), hydrogen (H_2), and CO_2 by both primary and secondary digestive microorganisms. Acetate, propionate, and butyrate, which are the major VFA are then absorbed, and utilized by the host animal. The major producers of H_2 are the organisms, which produce acetic acid in the fermentation pathway (Hegarty and Gerdes, 1998).

Even thoughH_2 is one of the major end products of fermentation by protozoa, fungi and bacteria, it does not accumulate in the rumen. Other bacteria mainly the methanogens, which are present in the mixed microbial ecosystem, use it. Moss *et al*. (2000), established that CH_4 production can be calculated from the stoichiometry of the mainVFA formed during fermentation i.e., acetate (C-2), propionate (C-3) and butyrate (C-4) as follows: CH_4 = 0.45C2 – 0.275C3 + 0.40C4. Thus, the molar percentage of VFA influences the production of CH_4. Acetate and butyrate production results in CH_4 production, while propionate formation serves as a competitive pathway for H_2 use

in the rumen. With an increased molar proportion of propionate, the molar proportions of acetate and/or butyrate are reduced.

2.2.Methanogens

Methanogens represent a unique group of microorganisms. They possess three coenzymes, whichhave not been found in other microorganisms. The three coenzymes are: coenzyme 420, involved in electron transfer in place of ferredoxin, coenzyme M, involved in methyl transfer, and factor B, a low molecular weight, oxygen-sensitive, heat-stable coenzyme involved in the enzymatic formation of CH4 from methyl coenzyme. Methanogens in all habitats differ from almost all bacteria in cell envelope composition: there is no muramic acid in the cell wall, and the cell membrane lipids are composed of isoprenoids ether-linked to glycerol or other carbohydrates (Baker, 1999). Analyses of the nucleotide sequence of the 16SrRNA indicate their very early evolutionary divergence from all other forms of life studied so far. They have therefore been classified in a different domain named the Archae (formerly Archaebacteria) within the kingdom Euryarchaeota (Baker, 1999).

Methanogens are nutritionally fastidious anaerobes and grow only in environments with a redox potential below -300mV (Stewart and Bryant, 1988). Most methanogens grow at neutral pH, between 6 and 8. Yet, some species can thrive in environments with pH extremes from 3-9.2 (Jones *et al*., 1987). Five species of methanogens were reported to have been isolated in the rumen (McAllister *etal*., 1996). These include *Methanobrevibacterruminantium*, *Methanosarcinabarkeri*, *Methanosarcinamazei*, *Methanobacteriumformicicum*and *Methanomicrobium mobile*. Only the first twohave been found in the rumen at populations greater than $10^6 mL^{-1}$, and are assumed to play a major role in ruminalmethanogenesis. In recent years, phylogenetic analysis of Archaeal16SrRNA genes cloned from the rumen showed that most of the organisms present differed from the cultivated species (Whitford*et al*., 2001). It has been suggested that there may still be more methanogens not yet identified, and more will be identified as 16SrRNA analysis progresses.

Methanogens use the process of formation of CH_4 to generate energy for growth. Substrates used in the process include H_2, CO_2, formate, acetate, methanol, methylamines, dimethyl sulfide, and some alcohols (McAllister *et al*., 1996). In the rumen, methanogens primarily use H_2, CO_2 and formate as substrates in methanogenesis (Jones, 1991). The unique biochemical ability of *Methanosarcinabarkeri*to use methanol, methylamines, and acetate in addition to CO_2 and H_2 as substrates enables the slow growing *Methanosarcina*organisms to flourish in ruminants fed diets containing ingredients like molasses that break down into methylamines, methanol and acetate.Only two species (*Methanosarcina*and *Methanosaeta*) are

known to degrade acetate to CH_4 in the rumen (Jones, 1991).

The interaction of methanogens with other bacteria through interspecies H_2 transfer in the fermentation process allows methanogens to gain energy for their own growth, while the accumulation of H_2 and other intermediates is prevented, which benefits the growth of H_2producing bacteria allowing further degradation of fibrous feed material (Hegarty and Gerdes, 1998). Methanogens are hydrophobic and therefore stick to feed particles as well as onto the surface of protozoa. Tokura*et al*., (1997) observed that the number of methanogens associated with protozoa reached a maximum (10-100 times pre-feeding levels) after feeding, when the rate of fermentation is the highest. It was shown that the symbiotic relationship of methanogens and protozoa might generate 37% of rumen CH_4 emissions (Finlay *et al.,* 1994).

Although methanogens are only directly involved in the terminal stages of fermentation, they are very important because they are capable of effectively utilizing electrons in the form of H_2 to reduce CO_2 to CH_4, therebymaintaining low H_2 pressure in the rumen. Thus, in their absence, organic matter could not be degraded as effectively in the gut (McAllister *et al*. 1996). However, since CH_4 has no nutritional value to the animal, its production represents a loss of dietary energy to the animal. In general, CH_4 production in cattle constitutes about 2-12% of dietary GEI (Johnson and Johnson, 1995). Reduction in CH_4 production can result from a decreased extent of fermentation in the rumen or from a shift in the VFA pattern towards more propionate and less acetate. Tamminga, (1992) noted that if decreased feed ruminal degradation is compensated for by an increased digestion in the small intestine instead of in the hindgut, it could be considered an advantage for the animal.

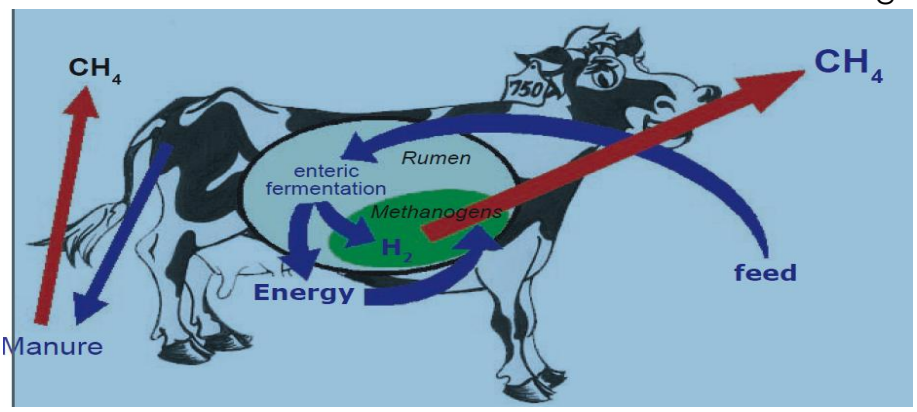

Fig. 1.The formation of methane in the rumen

2.3. Estimation of Enteric Methane Emission

The CH_4 emissions from enteric fermentation for Canadian cattle are

estimated by multiplying the population of various classes of animals by average emission factors derived for each type of domestic animal, which are set by the guidelines of IPCC (Neitzert*et al.,* 1999). The IPCCCH_4 emission values are based upon prediction equations and models, which are themselves based on the following relationship between CH_4 production, feed intake and digestibility (Blaxter and Clapperton, 1965)

CH4 (% of GEI) = 1.3 + 0.112 D + L (2.37 – 0.05D)

Where

GEI = gross energy intake,
L = level of feed intake
D = dry matter digestibility.

The prediction equation was developed from respiration calorimetry chamber experiments using mainly sheep, and is best suited for estimating CH_4 emissions when feed types and feeding levels are the same as those used to develop the model. The equation above predicts emission loss in the range of 5-8% of GEI. However, observed CH_4 emissions from a wide range of feeds and animals varied from 2-12% of GEI (Johnson and Johnson, 1995). Using an extensive database (*n* = 452), Johnson and Johnson, (1995) showed that the ability of the Blaxter and Clapperton's equation to predict CH_4 emissions was weak. As the relationship between the predicted and observed CH_4emissions was very poor (r^2 = 0.23).The literature also provides evidence that enteric fermentation can vary widely depending on factors such as the type of the animal, the amount and type of feed, environment and addition of dietary fat, feed additives and body weight of the animal (Moss *et al.,* 2000). Therefore, IPCC data, (1994) may over or under estimate emissions produced by Canadian cattle production systems where animals are under different feeding and environmental conditions from those under which IPCC data were derived.

3. Measurement of Methane Emission from Dairy Cattle

Different methods used to measure CH_4 from animals have been reported in the literature. These include use of respiration Calorimetry chambers (Murray *et al*., 1999), isotopic techniques (France *et al.,* 1993), tracer techniques [sulfur hexafluoride (SF6)], (Boadi and Wittenberg, 2002), and mass balance or micrometeorological techniques (Harper *et al.,* 1999). Johnson and Johnson, (1995) have reviewed the advantages and disadvantages of each method. Equations for predicting CH_4 emissions were developed mostly from data using the respiration Calorimetry chamber to define the relationship between energy intake and CH_4production, and are based mainly on the diet characteristics. The environment inside the respiration chamber is controlled and animals are under feed restriction during measurement. Therefore, data from the chamber cannot be applied under every farm situation, especially where animals are grazing and pasture quality is changing. Dynamic and mechanistic

models to predict CH_4 from ruminants have also been established to simulate ruminal fermentation under a variety of nutritional conditions (Mills *et al*., 2001).

Benchaar*et al*., (1998) showed that mechanistic models allow the prediction of CH_4 production more accurately than simple regression equations under a large variation of diet composition. Regression analysis showed good agreement between observed and predicted results by modeling experimental data taken from the literature (r^2 = 0.76, root mean square prediction error = 15.4%; Mills *et al*., 2001). Although these models have usefulness in the prediction of CH_4 production from animals under the conditions from which the equations or models are developed, they are of limited use in the prediction of CH_4 production when intake is unknown or when the rumen is disturbed (Johnson *et al.*, 2001).Recent studies have been directed towards measurement of enteric CH_4 emissions under typical farm conditions in order to reflect existing feeding and management conditions. The variations can be seen in CH_4 emission measurements and efficiency of CH_4 production (lkg^{-1} milk). These can be attributed to differences in diet quality and quantities fed, animal body weight, level of milk production and differences in methods used for estimating CH_4 emissions in each study.

4. Strategies for Reducing Methane Emissions from Dairy Cows

Environment Canada as part of the national greenhouse gas inventory (EC, 2008) calculates the enteric CH_4 emissions produced by the dairy sector annually. The calculation estimates gross energy intake of individual animals, applies a 6.5% CH_4 conversion rate (fraction of gross energy intake converted to CH_4), and then sums the daily emissions by animal category (lactating cows, replacement heifers, calves). Using this method of calculation, CH_4 reduction can be achieved either by reducing cow numbers or by reducing the conversion of feed to CH_4 in the rumen. The Canadian dairy industry has decreased its CH_4 emissions by about 24% since 1990 because cow numbers havedeclined because of increased milk production per cow. Because the increases in cow productivity have been accompanied by a decrease in cow numbers. Increasing animal productivity only reduces emissions if product output is capped (through supply management) because increased productivity increases CH_4 emissions per cow (due to increased feed intake).

Further reductions in CH_4 emissions from dairy cows can also occur by reducing the conversion of feed to CH_4 in the rumen (CH_4 conversion rate). Various research groups around the world are exploring the potential of strategically using feed ingredients and supplemental feed additives as a means of reducing conversion rates (Beaucheminet *al.,* 2008). In addition, non-dietary approaches are being examined including vaccination, biological controls (bacteriophage,

bacteriocins), chemical inhibitors that directly target methanogens, and promotion of acetogenic populations in the rumen to lower the supply of metabolic hydrogen to methanogens (McAllister and Newbold, 2008). While a number of ways of reducing CH_4have been proposed that, they must meet the following criteria before being adopted on-farm: documented effectiveness in reducing emissions, profitable (or at least revenue neutral), and feasible to implement on-farm. In most cases, there is a lack of information for dairy producers to properly evaluate profitability of the mitigation strategies proposed.

4.1. Nutritional Strategies that Reduces Enteric CH_4 Production

Diet modifications reduce CH_4 emissions by decreasing the fermentation of feed in the rumen, shifting the site of digestion from the rumen to the intestines, diverting hydrogen away from CH_4 production during ruminal fermentation, or by inhibiting the formation of CH_4 by rumen bacteria. The strategies in the table below have varying degrees of uncertainty associated with their estimated reduction in CH_4. A brief discussion of these strategies follows, but a more complete review of the impact of diet on CH_4 production can be found elsewhere (McAllister and Newbold, 2008). In addition, various models have been developed to predict CH_4 emissions based on diet composition (Pelchen and Peters, 1998).

Table 1. Dietary strategies that reduce enteric CH_4 production

Strategies	Reduction in CH_4	Comments
Strategies with higher certainty of reducing CH_4 production		
Fats and oil seeds	5-25	Level dependent
Ionophores	0-10	Dose dependent, response may decline after several months
Higher grain diets	5-20	Level dependent, increase the risks of acidosis
Replacing barely with corn	0-7	Depend on grain processing
Use of cereal and corn silages	5-10	Depend on grain content of silage
Use of legumes	5-10	Response often confounded with stage of maturity
Tannin containing forages	10-20	High potential but production often limited by agronomics
Strategies that are experimental		
Condensed tannin extracts	0-15	Depend on source, high level decrease milk production
Saponine	0-10	Depend on source
	0-5	Depend on strain, commercial strain have not been tested for their effectiveness
Essential oils	0-20	Promising results with garlic but further testing needed
Fiber depending enzyme	0-10	Commercial products have not been tested for their effectiveness

4.2. Feeding Fats and Oilseeds

Adding fats to the diet reduces CH_4 emissions by decreasing organic matter

fermentation in the rumen, reducing the activity of methanogens and protozoal numbers and lipids rich in unsaturated fatty acids, through hydrogenation of fatty acids (Johnson and Johnson, 1995). The effectiveness of adding lipids to the diet to reduce CH_4 emissions depends on many factors including level of supplementation, fat source, fatty acid profile, form in which the fat is administered (i.e., either as refined oil or as full-fat oilseeds) and the type of diet. However, level of added fat is by far the most important factor. (Beauchemin*et al*., 2008). Over a broad range of conditions, CH_4 (g/kg DMI) was reduced by 5.6% with each 1% addition of supplemental fat. In most cases, 2-3% fat can be added to dairy cow diets without negative effects. The total amount of fat in the diet (added fat plus fat in the basal diet) should not exceed 6-7% of the diet otherwise, a depression in DMI may occur, negating the advantages of increased energy density of the diet.

There is considerable variation in CH_4 reductions observed among fat sources. Higher reductions can be achieved with fats that contain medium chain fatty acids (i.e., C12:0 and C14:0). Examples of these types of oils are: coconut oil, myristic acid, palm kernel oil, high-laurate canola oil, and some genetically modified canola oils. Sources of long-chain fatty acids that can be effective CH_4 suppressants include animal fats, oilseeds, and refined oils. Pure oils are more effective against CH_4 than the same amount of lipid supplied via crushed oilseeds, but oilseeds are preferred because they have less adverse sideeffects on feed intake and fiber digestibility. Fats increase the energy density of the diet, which can improve cow productivity in some situations. However, high levels of added fat can reduce feed intake, fiber digestibility and milk fat percentage so care must be taken in choosing the appropriate level of supplementation.

4.3. Use of Ionophores

Ionophores such as monensin are antimicrobials typically used in dairy cattle diets to improve feed efficiency. Monensin decreases the proportion of acetate and increases the proportion of propionate in the rumen an effect that decreases CH_4 output. At times, monensin may also lower rumen protozoal numbers. This is important, as a direct relationship exists between rumen protozoal numbers and CH_4 formation in the rumen. Rumen protozoa are estimated to provide a habitat for up to 20% of ruminal methanogens while methanogens living on and within protozoa are thought to be responsible for about a third of the CH_4 emissions from ruminants.

The effect of monensin on lowering CH_4 production appears to be dosedependent. In recent studies, providing a dose of 10-15ppm had no effect on CH_4 production (g/d or g/kg DMI) in dairy cows (Waghorn*et al*., 2008), while a dose of 15-20ppm either had no effect on CH_4 production or reduced total CH_4 but not CH_4 per kg of DMI in dairy cows (VanVugt*et al.,* 2005). The higher the

doses (24-35ppm) fed to the dairy cows reduced the CH_4 production (g/d by 4-13% and g/kg DMI by 0-10% in beef cattle and dairy cows,respectively in North America (Odongo*et al.,*2007). Whilethis is with the short-term decreases in CH_4 of up to 30% being reported in beef cattle when 33ppm of monensin was included in high or low forage diets (Guan *et al.,*2006).Unfortunately, the inhibitory effects of ionophores on CH_4 production may not persist over time Guan *et al.* (2006) recently reported that monensin (33mg/kg) lowered CH_4 emissions in beef cattle by up to 30%, but levels were restored within 2 months. In that study, the effect of ionophores on CH_4 production was related to protozoal populations, which adapted to ionophores over time. In contrast, Odongo*et al.* (2007) provide evidence that adaptation to ionophores may not always occur; in their study monensin lowered CH_4 production in dairy cows over a 6-month period. It is evident that the long-term effects of monensin on CH_4 emissions require further study.

4.4.Feeding Higher Concentrate Diets

Increasing the grain content of total mixed rations (TMR) lowers the proportion of feed energy converted to CH_4 by decreasing the acetate: propionate ratio in the rumen fluid. Furthermore, methanogens are susceptible to the low pH conditions in the rumen that result from feeding high grain diets. However, the potential of using concentrates to lower CH_4 emissions from the dairy sector is limited because the increased incidence of rumen acidosis jeopardizes cow health and reduces milk fat content.

4.5.Forage-Related Strategies

Several forage-related strategies that reduce CH4 emissions have been identified, but the CH_4 response to implementing these strategies can be variable as many interacting factors can arise. In general, replacing grass and legume forages with corn silage and whole crop small grain silages reduces CH_4 emissions because grain silages favor the production of propionate rather than acetate in the rumen. Improved forage quality typically results in greater CH_4 output per day because high-quality forages have a faster passage rate from the rumen, which leads to greater feed intake and more fermentable substrate in the rumen. The result is greater daily enteric CH_4 production per day. However, the amount of CH_4 produced per unit of energy consumed or kg^{-1} of milk typically decreases as the quality of forages increases. Feeding legumes compared to grasses tends to reduce CH_4 but this relationship is also influenced by the maturity of the forage at the time of consumption. Legumes produce less CH_4because they have lower NDF content and pass more quickly through the rumen.

4.5.Feed Additives

4.5.1.Condensed tannin extracts

Condensed tannins are phenolic compounds extracted from the bark of black wattle trees (*Acacia mearnsi*; grown in South Africa) and Quebracho-Colorado trees (grown in South America). Adding *Acacia* tannin extract powder to the diet of sheep at a rate of 2.5% of DMI decreased enteric CH4 by about 12% with only a marginal decrease in fibre digestion (Carulla et al. 2005). However, Australian researchers used this same source of tannin extract in a dairy cow study and observed negative effects on milk production (Grainger et al., unpublished). In that study, the extract was mixed with water and provided to the cows twice daily as a drench at 1.5 and 3.0% of DMI. Within a few days, cows receiving the high dose dropped sharply in milk production (4 kg/d) and showed signs of ill health.

Consequently, the high rate was reduced to 2.25% of DMI for the remainder of the study. Averaged over the 5-week experiment, the low and high tannin levels reduced CH_4 emissions by 16 and 28%. However, the reduction in CH_4 was accompanied by a drop in the digestibility of the feed and a negative effect on milk yield (4.9 and 9.7% reduction in milk yield for the low and high tannin levels, respectively) and fat and protein yield (8 and 11% reductions in milk solids for the low and high tannin levels). At the Lethbridge Research Centre, we supplemented the diet of growing beef cattle with up to 1.8% condensed tannin extracted from Quebracho-Colorado trees and observed no effects on enteric CH_4 or digestibility of the dietary DM (Beauchemin*et al*. 2007).

These studies show that tannins hold some promise in terms of CH_4 abatement, but the source and optimum level of tannin need considerable refinement to ensure $CH_4$is lowered without negatively affecting milk production. Tannins have an additional advantage in that they are also highly reactive with protein and can affect the partitioning of nitrogen within the cow shifting the route of excretion away from urine towards feces. Reduced urinary nitrogen excretion would result in reduced environmental losses through nitrate leaching, ammonia volatilization and nitrous oxide emissions.

4.5.2.Yeast

Yeast cultures of *Saccharomyces cerevisiae*are widely used in ruminant diets to improve rumen function and milk production. Commercial products vary in the strain of yeast used and the number and viability of yeast cells present. Laboratory studies suggest that some live yeast strains can stimulate the use of hydrogen by acetogenic strains of ruminal bacteria, thereby enhancing the formation of acetate and decreasing the formation of CH_4 in the rumen. However, we conducted a study with growing beef cattle to evaluate two commercial yeast products, as commercial strains have not been selected for their effects on CH_4 (McGinn*et al*. 2004).

One product caused a 3% decrease in CH_4production (g/g DMI) while the other product increased CH_4production (g/g DMI) by 8%. These results indicate that

while it may be possible to select strains of yeast based on their anti-methanogenic effects, the commercially available strains of yeast likely have only minor, if any effects on CH_4. Because yeast products are modestly priced and used widely in ruminant production,acceptance of a CH_4-reducingyeast product would likely be high. However, considerable research and development would be needed to deliver such product to the marketplace. To date, commercial manufacturers have been reluctant to invest in such products because animal performance rather than CH_4abatement is the primary driver for product development.

4.5.3 Enzymes

Enzyme additives are concentrated fermentation products that contain fiber-digesting enzymes (cellulases, hemicellulases). The focus to date has been on developing enzyme additives that improve fiber digestion (Beauchemin et al. 2003), but it may also be possible to develop enzymeadditives that reduce CH_4 emissions. In a recent *in vitro* study in their lab, oneparticular enzyme candidate increased fiber degradation of corn silage by58%, with 28% less CH_4 produced per unit of fiber degraded (Beauchemin etal. unpublished). Furthermore, feeding dairy cows a diet containing cornsilage with added enzyme reduced CH_4 production (g/g DMI) by 9%. Enzymes that improve fiber degradationtypically decrease the acetate: propionate ratio in rumen fluid (Eun andBeauchemin, 2007), which is thought to be the primary mechanism wherebyenzymes decrease CH4 production. The potential of enzyme additives forCH4 abatement warrants further research, because enzymes are likely to havepositive effects both on milk production and on CH4 abatement.

4.5.4.Defaunation

Defaunation, which is the elimination of protozoa from the rumen by dietary or chemical agents, has been shown to reduce ruminalCH4 production by about 20 to 50% depending on the diet composition (Van Nevel and Demeyer 1996). Whitelaw et al. (1984) observed that faunated cattle fed barley diets at restricted levels lost about 12% of GEI as CH4 compared to 6–8% of GEI in ciliate-free animals. Protozoa in the rumen are associated with a high proportion of H2 production, and are closely associated with methanogens by providing a habitat for up to 20% of rumen methanogens (Newbold et al. 1995). Finlay et al. (1994) reported that protozoa could account for 37% of the total CH4 production. It is assumed that there is a symbiotic H2 transfer between anaerobic protozoa and methanogens (Ushida and Jouany 1996).

The reduced ruminalmethanogenesis observed with defaunation can be attributed to factors such as a shift of digestion from the rumen to the hind gut (Van Nevel and Demeyer, 1996) or the loss of methanogens associated with protozoa during defaunation(Hegarty, 1999).It has been shown that defaunation may depress fiber digestion, thus complete elimination

of protozoa (rather than selective defaunation) is not recommended as a method for reducing CH4 (Itabashi 2001). On the other hand, protozoa have been reported to negatively affect ruminal protein metabolism through predation of bacteria, which reduces the flow of microbial protein leaving the rumen (Koenig *et al.* 2000).

Therefore, the use of defaunation to mitigate CH4 production from ruminants should be weighed against its possible impact on the efficiency of the whole ruminal system. Defaunating agents or protozoal inhibitors are not currently available for commercial or practical use as many of the defaunating agents are toxic to the animal. The control of protozoa is unlikely to lead to H2 accumulation or inhibition of fermentation;therefore, it represents a promising method of CH4 reduction. Further work is needed in this area to develop commercial means of controlling rumen protozoa (Klieve and Hegarty, 1999).

5. New Potential Mitigation Options

5.1.Probiotics

There is very little information on the effects of probiotics on CH_4 production in dairy cattle. The effects of the most widely used microbial feed additives, *Saccharomyces cerevisiae*and *Aspergillusoryzae,* on rumen fermentation were earlier studied in vitro (Mutsvangwa*et al*. 1992). *Aspergillusoryzae*was shown to reduce CH_4 by 50% as a result of a reduction in the protozoal population (Frumholtz*et al*. 1989). The addition of *Saccharomyces cerevisiae*reduced CH_4 by 10% in vitro, but was not sustained over a long period (Mutsvangwa*et al.* 1992). It has been shown that yeast culture influenced microbial metabolism and improved DMI, fiber digestion, and milk production in lactating cattle (Dann*et al*. 2000).

However, the specific mode of action is still unknown. It has been proposed that probiotics provide nutrients, including metabolic intermediates and vitamins that stimulate the growth of ruminal bacteria, resulting in increased bacterial population (Newbold*et al*. 1996). Another theory indicates that probiotics stimulate lactic-acid-utilizing bacteria, resulting in a reduction of lactic acid and a more stable ruminal environment. A less acidic ruminal environment favors the growth of cellulolytic bacteria, which in turn improves fiber digestion, feed intake, and production response (Yoon and Stern 1996).

Miller-Webster et al. (2002) recently showed that the inclusion of yeast culture products (YC1, Diamond-V XP, and YC2, A-Max) in a continuous culture system increased DM digestion and propionic acid production whereas it reduced acetic acid production and protein digestion compared with the control. Eun*et al.,* (2003) reported that brewer's yeast culture enhanced the activity of bacteria that convert H_2 to acetate and decreased CH_4output by 25% in a continuous culture of ruminal

microorganisms. In a previous study, Chiquette and Benchaar, (1998) reported no effect on molar proportions of ruminalVFA when a mixture of *Saccharomyces cerevisiae*and *Aspergillusoryzae*was added to the diet of dairy heifers. The effects of probiotics on fermentation pattern are not consistent across experiments and between strains of yeast (Newbold*et al*., 1995). Doreau and Jouany, (1998) found no effect of *Saccharomyces cerevisiae*on fermentation in lactating dairy cows, while Takahashi *et al*., (1997) observed that a probiotic preparation significantly increased (+18%) CH_4 production in sheep. Although microbial preparations are commercially available as ruminant feed additives, there is a need for further research to establish the potential of probiotics for reducing CH_4 production in vivo. Producers are skeptical about the benefits of probiotics and there is a need to identify the dietary and management situations in which probiotics can give consistent production benefits as well as the added effect of reducing CH_4 emissions (Moss *et al*.2000).

5.2.Bacteriocins

Direct suppression of methanogens may be possible through stimulation of natural or introduced ruminal organisms to produce bacteriocins as a means of biological control (Klieve and Hegarty, 1999). Bacteriocins are bacteriocidal compounds that are peptide or protein in nature, and are produced by bacteria. However, little information is available concerning their effect on methanogenesis. They often display a high degree of target organism specificity, although many have a very wide spectrum of activity (Kalmokoff*et al*. 1996). Nisin, an exogenous bacteriocin produced by *Lactococcuslactis*, is the best studied and understood bacteriocin. It has similar actions to monensin and is widely used in the food industry as a preservative in controlling food borne pathogens (Lee *et al*. 2002). In vitro, nisin stimulated propionate production, increased the ratio of propionate to acetate and reduced methanogenesis by 36% (Callaway et al. 1997).

However, recent work indicated that some ruminal bacteria become nisin-resistant (Mantovani and Russell, 2001) and an in vivo feeding trial indicated that nisin could not decrease the acetate: propionate ratio as observed with cattle consuming the same amount of monensin ($350 mg d^{-1}$) (Russell and Mantovani, 2002). This suggests that nisin was either being degraded or the bacteria were becoming nisin-resistant. The HC5bovicinbacteriocin from *Streptococcus bovis*(*S. bovis*) has also been shown to inhibit CH4 by as much as 50% (Lee *et al.,* 2002). Although exogenous bacteriocins may be safe and can be incorporated into feed, a limitation may be the degree of stability of these peptides in the ruminal environment, as rapid degradation by proteolytic enzymes could reduce their effectiveness (Klieve and Hegarty, 1999).

Endogenous bacteriocinshave been identified in the rumen (Teather and

Forster, 1998). A survey of 50 strains of *Butyrivibrio*spp. isolated from a variety of sources (sheep, deer and cattle) for bacteriocin production indicated a high incidence of bacteriocin-like activity (50%) (Kalmokoff*et al*., 1996). Although the potential for ruminally-producedbacteriocins to suppress methanogens is unknown, their potential to improve ruminant production and modify microbial populations has been suggested by Teather and Forster, (1998). Bacteriocins may therefore provide an alternative to ionophore antibiotics for manipulation of ruminal microbial populations. They have advantages over other antibiotics in terms of target specificity, broad spectrum of activity, and possibility of genetic transfer and manipulation into other organisms (Kalmokoff*et al*. 1996).

Bacteriocinscould possibly be delivered as microbial inoculants for in situ production of the bacteriocin in the rumen or in silage (Kalmokoff*et al*., 1996). Given the fact that *S. bovis*produces a very potent bacteriocin (bovicinHC5), which reduces methanogenesis (Lee *et al*., 2002) silage fermentation can be a vehicle for delivering bacteriocins to the rumen (Kalmokoff*et al*. 1996). Controlled colonization of the rumen by genetically engineered ruminal bacteria is a great challenge (Teather and Forster, 1998). In addition, there is a need to develop rapid and accurate techniques to characterize the existing ruminal populations in terms of bacteriocin production and resistance. Efforts are under way to clone bacteriocin genes and develop DNA probes for the detection of these genes in rumen samples (Teather and Forster, 1998).

Currently, the genomes of several lactic acid bacteria that produce bacteriocins have been sequenced (Koningse*t al*. 2000). These organisms have found wide application in the manufacturing of fermented foods and drug industry. Recent progress has been made in the construction of genetically modified lactic acid bacteria used in food products (Koningse*t al*. 2000). It can be concluded that bacteriocins have the potential to reduce CH_4 production, but further studies in vivo are needed to establish their adaptability and long-term effectiveness as a feed additive.

5.3.Immunization

In the past 3 years, researchers in Australia have vaccinated sheep with a number of experimental vaccine preparations against methanogens, so that the animals produce antibodies to methanogens (http://www.csiro.au). Methane production was reduced between 11 and 23% in vaccinated animals and productivity was improved. No long- or short-term adverse effects on sheep were found. Researchers anticipate that commercial vaccines will allow a 3% gain in animal productivity and a 20% reduction in CH_4 production (http://www.csiro.au). It is important to note that the vaccines currently under development are based on cultivable methanogens. However, the work of Whitforde*t al.* (2001) showed that most ruminal methanogens have not yet been cultivated. Hegarty, (2001) noted

that vaccine preparations are likely to work on some methanogens and not on others; thus, monitoring and assessment of efficacy will be required for novel control measures such as vaccines.

5.4.Reductive Acetogenesis

A technology that may hold some promise in the long-term of diverting electrons from methanogens is the production of acetic acid by acetogens (Joblin, 1999). In the gut of termites and rodents, acetogens convert excess H_2 to acetic acid, which is then utilized by the host (Joblin, 1999). However, in the rumen, acetogens are few and cannot compete effectively with methanogens for H_2 ions, because they have a lower affinity for H_2 than methanogens (Nollet*et al*., 1998).Carbon flux studies in the rumen of sheep revealed that rumen acetogenesis occurs in the first 24 hrs after birth, but is subsequently displaced by methanogenesis (Morvan*et al*., 1994); methanogens easily out-compete the acetogens for the low concentration of H_2 normally encountered in the rumen (Joblin, 1999). Thus, methanogens have to be inhibited to allow H_2 pressure to rise before acetogenesis can be significant as an alternate H_2 sink in the rumen. Increasing the populations of acetogens through exogenous inoculations into the rumen could be useful for competing against methanogens (Joblin, 1999). However, previous attempts at inducing acetic acid by inoculation with acetogens were not successful (Nollet*et al*. 1998).

5.5.Methane Oxidizers

CH_4 oxidizing bacteria have been isolated from different environments, including the rumen (Moss et al., 2000). In vitro studies with stable carbon isotopes suggest that the extent of CH4 oxidation to CO2 is quantitatively minor (0.3-8%) in the rumen (Kajikawa and Newbold, 2000). Valdez et al., (1996) isolated a CH_4 oxidizing bacterium from the gut of young pigs, which decreased CH_4accumulation when added to rumen fluid in vitro. However, this approach has not been validated in vivo. In the long-term, CH_4 oxidizers from gut sources could be screened for their activity in the rumen to reduce the proportion of ruminal gas in the form of CH_4.

5.6.Propionate Enhancers

Because of the growing awareness of the threat of microbial resistance to antibiotics, there is an increasing interest in alternatives to antibiotics as growth promoters (Moss *et al*., 2000). Dicarboxylic acids such as fumaric and malic acids have been studied in vitro as feed additives in ruminant diets (Asanuma *et al.,* 1999). Fumaric acid is an intermediate in the propionic acid pathway, in which it is reduced to succinic acid. In this reaction, H_2 ions are needed and therefore reducing fumaric acid may provide an alternative electron sink for H_2. It was foundthat the addition of up to 500 and mol of sodium fumarate in vitro decreased CH_4 production by 6% and increased DM

digestibility of the basal diet by 6% after 48 h incubation (Lopez *et al*. 1999).

Asanuma *et al*., (1999), showed that the addition of 20mM of fumarate to cultures that were fermenting hay powder and concentrate incubated for 6hrs significantly decreased CH_4 production by 5% and increased propionate production by 56%, while with the addition of 30 mM of fumarate, CH_4 declined by 11%, and propionate production increased by 58% compared to the control. Their data suggested that most of the fumarate consumed was metabolized to propionate with little production of acetate and succinate, whereas a much larger amount of succinate accumulated with the addition of 30 mM of fumarate. However, when incubation time was prolonged to 12 hrs, most of the succinate was metabolized to propionate.

There is little information available on the actual effects of fumaric acid on fermentation and animal performance in vivo. Isobe and Shibata, (1993) observed that the proportion of acetic acid and propionic acid increased following the addition of fumaric acid whereas the proportion of the higher acids decreased. The effects of salinomycin (15ppm) plus fumaric acid (2%) supplemented to diets of Holstein steers increased the molar proportion of propionic acid anddecreased CH_4 production ($lkgDMl^{-1}$) by 16% and had no effect on DM digestibility (Itabashi *et al*. 2000). Bayaru*et al*., (2001) found that CH_4 production was reduced by 23% when fumaric acid added to sorghum silage was fed to Holstein steers.

The authors observed that the addition of fumaric acid increased propionic acid formation and had no effect on DM digestibility. Fumaric acid was also shown to increase concentration of plasma glucose and milk protein synthesis in dairy cows due to an increase in propionic acid production (Itabashi, 2001). The authors concluded that fumaric acid may be put to practical use for ruminant diets since it has the dual benefit of decreasing CH_4 production and increasing net energy retention. Malate, which is converted to propionate via fumarate, also increased propionate production and inhibited CH_4 production in vitro (Martin *et al.,*1999). However, malate failed to increase ruminal propionate concentrations in feedlot cattle and did not affect CH_4 production (Montano *et al.,* 1999) although it stimulated daily gains in steers (Martin *et al*., 1999). There is a need for further testing and evaluation of these enhancers in vivo to assess their potential as feed additives in the industry.

5.7.Essential Oils

There is an increasing interest in exploiting natural products as feed additives to manipulate enteric fermentation and possibly reduce CH_4 emissions from livestock production (Wenk, 2003). Essential oils are a group of plant secondary compounds that hold promise as natural additives for ruminants (Wallace et al., 2002). Essential oils are any of a class of

steam volatile oils or organic-solvent extracts of plants (e.g., thyme, mint, oregano, sage) possessing the odor and other characteristic properties of the plant (mainly antimicrobial), used chiefly in the manufacture of perfumes, flavors, food preservatives, and pharmaceuticals (Wenk, 2003). Essential oils are present in many plants and may play a protective role against bacterial, fungal, or insect attack. The antimicrobial activity of essential oils can be attributed to a number of small terpenoids and phenolic compounds, e.gmonoterpenes, limonene, thymol, carvacrol(Wallace *et al*. 2002). The specific mode of action of essential oil constituents remains poorly characterized or understood (Helander*et al*., 1998).

The antimicrobial properties of essential oils have been shown through in vitro and in vivo studies to inhibit a number of bacteria and yeasts and to control fermentation gases, VFA, livestock waste odors and human pathogenic bacteria such as *Escherichia coli 0157:H7*, *Enterococcus faecalis and Salmonella* sp. (Wallace *et al*., 2002). For the purposes of controlling ruminal fermentation and CH_4 production, Lee and Ha, (2002), examined the effect of adding 0, 1 and 10% essential oil to 0.5 g of ground tall fescue and concentrate in the ratio of 2:8 or 8:2 on in vitro gas production and fermentation. The authors showed that supplementing 10% of essential oil increased ruminal pH and lowered NH3-N, VFA concentration and cumulative CH_4 production over 48hrs of incubation, when compared with the 0 or 1 % levels. There was no effect on CH_4 production following the addition of 1% essential oil to both substrates (Lee and Ha, 2002).

Broudiscou*et al.* (2000) screened 13 plant extracts for their action on fermentation in vitro and observed that protozoa numbers were little affected. On the other hand, methanogenesis decreased by 8.2% with *Salvia officinalis*and by 14.2% with *Equisetum arvense*, while it increased by 13.7% with *Lavandulaofficinalis*and 7.7% with *Solidagovirgaurea*, indicative of diverse modes of action among plant extracts.When sheep diets (60:40 silage:concentrate) were supplemented with 100mg of essential oils $head^{-1}$ d^{-1}, Wallace *et al*. (2002) reported no effects on the ruminal concentration of VFA and protozoa numbers. Recently, Benchaar*et al*. (2003) did not observe any effects of dietary addition of essential oils on VFA concentrations, acetate propionateratio or rumen microbial counts in lactating cows. The potential of essential oils for modulating ruminal function on a long-term basis has not been evaluated. It is also important to know the most effective level of inclusion of essential oils in the diet, as well as the possible adaptation of ruminal microorganisms to this feed additive.

5.8.Genetic Selection

Robertson and Waghorn, (2002) observed that Dutch/US cross Holstein cows produced 8-11% less CH_4 (% of GEI) than New Zealand Friesian cows for about 150 d post calving, either when grazing or receiving a TMR. Hegarty, (2001) noted

that the natural variation among animals in the quantity of feed eaten per unit of liveweight gain could be exploited to breed animals that consume less feed than the unselected population while achieving a desired rate of growth. Accordingly, to exploit such traits, the concept of Residual (Net) Feed Intake (RFI) was developed and used(Basarab*et al.* 2003). The RFI is moderately heritable (h^2 = 0.39), and is independent of the rate of gain (Arthur *et al.* 2001).

Okine*et al.* (2002) calculated annual CH_4 emissions from Canadian high NFE steers to be 21% lower than that for low NFE steers. Selection for high NFE in beef cattle also decreased manure N, P, K output due to a reduction in daily feed intake and more efficient use of feed, without any compromise in growth performance (Okine*et al*. 2002). The mean retention time of digestahas also been shown to be selectable among animals (Hegarty, 2001). Selecting animals for a faster passage rate of feed from the rumen would reduce CH_4 emissions per unit of food ingested. Faster passage rate of feed also affects propionate and microbial yield; thus, selection of animals for this would also have major production benefits. Selecting animals with high NFE offers an opportunity to reduce daily CH_4 emissions without reducing livestock numbers.

Conclusion

Mitigation of CH_4 emissions can be effectively achieved by strategies that improve the efficiency of animal production, reduce feed fermented per unit of product, or change the fermentation pattern in the rumen (Table above). Many current and potential mitigation strategies have been evaluated, but not all of them can be applied at the farm level and in many cases, the potential negative effects and associated costs have not been fully researched. Strategies that are cost effective, improve productivity, and have no potential negative effects on livestock production hold a greater chance of being adopted by producers.

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