



## Alternatives for methane emission mitigation in livestock systems

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**ABSTRACT** - Human activities are contributing to Global Climate Change through the production of Green House Gases (GHG), which result in increased air, land and ocean temperatures and extreme changes in precipitation in regions of low and high rainfall. The most important GHG's are carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O). It is estimated that 18 % of the annual GHG emissions come from different types of livestock and that 37% of CH<sub>4</sub>, with higher global warming potential (23) relative to CO<sub>2</sub> (1), comes from fermentation processes in ruminants. It is possible that in the future beef and milk exports from producing countries is subject to bans if cattle systems do not comply with measures to reduce GHG. There are several alternatives available and being researched to reduce enteric CH<sub>4</sub> emissions from cattle that range from manipulating diet composition, supplementing feed additives (i.e. ionophores, organic acids, halogenated compounds, oils) and selection of forage plants of high quality and containing secondary metabolites (i.e. tannins and saponins) to animal breeding, immunization and genetic transformation of rumen microorganisms. Results show that inhibition of enteric CH<sub>4</sub> emission is possible through the use of ionophores, organic acids and oils. The use of ionophores can result in resistance of rumen microbes and as a result the effect is short term. The high cost of organic acids makes it unlikely that there direct supplementation in ruminant diets is economically viable. However, organic acids are present at relatively high concentrations in the leaf tissue of plants and attempts should be made to select and breed forages with higher levels of these compounds. It is argued that a more efficient strategy to reduce enteric CH<sub>4</sub> in ruminants is through selection of grasses of high quality (i.e. high concentration of water soluble carbohydrates), of forage legumes containing secondary metabolites like tannins and of fruits/plants containing saponins, provided that they do not affect intake and digestibility. Improved nutrition of cattle through feeding high quality forages can result in high animal performance and in reductions of CH<sub>4</sub> emitted per unit of dry matter intake and per unit of product.

Key Words: climate change, defaunation, feed chemical additives, genetic transformation of bacteria, greenhouse gases, immunization, saponins, tannins

## Alternativas para mitigação de emissão de metano em sistemas de criação de animais domésticos

**RESUMO** - As atividades humanas têm contribuído para a mudança do clima global, pela produção de gases de efeito estufa, dos quais podem resultar no aumento da temperatura atmosférica e mudanças extremas de precipitação em regiões de altas e baixas pluviosidades. Os gases de efeito estufa mais importantes são o dióxido de carbono (CO<sub>2</sub>), o metano (CH<sub>4</sub>) e o óxido nitroso (N<sub>2</sub>O). Estima-se que 18% das emissões anuais de GEE são provenientes de diferentes tipos de animais e que 37% do CH<sub>4</sub>, com maior potencial de aquecimento global (23) em relação ao CO<sub>2</sub> (1), decorrem dos processos fermentativos dos ruminantes. Possivelmente, no futuro, os países produtores de carne e leite estarão sujeitos a proibições, caso os sistemas de bovinocultura não cumpram as medidas de redução de gases de efeito estufa. Há diversas alternativas disponíveis e em estudo para reduzir as emissões de CH<sub>4</sub> entérica de bovinos, que vão desde a manipulação da composição da dieta, suplementação com aditivos (ionóforos, ácidos orgânicos, compostos halogênicos, óleos) e seleção de plantas forrageiras de alta qualidade contendo metabólitos secundários (taninos e saponinas) ao melhoramento animal, imunização e transformação genética dos microrganismos ruminais. Os resultados mostram que é possível a inibição da emissão entérica de CH<sub>4</sub> utilizando-se ionóforos, ácidos orgânicos e óleos. O uso de ionóforos pode resultar em resistência dos microrganismos ruminais e, como resultado, o efeito é de curta duração. O custo elevado dos ácidos orgânicos torna-os economicamente inviáveis se adicionados diretamente na dieta de ruminantes. No entanto, os ácidos orgânicos estão presentes em concentrações relativamente elevadas no tecido foliar das plantas, e devem ser feitas tentativas para selecionar e produzir forragens com altos níveis destes compostos. Argumenta-se que uma estratégia mais eficiente para redução entérica de CH<sub>4</sub> em ruminantes seja com a escolha de forragens de alta qualidade (alta concentração de carboidratos solúveis), de leguminosas contendo metabólitos secundários como taninos e de frutos e/ou plantas contendo saponinas, desde que estes não afetem o consumo e a digestibilidade. Melhora da nutrição de bovinos por meio da alimentação com forrageiras de alta qualidade pode resultar em bom desempenho dos animais e na redução da emissão de CH<sub>4</sub> por unidade de consumo de matéria seca e por unidade de produto.

Palavras-chave: aditivos químicos dos alimentos, defaunação, gases de efeito estufa, imunização, mudança climática, saponinas, taninos, transformação genética de bactérias

## Introduction

The Intergovernmental Panel on Climate Change (IPCC), convened by the United Nations, has reported evidence that human activities over the past 50 years have influenced Global Climate through the production of Green House Gases (GHG), which results in increased absorption in the atmosphere of infrared radiations emitted from the earth's surface. The accumulation of GHG results in increased global temperature (approximately 0.6 to 0.7°C), which in turn can increase annual precipitation in high rainfall regions and decrease precipitation in regions of low rainfall (Gerstengarbe & Werner, 2008). The most important GHG's are carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O), which have increased in the last 150 years (Monteny et al., 2006) and have different global warming potential. According to Ramaswamy et al. (2001) and Solomon et al. (2007), the warming potential of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O is 1, 23, and 298, respectively. Burning of fossil fuels is the main source of CO<sub>2</sub> emissions, while agriculture activities are the main contributors of global emissions of CH<sub>4</sub> and N<sub>2</sub>O (Wheeler et al., 2008). Thus, adoption of agricultural practices and technologies aimed specifically at reducing emissions from this sector will have a significant impact on total GHG emissions.

Livestock are well-known to contribute to GHG emissions. In the widely - cited 2006 report (Livestock's Long Shadow) by the United Nations Food and Agriculture Organization (FAO), it is indicated that 18 % of annual worldwide GHG emissions, are attributable to cattle, buffalo, sheep, goats, camels, horses, pigs, and poultry. Agriculture and in particular enteric fermentation in ruminants (predominantly cattle and sheep) produces between 21 and 25% of the total anthropogenic emissions of CH<sub>4</sub> on a global scale. The two major sources of agricultural CH<sub>4</sub> emissions are enteric fermentation in ruminants and livestock manure. In this paper we will focus on enteric CH<sub>4</sub>.

Many countries with large cattle populations are signatory to the Kyoto Protocol Framework Convention on Climate Change (1997), which requires that signatory countries maintain greenhouse gas emissions at 1990 levels by 2012. A non binding agreement was reached in Copenhagen (2009) by which 25 countries agreed to reduce GHG by 50% in 2050 in order to prevent that global temperature increased more than 2°C.

It is conceivable that if a carbon tax for greenhouse gas emissions is introduced as part of an agreement among countries to reduce GHG emissions, the cattle industry in many countries stands to lose profits. In addition, it is possible that beef and milk exports from producing countries

are subject to bans if cattle systems do not comply with measures to reduce GHG. Hence the need to investigate how we can reduce CH<sub>4</sub> emissions from ruminants should be of high priority in cattle producing countries.

In this paper we review alternatives to reduce CH<sub>4</sub> emissions from cattle through the use feed additives and of forage plants and fruits containing secondary metabolites.

### *Mechanism of enteric methane production in ruminants*

The major factors influencing CH<sub>4</sub> emissions from ruminants are: a) level of feed intake, b) type of carbohydrate fed and c) alteration of the ruminal microflora (Johnson & Johnson, 1995). Feed consumed by cattle is fermented in the rumen by bacteria, protozoa, and fungi and as a result polysaccharides in the feed are converted into volatile fatty acids (VFA) and microbial protein accompanied by the release of gaseous by-products (carbon dioxide and hydrogen) (Kamra, 2005). In adult cattle molecular hydrogen is produced every day, which does not accumulate as gases in the rumen given the presence of methanogenic archaea and other hydrogen utilizing microbes in the rumen. The symbiosis between bacteria that ferment polysaccharides and produce hydrogen and the methanogens which utilize hydrogen to reduce CO<sub>2</sub> and produce CH<sub>4</sub> results in an enhanced digestion of feed and microbial biomass production. As a result of this process, ruminants' loose between 2–12% of the gross dietary energy in the form of CH<sub>4</sub>, depending on the quality and quantity of diet offered and consumed (Johnson & Johnson, 1995). Approximately 87% of the enteric CH<sub>4</sub> is produced in the rumen and the remaining 13% is released in the large intestine through fermentation (Lockyer & Jarvis 1995; Lasey et al., 1997)

In summary, methane produced by cattle (250-500 l/day) not only affects the efficiency of energy utilization by ruminants, but also contribute significantly to environmental pollution. Thus it is essential to look for alternatives to reduce CH<sub>4</sub> emissions in cattle and by doing so contribute to less GHG and at the same time improve feed conversion efficiency, which should translate into economical profits for producers.

### *Strategies to reduce enteric methane emissions from ruminants*

Different strategies available to reduce CH<sub>4</sub> emission from enteric fermentation were reviewed by Hopkins & Del Prado (2007). They categorize them as: dietary changes, direct rumen manipulation and systematic changes. The latter include considerations of breed, livestock numbers and intensiveness of production. More intensive production may result in lower CH<sub>4</sub> emission, but may be less desirable in terms of other environmental impacts.

An overall reduction in CH<sub>4</sub> production (liters/day) per individual animal is the ideal goal. However, given the nature of livestock production systems, the immediate goal should be to reduce CH<sub>4</sub> per unit of product (milk or beef). Decreasing livestock numbers as an approach to reducing CH<sub>4</sub> implies reducing numbers, but holding productivity per animal constant so that CH<sub>4</sub> emissions fall. This strategy has economic consequences as the profit from livestock farms will decline in direct proportion to the reduction in numbers of animals. As milk or beef production per animal increases, CH<sub>4</sub> output per animal also increases, but both the proportion of gross energy used in the production of CH<sub>4</sub> and the amount of CH<sub>4</sub> emitted to produce a given quantity of milk or beef falls (Blaxter & Clapperton, 1965).

In summary, improvements in the efficiency of conversion of feed into animal product will reduce the amount of CH<sub>4</sub> emitted per unit of product, but will not necessarily reduce the amount of CH<sub>4</sub> produced in total.

In what follows we summarize feeding strategies and use of feed additive that have been evaluated to reduce CH<sub>4</sub> in ruminants and will highlight some areas of research that could be considered promising to reduce enteric CH<sub>4</sub>.

#### *Alternative feeding strategies to reduce methane in ruminants*

##### *Manipulating nutrient composition of the diet*

Manipulating the nutrient composition of the diet of ruminants can directly reduce enteric CH<sub>4</sub> output. For example, a high proportion of concentrates (grain based feeds) in the diet tends to reduce the protozoa population in the rumen, reduce rumen pH, alter the acetate: propionate ratio and decrease the amount of CH<sub>4</sub> produced per unit of feed intake (Blaxter & Clapperton, 1965). The proportion of concentrates in the diet needed to bring about this effect may well be over 50%. The direct manipulations of the diet in pasture - based systems by feeding concentrate supplements has economical consequences, which limit their use in many cattle production systems. Developing forages that directly reduce CH<sub>4</sub> is likely to be a better option for reducing CH<sub>4</sub> than feed supplementation based on concentrates.

##### *Selection of plants with secondary compounds*

In many studies (in vitro and in vivo) it has been demonstrated that with temperate legumes (*Hedysarium coronarium*, *Lespedeza cuneata*, *Lotus corniculatus* and *L. uliginosus*) and tropical legumes (*Calliandra calothyrsus*, *Flemingia macrophylla*) that contain secondary compounds such as condensed tannins (CT) it is possible to reduce methanogenesis. Tannins and phenolic monomers

have been found to be toxic for some of the rumen microbes, especially ciliate protozoa, fiber degrading bacteria and methanogenic archaea, and as a result methanogenesis in the rumen can also be reduced. However, Tiemann et al. (2008) indicated that with some tropical legumes with tannins (i.e. *Calliandra calothyrsus* and *Flemingia macrophylla*) their low fiber digestibility also contributes to the reduced in vitro CH<sub>4</sub> production measured with these legumes.

Reports in the literature provide evidence that by feeding legumes with CT there is a reduction of CH<sub>4</sub> production in different ruminant animals. In a review by Ramirez-Restrepo & Barry (2005) on alternative forages containing secondary compounds for improving sustainable production of grazing ruminants, they indicated that the condensed tannin-containing legumes *Lotus corniculatus* and sulla (*Hedysarum coronarium*) promoted faster growth rates in young sheep and deer in the presence of internal parasites, and showed reduced methane production relative to forages without tannins (*Chicorium intybus*). They also reported that grazing on *L. corniculatus* with CT was associated with increases in reproductive rate in sheep, increases in milk production in both ewes and dairy cows and reduced CH<sub>4</sub> production.

In other studies, *Lotus pedunculatus* was compared with ryegrass silage diets in Holstein cows and results showed that production of CH<sub>4</sub> was 27 and 35 per kg of dry matter intake for *Lotus* and silage ryegrass, respectively (Woodward et al., 2001). In a study carried out with sheep Carulla et al. (2005), found that the addition of *Acacia mearnsii* with CT to diets of *Lolium perenne* reduced by 13% the emissions of CH<sub>4</sub>. In goats consuming different levels of CT from *Lespedeza striata* there was a reduction in the emission of CH<sub>4</sub>, while in the same study feeding *Sorghum bicolor* with lower levels of CT showed no reduction of enteric production of CH<sub>4</sub> (Animut et al., 2008).

Studies reported in the literature on strategic use of tropical legumes with tannins to reduce CH<sub>4</sub> in ruminants are limited. Hess et al. (2006) reported reductions in CH<sub>4</sub> emissions when feeding *Calliandra calothyrsus* high in tannins as compared to *Cratylia argentea* low in tannins.

In summary, experimental evidence with temperate and to a lesser extent with tropical plants suggests that by using legume species with tannins it is possible to decrease the amount of enteric CH<sub>4</sub> produced by ruminants. It should be kept in mind that the methanogenic effect produced is not the same for all CT, but rather depends on the concentration and structure of the CT being fed (Min et al., 2003). Finally, the impact of legume forages with CT to reduce CH<sub>4</sub> could be constrained by the area sown each year in livestock producing regions of the world.

### Oils

Vegetable and animal oils have been used in ruminant rations to increase the energy density of diets. However, the use of oils is considered by some as very promising dietary alternatives to depress ruminal methanogenesis. It has been shown that vegetable oils can decrease CH<sub>4</sub> production in vitro (Broudiscou & Lassalas, 1991) as well as in vivo in sheep at maintenance (Czerkawski et al., 1966), in growing lambs (Machmüller et al., 2000) and in dairy cattle (Martin et al., 2008). Other studies have reported a 27% reduction in CH<sub>4</sub> emission with the supplementation of fish oil and sunflower oil in quantities of 500 mg/d when fed to dairy cows in short periods (14 days) (Woodward et al., 2006). However, when these same oils were fed for longer periods of time (12 weeks) there was no reduction of CH<sub>4</sub>.

The reduction in methanogenesis with oils/lipids appears to be the result of inhibition of microbial flora especially protozoa (Hu et al., 2005). Recent studies by Mao et al. (2010) showed a direct effect of soybean oil on reduction of protozoa and ruminal CH<sub>4</sub> production in sheep. The addition of coconut oil to forage and concentrate rations supplemented to Charolais steers showed a reduction in voluntary intake and protozoa population and this was reflected in low CH<sub>4</sub> emissions, without affecting livestock production (Lovett et al., 2003).

In summary, from the results reviewed it is evident that vegetable and animal oils are a good alternative to reduce CH<sub>4</sub>, but it is not clear if this reduction is long or short term. It is also not well defined if the effect of oils on methanogenesis is due to reduced intake, to the inhibiting effect of oils on protozoa or on the reduction of digestion of dietary fiber.

### Ionophores

Antibiotics that are used as feed additive and that affect several pathways of fermentation in ruminants. Among, the ionospheres, monensin is the most studied in ruminants, although other such as lasalocid, salinomycin, nigericin and gramicidin are also available. When added to the diet, ionophores affect CH<sub>4</sub> production in two ways: a) increased feed conversion efficiency and this reduces CH<sub>4</sub> output per unit of product and b) reduced amount of CH<sub>4</sub> produced per unit of dry matter consumed because of their effect on rumen fermentation.

In relation to feed conversion efficiency, a common result is that ionophores reduce intake but maintain or increase animal productivity. On high concentrate diets, results from a number of trials indicates that dry matter intake can be reduced by 5-6% and feed conversion efficiency increased by 6-7% (van Nevel & Demayer, 1996;

Raun et al., 1976; Goodrich et al., 1984). Less data is available on the effects of ionophores on forage based diets and the results tend to be more variable (O'Kelly & Spiers, 1992). Herbage intake has been measured less frequently on forage diets, but is usually unaffected or reduced when ionophores are supplemented (O'Kelly & Spiers, 1992).

In a review of in-vitro studies, van Nevel & Demayer (1996) found that ionophores reduce CH<sub>4</sub> output, but the percentage inhibition showed a wide range (0 - 76%) and this seem to be related to ionophore type and dose rate. The same authors give a figure of 18% as average reduction of enteric CH<sub>4</sub> emissions from in-vivo trials. In other studies, O'Kelly & Spiers (1992) working with steers fed Lucerne found that 55% of the reduction in CH<sub>4</sub> due to supplementation of ionophores was related to reduced intake and 45% to direct effect on rumen fermentation. One concern from some in-vivo trials is evidence of adaptation of rumen microorganisms to ionophores in a way that enteric CH<sub>4</sub> reduction per unit of feed is short term (Johnson & Johnson, 1995).

In general, due to the dual impact (reduced intake and changes in rumen fermentation patterns) of ionophores on enteric CH<sub>4</sub> production, the feeding of ionophore is an alternative for reducing CH<sub>4</sub>. However, studies with grazing ruminants need to be carried to confirm the utility and short or long term effects of ionophores for reducing enteric CH<sub>4</sub>. Of particular concern is that ionophores could accumulate in animal products, that rumen bacteria could get adapted to the antibiotic and that they need to be fed at frequent daily intervals unless they can be delivered by a slow release delivery device.

### Probiotics

Are microbial feed additives that have been developed to improve animal productivity by directly influencing rumen fermentation. Wallace & Newbold (1993) reviewed data from trials involving dairy cows and growing cattle fed high concentrate diets and calculated that probiotics improved productivity by 7 - 8%. Interest in probiotics as a potential technology to reduce CH<sub>4</sub> came from findings that in vitro they can directly reduce CH<sub>4</sub> production (Frumholtz et al., 1989). However, in vitro results on CH<sub>4</sub> reduction have not been consistent (Martin et al., 1989) and there are no reports in the literature on in vivo CH<sub>4</sub> production after supplementation of probiotics.

Given that probiotics are feed additives that need to be fed daily, they would appear to be only suitable for systems where feed supplements are given on a routine basis or for lactating dairy cows. This combined with the limited evidence that probiotics directly influence CH<sub>4</sub>

emissions indicate that they have limited utility to reduce CH<sub>4</sub> in ruminants.

#### *Organic acids*

As indicated earlier, enteric CH<sub>4</sub> arises from the conversion of hydrogen to CH<sub>4</sub> by a specific group of microorganisms, collectively described as methanogens. Other microorganisms break down feed to produce VFA, carbon dioxide and hydrogen. Increasing the production of one of these fatty acids (propionate) reduces hydrogen production, resulting in less being available for conversion to CH<sub>4</sub>. A number of organic acids (malate, fumarate, and pyruvate) are needed as precursors to propionate and if the rumen concentrations of these acids could be increased, propionate production would increase and methane production would fall. Malate is the organic acid most studied in relation to CH<sub>4</sub> production although fumarate has also been the subject of some limited work.

In vitro studies conducted by Martin & Streeter (1995) demonstrated that malate increases propionate production and decreases CH<sub>4</sub> output. The same workers (Martin et al., 1999) also found that direct supplementation of malate to the diet of finishing steers improved feed conversion efficiency. No reports were found in the literature of studies where CH<sub>4</sub> output has been measured from ruminants receiving malate supplementation. Other organic acids such as fumarate has not been shown to decrease CH<sub>4</sub> production in vivo (Beauchemin & McGinn, 2006).

The high cost of organic acids makes it unlikely that direct supplementation of ruminant diets is an economic proposition. However, organic acids are present at relatively high concentrations in the leaf tissue of plants and it may be possible to select and breed forages with higher levels of these compounds. Studies from the USA with Lucerne, Bermuda grass and Tall Fescue indicate that concentrations of organic acids vary among species and cultivars of the same species (Callaway et al., 1997). However, from the information available in the literature it is not possible to conclude if differences in organic acid concentrations found among forage species and cultivars are large enough to influence CH<sub>4</sub> production by ruminants.

#### *Halogenated compounds*

Chemical products such as bromochloromethane are potentially strong inhibitors of CH<sub>4</sub> production in ruminants. For example, when added to ruminant diets at a rate of 5 g per day, bromochloromethane was shown to reduce CH<sub>4</sub> for up to 15 hours after treatment (McCrabb et al., 1997). In addition to reducing CH<sub>4</sub> these compounds reduce intake and have little effect on live weight gain, which results in increased feed conversion efficiency (McCrabb, 2000).

In Australia, a compound containing bromochloromethane and cyclodextrin has been found to have a very large impact on enteric CH<sub>4</sub> production (May et al., 1995). When fed to cattle at hourly intervals it completely reduced CH<sub>4</sub> production (McCrabb et al., 1997) and when fed twice daily to cattle over an eight week period, it reduced CH<sub>4</sub> output by 54% (McCrabb, 2000).

A potential problem with halogenated compounds is that microbial populations may adapt and as result CH<sub>4</sub> inhibition may be short term (van Nevel and Demeyer 1996). They are also unstable compounds which are potentially toxic to ruminants and humans. Much more work needs to be done to define the utility of halogenated compounds as a CH<sub>4</sub> mitigation tool.

#### *Other alternatives being researched to reduce methane emissions from cattle*

##### *Animal breeding and selection*

There is plenty of evidence that indicates that improving individual animal performance reduces CH<sub>4</sub> produced per unit of product. It is also possible that some animals have intrinsic lower CH<sub>4</sub> emissions per unit of intake than others at the same level of performance. In trials with grazing sheep, Pinares-Patiño et al. (2003) identified some animals as 'high' and 'low' emitters per unit of feed intake in a single trial and then confirmed in a second trial that these differences persisted when the same type of diet was fed. The reasons why particular animals emitted less CH<sub>4</sub> per unit of feed intake in these trials is not known, but it does raise the possibility of genetic differences between animals in CH<sub>4</sub> production. Breeding animals with higher levels of individual performance will counteract the adverse consequences for CH<sub>4</sub> production resulting from increases in cattle numbers in livestock producing regions.

Other researcher have indicated that selection of animals with low potential to emit CH<sub>4</sub> should be based on differences in the gastrointestinal tract (i.e. feed retention time), which has an effect on digestion. For example, Waghorn et al. (2006) reported that when compared at the same stages of lactation (60 and 150 days), Holstein cows from the Northern Hemisphere consuming mixed diets produced 15% less CH<sub>4</sub>/kg of dry matter intake than cows from New Zealand also consuming mixed diets. However, in a recent study Munger & Kreuzer (2008) compared the emission of CH<sub>4</sub> in Jersey and Simmental cattle fed ad libitum in open gas exchange chamber and found no differences in CH<sub>4</sub> production between breeds.

### Defaunation

The elimination of protozoa from the rumen has been shown to reduce the amount of enteric CH<sub>4</sub> produced in a number of ways: a) lowered fiber digestion, b) reduced methanogen populations that are symbiotically associated with protozoa and c) reduced hydrogen production (Hegarty, 1999).

It is well documented that the secondary compounds known as saponins have antiprotozoal activity, but the level of antiprotozoal activity may vary with the type of saponin (Hess et al., 2003). In the rumen, methanogens are associated with protozoa (Lange et al., 2005), thus any additive which reduces the protozoa population will inhibit CH<sub>4</sub> production indirectly.

In vitro studies carried out in India using extracts of *Sapindus mukorossi* (a seed rich in saponins) showed a 52% reduction in protozoa population when ethanol extract was added in the incubation medium and this was associated with 96% inhibition in CH<sub>4</sub> production by rumen microbes of buffalos (Agarwal et al., 2009). One drawback was that this extract also reduced in vitro feed degradability by 48%. In other studies, Abreu et al. (2004) observed that with the addition of the saponin-rich fruit *S. saponaria* in the diet of sheep fed a low quality grass there was an increase in propionate relative to acetate, but without affecting the protozoa population. Hess et al. (2003) also evaluated in vitro the fruit of *S. saponaria* and found decreased protozoal count (by 54%) and daily CH<sub>4</sub> release (by 20%) relative to the control (grass + legume hay + straw + urea), but without affecting the methanogen count. In this study defaunation suppressed methanogenesis by 43% over all and the effect of *S. saponaria* on CH<sub>4</sub> was greater in defaunated (29%) than in faunated rumen fluid (14%).

In general, there is evidence that saponins from tropical fruits suppress the protozoa population and by doing so reduce methanogenesis, but the effect would not seem to be exclusively due to protozoal count depression. It would also appear that for practical use of *Sapindus* spp. as a feed additive to control enteric CH<sub>4</sub> emission there is a need to standardize a dose based on saponin type and concentration in order to achieve maximum inhibition in CH<sub>4</sub> production with minimum adverse effect on feed utilization and animal performance.

### Immunization

A team of researchers in Western Australia have taken out two patents on a vaccine that is claimed to improve animal performance and directly reduce CH<sub>4</sub> by invoking an

immune response in the rumen to protozoa and methanogens. Details in the scientific literature on the product are not available, but publicity material available from CSIRO (<http://www.csiro.gov.au>), claims that based on animal trials in sheep it will reduce methane production in sheep and cattle by 11 - 23% and, in addition, increase animal productivity. However, some authors have considered that the anti-methanogen activity of antibodies present in a vaccine for reducing methane in ruminants could be of short term nature given that proteolytic degradation in the rumen could limit their persistence (Li et al., 2007; Cook et al., 2008).

The vaccine is still at the development stage and if proven successful it will not likely be commercially available in the near future.

### Acetogens

These are bacteria present in adult ruminants that produce acetic acid by the reduction of CO<sub>2</sub> with hydrogen in the rumen. Although acetogenic bacteria can utilize H<sub>2</sub> and CO<sub>2</sub> to form acetate in the rumen, even large concentrations of acetogenic bacteria cannot compete for H<sub>2</sub> with methanogenic archaea under normal circumstances (Lopez et al., 1999).

In general, research carried out in Europe is attempting to increase the populations of acetogenic bacteria at the expense of methanogenic bacteria. If this approach is successful it would reduce CH<sub>4</sub> and increase the efficiency of production since acetic acid is an important energy source for ruminants. The research is at a very early stage and it is not possible to assess how successful this approach will be to reduce CH<sub>4</sub>.

### Genetic transformation of bacteria

Altering the fermentation characteristics of rumen microorganisms by genetic modification was identified as a mechanism whereby ruminant CH<sub>4</sub> emissions could be reduced (Armstrong & Gilbert, 1985). Research is at an early stage and has so far concentrated on the use of molecular biology techniques to quantify and characterize rumen microbial populations (Greg et al., 1996).

Many of the persistent doubts about rumen bacterial genetic manipulation and the viability of altered organisms in a competitive environment have been shown to be capable of resolution. In addition, the technology now available will allow extensive characterization of the molecular genetics of rumen bacteria with a precision that was not previously possible. However, it should be kept in mind that even if genetically altered rumen microbes did become available their acceptance by both producers and consumers is debatable. The approval of any

product/organism would have to meet both national and international regulatory standards for GM organisms and products.

### Conclusions

The inhibition of enteric CH<sub>4</sub> emission in ruminant animals is possible through the use of vegetable and animal oils, chemical feed additives like organic acids or antibiotics (ionophores), but high cost and/or short term nature of their effects may limit their use. Therefore, it would seem that efforts should be made to select feed ingredients and to identify forage plants containing secondary metabolites (tannins and saponins) that can be used to inhibit methanogenesis selectively, but without adversely affecting feed utilization. Genetic differences between animals in CH<sub>4</sub> production should also be explored in current animal breeding programs in the tropics.

There is evidence that shows that improved grass cultivars can increase animal performance without changing the quantity of feed consumed (Woodfield & Easton, 2004). This would imply a reduction in CH<sub>4</sub> production per unit of product and per animal. One option that should be explored is the development through breeding of tropical grass cultivars containing high levels of water soluble carbohydrates to increase animal performance and reduce CH<sub>4</sub> per animal as has been shown with ryegrass genotypes in the UK (Lovett et al., 2006). The potential for CH<sub>4</sub> mitigation through the genetic improvement of forage species remains largely unexplored and has been the subject of a review recently published by the FAO (2007) entitled "The genetic improvement of forage grasses and legumes to reduce greenhouse gas emissions".

In summary, the range of technical options available at present to farmers to reduce CH<sub>4</sub> emissions in cattle is limited and no single option appears to provide a simple solution. However, selection and breeding of animals with low capacity to produce CH<sub>4</sub>, selection and utilization of high quality forages, strategic supplementation forages with tannins, and better management of livestock in pasture-based systems are options that should be considered for reducing enteric CH<sub>4</sub> emitted per animal and per unit of product.

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