Factors affecting mitigation of methane emission from ruminants: Microbiology and biotechnology strategies

Fatores que afetam a mitigação das emissões de metano a partir de ruminantes: Estratégias de microbiologia e biotecnologia

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Abstract Climate change is a subject of global environmental concern. Increased anthropogenic Greenhouse Gas (GHG) emissions have increased the global temperature the last 100 to 200 years. Carbon dioxide and methane are the main greenhouse gases related to animal nutrition and methane has greater global warming potential than carbon dioxide. Among greenhouse gases, methane is considered a potent greenhouse gas with 21 times more global warming potential than carbon dioxide. Worldwide, ruminant livestock produce about 80 million metric tons of methane each year, accounting for about 28% of global emissions from human related activities. Therefore it is impelling animal scientists to finding solutions to mitigate methane emission from ruminants. It seems that solutions can be discussed in four topics including: nutrition (feeding), biotechnology, microbiology and management strategies. We have already published the first and second review articles on feeding strategies and management strategies. In the current review, Microbiology and biotechnology such as emphasizing on animal breeding, genetic merit, bovine somatotropin (BST), unproductive animals, vaccination, immunisation and biological control (bacteriophage, acetogenesis reductive), chemical defaunation that can be leads to decreasing methane production from ruminant animal production are discussed.

Keywords: enteric fermentation, greenhouse gases, methane, nutrition, ruminant

Resumo A mudança climática é um assunto de preocupação ambiental global. O aumento dos gases de estufa antropogênicos (GEA) tem elevado a temperatura global nos últimos 100 a 200 anos. O dióxido de carbono e metano são os principais gases do efeito estufa e estão relacionados com a nutrição animal, onde o metano tem maior potencial de aquecimento global do que o dióxido de carbono. Entre os gases do efeito estufa, o metano é considerado um gás 21 vezes com maior potencial de aquecimento global do que o dióxido de carbono. Em todo o mundo, animais ruminantes produzem cerca de 80 milhões de toneladas de metano por ano, respondendo por cerca de 28% das emissões globais das atividades humanas relacionadas. Por isso é impelindo aos zootecnistas encontrar soluções para mitigar emissões de metano a partir de ruminantes. Parece que as soluções podem ser discutidas em quatro temas, incluindo: nutrição (alimentação), biotecnologia, microbiologia e estratégias de gestão. Nós já publicamos o primeiro e segundo artigos de revisão sobre estratégias de alimentação e estratégias de gestão. Na avaliação atual, microbiologia e biotecnologia, tal como com ênfase na criação de animais, mérito genético, somatotropina bovina (BST), animais improdutivos, vacinação, imunização e controle biológico (bacteriófago, acetogênese redutora), defaunação química que pode conduz à diminuição da produção de metano na produção de ruminantes são discutidos.

Palavras-chave: fermentação entérica, gases do efeito estufa, metano, nutrição, ruminantes

Introduction

Climate change is a subject of global environmental concern. Increased anthropogenic Greenhouse Gas (GHG) emissions have increased the global temperature the last 100 to 200 years (Mirzaei-Aghsaghali and Maheri-Sis 2011). Carbon dioxide and methane are the main greenhouse gases related to animal nutrition and methane has greater global warming potential than carbon dioxide (Mirzaei-Aghsaghali et al 2015; Yurtseven et al 2009). Livestock, produced throughout the world, are an important agricultural product in virtually every country. Ruminant animals (particularly cattle, buffalo, sheep, goat and camels) produce significant amounts of CH_4 under the anaerobic conditions of the

digestive processes (Sejian et al 2011). Domestic livestock produce 80-115 million tons of methane per year. Ruminants typically lose 2-12% of their gross energy intake as emissions of methane (Yurtseven and Ozturk 2009). The mean volume of methane produced by a dairy ewe each year is 17.8 m^3, which is similar to that of a dairy goat (Yurtseven et al. 2009). Several factors play a major role in enteric fermentation and in controlling the overall CH$_4$ emission from livestock (Sejian et al 2011).

Methane is produced in the rumen and hindgut of animals by a group of *Archaea* known collectively as methanogens, which belong to the phylum Euryarcheota. Among livestock,methane production is greatest in ruminants, as methanogens are able to produce methane freely through the normal process of feed digestion (Hook et al 2010).

Methane production in ruminants has received global attention in relation to its contribution to the greenhouse gas effect and global warming. In the last two decades, research programs in Europe, Oceania and North America have explored a variety of approaches to redirecting reducing equivalents towards other reductive substrates as a means of decreasing methane production in ruminants. Some approaches such as vaccination, biocontrols (bacteriophage, bacteriocins) and chemical inhibitors directly target methanogens. Other approaches, such as defaunation, diet manipulations including various plant extracts or organic acids, and promotion of acetogenic populations, seek to lower the supply of metabolic hydrogen to methanogens (McAllister and Newbold 2008a).

In this paper we review alternatives to reduce CH$_4$ emissions from ruminants through Microbiology and biotechnology strategies.

Animal breeding

Traditional animal breeding has focussed on production traits, such as milk production and growth rate, since these are relatively easy to measure in the national herd or flock. One of the key challenges for the industry, and opportunities for bioscience, is the ability to incorporate a much wider range of traits into breeding indices. Examples include product quality, animal health, efficiency and environmental impact. Genetic gain tends to be poor in traits that are lowly heritable (e.g., fertility, health and survival), not recorded on a large scale (e.g., feed intake, product quality, animal health traits), sex linked (e.g., daughter fertility, milk quality), and/or require a long time horizon to measurement (e.g., survival). Many of these traits have not been recorded routinely and may be difficult to record outside research centres. This requirement is driven by growing demands from the consumer and the need to develop and differentiate higher-value ruminant products in

the face of low-cost commodity production, particularly in Southern Hemisphere countries. The use of simple gene markers to identify deleterious alleles is well established in the animal breeding industry, for examples with bulls being screened for known recessive alleles prior to entering progeny testing schemes.

Within groups of sheep or cattle fed fresh forages, about 10% have very high and 10%low methane emissions (per kg DMI) and the difference between the two groups is about 40%. One group researchers showed mean methane production from four highest and four lowest producing sheep (selected from a random group of 20 animals) over a four month period was 3.75 vs. 5.15% of GEI. Earlier reports found 86% of variation in methane production by sheep consuming 900-1700 g DM day^{-1} was due to animal variation and only 14% was attributable to diet (Waghorn and Clark 2004).

Ulyatt et al (1999) summarized existing validations of the SF6 technique and found SF6 estimates were 93 to 95% of chamber-based measurements of methane emissions and showed that variation between days was attributable to animals (sheep or cattle fed forages) even though intakes and composition of each diet were relatively constant (Ulyatt et al 1999).

Robertson et al (2002), reported that Overseas genotypes produced 8-11% less methane, as a percentage of gross energy (GE) intake, compared to New Zealand genotypes and total mixed rations (TMR) resulted in similar energy losses to methane (% of GE) as pasture. Genotype differences had disappeared by day 240. The result of this experiment demonstrates a persistent high or low methanogenesis for some, but not all cows fed pasture. A similar variation between individuals was evident for TMR diets fed to cows (Robertson and Waghorn 2002).

Sheep with high CH$_4$ yields had larger rumen volumes, a slower particulate outflow rate, higher fibre digestibility and longer retention times than sheep with low CH$_4$ kg^{-1} DMI. Methane yield is best predicted as a function of particulate fractional outflow rate, organic matter intake (g kg LW-0.75) and molar proportion of butyrate (Pinares-Patiño et al 2003). Retention time of digesta in the rumen is a repeatable characteristic, which contributes in part to differences in wool growth rate. Because digestibility, as influenced by retention time, do not affect wool growth rate, the positive effect of retention time on wool production is accounted for by the effect of retention time on level of intake. Both retention time and wool growth rate may be affect by the same physiological controls, although they are not directly related to each other. Progress in wool production using retention time as selection criteria, is thus expected to be poor (Smuts et al 1995). Animal effects on rumen microflora have been demonstrated by widely differing *in sacco* degradation rates and contrasting

populations of cellulolytic bacteria. Variation in susceptibility to bloat appears affected by salivary proteins and bloat prone cattle produce bloat prone offspring. This capacity to affect their microflora offers potential for development of anti-methanogen or anti-protozoal vaccines (Waghorn et al 2004).

A number of experiments have reported variation between animals in CH_4 emissions per unit of feed intake. In a trial involving 302 grazing dairy cows mean CH_4 emissions of 19.3 ±2.9 g/kg DMI were reported (Clark et al 2005); the 15% variance suggesting heritable differences in methanogenesis. Similar responses were reported in sheep on an unlimited pasture diet (Eckard et al 2010).

Hegarty et al (2007) also reported a significant relationship existed between methane production and residual feed intake when residual feed intake (RFI) (RFI15d) was estimated over the 15 d when steers were harnessed for methane collection. Although in this experiment the opportunity to abate livestock methane production by selection against residual feed intake seems great, residual feed intake explained only a small proportion of the observed variation in methane production. A genotype × nutrition interaction can be anticipated, and the methane production: residual feed intake EBV (estimated breeding value) relationship will need to be defined over a range of diet types to account for this (Hegarty et al 2007)

One group of researchers suggested that animal breeding could achieve a 10 to 20% reduction in CH_4 losses from DM during digestion (Waghorn et al 2006).

However, breeding for reduced methanogenesis is unlikely to be compatible with other competing breeding objectives. In contrast, breeding for improved feed conversion efficiency (lower net feed intake) should be compatible with existing breeding objectives and likely to both reduce CH_4 and the ratio of CH_4 per unit of product produced (Eckard et al 2010).

Genetic merit

Improving the genetic merit of dairy cows has escalated in the last decade with the import of Holstein genetic material from US and Canada for use on the EU native dairy breeds. As a result, average national yields have increased. One of the major improvements is the ability of the cow to partition nutrients into milk preferentially to maintenance and/or growth. This has undoubtedly resulted in increased efficiency. The UK dairy herd has increased its average yield by 8.8% from 1995 to 1997 and the top 10% of herds are averaging 8351 litres per cow. There are additional benefits which include the following: (i) a cow's lifetime production can be achieved in less lactations, therefore there are less maintenance costs e.g. lifetime production of 30000 litres achieved as 5 lactations of 6000 litres or 3 lactations of

10000 litres; (ii) a 100 cow herd producing average yield of 6 000 litres = 600 000 l.y^{-1} or 60 cow herd producing 10 000 litres, therefore less cows to maintain; (iii) less replacement heifers to maintain (Moss et al 2000). Kirchgessner et al (1995) suggested that increasing milk production of dairy cows from 5000 to 10000 litres milk annually would only increase methane production by 5% (i.e. from 110 to 135 kg methane per year) (Kirchgessner et al 1995). The genetic merit of livestock within the EU is rapidly improving and this will undoubtedly bring with it increased efficiency. The management of these high genetic merit cows will also become more complex and the overall implementation of this may be stalled by animal welfare implications. High genetic merit cows can have increased problems with fertility, lameness, mastitis and metabolic disorders. All these issues will have to be addressed if genetic progress is to be successfully continued (Moss et al 2000).

Bovine somatotropin (BST)

Bovine somatotropin (BST) is a genetically engineered metabolic modifier approved for use in some countries to enhance milk production from dairy cows. BST does not affect digestibility, maintenance requirements or the partial efficiency of milk synthesis, nor does it act directly on the mammary gland. BST affects mammary tissue indirectly by its action on the liver and the kidney to stimulate production of insulin-like growth factors which act on the mammary gland to increase milk synthesis. Nutrients for increased milk yield are provided by increased intake and coordination of metabolism to increase supplies to the mammary gland of glucose, amino acids and fatty acids. Given a 15% increase in productivity per animal, there would be are reduction in methane production per unit product. Again, this is not a popular consumer choice for enhancing animal productivity and is actually banned by all EU member states. It is therefore not worthy of pursuance (Moss et al 2000).

Residual feed intake

Hegarty et al (2007) showed that Daily DMI of steers was lower during the methane measurement period than when methane was not being measured (11.18 vs. 11.88 kg) (Hegarty et al 2007). A significant relationship existed between methane production and residual feed intake (RFI) when RFI (RFI15d) was estimated over the 15 d when steers were harnessed for methane collection. Animals expressing lower RFI had lower daily methane production. The relationship established between methane production and RFI15d was used to calculate a reduction in daily methane emission of 13.38 g accompanied a 1 kg/d reduction in RFIEBV in cattle consuming *ad libitum* a diet of 12.1 MJ of

ME/kg. The magnitude of this emission reduction was between that predicted on the basis of intake reduction alone (18 g_d^{-1}_kg of DMI^{-1}) and that predicted by a model incorporating steer midtest BW and level of intake relative to maintenance (5 g_d-1_kg of DMI-1). Comparison of data from steers exhibiting the greatest (n = 10) and lowest (n = 10) RFI15d showed the low RFI15d group to not only have lower MPR but also reduced methane cost of growth (by 41.2 g of CH_4/kg of ADG). Results of this study have confirmed that breeding of cattle on the basis of estimated breeding values for RFI offers a definite mechanism to reduce enteric methane emissions without compromising animal productivity.

Alford et al (2006) reported the annual methane abatement in year 25 of selection was 15.9% lower than in year 1. For the national herd, differential lags and limits to adoption were assumed for northern and southern Australia. The cumulative reduction in national emissions was 568 100 t of methane over 25 years, with annual emissions in year 25 being 3.1% lower than in year 1. It is concluded that selection for reduced RFI will lead to substantial and lasting methane abatement, largely as a consequence of its implementation as a breeding objective for the grazing beef herd (Alford et al 2006).

Contrary to beef cattle, it seems that the genetic determination of CH_4 production is of minor importance in taurine dairy breeds and selection for RFI may not be effective (Münger and Kreuzer 2008). Options for selection in ruminants lie in the host components of rumen function, in post absorption nutrient utilization and in disease resistance Such approach is promising but requires additional research (Hoffmann 2008).

Unproductive animals

Reducing the number of unproductive animals on farm has potential to both improve profitability and reduce CH_4. Strategies like extended lactation in dairying, where cows calve every 18 months rather than annually, reduces herd energy demand by 10.4% (Trapnell and Malcolm 2006) and thus potentially reduces on-farm CH_4 emissions by a similar amount. Through earlier finishing of beef cattle in feedlots, slaughter weights are achieved at a younger age, with reduced lifetime emissions per animal and thus proportionately less animals producing CH_4 (Eckard et al 2010).

Vaccination

A vaccine developed from a three-methanogen mixture produced a 7.7% reduction (kg^{-1} DM) in methane emissions from sheep despite only one antigen being effective against the methanogenic species in the sheep. The vaccine was much more effective than the seven methanogen mix tested previously and was able to increase saliva and plasma antibody titres by 4 – 9 folds over the seven methanogen mixture. Successful elevation of antibody titres in saliva and a significant reduction in methane emissions offers real potential for a widespread application to ruminants in all environments. At present vaccines do not have sufficient efficacy for commercial use and funding has recently been curtailed. Opportunities through rumen additives, defaunation and specific compounds targeting methanogens provide several routes for reducing methane production. However these agents have not addressed the inevitable production of hydrogen from fermentation of fibre (Waghorn and Woodward 2006).

Chemical defaunation

The removal of protozoa from the rumen (defaunation) has been shown to reduce CH_4 production by up to 50% depending on the diet (reviewed by (Hegarty 1999)).

It has been estimated that the methanogens associated with the ciliate protozoa, both intracellularly and extracellularly, are responsible for 9 to 37% of the methane production in the rumen (Hook et al 2010).

The effect of rumen protozoa on methane production and on methanogens has been recently investigated by molecular biology. The decrease in methane production of 26% per kg DM intake in protozoa-free lambs was related to a decrease in the proportion of methanogens in the total bacterial population of the whole ruminal content (McAllister and Newbold 2008a).

Methanogens are known to have symbiotic relationships involving interspecies hydrogen transfer with rumen microorganisms, especially with rumen protozoa where the methanogens can be associated intracellularly and extracellularly. Common protozoa in the bovine rumen found to have such a relationship are from the genera Entodinium, Polyplastron, Epidinium, and Ophryoscolex, while the methanogens most often associated with protozoa are from the orders Methanobacteriales and Methanomicrobiales. Anaerobic fungi, such as Neocallimastix frontalis, have also been found to have a relationship with methanogens involving interspecies hydrogen transfer whereby the fungi's enzymatic activity has increased and metabolism has shifted towards acetate production (Hook et al 2010).

In another study, whereas CH_4 production decreased by 20% in protozoa-free sheep (Morgavi et al 2008), the quantity of methanogens estimated by qPCR, as well as their diversity estimated by PCR-DGGE was not different between faunated and defaunated animals suggesting that the decreased methanogenesis might be due to a reduction in the amount of hydrogen substrate (Martin et al 2008).

Methanogenic bacteria have been observed on the exterior surface of rumen ciliate protozoa and as endosymbionts within the ciliates. Newbold et al (1995) estimated that methanogens associated with ciliate protozoa were responsible for between 9 and 25% of the methanogenesis in rumen fluid and the removal of protozoa from the rumen (defaunation) has been associated with decreases in methane production (Newbold et al 1995a). Ushida et al (1987) reported that the use of defaunation to decrease methane production from ruminants would have to be balanced against the effects on fibre and protein metabolism in the rumen (Ushida et al 1986).

A wide range of chemicals are available that will reduce rumen methanogenesis:

a. Alternative hydrogen acceptors, such as fumarate, sulphate/sulphite, nitrate/nitrite and unsaturated fatty acids. Generally the amount required to be effective in reducing methane emission is likely to be either toxic to the animal, cause disruptions to digestion, or be uneconomic.

b. Halogenated methane analogues, such as, chloroform, carbon tetrachloride, chloral hydrate, bromochloromethane and bromoethanesulphonic acid can be very potent methane inhibitors. While some of these compounds are volatile and difficult to administer, McCrabb et al (1997) claimed success in inhibiting methane in cattle with bromochloromethane complexed with -cyclodextrin, which reduced volatility (McCrabb et al 1997). Mathison et al (1998) concluded that halogenated methane analogues have potential as methane inhibitors, provided that problems such as adaptation by rumen microbes, host toxity and suppression of digestion can be overcome (Mathison et al 1998).

c. Ionophores, such as monensin and lasalocid have been shown to reduce methane emission (Johnson and Johnson 1995), though the affect appears to be short-lived as the rumen microbes adapt to the additive within two weeks.

d. Defaunating agents, like manoxol, teric, alkanate 3SL3 and sulpho succinate can reduce methane emission (Mathison et al 1998). They appear to act by disrupting the close symbiotic relationship between methanogenic bacteria and protozoa. Many of these defaunating agents are toxic to the host animal and this restricts their routine use. The main problems with chemical additives are that many are toxic to the animal, toxic to rumen microflora and therefore reduce digestion and food intake, have short lived effects because the rumen microbes adapt, are volatile and thus difficult to administer,

are expensive, or would fail to meet consumer product acceptance (Ulyatt and Lassey 2001).

Hegarty (1999) reviewed the impact of total or partial defaunation to improve ruminant performance and lower methane emissions. Improved performance has been associated with increased microbial flow to the intestine (protozoa consume bacteria) and increased proportions of propionate (protozoa produce acetate, butyrate as well as hydrogen gas). There is also a close (symbiotic) association between protozoa and methanogens, and defaunation is likely to lower methane emissions by 20–50%. Defaunation is somewhat risky, and is frequently incomplete, with a return of protozoa within weeks or months even if defaunated animals are kept separate from faunated livestock. However even partial defaunation is likely to achieve substantial benefits for CH_4 reduction and animal performance, especially when grazing diets with a medium-low protein content (Hegarty 1999).

Most of the small ciliate protozoa, including Dasytricha ruminantium and Entodinium spp. living in the rumen of sheep, were found to have intracellular bacteria. Hydrogen is one of the major end products of the rumen protozoa metabolism and a physical association between protozoal cells and methanogens exist in the rumen ecosystem (Finlay et al 1994).

The absence of protozoa, rumen methane emissions are reduced by an average of 13% but this varies with diet. Decreased methane emissions from the protozoa-free rumen may be a consequence of: (1) reduced ruminal dry matter digestion; (2) a decreased methanogen population; (3) an altered pattern of volatile fatty acid production and hydrogen availability; or (4) increased partial pressure of oxygen in the rumen (Hegarty 1999).

The effect of rumen protozoa on methane production and on methanogens has been recently investigated by molecular biology. The decrease in methane production of 26% per kg DM intake in protozoa-free lambs was related to a decrease in the proportion of methanogens in the total bacterial population of the whole ruminal content (Martin et al 2008).

Newbold et al (1995) was estimated the importance of methanogenic bacteria associated with ciliate protozoa either by removing protozoa from whole rumen fluid (using defaunated rumen fluid to correct for the effects of centrifugation on bacteria) or by isolating the protozoa and reported that methanogenic bacteria associated with rumen ciliates were apparently responsible for between 9 and 25% of methanogenesis in rumen fluid (Newbold et al 1995b).

Recently, there has been an increased interest in plant secondary metabolites for use as possible defaunating agents. In particular, saponin-containing plants show promise as a possible means of suppressing or eliminating protozoa in the

rumen without inhibiting bacterial activity. Saponins are glycosides which apparently interact with the cholesterol present in eukaryotic membranes but not in prokaryotic cells (Moss et al 2000).

Lu and Jorgensen (1987) reported that microbial fermentation and nutrient degradation in the rumen were reduced by saponins and Total protozoa! count in the rumen was reduced 34 and 66% by saponins at levels of 2 and 4% dietary dry matter, respectively (Lu and Jorgensen 1987).

Coleman (1986) reported that rumen ciliate protozoa play an active role in ruminal fibre break-down because the rumen CMCase was lowest in defaunated animals and tended to be higher in animals containing the amylolytic *Entodinium caudatum* or a natural mixed protozoal population and highest in sheep containing single cellulolytic species. In animals containing the cellulolytic species, *Epidinium ecaudatum caudatum* or *Eudiplodinium maggii*, 70% of the CMCase was associated with the protozoal fraction whereas less than 15% was present in a fraction prepared in the same way from sheep containing no protozoa or only *Entodinium caudatum* (Coleman 1986).

Wallace and McPherson (1987) investigate the factors affecting the rate of breakdown of bacterial protein in rumen fluid and based on the results this experiment; one might expect defaunation to be of great benefit to the efficiency of microbial protein synthesis in the rumen, by avoiding wasteful protein turnover. Enhancement of the microbial growth yield by defaunation has been observed both in and in vivo. However, the finding that defaunation is actually of little benefit in vivo unless dietary protein is exceptionally low implies that other influences, such as on the rate of fibre degradation, intervene to counteract the advantageous one of removing the most important cause of microbial protein turnover in rumen fluid. Thus the use of defaunation to decrease methane production from ruminants would have to be balanced against the effects on fibre and protein metabolism in the rumen (Wallace and McPherson 1987).

The number of protozoa in the rumen of sheep during the period on the high-fat diet decreased considerably but the total number of bacteria increased. The decrease in the number of protozoa could have been due to toxicity of linseed-oil fatty acids to these organisms, but it could also have been the result of reduced sequestration of these micro-organisms in the rumen (Czerkawski et al 1975).

Pilajun and Wanapat (2011) investigate the methane production and methanogen population in rumen liquor of swamp buffalo as influenced by coconut oil and mangosteen peel powder supplementation. The result of this experiment showed that coconut oil and mangosteen peel powder supplementation decreased methane production by changing methanogen population. Although, methanogen population was not changed, methanogen diversity was relatively changed by dietary supplementation. Combination of coconut oil and mangosteen peel powder had pronounced effect than those of single supplementation. Supplementation by high level of coconut oil or mangosteen peel powder in this experiment did not show positive effects. Thus, appropriate level should not exceed 6% DM for coconut oil and 4% DM for mangosteen peel powder supplementations (Pilajun and Wanapat 2011).

Immunisation and biological control (bacteriophage, acetogenesis reductive)

Baker (1995) has proposed that it may be possible to immunize ruminants against their own megthanoens with associated decrease in methane output and that such an approach can successfully reduce the members of streptococci and lactobacilli in the rumen (Baker 1999). An alternative strategy to reduce ruminal methanogenesis would be to re-channel substrates for methane production into alternative products. Acetogenic bacteria in the hindgut of mammals and termites, produce acid by the reduction of carbondioxide with hydrogen and reductive acetogenesis acts as an important hydrogen sink in hindgut fermentation (Gworgwor et al 2006).

The addition of acetogens into normal ruminal fermentations has not increased acetate production, probably because acetogens have much lower affinities for hydrogen than methanogens. Thus, they are unable to compete for substrate when the methanogens are present. However, when combined with methane inhibitors, acetogens have been able to alter ruminal fermentations (Kung et al 2003).

Le Van et al (1998) evaluate the role of reductive acetogenesis as an alternative H_2 disposal mechanism in the rumen and reported that inhibition of methanogenesis with 2-bromoethanesulfonic acid (BES) and the addition of Acetitomaculum ruminis 1904A to obtain $>10^5$ cfu/mL, resulted in reductive acetogenesis (Le Van et al 1998).

Attempts to use acetogenic bacteria to outcompete methanogens for reducing equivalents have also been reported but again with limited success (Lopez et al 1999).

In a study by Wright and colleagues, two vaccines were developed, named VF3 (based on three methanogen strains) and VF7 (based on seven methanogen strains), which produced a 7.7% methane reduction per dry matter intake, despite only approximately 20% of the methanogen population being targeted (Wright et al 2004a).

Williams et al (2009) methane output levels corrected for dry-matter intake for the control and treatment groups were not significantly different, and real-time PCR data also indicated that methanogen numbers were not significantly different for the two groups after the second vaccination. However, clone library data indicated that methanogen diversity was significantly greater in sheep receiving the anti-methanogen vaccine and that the vaccine may have altered

the composition of the methanogen population (Williams et al 2009).

Scientists in Australia have registered patents for immunization procedures that are claimed to reduce methane emission. They have developed a vaccine containing an antigen derived from methanogenic bacteria and an immunogenic preparation which reduces the activity of rumen protozoa. The antimethanogenic vaccine is claimed to reduce methane in in vitro incubations, and significantly increase DM intake and wool growth. Such vaccines have the potential to provide a cost-effective treatment to reduce methane emission and enhance animal production (Ulyatt and Lassey 2001).

A new generation of designer inhibitors may be developed based on knowledge of the genome of rumen methanogens (Attwood and McSweeney 2008).

Cook et al (2008) reported that the transient nature of the inhibition of methane production by the antibodies may have arisen from instability of the antibodies in ruminal fluid, or to the presence of non-culturable methanogens unaffected by the antibody activity that was administered. More recently, passive immunization was also assayed using antibodies prepared from hen's eggs. Antibodies decreased metehan production in vitro but the effect was short-lived (Cook et al 2008).

Nisin is thought to act indirectly, affecting hydrogen producing microbes in a similar way to that of the ionophore antibiotic monensin. However, there is no published in vivo trial of the effects of this bacteriocin on methane. Nisin is widely used in the food industry as a conservative and fears of microbial cross-adaptation might prevent its approval as a feed additive. A bacteriocin obtained from a rumen bacterium, bovicin HC5, decreased methane production in vitro up to 50% without inducing methanogens' adaptation (Martin et al 2008).

Klieve and Hegarty (1999) also suggested the use of archaeal viruses to decrease the population of methanogens but, to our knowledge no bacteriophages from rumen methanogens have been isolated (Klieve and Hegarty 1999).

Manipulation of the rumen microbial ecosystem

Manipulating microbial populations in the rumen, through chemical means, by introducing competitive or predatory microbes, or through vaccination approaches, can reduce CH_4 production (Eckard et al 2010)

Wright et al (2004), investigate the methane emissions from sheep immunized with an anti-methanogen vaccine were significantly lower than methane emissions from non-immunized sheep, to test the effectiveness of two different vaccine formulations (VF) on methane abatement, and to compare methane emissions measured using a closed-circuit respiration chamber and the sulphur-hexafluoride

(SF6) tracer technique. The result of this experiment showed that a significant 7.7% reduction in methane production per kg dry matter intake, although results were not repeatable with subsequent vaccine preparations (Wright et al 2004b).

The decrease in rumen methane formation can be attained through several mechanisms of action. The direct inhibition of methanogens is one of them. Some approaches such as vaccination, biocontrols (bacteriophage, bacteriocins) and chemical inhibitors directly target methanogens. Development of a vaccine against cell-surface proteins, common to a broad range of methanogen species may improve the efficacy of vaccination as a CH_4 mitigation strategy (McAllister and Newbold 2008b).

The microbial ecology of the rumen ecosystem is exceedingly complex and the ability of this system to efficiently convert complex carbohydrates to fermentable sugars is in part due to the effective disposal of H_2 through reduction of CO_2 to methane by methanogen (McAllister and Newbold 2008b).

Tomkins and Hunter (2003) reported that loss of GE tomethane decreased from 3.9% to 0.6% in steers using acompound containing bromochloromethane. Microbial adaptation, decreased fiber digestion and toxicity to the animal aredrawbacks of these inhibitors.

The chemical reduction of protozoal numbers has been shown to reduce CH_4 by up to 26% (DMI basis), because methanogens are often attached to the surface of or are endosymbionts within rumen ciliate protozoa (McAllister and Newbold 2008b).

Monensin is a polyether ionophore antibiotic that decreases the acetate-to-propionate ratio in the rumen, effectively decreasing CH_4 production. The effect of monensin on lowering CH_4 production appears to be dose-dependent, with lower doses (10-15 ppm) producing a profitable milk response but showing no effect on CH_4, but with higher doses (24 to 35 ppm) reducing CH_4 production by up to 10% (g/kg DMI). However, there have been questions over the persistence of CH_4 suppression, plus questions over the future use of antibiotics in animal production systems (Eckard et al 2010).

The methanogenic bacteria, which are highly efficient scavengers of hydrogen, are the main, but not the only, agents for converting hydrogen to methane in the rumen. There is also evidence that the rumen can function satisfactorily the absence of methanogens. There are many potential opportunities for mitigating methane through microbial intervention in the rumen such as: targeting methanogens with antibiotics, bacteriocins, or phage; removing protozoa from the rumen; development of alternative sinks for hydrogen such as reductive acetogenesis. Acetogens do not compete well in the rumen compared to methanogens, so experiments are in progress to see if the microbial ecosystem can be manipulated to enhance acetogen

activity. One strategy is to genetically modify acetogens so that they can competemore effectively in the rumen (Ulyatt and Lassey 2001).

There are a number of mechanisms that can affect the rumen fermentation process within the animal that subsequently will reduce enteric CH_4 emissions. A number of recent reviews on this subject (Boadi et al 2004; Ominski and Wittenberg 2006) are available that evaluate the pros and cons of the addition of fats to cattle diets, ionophores, defunation, bacteriocins, probiotics, and use of alternative hydrogen acceptors or sinks (e.g., organic acids: malate and fumarate etc) to mitigate CH_4 emissions (Iwaasa 2007).

Final Considerations

Carbon dioxide and methane are the main greenhouse gases related to animal nutrition and methane has greater global warming potential than carbon dioxide. Livestock, produced throughout the world, are an important agricultural product in virtually every country. Ruminant animals (particularly cattle, buffalo, sheep, goat and camels) produce significant amounts of CH_4 under the anaerobic conditions of the digestive processes. Domestic livestock produce 80-115 million tons of methane per year. Significant mitigation of greenhouse gas emission is a critical subject of biological, ecological and environmental research area in the world. Due to higher global warming potential of the methane, it is subjected to many studies in recent years. From the view point of methane emission, ruminant animals are consequential than that of other animal species owing to higher fermentation activities. Integral microbiology and biotechnology strategies in ruminant production such as animal breeding, genetic merit, bovine somatotropin (BST), unproductive animals, vaccination, immunization and biological control (bacteriophage, acetogenesis reductive), chemical defaunation; can be results in mitigating methane production. It is notable that, other than microbiology and biotechnology related strategies, three important strategies including nutritional, management strategies are required for controlling and decreasing methane emission.

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