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A Review on the Mitigation Strategies for Reducing Enteric Methane Emissions from Dairy Cows

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Abstract
The objective of this paper is to provide updated information on current management practices and new dietary strategies recently developed to reduce CH4 emissions from ruminants. Enteric methane (CH4) emission is a major contributor to greenhouse gas emissions, and also a loss of feed energy during production. The Existing mitigation strategies for dairy cattle are 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 CH4 emissions by manipulating ruminal fermentation, directly inhibiting methanogens and protozoa, or by diverting hydrogen ions away from methanogens. Currently new CH4 mitigation options have identified. 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 CH4 production by dairy cows. It is also important to evaluate CH4 mitigation strategies in terms of the total greenhouse gas budget and to consider the cost associated with the various strategies. 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 CH4 mitigation strategies for dairy cattle production.
Keywords: *Dairy Cow, Methane and Rumen*

1. Introduction

Methane is a potent greenhouse gas that contributes to global warming. Over the past three centuries, the amount of atmospheric CH_4 has grown by 2.5-fold (Lassey, K.R., 2008). The world's estimated 1.3 billion cattle, 75% of which are found in developing countries, account for one fourth of the total CH_4 that arises from human activity (Lassey, K.R. 2008). Most CH_4 that is emitted from livestock originates in the fore stomach, also called the rumen, of ruminants. This source of methane is called enteric CH_4. Typically, about 6 to 10% of the total gross energy consumed by the dairy cow is converted to CH4 and released via the breath. Reducing CH_4 losses is an environmentally sound practice that can improve production efficiency (Karen A et al., 2008). 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. Enteric CH_4 emission is produced as a result of microbial fermentation of feed components. Methane, a colorless, odorless gas, 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 fermented to 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). 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 recent FAO report 'Livestock's Long Shadow', enteric methane emissions amount to almost 86 million tonnes of methane each year (FAO, 2006). With an extra 17.5 million tonnes of methane produced from manure, livestock are responsible for 37% of anthropogenic methane (FAO, 2006). The total share of livestock in CO_2-emissions is 9%. 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. Emissions into the air by any animal production system can be problematic in terms of pollutants and toxicity and in terms of odour and the perception of air quality by human neighbours. The three major greenhouse gases

are carbon dioxide, methane and nitrous oxide. Methane has a positive radiative force on the climate; the global warming potential of methane is 21-times that of CO_2 over 100 years UNFCCC (2007) even though it is much shorter-lived in the atmosphere. It also has serious impact on high atmosphere ozone formation. It is important to reduce methane production from the rumen, because methanogenesis corresponds to 2-12% of dietary energy loss as well as contributing to global warming. Enteric methane emissions represent an economic loss to the farmer where feed is converted to CH4 rather than to product output (CCTP, 2005).

Livestock accounts for 35-40% of the global anthropogenic emissions of methane, via enteric fermentation and manure (Steinfeld et al., 2006). Recent estimates by Herrero et al. (2008) indicate that methane emissions from African cattle, goats and sheep are likely to increase from their current level of about 7.8 million tons of methane per year in 2000 to 11.1 million tons per year by 2030; largely driven by increase in livestock numbers. Again, there are considerable differences in methane emission per tropical livestock unit (TLU, 250 kg body weight), depending on the production system and diet, from 21 (less productive systems) to 40 (more productive systems) kg per TLU per year. Developing countries are now responsible for almost three-quarters of the enteric methane emissions which have important implications in terms of mitigation strategies. The aim of this paper is to review some of the current management practices available for mitigation and new strategies proposed to mitigate enteric CH4 emissions from ruminants, as they relate in particular to dairy cattle.

2. Methane Production in the Rumen

2.1. Methanogenesis

Hydrogen is one of the major end products of fermentation by protozoa, fungi and bacteria; it does not accumulate in the rumen. It is used by other bacteria, mainly the methanogens which are present in the mixed microbial ecosystem. Moss et al. (2000) established that CH4 production can be calculated from the stoichiometry of the main VFA formed during fermentation, i.e., acetate (C_2), propionate (C_3) and butyrate (C_4) as follows: $CH_4 = 0.45\ C_2 - 0.275\ C_3 + 0.40\ C_4$. 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

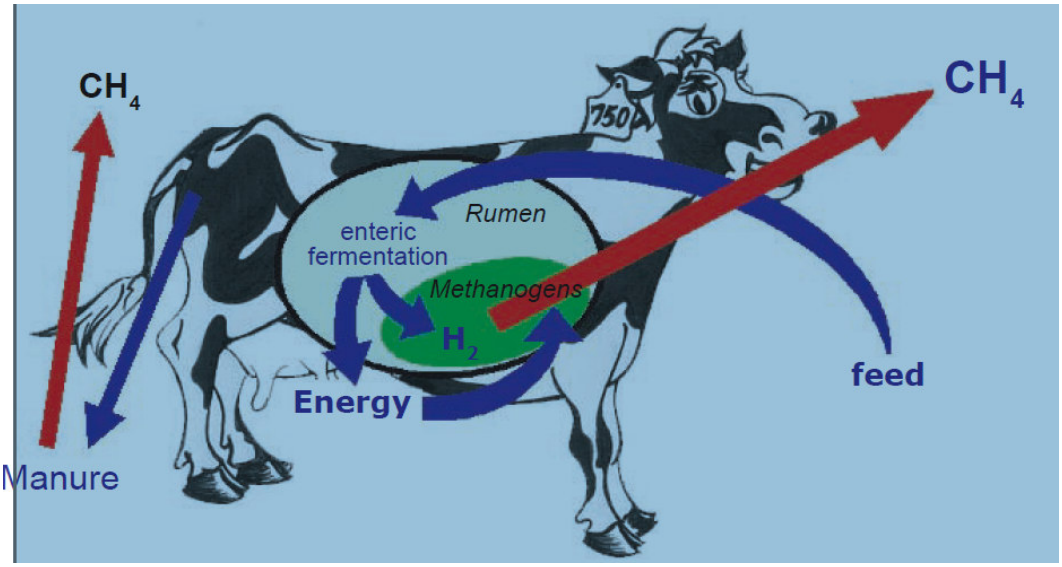
Methanogens represent a unique group of microorganisms. They possess three coenzymes which have not been found in other microorganisms. The three coenzymes are: coenzyme 420, involved in electron transfer in place of ferredoxin, coenzyme M, involved in methyl transfer, and factor B, a low molecular weight, 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 16S ribosomal RNA indicate their very early evolutionary divergence from all other forms of life studied so far. Therefore they have 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 –300 mV (Stewart and Bryant, 1988). Most methanogens grow at neutral pH, between 6 and 8. However some species can thrive in environments with pH extremes from 3 to 9.2 (Jones et al., 1987). Five species of methanogens were reported to have been isolated in the rumen (McAllister et al., 1996). These include *Methanobrevibacter ruminantium*, *Methanosarcina barkeri*, *Methanosarcina mazei*, *Methanobacterium formicicum* and *Methanomicrobium mobile*. Only *Methanobrevibacter ruminantium* and *Methanosarcina barkeri* have been found in the rumen at populations greater than 106 mL^{-1}, and are assumed to play a major role in ruminal methanogenesis. In recent years, phylogenetic analysis of Archaeal 16S rRNA 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 16S rRNA 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 *Methanosarcina barkeri* 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_2-producing 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 to 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 may generate 37% of rumen CH_4 emissions (Finlay et al., 1994).

Although methanogens are only directly involved in the very 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, thereby Maintaining 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.



Formation of methane in the rumen

3. Estimation of Enteric Methane Emission

Currently, CH4 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 IPCC CH4 emission values are based upon prediction equations and models, which are themselves based on the following relationship between CH4 production, feed intake and digestibility (Blaxter and Clapperton, 1965).

CH_4 (% of GEI) = 1.3 + 0.112 D + L (2.37 – 0.05D)

Where GEI = gross energy intake, L = level of feed intake and 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 to 8% of GEI. However, observed CH_4 emissions from a wide range of feeds and animals varied from 2 to 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; i.e., the relationship between predicted and observed CH_4 emissions was very poor ($r2$ = 0.23).

The literature also provides evidence that enteric fermentation can vary widely depending on factors such as 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.

Different methods used to measure CH_4 from animals have been reported in the literature. These include the 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/micrometeorological techniques Harper et al. (1999). The advantages and disadvantages of each method have been reviewed by (Johnson and Johnson, 1995).

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_4 production, 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 ($r2$ = 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 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. Variations can be seen in CH_4 emission measurements and efficiency of CH_4 production (L kg^{-1} milk). These can be attributed to differences in diet quality and quantities fed, animal body weight, level of milk production and also differences in methods used for estimating CH_4 emissions in each study

4. Strategies for Reducing Methane Emissions from Dairy Cows

The enteric CH_4 emissions produced by the dairy sector are calculated by using the estimates of 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 have declined as a result of increased milk production per cow. Because the Supply Management System in Canada imposes quotas on production, increases in cow productivity have been accompanied by a decrease in cow numbers. Increasing animal productivity only reduces emissions if product output is capped (e.g. 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 CH4 in the rumen (i.e., 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 (Beauchemin et 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_4 have been proposed, they must meet the following criteria before being adopted on-farm: 1) documented effectiveness in reducing emissions, 2) profitable (or at least revenue neutral), and 3) 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 Reduce Enteric CH_4 Production

Some dietary strategies that reduce enteric CH_4 production are listed in Table below. 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 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).

4.1.1. 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 for 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, CH4 (g/kg DMI) was reduced by 5.6% with each 1% addition of supplemental fat. In most cases, 2 to 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 to 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 the 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 side effects 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, fibre digestibility, and milk fat percentage, so care must be taken in choosing the appropriate level of supplementation.

4.1.2. 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.

4.1.3. Forage-Related Strategies

Several forage-related strategies that reduce CH_4 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 per kilogram 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_4 because they have lower NDF content and pass more quickly through the rumen.

4.1.4. Feed Additives

4.1.4.1. 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, they 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 volatilisation and nitrous oxide emissions.

4.1.4.2. 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, they conducted a study with growing beef cattle to evaluate two commercial yeast products,

as commercial strains have not been selected for their effects on CH4 (McGinn et al., 2004). One product caused a 3% decrease in CH_4 production (g/g DMI) while the other product increased CH4 production (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 generally modestly priced and already widely used in ruminant production, acceptance of a CH_4-reducing yeast product would likely be high. However, considerable research and development would be needed to deliver such a product to the marketplace. To date, commercial manufacturers have been reluctant to invest in such products because animal performance, rather than CH_4 ab atement, is the primary driver for product development.

4.1.4.3. Enzymes

Enzyme additives are concentrated fermentation products that contain fiber digesting enzymes (e.g., 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 enzyme additives that reduce CH_4 emissions. In a recent *in vitro* study in their lab, one particular enzyme candidate increased fiber degradation of corn silage by 58%, with 28% less CH_4 produced per unit of fiber degraded (Beauchemin et al. unpublished). Furthermore, feeding dairy cows a diet containing corn silage with added enzyme reduced CH_4 production (g/g DMI) by 9% (Beauchemin et al. unpublished). Enzymes that improve fiber degradation typically decrease the acetate: propionate ratio in rumen fluid Eun and Beauchemin (2007), which is thought to be the primary mechanism whereby enzymes decrease CH_4 production. The potential of enzyme additives for CH_4 abatement warrant further research, because enzymes are likely to have positive effects both on milk production and CH_4 abatement.

4.2. Non-Dietary Strategies that Reduce Enteric Methane Production

4.2.1. 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 dose dependent. In recent studies, providing a dose of 10-15 ppm had no effect on CH_4 production (g/d or g/kg DMI) in dairy cows Waghorn et al. (2008) while a dose of 15-20 ppm either had no effect on CH_4 production or reduced total CH_4 but not CH_4 per kilogram of DMI in dairy cows (VanVugt et al., 2005). Higher doses (24 to 35 ppm), which are typically fed to dairy cows in North America, reduced CH4 production (g/d by 4 to 13% and g/kg DMI by 0 to 10%) in beef cattle and dairy cows Odongo et al. (2007), with short-term decreases in CH_4 of up to 30% being reported in beef cattle when 33 ppm of monensin was included in high or low forage diets (Guan et al*.,* 2006).

Ionophores such as monensin cause a moderate but transitory inhibition of rumen methanogenesis. Decreases in CH_4 to ionophores are related to a reduction in rumen protozoal numbers Guan et al. (2006), and alterations in ruminal bacterial populations, i.e. inhibition of the growth of Ruminococci without affecting F. Succinogenes (Chen and Wolin, 1979). Since January 2006 the use of ionophores in animal feeds has been banned in the European Union. It has been suggested that the relationship between the diversity of cellulolytic microorganisms in the rumen and CH_4 production merits further investigation, based on evidence that metabolic hydrogen and CH4 production can be decreased in the absence of lowered fibre digestion (Morgavi et al., 2010).

Unfortunately, the inhibitory effects of ionophores on CH4 production may not persist over time Guan et al. (2006) recently reported that monensin (33 mg/kg) lowered CH4 emissions in beef cattle by up to 30%, but levels were restored within 2 months. In that study, the effect of ionophores on CH4 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.

4.2.2. Defaunation

Defaunation, which is the elimination of protozoa from the rumen by dietary or chemical agents, has been shown to reduce ruminal CH4 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 CH_4 compared to 6–8% of GEI in ciliate-free animals. Protozoa in the rumen are associated with a high proportion of H_2 production, and are closely associated with methanogens by providing a habitat for up to 20% of rumen methanogens (Newbold *et a*l., 1995). Finlay *et al*. (1994) reported that protozoa could account for 37% of the total CH_4 production. It is assumed that there is a symbiotic H_2 transfer between

anaerobic protozoa and methanogens (Ushida and Jouany, 1996). The reduced ruminal methanogenesis 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 CH_4 (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 al., 2000). Therefore, the use of defaunation to mitigate CH_4 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 H_2 accumulation or inhibition of fermentation; therefore it represents a promising method of CH_4 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

A meta-analysis concluded that probiotic live yeasts have no effect on CH_4 production (Sauvant, 2005). However, the findings of other studies indicate that probiotic yeasts have variable effects on CH_4 emissions Chaucheyras-Durand et al. (2008), due to functional and metabolic diversity between specific strains (Newbold and Rode, 2006). In light of the significant genetic diversity between yeast strains, the potential of these feed additives to lower CH_4 emissions merits further investigation (Martin et al., 2010).

5.2. Bacteriocins

Certain bacteriocins including nicin and bovicin have been tested in vitro or in vivo. Most evaluations are based on functional studies in vitro with few data in vivo, highlighting that much more information on the stability and efficacy of bacteriocins in ruminants is required before these can be used on-farm (Martin et al., 2010). Some time ago, it was suggested that archaeal viruses that act against rumen methaogenes could be used to decrease CH_4 production Klieve and Hegarty, (1999), but thus far, these have not yet been isolated and/or identified in the scientific literature (Martin et al., 2010).

5.3. Propionate Enhancers

Dietary supplementation of 100 g fumaric acid/kg diet DM in free or encapsulated form was shown to decrease CH_4 by 62% and 76%, respectively in growing lambs (Wood et al., 2009). In contrast, other studies have reported that fumaric acid supplements had no effect on CH_4 emissions when fed at 175 g/d to growing beef cattle (Beauchemin and McGinn, 2006), at 80 g/d to steers (McGinn et al., 2004) or between 4–10 g/100 g (diet DM) in lambs (Molano et al., 2008). Other investigations have examined the potential of organic acids to serve as alternative hydrogen sinks to CH_4 in the rumen. Dietary supplements of DL-malic acid (from 0 to 75 g/kg diet DM) were reported to decrease linearly CH_4 production in beef cattle, changes that were also accompanied by lowered DM intake, total rumen VFA production and molar acetate to propionate ratios (Foley et al., 2009a). It has been speculated that the potential of organic acids to lower CH_4 may depend on the forage to concentrate ratio of the diet (Foley et al., 2009b). Further experiments are required to define conditions that optimize the efficacy of organic acids in the rumen and the persistency of their effects on rumen methanogenesis (Hook et al., 2010).

As a result 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 found that the addition of up to 500 mol of sodium fumarate in vitro decreased CH_4 production by 6% and increased DM digestibility of the basal diet by 6% after 48h incubation (Lopez et al., 1999). Asanuma et al. (1999) showed that the addition of 20 mM of fumarate to cultures that were fermenting hay powder and concentrate incubated for 6h significantly decreased CH4 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 30mM of fumarate. However, when incubation time was prolonged to 12h, 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 (15 ppm) plus fumaric acid (2%) supplemented to diets of Holstein steers increased the molar proportion of propionic acid and decreased CH_4 production (L kg DMI–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.

5.4. 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.g monoterpenes, 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 CH4 production, 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 was examined on in vitro gas production and fermentation by (Lee and Ha, 2002). The authors showed that supplementing 10% of essential oil increased ruminal pH and lowered NH3-N, VFA concentration and cumulative CH4 production over 48 h 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 *Lavandula officinalis* and 7.7% with *Solidago virgaurea*, indicative of diverse modes of action among plant extracts.

When sheep diets (60:40 silage:concentrate) were supplemented with 100 mg 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:propionate ratio, 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.

5.5. 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 Whitford et 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.

5.6. 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 days 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 can 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 ($h2 = 0.39$), 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 digesta has 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.

Table 1. Summary of Methane Mitigations Strategy for Dairy Cattle

Strategy	Potential CH4 reduction	Technology availability/feasibility	Cost/production benefit
Improving animal productivity	20-30%	Feasible and practical	Increased feed cost increased milk production use of fewer animals less feed per kg of milk
Increasing concentrate level at high levels of intake	25% or more	Feasible, for high producing cows, but may increase N_2O and CO_2 emissions	Increased feed intake Increased feed cost, Machinery/fertilizer use increased milk production
Processing of forages, grinding/ pelleting	20-40%	Feasible	Increased cost of processing improved feed efficiency increased milk production
Forage species and maturity	20- 25%	Feasible	Increased feed efficiency increased milk production
Rotational grazing of animals/early grazing	9% or more	Feasible	Increased cost of fencing increased management of animals increased feed intake increased milk production
Managed intensive grazing vs. confined feeding		Feasible needs more investigation	Cheaper feed cost May need supplements Reduced milk fat/protein content higher net return
Use of high quality forage/pastures	25% or more	Feasible	Increased feed intake increased milk production
Preservation of forage as silage vs. hay/additives	up to 33%(model prediction)	Feasible	Limited studies
Addition of fats	Up to 33%	Feasible and practical, but usage limited to 5-6 % in diet	Increased cost of diet increased or no effect on milk production May or may not affect milk fat
Use of ionopheres, e.g., monensin, lasolocid	11-30%	Feasible , but not long lasting public concerns	Increased feed efficiency decreased feed intake increased milk production
Use of probiotics	10-50% (*in vitro*)	Feasible, needs more investigation	May increase feed intake may increase milk production or no change
Use of essential oils	8-14% (*in vitro*)	Feasible, needs more investigation	Not quantified
Use of bovine somatotropin (bST)	9-16%	Not approved for use in canada	Reduced feed cost
Protozoa inhibitors	20-50% (*in vitro and in vivo*)	Not available for practical use	Practicability and cost to be assessed
Propionate enhancer (fumarate, malate)	5-11% (invitro) Up to 23% (*in vivo)*	Possible microbial adaption to fumaric acid	Economic feasibility ruminal adaptation and level of inclusion need to be evaluated
Use of acctogens	not qualified	Not available, needs more investigation	Needs further investigation
Use of bacteriocins, e.g., Nisin, bovicin HC5	Up to 50% (in vitro)	May provide alternatives to ionophores needs more investigation	Production effects are to be evaluated
Use of methane inhibitors, e.g., BES, 9.10-anthraquinone	up to 71% (in vitro)	No compound registered for use no long lasting effects identified	Increased cost of chemicals Production effects not established
Immunization	11-23%	Not available, needs more investigation	May increase cost of production increased gain
Genetic selection (Use of High Net Feed Efficiency animals)	21%	Long term feasibility	Decreased feed intake increased feed efficiency

6. Conclusion

Mitigation of CH4 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. 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. Existing strategies to lower enteric CH_4 emissions include increasing feed intake, proportion of concentrates in the diet, feeding high-quality forages or dietary supplements of plant and marine oils, oilseeds or specific fatty acids and ionophores. Recent research has focused on the potential of novel feed ingredients (probiotics, acetogens, bacteriocins, archaeal viruses, organic acids and plant extracts), vaccination of host animal against some methanogenic bacteria and the selection of cows with inherently lower losses of CH_4 as a proportion of dietary energy intake.

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